THE HOST RELATIONSHIPS OF THE TICK *AMBLYOMMA VARIEGATUM* IN RABBITS AND CATTLE

By

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ABSTRACT

Repeated feeding of the different life stages of Amblyomma variegatum and Rhipicephalus appendiculatus on four groups of rabbits induced resistance in these animals. Resistance to the adults was manifested by reduced engorged weights, subsequent reduction in the egg-conversion factor and fewer ticks that were able to lay eggs. Similarly, resistant hosts allowed significantly less immatures to complete their engorgement and these also dropped with reduced engorged weights. The feeding of ticks was also greatly inhibited. Thus, some ticks were observed to die and shrivel while still on the hosts; others died immediately after detachment; while a third group fed partially but were unable to complete their blood meal or detach as the mouth-parts were masked by host scar tissue. It was also found that 72% of nymphs fed on resistant animals dropped prematurely, with body sizes in feeding categories N2 and N3, and only 28% were able to complete their blood meal and reach N4 (engorged). Resistance in rabbits also seem to interfere with some vital physiological processes in the tick and possibly inhibited some factors or hormones which are responsible for the scutal pigmentation pattern. Thus, two additional spots were regularly observed on male A. variegatum which had fed as nymphs on resistant animals.

High levels of intra-specific cross-resistance between the life stages of ticks have been demonstrated for the two genera. It was also demonstrated that rabbits sensitized to R. appendiculatus showed appreciable level of resistance to A. variegatum nymphs. However, R. appendiculatus appeared unaffected in its ability to engorge on A. variegatum resistant rabbits.

Cattle also became resistant to R. appendiculatus after repeated infestations. They also showed considerable resistance to A. variegatum nymphs. Although these results substantiate the findings from rabbits, data from control cattle susceptible to ticks would be required to confirm these observations fully.

Antibodies have been demonstrated in the serum of all animals within the four groups of rabbits which had been exposed to natural tick feeding. The immuno-diffusion tests also demonstrated intra-specific cross-reactivity. Thus, sera from rabbits infested with adults nymphs, and to a lesser extent larvae, reacted to give intense precipitin lines against various adults organs (salivary glands, midgut, ovaries, whole internal organs) and whole nymphal and larval extracts. However, the test did not reveal detectable inter-generic cross-reactivity between these two species of ticks.

The histophathological studies on the attachment sites of nymphs, 48 hours post attachment, have revealed differences between the three groups of rabbits which were related to the nature of their resistance. Thus, rabbits which were made resistant to A. variegatum and R. appendiculatus by repeated infestations showed epidermal vesiculation and significant mobilization of eosinophils at tick feeding sites, which was not apparent with tick-naive rabbits. The feeding of A. variegatum nymphs on rabbits resistant to R. appendiculatus produced a similar type of reaction. On the other hand, the cellular response and tissue reaction of R. appendiculatus in the skin of rabbits resistant to A. variegatum was negligible.

Paralysis in rabbits due to feeding of adults and nymphs of A. variegatum was observed. However, removal of the feeding ticks after the symptoms started to appear caused them to regress.

Abnormal individuals of A. variegatum were identified in culture and their characteristic features, anatomy and life cycle are described.

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INTRODUCTION

Ticks are members of the class Arachnida which also includes the spiders, scorpions and mites. In this class ticks and mites are grouped together in the order Acarina. Ticks belong to the superfamily Ixodoidea with 3 families: Argasidae (soft ticks) with about 150 species in 5 genera, Nuttallielidae with one genus and one species and Ixodidae (hard ticks) with about 650 species in 4 subfamilies and 13 genera (Hoogstraal, 1976). Nine genera of the family Ixodidae are recorded in Kenya (Walker, 1974). These are Amblyomma (11 species); Aponomma (3 species); Boophilus (2 species); Cosmiomma (1 species); Dermacentor (1 species); Haemaphysalis (7 species); Hyalomma (5 species); Ixodes (20 species) and Rhipicephalus (23 species).

1. ECONOMIC IMPORTANCE OF TICKS

The role of ticks in the human economy merits special consideration, for not only are they annoying pests, but in both the temperate and the tropical countries they surpass all other arthropods in number and variety of diseases which they transmit to man and his domestic animals (Arthur, 1962). Ticks and tick-borne diseases cause enormous economic losses even in developed countries. In the USA, it was estimated that Boophilus annulatus caused losses in excess of US $ 130 million, and in 1965 even after eradication of B. annulatus, losses in production of cattle due to Amblyomma, Dermacentor and other tick species were calculated to be

US $ 60 million. In Australia in 1972-73, losses caused by Boophilus microplus to the cattle industry were calculated to be one million dollars (FAO, 1977). In developing countries where there are plans for importing exotic breeds of cattle, or of distribution of cattle of high quality from well managed ranches into tick infested areas they can still have considerable problems from ticks and tick-borne diseases. There are many examples of the disastrous results of importation or distribution when no efforts are made to anticipate and prevent the massive losses from theileriosis, babesiosis and cowdriosis that can occur (Latif, 1985). It is a waste of time and resources to import or distribute disease-susceptible cattle, unless the operation is supervised closely by competent staff who can immediately give appropriate treatment. However, even when such treatment is available, it is often too late (Barnett, 1961). Therefore no importation should be made unless it has been preceeded by a thorough evaluation of the situation regarding ticks and tick-borne diseases of livestock (FAO, 1976).

Interest in arthropods as carriers and transmitters of disease organisms has long overshadowed the equally, and sometimes more, important fact that these arthropods themselves can produced pathological changes in their hosts without transmitting any organisms (Gaafar, 1972). The effect of tick infestation on cattle has been studied by many workers (Francis, 1960; Little, 1963; Johnston and Haydock, 1969; Williams et al., 1977; 1978). The mean daily losses in livestock gain of steers, from a cross between Bos

indicus and Bos taurus infested with B. microplus in an experiment inQueensland were 0.72g/engorged female tick in summer, 0.47 in autumn and winter and 1.52 in the following summer (Sutherst et al., 1983). These losses were independent of tick density. The loss per tick was unaffected by a dietary supplement of molases and urea. Mellor et al. (1985) did not find any differences in cummulative weight gain over a period of 24 weeks but animals free of ticks performed significantly better during the first 12 weeks of the experiment.

2. CONTROL OF TICKS BY CHEMICALS

Chemicals have been used in routine tick control for many years. Dipping of livestock in acaricides has been the traditional method for control. The method is easy to operate and while the dips need little maintenance, they can also be used continuously for a longer period and larger groups of animals. On the other hand, hand spraying by a skilled operator is the most efficient form of application, but it is slow and also the most expensive as it is usually impossible to collect and re-use the acaricide. A mechanical spray race has the advantage of being more rapid. The acaricide can be freshly prepared (thus avoiding loss of activity) and in the event of resistance it is easy to change from one chemical to another. The disadvantage of the method is that maintenance problems and non-availability of spare parts render its recommendation difficult in developing countries (Tatchell, 1981).

The cost of acaricide and labour, the rapidity with which resistance has developed to new acaricides in recent years, the increased cost involved in the testing, development and marketing of a new product and finally the chemical hazards all create serious difficulties in the long term usage of chemicals to control ticks. When a tick population is subjected to chemical control only the most resistant individuals survive, but these reproduce each time, to form an even more resistant population. Resistance of ticks to chemicals has been reviewed by Wharton and Roulston (1970). Arsenical dips were the first to be used and they were highly effective against cattle ticks but in many countries this is no longer so. In South Africa resistance appeared after 50 years of arsenic usage; it appeared in 1963 in Rhodesia (Zimbabwe) and was confirmed in 1969 in Malawi (Jones-Davis, 1972). Lindane, malathion, BHC and DDT were also effective against ticks and their use was widespread until the ticks also became resistant to them in many places. Australia has fared worst with sequential development of resistance in Boophilus to arsenic, DDT, BHC and the organophosphates, to the point where they are of little value over large areas (Wharton, 1979). If acaricides continue to be employed in the same way as they have been in the past, it is likely that resistance will develop against whatever acaricide is used to control B. microplus (Anon, 1973).

3. ALTERNATIVE METHODS FOR TICK CONTROL

3.1 Pasture Spelling

Prevention, or sufficient delay, in host finding by ticks results in their dehydration to death. In Australia rotational grazing is practised to control B. microplus and has been called "pasture spelling" (Wilkinson, 1957; Wharton et al., 1969). Pasture spelling offers an alternative approach for owners of B. taurus cattle to control ticks without complete reliance on acaricides (Anon, 1973). Though technically efficient, pasture spelling for tick control has had limited appeal for a number of reasons, including the cost of additional fencing and watering facilities and the fact that pastures not grazed for several months may deteriorate and become less productive.

3.2 Habitant Modification

The unfed stages of ticks, particularly the eggs, need ready access to a humid microclimate. Reduction in bushes, trees and the mat of vegetation is likely to reduce tick numbers (Hair and Howell, 1970). This can be achieved by burning of pastures, heavy grazing and by clearance and drainage in some cases. These measures can be used to reduce a tick population on a local scale

if there is good policy restricting livestock movement, but are more difficult to apply on a large scale especially where cattle are kept on open range.

4. NOVEL METHODS FOR TICK CONTROL

4.1 Genetic Control

Genetic control methods of arthropods, such as cytoplasmic incompatibility, hybrid sterility, distorted sex ratio and lethal factors if employed could play a role in integrated control programmes (Knipling et al., 1968). Interspecific crosses between B. annulatus and B. microplus produce viable F1 progeny in which all males are sterile (Graham et al., 1972; Thompson et al., 1981), but females are fertile when backcrossed to the male parent species. However, the eggs resulting from F1 brother and sister matings generally did not hatch (Graham et al., 1972). On the other hand, the progeny resulting from an F1 female backcrossed to parent male consisted of sterile males but fertile females and this pattern continues for several generations. These sterile males mate with a greater number of females on the host (average 26.2) than fertile males (average 13.2). Moreover, they have longer survival times. These two factors indicated that they could successfully be used in a sterile male release programme (Davey et al., 1983). Spickett and Malan (1978) also showed that females of

the South African strain of B. microplus when mated with males of an Australian strain of B. microplus yielded 62% of viable progeny, where as the reciprocal cross produced non-viable larvae. The potential of sterile hybrid Boophilus ticks as a supplementary eradication technique has been discussed by Osburn and Knipiling (1982).

4.2 Pheromones

Pheromes are chemicals released by an animal to influence the behaviour of other individuals of the same species (Karlson and Luscher, 1959). Pheromones, in particular aggregation - attachment pheromone which are known only from Amblyomma spp., have been used in tick control programmes. These species specific compounds are active only during the parasitic phase, attracting unfed ticks to the feeding sites. Gladney et al. (1974), Rechav et al. (1977), Rechav and Whitehead (1978; 1981) and Sonenshine et al. (1979) applied aggregation - attachment pheromone baited acaricide to single locations on bovine hosts. Ticks released on the animal were attracted to this site where they attached and got killed. In another study, high pheromone concentrations saturating the environment confused the sexually mature adults to such as extent that they were unable to locate or to contact natural pheromone donors (Sonenshine et al., 1979). As a further strategic measure for tick control, which at the same time permits population dynamic surveys is the pheromone traps on the ticks

habitat. Ticks are then trapped even before an infestation occurs (Rechav et al., 1977; Rechav and Whitehead, 1978). Refinement of these methods may make it possible to kill attracted ticks before they attach, thereby preventing transmission of diseases. One drawback of such a technique is that the species specificity of each aggregating pheromone for a target species limits the range of use of the pheromone baited acaricide system.

Sex pheromones are also active during the parasitic phase of adult life. These compounds are phenolic in nature (2, 6_dichlorophenol) and most are produced by the female to attract the male. This compound has been identified for R. appendiculatus (ICIPE Annual Report, 1983, 1984). Although promising results have been achieved with mixtures incorporating aggregation - attachment pheromone for control of Amblyomma spp., mixtures including the sex pheromone are less effective and failed to prevent mating (Sonenshine et al., 1982).

5. BIOLOGICAL CONTROL OF TICKS

5.1 Anti-tick Grasses

Resistant varieties of crops have been widely used to control phytophagous insects (Norris and Kogan, 1980). Unfed stages of most species of hard ticks ascend plants to gain

access to a passing host, which is often infrequent. Thus, the ticks must often wait in the pasture for long periods (up to one year). A substantial reduction in the life expectancy of such ticks would lower populations of the parasitic stage. Molasses grass Melinis minutiflora and gamba grass Andropogon sp. have been shown to reduce tick survival with low infestation on cattle (Thompson et al., 1978). Some highly productive, nutritious varieties of the tropical pasture legume Stylosanthes sp. are covered with glandular trichomes or hairs which secrete a viscous fluid. These legumes produce sticky secretions that immediately immobilize larvae of B. microplus (Sutherst et al., 1982). Similar results have also been obtained with A. variegatum (Zimmerman et al., 1984). The tick larvae were poisoned within 24h by an unidentified vapour from the secretions. If these results are reproducible in the field, it may be possible to substantially reduce tick populations throughout much of the tropics and subtropics. The legumes not only trap questing ticks but also improve cattle nutrition, a factor which is important in their immune response to ticks (Sutherst et al., 1982).

5.2 Parasites and Predators

While parasitic wasps (Hunterellus spp.: Hymenoptera) of ticks do occur, the efforts to use them as biological control agents in the United States were unsuccessful (Anon, 1973). In the same report ants were found to be predators of

engorged ticks in some areas but their use as biological control agents appears to be unpractical and undesirable. The oxpecker or 'tick bird' (Buphagus spp.) in Africa does not control ticks effectively. They feed on ticks on the host, but also consume blood and scabby material from any superficial wounds and probaby exacerbate such injuries.

5.3 Tick Resistant Cattle

It has been recognized for many years that some individual animals, or whole breeds, kept under similar conditions, consistently carry fewer ticks than others (Roberts, 1968a; Wagland, 1975). It is now known that such differences are caused by variation in their ability to respond immunologically to tick infestation (Roberts, 1968b). Host resistance is accepted as the most significant parameter that could be utilized to manage B. microplus (Wharton, 1974). The broad picture of resistance is that the ability to develop it is heritable (Hewetson, 1972; Seifert, 1984) and therefore the actual manifestation is acquired (Riek, 1962; Roberts, 1968a). It is relatively stable over longer periods, although stresses such as lactation or sickness cause a drop in resistance (Wharton et al., 1970; Seifert, 1971; Utech et al., 1978a). A wide range of resistance occurs in all breeds, but it is expressed most strongly in Zebu cattle and their crosses (Riek, 1962; Wilkinson, 1962; Wharton et al., 1969; Seifert, 1971; Hewetson, 1979).

The improved tick control following the use of tick resistant cattle has been demonstrated on different breeds of cattle and crossbreeds (Riek, 1962; Wilkinson, 1963; Little, 1964). Kelly (1943) suggested that the tick resistant quality of Zebu cattle should be utilized for tick control. Moreover, a cross between Zebu and British cattle was shown to carry fewer ticks and require less dipping than British cattle on similar pastures (Wharton et al., 1969).

The Australian pest management approach has stimulated other workers elsewhere to investigate the tick-host relationship. Strother et al. (1974) and Garris et al. (1977) compared the level of resistance in 2 breeds of cattle. Both the Brahman and European breeds acquired a high level of resistance to the lone star tick, Amblyomma americanum, by the third infestation. Resistance was manifested by significant reduction in number and weight of replete female ticks. Similar results were found with purebred and crossbred B. indicus calves (Brown et al., 1984; George et al., 1985). Comparisons of the natural tick burdens between different breeds of cattle or within individuals of the same breed revealed two important features. Firstly, resistant cattle respond to attaching ticks of different species and stages by rejecting them (Kaiser et al., 1982; Latif et al., 1984a). Secondly, 9-25% of a herd was found to carry 50% of the total tick burden

borne by the herd (Kaiser et al., 1982; Latif, 1984b). Latif (1984b) confirmed this natural resistance to tick infestation in cattle by artificially infesting them with two tick species.

Cattle have been shown to respond with varying degree of immunity to other tick species like Ixodes holocyclus (Doube and Kemp, 1975), Haemaphysalis longicornis (Sutherst et al., 1979; 1983) and R. appendiculatus (Newson et al., 1980; Fivas et al., 1984). The relevance of the Australian tick control approach in Africa was highlighted by Sutherst (1981).

5.3.1 Assessment for resistance

Johnson and Bancroft (1918; cited by Riek, 1962) gave the following criteria for the assessment of resistance in cattle to ticks; a failure of a particular species of ticks to complete their development, a tendency towards light infestation when other animals become heavily infested, the number of female ticks completing engorgement on resistant animals under similar conditions, is fewer than those from susceptible animals, and a failure of engorged ticks to either lay a normal number of eggs or to lay eggs of normal viability. Riek (1962) and Hewetson (1971) have since added two more criteria for defining resistance; an increase in the time taken for the female to complete the parasitic

lifecycle and a decrease in the mean weight of replete female ticks.

Resistance to B. microplus is manifested by rejection of larvae in the first 24h of the tick lifecycle (Roberts, 1968b). Similar results with the same tick on Zebu cattle were observed by Wagland (1979) and a gradual loss of ticks occurring throughout the instars was also noticed. The weights of fully engorged ticks were reduced on B. indicus immune hosts (Wagland, 1978) whereas those ticks which completed engorgement on pure B. taurus breed were significantly heavier and laid a greater number of eggs (Riek, 1962).

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

Responses of hosts to ectoparasites are recognized as immunoallergic in addition to being traumatic, toxic or directly irritant (Nelson et al., 1977). Various protective mechanisms against ticks have evolved amongst farm animals; the hairs prevent many arthropods from coming in direct contact with the skin surface; long wool and grease of sheep prevent the ticks, Rhipicephalus bursa and Hyalomma plumbeum, from attaching (Musatov, 1967). Cattle by active grooming can also limit the number of attaching ticks (Snowball, 1956; Bennett, 1969; Koudstaal et al., 1978). Rodents actively

clear their body of ticks by combing their skin with their claws or gnawing the ticks with their teeth (Musatov, 1967). The immunological responses of the host include the regional lymphnodes, antibodies, complement and hypersensitivity reactions (Gaafar, 1972).

5.3.2.1 Humoral response. Demonstration of ciruclating antibodies in host animals after injection of protein extracts or purified protein fractions is relatively easy. Such information provides little to explain the processes involved in the host animals with naturally acquired resistance (Nelson, 1977). The production of antibody in response to tick infestation has been reported in a number of cases. Partial immunity to Dermacentor variabilis larvae could be conferred by injecting immune serum into guinea pigs at the time of tick infestation (Trager, 1939a). As a result of this passive transfer of immunity Trager (1939a) observed, the number of ticks engorging was reduced by 50%. Similar results were demonstrated for Ixodes ricinus (Brossard, 1977), B. microplus (Riek, 1959) and H. leporispalustris (Boese, 1974).

Riek (1958) produced both humoral and homocytotropic (skin sensitizing) antibodies in mice, guinea pigs and rabbits by injection of Haemaphysalis

bispinosa egg-antigen. He also (1962) demonstrated homocytotropic antibodies in cattle against B. microplus. Although, Boese (1974) demonstrated homocytotropic antibodies in rabbits to H. leporispalustris he was unable to correlate the appearance of antibody with onset of resistance to tick feeding. Transfer of immune serum into recipient guinea pigs hours before infestation failed to confer any significant resistance to the feeding of D. andersoni larvae (Wikel and Allen, 1976a). The same authors found that if cyclophosphamide was given to immune guinea pigs before an infestation with ticks, then the expression of resistance was largely blocked (Wikel and Allen, 1976b). This is indirect evidence of the involvement of humoral component. Passive intravenous transfer of serum or peritoneal exudate cells from actively sensitized and challenged guinea pigs to naive recipients conferred significant resistance to Amblyomma americanum (Brown and Askenase, 1981; Brown, 1982; Brown et al., 1982b; Brown and Askenase, 1985). Similar antibody and cell-mediated immune resistance responses have been described in guinea pigs infested by R. sanguineus (Brown and Askenase, 1981), R. appendiculatus and I. holocyclus (Askenase et al., 1982).

There is also evidence for antibody involvement in cattle with B. microplus. Brossard (1976) found that serum gamma-globulin concentration increased significantly following tick infestation. The appearance of antibody coincided with the acquisition of resistance to the tick. Roberts and Kerr (1976) were also able to transfer significant levels of resistance to tick-naive cattle with large volumes of immune plasma.

The production of antibodies in response to tick infestation has also been reported by Kohler (1967); Weiland and Emokpare (1968); Schneider et al. (1971) and Fujisaki (1978). However, these workers did not relate the presence of circulating antibody to the onset of resistance or skin reactions.

5.3.2.2 Cell-mediated immune response (delayed hypersensitivity). Wikel et al. (1978) found that intradermal injection of 50 ug of salivary gland antigenic material from partially fed D. andersoni into tick resistant guinea pigs gave a significant delayed hypersensitivity reaction, reaching a maximum by 48 hours after the injection. Tick-naive controls showed only little reaction. Lymphocyte blastogenesis stimulated by the same antigen gave further evidence of hypersensitivity. The peak lymphocyte response was observed during the second infestation. Wikel and Osburn

(1982) in a similar experiment with cattle obtained similar results. Several successful attempts have also been made to tranfer immunity with cells from lymphnodes and perineal exudate (Bagnall, 1978; Wikel, 1976; Wikel and Allen 1976a; McTier et al., 1981; Brown, 1982; Brown and Askenase, 1985).

5.3.2.3 Immediate hypersensitivity. Resistant cattle exposed to B. microplus were intensely irritated by larvae; papular reactions were seen around nymphs and adults and there was a transient increase in blood histamine levels (Riek, 1956; 1962). Intradermal injection of eggs or larval antigens gave immediate oedematous dermal reactions (Riek, 1962; Binta and Cunningham, 1984). On intradermal injection of 3 purified allergens from unfed B. microplus larvae in sensitized cattle all gave oedematous reactions which reached a maximum after 20 minutes. There was no delayed response (Willadsen et al., 1978). The immediate response could be quantified by diluting the antigen and measuring the size of the resultant oedematous reactions. The sensitivity to allergen was correlated with the level of immunity; the higher the level of immunity the more sensitive the animal was to small amounts. It was mentioned earlier that tick-induced grooming activity is positively correlated to the level of immunity (Koudstaal et al., 1978). Injection of histamine beneath an attached tick or its natural release from highly resistant animals will cause earlier detachment of B. microplus larvae (Kemp, 1978; Kemp and Bourne, 1980). Cellular responses

after 3 hours of larval attachment on resistant animals were aggregation of eosinophils and mast cell degranulation beneath the site of tick attachment. This picture is typical of an immediate hypersensitivity reaction (Schleger et al., 1976). Immediate hypersensitivity reactions to other tick species have also been reported by Allen (1973), Boese (1974) and Allen et al. (1979).

5.3.2.4 Skin cellular reactions of tick bite. Histological examination of the host skin at the site of tick attachment is very useful in elucidating the feeding mechanisms of the ticks, the extent of host tissue damage (mechanical or immunological) and the nature of the host response, including changes after intermittent or continuous exposure of the host (Nelson et al., 1977).

The pattern of inflammatory cells invading the tick feeding lesion varies depending on the species of tick and host, the time after attachment, and whether or not the animal is sensitized. Nelson et al. (1977) reviewed the histopathology of tick bite reactions and speculated on the nature of resistance. The main cells found in the early lesion are neutrophils, which may be found either occluding or cuffing the venules and may be accompanied by varying

numbers of lymphocytes and eosinophils. As feeding progresses, neutrophils invade the tissue beneath the hypostome. Lysis of collagen fibres beneath the hypostome follows neutrophil invasion, resulting in the formation of a cavity.

Working with guinea pigs, Trager (1939b) came to the conclusion that resistance to D. variabilis larvae and nymphs was due to walling off the feeding lesion by rapid regrowth of the hyperplastic epidermis beneath it. Masses of inflammatory cells were trapped within the epidermal vesicle. Riek (1962) suggested that acquired resistance of Shorthorn cattle to B. microplus was associated with a strong hypersensitivity response in the skin. The number of eosinophils in this case was increased. Schleger et al. (1976) also reported a significant difference between susceptible and resistant cattle. He found that the degree of mast cell disruption, eosinophil concentration and degranulation, and the extent of epidermal vesiculation were all significantly greater at the site of attachment on highly resistant cattle. With guinea pigs, resistance to D. andersoni has been characterized by predominance of basophils in the vesicle and the reaction is typical of cutaneous basophil hypersensitivity (Allen et al., 1973). Rabbits resistant to R. appendiculatus develop epidermal hyperplasia and eosinophil infiltrates (Rubaire-Akiki and Mutinga, 1980) while in guinea pigs the lesions were characterized by basophilia and the eosinophils became prominent in the lesion later (McLaren et al., 1984). The recruitment of eosinophils and basophils from blood to the tissue site of tick feeding may contribute to the resistance mechanism (Brown and Askenase, 1982).

5.3.3 Artificial induction of host resistance to Ixodidae.

Immunomanipulation of the host animal to induce resistance to tick infestation has a potential role in integrated pest management schemes (Wikel, 1984). Artificial immunization to prevent tick infestation has recieved the most attention.

By immunizing guinea pigs with D. variabilis larval extracts, Trager (1939a) was able to induce partial protection against infestation by these ticks. Gregson (1941) infested two guinea pigs with D. andersoni nymphs and one of these animals was subsequently given a subcutaneous injection of nymphal extract. The infested and immunized animal when challenged with nymphs allowed fewer nymphs to engorge. Whole egg extracts of H. bispinosa and B. microplus have been used to immunize laboratory animals and cattle respectively. The protection against ticks was not as great as that induced by natural tick challenge (Riek, 1958; 1962). Protein extracts of I. holocyclus larvae have also been used to immunize guinea pigs and the resistance obtained was manifested by 38-68% reduction in the number of ticks successfully engorging. Different preparations of tick antigens have been used by different workers to immunize animals e.g. salivary gland extract antigen from partially

fed ticks (Brossard 1976; Wikel, 1976; 1981;Brown et al., 1984), extracts of whole tick homogenates (McGowan et al., 1981; Wilkinson and Allen, 1983), extracts from tick internal organs (Allen and Humphreys, 1979) and midgut extracts (Allen and Humpreys, 1979; Ackerman et al., 1980).

Attempts to artificially immunize animals against tick infestation have achieved partial success and the number and tissue source of antigens from the tick is not yet well known (Willadsen, 1980). Recently, however, Brown et al. (1984) have recognized a 20,000 m.w. protein derived from A. americanum salivary glands as being responsible for the induction and, perhaps, elicitation of host immune resistance responses to the tick. Better results by immunization against ticks can be obtained from using pure protein preparations rather than crude protein extracts from whole ticks (Nelson et al., 1977). Concentration of antigens, selection of adjuvant and route of administration are all critical factors in the induction of resistance (Wikel and Allen, 1981). Lastly, application of modern immunological and molecular biological techniques will facilitate these studies (Wikel, 1982).

6. OBJECTIVES

The two most important tick species in Kenya and Sudan are, R. appendiculatus and A. variegatum. Both species cause costly losses for the livestock industry. The present study deals with some aspects of their host relationship in laboratory animals.

Amblyomma variegatum, a three-host tick, is commonly found on cattle. Nymphs feed on moderate-size animals and large animals, while larvae attack mostly birds, small mammals and goats (Hoogstraal, 1956). Adults and nymphs are normally found on the udder, scrotum, flanks, dewlap, axillae and brisket, while larvae feed on the ears and heels of the host. The majority of records for this tick came from forests, woodland, bushland, bushed grassland and grasslands which are relatively moist, i.e. the mean annual rainfall in these places is 500 mm or more (Walker, 1974). The tick is very prevalent throughout Western and Nyanza Provinces of Kenya. In Sudan it is also considered as a very important species on cattle. It is prevalent in the south of Sudan, Blue Nile Province in the north and Kordofan and Darfur Provinces in the west (Hoogstraal, 1956). Amblyomma variegatum is regarded as the most efficient vector of Cowdria ruminantium, the causal organism of heart water in ruminants. The tick also transmits Theileria mutans and Nairobi sheep disease virus. Due to its long mouth-parts, feeding behaviour and/or host reaction, the tick causes severe inflammation and large abscesses and damage to udder

quarters. Hence it is considered as the most important species of Amblyomma infesting livestock in Kenya.

Rhipicephalus appendiculatus is the most important species of tick in Kenya since it is the principal vector of Theileria parva, the causative agent of a killer disease of cattle, East Coast fever (ECF). It is found in the southern corner of the country, in the Rift Valley, around Mt. Kenya, Nairobi areas, Kitui District and along the coast (Walker, 1974). Over 80% of cattle are believed to be within these areas. The northern limits of ECF are in the Equatorial Province of the Sudan. Morzaria et al. (1981) found that the ECF infection rate in cattle in some areas in that province ranged from 12-100%. All areas surveyed showed the presence of R. appendiculatus. Interestingly, two Amblyomma species were also present in these areas i.e. A. variegatum and A. lepidum.

Control measures such as regular dipping, spraying or immunization have never been practiced in the Sudan against ticks and tick-borne diseases. Hand dressing using BHC (Gammatox) is practised in individual cases. The method for ECF control in Kenya is the close interval application of acaricides to cattle in dips or sprays. This rigorous usage of acaricides has resulted in acaricide resistant ticks. Besides the cost of this control system, dipped cattle remain completely susceptibe to both ticks and tick diseases.

The results of the studies on host parasite relationship of Sudanese cattle and ticks confirmed that cattle are able to acquire a significant degree of resistance to ticks, and indigenous breeds do so more effectively than exotic, crossbred cattle, although some of the latter are highly resistant (Latif, 1984a). Therefore, in the Sudan where the cattle population is not exposed to chemicals, the future for tick control is promising if balanced and ecologically sound methods of control are used. An integrated tick control programme should now be considered in Kenya to reduce the costs of dipping and eliminate the danger of heavy losses of cattle if chemical control breaks.

The integrated pest management approach requires a thorough knowledge of the host-parasite relationship. Rhipicephalus appendiculatus is a well studied tick in Kenya while A. variegatum is the least. Lutu (1982) and De Castro et al. (1985) showed the effect of repeated infestations of R. appendiculatus on cattle. Newson (1978) studied its seasonality while others investigated its host resistance in rabbits (Branagan, 1974; Rubbaire-Akiki and Mutinga, 1980). Resistance of cattle and laboratory animals to A. variegatum is not well studied. Varying results have been obtained with other Amblyomma species.

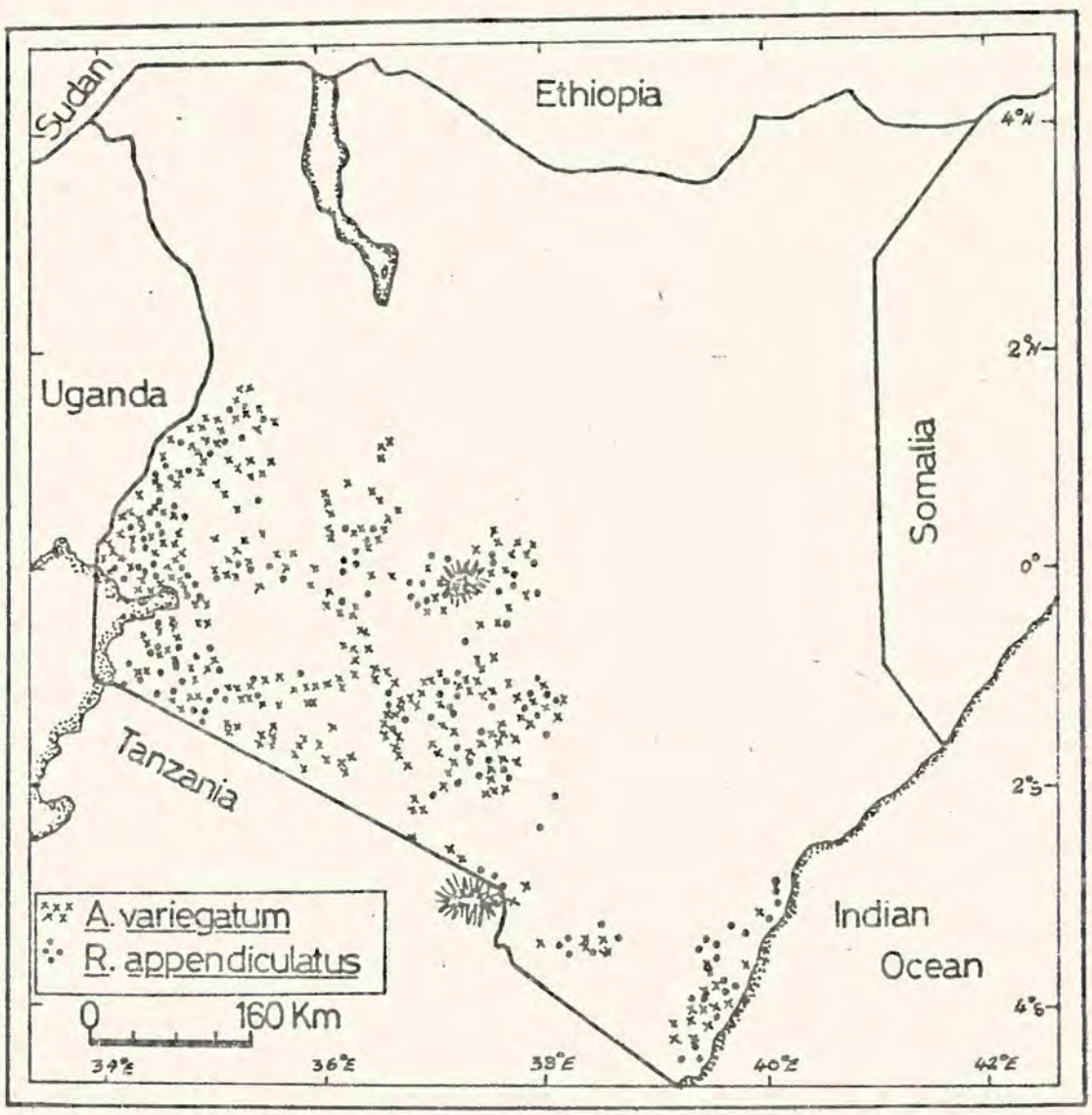
Infestation with nymphs of A. variegatum and A. hebraeum on laboratory animals conferred resistance to a second

infestation with the same species (Heller-Haupt et al., 1981). Norval (1978) found different results with A. hebraeum. Sheep and rabbits were unable to acquire resistance to the immature stage of the tick, even after repeated infestations. Guinea pigs acquire a significant level of resistance to the feeding of A. americanum after a single infestation (Brown and Askenase, 1981; Brown and Knapp, 1981; Brown, 1982). However, in another study, the same laboratoryanimals showed considerably less resistance to the same tick species the third time they were exposed (McTier et al., 1981). Norval (1978) suggested that ticks with long-mouth parts e.g. Amblyomma, generally do not evoke host response and fail to induce resistance. Literature on the host immune response of Amblyomma and Hyalomma species is scanty with the exception of A. americanum.

The purpose of the present study, therefore, was to investigate whether rabbits could acquire resistance to A. variegatum through natural tick feeding. If so, to study the host-immune response. Amblyomma variegatum and R. appendiculatus coexist (Fig. 1) under natural conditions (Morzaria et al., 1981; Walker, 1974).). It was very important to know if the acquired resistance to one tick species was specific to that species or if there was any cross-resistance between the two species. With these questions in mind, this work was started with the following specific objectives:

Fig. 1

Map of Kenya showing distribution of Amblyomma variegatum and Rhipicephalus appendiculatus



Modified from J.B. Walker (1974)

a) To induce resistance in rabbits to A. variegatum and R. appendiculatus by repeated infestation of all instars.

b) To study possible cross-resistance between the tick instars within one species and between the two tick species.

c) To study the cellular responses in the skin, and humoral antibody response in tick-naive and resistant rabbits when infested with the two species of at the same time.

GENERAL MATERIALS AND METHODS

1. BIOLOGICAL MATERIALS

1.1 TICKS

1.1.1 Tick Colony

Amblyomma variegatum and R. appendiculatus came from a disease-free laboratory colony which has been maintained at the Veterinary Research Department, Kenya Agricultural Research Institute, Muguga. All tick stages were fed on tick-naive rabbits.

1.1.2 Tick Breeding

1.1.2.1 Temperature and humidity

The tubes containing A. variegatum were put in "Kilner" preserving jars. The bottom of each jar was lined with a layer of 5 cm of plaster of Paris moistened with physiological saline. This prevented fungal and bacterial growth inside the container and when sealed by screwing on the lid it provided a relative humidity of more than 90%. They were stored in an incubator at 28°C. Tubes containing R. appendiculatus were placed in a dessicator over saturated potassium chloride solution to give a relative humidity of approximately 85% (Winston and Bates, 1960), and were stored also at 28°C.

1.1.2.2. Engorged stages and eggs

Each engorged female tick was put in a 7.5 x 2.5 cm flat bottomed glass tubes, or a plastic vial 7.5 x 5 cm, containing a U-shaped piece of filter paper which facilitated removal of the egg batch. These tubes and vials were covered with a layer of muslin gauze. The eggs layed by three or four females were mixed, then divided into 0.15 g aliquots and put into 7.5 x 1.9 cm glass tubes. The tubes were plugged tightly with cotton wool wrapped in gauze. Engorged larvae were treated similarly in batches of 100 in 7.5 x 1.9 cm flat bottomed tubes and engorged nymphs were kept in groups of 50 in 7.5 x 2.5 cm flat bottomed tubes. All moulting ticks and developing eggs were incubated at 28°C.

1.1.2.3. Unfed larvae, nymphs and adults

After emergence, larvae, nymphs and adults were stored at 18-22°C. They were kept in aluminium canisters at the bottom of which was a 10 cm layer of moistened sand. Humidity of the room in general was maintained at 70% by means of a humidifier (Defensor Model 550).

1.1.3. General handling of ticks

Handling and counting of eggs, engorged larvae and nymphs were done with a fine camel-hair water-colour brush. Egg batches were divided and transfered into tubes with a small spatula and a brush. Unfed larvae were not handled and it was convenient to transfer eggs (with numbers estimated by means of weighing),

into the tubes in which the hatched larvae could later be applied to the host. In a similar way, the unfed nymphs were not handled as it was easier to count the engorged larvae in the waxy stage i.e. 2-3 days post dropping. This ensured that only the healthy ones were put to moult. The dates of dropping, moulting, oviposition or hatching and of application to the host and days to engorgement were all recorded.

1.1.4. Preservation of ticks and measurements

Ticks were preserved in 70% ethanol for measurement. The ticks were mounted ventral side down on double-sided adhesive tape on glass slides. The length of each tick was measured from the anterior margin of the basis capituli to the posterior margin of the body. The mouth parts were omitted because they might be destroyed when removing the attached ticks. A dissecting microscope with a micrometer mounted in the eyepiece was used for measurements and the calibration of the ocular micrometer was done with a stage micrometer.

1.1.5. Weighing and counting

Ticks were weighed immediately after they had dropped. Engorged females, of both species, and nymphs of A. variegatum were weighed individually while the engorged larvae, of both species, and nymphs of R. appendiculatus were weighed in batches of ten and five respectively. Statistical analysis was later performed on the group data. Weights were taken with an electric balance (Mettler 163) sensitive to 0.0001g.

1.2. Experimental animals

1.2.1. Rabbits

These were either from the ICIPE colony or purchased from commercial breeders, and all were of the white New Zealand breed. They were caged separately and weighed 2.0 - 3.5 kg. They were maintained on rabbit pellets supplemented with green vegetables and carrots. Occasionally they were given prophylactic doses of a coccidiostat (Esb $\frac{R}{3}$- 30%, Ciba-Geigy) at 1g/litre in the drinking water.

1.2.2. Cattle

Bos taurus type of cattle, 1 1/2 - 2 years of age were used. They were pastured when not under experiment and were then dipped regularly in Delnav (Wellcome E.A. Ltd.). The animals were housed in indoor pens during experiments. Here the diet was hay, supplemented with concentrates.

1.3. Feeding of ticks

1.3.1. On rabbits

1.3.1.1. Ear-bags

Rabbits were infested with ticks according to the method of Bailey (1960), modified by Branagan (1974). The ticks were

contained in cloth sleeves glued with Evo-stick Impact Adhesive, Evode, Stafford, England) to the base of the ears. The outer end was opened daily to inspect the ticks and closed afterwards with two rubber bands. When it was open, the sleeve was shaken out into a polythene bag, or else into a white enamel tray, to collect all the detached ticks, or engorged. The outer ends of the two sleeves were also stuck together with adhesive tape (Leukoplast ^{R} Beiersdorf) and the rabbits found it difficult to remove them. The only preparation for the rabbit was to clip the hair on the ears, and the claws with scissors.

1.3.1.2. Capsules

These were used mainly to protect and restrain feeding A. variegatum adults. The covers of the leukoplast tape rolls (Leukoplast ^{R} Beiersdorf, 5 cm) which was fabricated out of sheet metal constituted the feeding chambers or capsules. These capsules measured 5 cm in diameter and 6 cm. in height. The hair on the container part of the rabbit's back was clipped with scissors and cleaned with 70% ethanol. Two holes, 5 cm diameter, were made in a piece of sponge 16 x 9 cm and two capsules were fixed over these holes with adhesive tape. The sponge, with two feeding chambers was glued to the prepared area on the rabbit. The free end of the capsules were covered with nylon gauze and fixed with adhesive tape. This would facilitate free ventilation since feeding ticks excreted water, during the last stage of

feeding, which tended to collect around the walls of the capsule. Engorged ticks could be removed by means of blunt forceps. Each capsule allowed the feeding of five females.

1.3.2. Feeding ticks on cattle

1.3.2.1. Earbags

The method was similar to that used for rabbits (Section 1.3.1.1.). The hair on the ears was clipped with scissors, and the skin was cleaned with 70% ethanol and followed by thorough washing with soap and water for two consecutive days to remove any residual acaricide.

1.3.2.2. Scrotal bag

This procedure was used mainly for partial feeding of adult A. variegatum needed in antigen preparation. Uncastrated bulls were used. The scrotum was washed thoroughly with soap and water for two days consecutively to remove any residual acaricide. A cloth sleeve was secured around the neck of the scrotum and used in exactly the same manner as on the ears (Section 1.3.1.1.).

1.3.2.3. Body tailored bag

The preferred sites for the feeding of A. variegatum adults on cattle are the dewlap, chest and axillae. A tailored bag was designed which enclosed these parts of the body (Fig.2).

Fig. 2. The tailored-bag which covers the neck, dewlap, the fore-arms and chest to restrain the feeding adults of A. variegatum. The arrows show the site of tick application and collection of engorged ones by releasing the adhesive tape and securing it.

It consisted of 2 sleeves, one for each forearm, and this part also covered the dewlap. Another part surrounded the chest and was fixed in position on the animal with Evostik. There were 2 flank patches which were stitched together, along the neck and back, with thin rope. The sleeves at the forearms were secured by adhesive tape to the upper part of the knee joints. Tubes containing ticks were introduced by releasing the tape at one forearm and the contents were released after refixing. In the same way the engorged ticks could easily be collected by introducing a hand through one sleeve and both sides were inspected. Feeding ticks could be felt through the cloth and their degree of engorgement could be assessed.

2. Histopathological reactions

2.1. Preparation of animals

Three groups of five rabbits each, were treated as follows; animals in group one were tick-naive rabbits, group 2 was sensitized to A. variegatum through three repeated infestations with 100 nymphs and rabbits in group 3 were sensitized to R. appendiculatus by three successive infestations with 100 nymphs.

2.2. Sampling

Animals in the three groups were left free of ticks for one month. Fifty nymphs of A. variegatum and 50 nymphs of R. appendiculatus were applied simultaneously and separately to each

ear of each rabbit from the three groups. The ticks were fed in ear-bags. The tubes and unattached ticks were removed after six hours. Samples were taken from both ears about 48 hours after tick application. The rabbits were killed by an intracardiac injection of 2 ml of sodium phenobarbitone (Sagatal, 200 mg/ml; M & B). Four to five ear biopsies with attached ticks were taken from each ear using a scalpel blade. The biopsies were trimmed to within 4 mm of the mouth parts of attached ticks and fixed in 2.3% or 2.5% glutaraldehyde solution buffered in sodium cacodylate (pH 7.4).

2.3. Paraffin embedding

The tissues were dehydrated through ascending concentrations of ethanol starting at 50% and through 70%, 80% and 90% and two changes of the absolute alcohol. Then they were transferred to methyl benzoate, cleared in two changes of xylene and infiltrated with paraplast (60^{o}C). These processes were carried in an automatic tissue processor. The tissues were removed from the clearing agent and placed in three successive baths of melted paraplast, being embedded and blocked in the third paraffin bath.

2.4. Sectioning

During embedding the tissues had been arranged to give saggital sections of tick mouth parts and transverse sections of the skin. The embedded tissues were soaked overnight in Mollifex (B.D.H.), with the side containing the tissue downwards. This

reagent makes the tissues softer so that they can be easily cut and flattened. The tissues were serially sectioned at 6-7 µm thickness using a rotary microtome (R. Jung-Herdelberge).

Microscopic slides were cleaned in 90% ethanol, dried and smeared with a glycerin-egg-albumin (one part to one part by volume) adhesive mixture. Individual ribbon sections were flattened by heating (45^{o}C) the slide bearing the section floated on distilled water. The slides were left to dry on the slide warmer (Fischer) overnight before staining.

2.5. Staining

2.5.1. Ehrlich's haematoxylin and eosin (H & E)

The staining essentially involved dewaxing for 10 minutes in 2 changes of xylene rehydrating in descending grades of ethanol and running water for 5 minutes, staining in Ehrlich's haematoxylin for 15 minutes, differentiating by immersing in acid alcohol, bluing in running water for 5 minutes, counter staining in 1% eosin for 3 minutes and rinsing in water to remove most of eosin. The specimens were then dehydrated by ascending grades of ethanol up to absolute, cleared in xylene (2 changes) and mounted using Distrene - Plasticezm-xylene (D.P.X.) as a mountant.

2.5.2. Ehrlich haematoxylin-eosin-azur II mixture

The mixture was made just before use by adding 10 ml of azur II slowly to 100 ml of 0.1% boiled eosin. The solution, ready for use, was deep violet. The sections were hydrated and processed the same as for H & E (Section 2.6.1) up to the bluing stage. The tissues were then left for 24 hours in the mixture of 0.1% azur II and 0.1% eosin. Dehydration and mounting were as outlined in section 2.5.1.

2.5.3. Toluidine blue

Sections were hydrated as described in section 2.5.1. and then stained in 1% toluidine blue for one minute and rinsed in distilled water. They were dehydrated in 95% and absolute ethanol, two successive changes of one minute each. The sections were subsequently cleared in xylene and mounted in D.P.X.

2.6. Microscopy

The following features were observed and recorded: the degree of cellular reaction in terms of cell number per 0.01 mm^2 of tissue, the degree of eosinophil degranulation, the magnitude of inflammation as measured by epidermal hyperplasia, proliferation and lengthening of hair follicles, presence or absence of vesicles and their contents.

2.7. Cell counting and micromeasurements

Cell counting and micromeasurements were carried out with a 100-squares Zeiss integrating graticule mounted in an eyepiece of magnification X10. The calibration of the graticule was done with a stage micrometer. From each rabbit five separated 6-7 µm thick sections, showing the mouthparts in a tick feeding lesion, were studied. In each section the cellular reactions in 5-8 fields of 100 squares each were recorded. These areas included the centre of the vesicle, its periphery and the area immediately outside the vesicle. In cases where the vesicle was not formed, then the areas immediately below the mouth parts were studied. The mean number of each cell type from the five fields of each of the 5 sections, was obtained. Epidermal thickness (hyperplasia) was measured in 20 squares of the graticule, 10 squares being on each side of the mouth parts. The number and length of hair follicles was similarly determined. Multiplying the mean of units by 0.01 gave the measurements in mm of thickness of epidermis and length of hair follicles.

In each group of rabbits comparisons were made between the intensity of tick bite reactions resulting from the two tick species i.e. A. variegatum and R. appendiculatus on the same animal. Comparisons were also made of the intensity of cellular reactions on tick resistant and tick-naive rabbits to the two species of ticks.

3. Immunological tests

3.1. Preparation of protein extracts and antisera

Protein extracts from the tick internal organs were prepared from partially fed (5-6 days) females A. variegatum and R. appendiculatus. Ticks were washed with water, dried, partially embedded in paraffin wax and covered with cold (4°C) phosphate buffer saline (PBS 0.01M sodium phosphate; 0.15M sodium chloride; pH 7.2). To dissect the internal organs, the scutum of embedded ticks was first cut along the lateral edge with a number II surgical blade. The midgut diverticulae were carefully dissected free from other tissues using watch-makers forceps and excised. The salivary glands were exposed and were also dissected out and the ovaries were removed separately. In another batch of ticks all the internal organs were removed without separating them. The dissected tissues were kept separate and held in ice-cold PBS. Proteins from all the tissues were extracted in cold PBS containing 50 mM glutathione and 20 mM aproteinin. The tissues were homogenized by hand using a glass homogenizer on ice or by Sorval® Omni-mixer fitted with microattachment (setting for 2 minutes with one minute interval). One hundred salivary glands were homogenized in 3ml PBS, 100 midguts in 3ml, 100 ovaries in 2 ml and internal organs of 25 ticks in 2 ml. The homogenates were

centrifuged at 12,000xg for 10 minutes at 4°C. The supernatants containing soluble proteins were collected and their protein concentration determined by the method of Lowry et al (1951) using BSA as standard. The protein samples were diluted to 1.0 mg/ml with PBS and stored at -20°C until used.

Antigens from whole tick homogenates were prepared from larvae and nymphs which had fed on rabbits for 3-4 days. Ticks were washed with water, PBS and then homogenized. The antigens were extracted in the same way as described above. The spinning in this case was done twice at 12,000 xg for 10 minutes each and the clear supernatants were collected and stored at -20°C.

Rabbits were bled for antisera each time they were infested with ticks. The blood was allowed to clot at room temperature for 1 hour and stored at 4°C overnight, after which the serum was collected. The serum was centrifuged at 10,000 xg for 10 minutes at 4°C to pellet the lysed cells. Sodium azide (0.05% w/v) was added to the sera and then stored at -20°C until needed. Sera were obtained from groups of rabbits infested separately with larvae, nymphs and adults of A. variegatum and from rabbits infested with the three tick stages of R. appendiculatus together with sera from rabbits infested repeatedly with nymphs.

3.2. Immunological tests

Ouchterlony's (1964) double immunodiffusion tests were performed using the tick antigen extracts and the various sera. Ten ml of 1.5% Noble Agar in PBS (pH 7.2) was poured on clean glass slides (9 x 6 cm). Five wells were cut in the solidified agar using a gel punch attached to a vacuum pump. The wells were filled with the tick antigen and the antisera from infested rabbits. The slides were placed in a humid chamber (Shandon) and incubated overnight at room temperature. They were transferred in physiological saline containing 0.02% sodium azide to remove residual proteins. This was repeated a number of times before washing with distilled water to remove crystallizable salts. The gels were dried on the glass slides followed by staining with Coomassie brilliant blue stain (G250-Sigma) for 30 minutes. The gels were destained in a solution of acetic acid and methanol in distilled water (8:25:67 v/v/v respectively).

4. Statistical analysis

Data were examined as means and as percentages. Analysis of data was carried out in a computer (Wang 2200 VP). The percentages were transformed using angular transformation (arcsine) tables. One-way analysis of variance (ANOVA) and two-way ANOVA were used. Correlation regression analysis was used to study the degree of covariance between tick weight, tick length and egg-batch weight.

CHAPTER 1

Resistance and cross resistance of rabbits to *Amblyomma variegatum* and *Rhipicephalus appendiculatus*: Tick feeding performance

Rabbits have been used frequently in laboratory studies of immunity to ticks, and in many cases they are capable of showing an effective response (Willadsen, 1980). Resistance to tick infestation was expressed by reduced numbers of ticks obtaining a blood meal and/or by reduced engorged weights, when compared to ticks infesting non-resistant control animals. Repeated infestations with *I. ricinus* produced a significant reduction the in percentage of ticks engorging, the engorgement weight of the females and the viability of the eggs (Bowessidjaou *et al.*, 1977). Similar results have been observed for *D. variabilis* (Trager, 1939a), *Hyalomma anatolicum excavatum* (Kohler et al., 1967), *R. sanguineus* (Garin and Grabarev, 1972) and *R. appendiculatus* (Branagan, 1974). Immunity to *H. longicornis* was reported by Fujisaki (1978) in which the engorged weights of female ticks were reduced. Norval (1978) found that sheep and rabbits were unable to acquire resistance to repeated feedings of *A. hebraeum* immatures but Heller-Haupt *et al.* (1981) demonstrated resistance to the same tick species in rabbits after a second infestation.

Guinea pigs have also been used as models to examine the mechanisms of immunity to ticks. These animals can acquired resistance to *A. americanum* after a single infestation (Brown and Askenase,

1981; Brown and Knapp, 1981; Brown, 1982) A single infestation of D. andersoni produced almost complete immunity as the percentage of larvae engorging was reduced to less than 20% (Allen, 1973; Wikel and Allen, 1976a). A single infestation with I. holocyclus on the same laboratory animals also conferred a high level of immunity to a second infestation (Bagnall, 1978).

Though livestock are subject to challenge by more than one tick species under field conditions, there are only a few cases in which cross-resistance between ticks has been studied (Trager, 1939a; Heller-Haupt et al., 1981; McTier et al., 1981). There is a close association in the field between A. variegatum and R. appendiculatus. Their host relationship has, therefore, been studied in detail but using rabbits instead of cattle.

MATERIALS AND METHODS

1. REPEATED FEEDS OF A. VARIEGATUM

1.1 Adult Feeding

Six rabbits were infested three times on the back. A fourth infestation was carried out on three of these rabbits. Three tick-naive controls were used with the second and third infestations. Twenty male ticks were applied in capsules and six days later ten females were also put to feed. During successive infestations males and females were continuously applied

until at least five females had attached and were feeding. The percentage of females engorging was therefore not recorded. The weight and length of the engorged, detached females were recorded. The engorged females were then kept individually and each egg-batch was weighed 18 days post oviposition.

1.2 Nymphal Feeding

A total of 200 nymphs was applied in bags fixed to both ears of 9 rabbits (100/ear). They had not experienced ticks feeding on them before. Six of them were infested repeatedly with the same number of ticks for five infestations. The weight and percentage of nymphs engorging were recorded.

1.3 Larval Feeding

Approximately 1500 larvae (emerged from 0.15 g of eggs) were applied on each ear of 5 tick-naive rabbits in ear bags. They were infested six times at 3 week intervals. The weight of the engorged larvae was recorded.

2. INTRA-SPECIFIC AND INTER-SPECIFIC CROSS RESISTANCE EXPERIMENTS

The two groups of rabbits infested repeatedly with the adults and nymphs of A. variegatum were challenged after the fourth

infestation with 1,000 larvae on one ear and 100 nymphs on the other ear. The group of rabbits which was infested repeatedly with nymphs and exposed to larval challenge was then also challenged with 100 nymphs of the same species on one ear and 100 nymphs of R. appendiculatus on the other ear. For all experiments, two or three tick-naive rabbits were used as controls and the nymphal and/or larval challenges were done simultaneously.

A group of five rabbits was exposed to 3 weekly infestations with R. appendiculatus larvae and then challenged with 20 males and 20 females of the same species. Adults were confined in capsules fixed on the backs of the rabbits. This group was later challenged with 1000 larvae of the same species. When rabbits in this group proved to be resistant to R. appendiculatus, they were then exposed simultaneously to 100 R. appendiculatus nymphs on one ear and 100 nymphs of A. variegatum on the other. Control tick-naive rabbits were used for all experiments.

3. GROWTH OF A. VARIEGATUM NYMPHS ON TICK-NAIVE AND TICK RESISTANT RABBITS

One hundred nymphs of A. variegatum were applied on each ear of 9 tick-naive rabbits. Ten nymphs were picked at random from one ear of each rabbit. They were weighed and then preserved in 70% ethanol for measurements. Ticks fed on the other ear were left undisturbed and their weights and lengths were taken after they detached. This procedure of infestation, weighing and measurement was also carried out on a group of five rabbits resistant to A. variegatum nymphs and on three tick-naive controls.

RESULTS

1. REPEATED FEEDS OF A. VARIEGATUM

1.1 Females

First Infestation: The mean engorged weight was 2.076 ± 0.660 g with an average length of 17.5 ± 3.2 mm. The mean number of days to engorgement was 16.5 ± 2.5 and the mean interval between dropping and the appearance of the first eggs was 10.7 ± 2.2 days (n = 40). The regression analysis of length versus weight in engorged females is shown in Fig. 3. These two parameters had a correlation coefficient of ± 0.9280 ($P < 0.001$). A similar regression analysis between egg-batch weight and engorged weight of female ticks is shown in Fig. 4. The correlation coefficient was + 0.9701 ($P < 0.001$).

Subsequent Infestations: The feeding performance for three successive infestations is shown in Table 1. The mean engorged weight on the experimental rabbits, was significantly reduced at each infestation ($P < 0.01$). There was no significant difference within the resistant group or the control group. In the second and third infestations the difference between the resistant and control animals was significant ($P < 0.05$ and $P < 0.01$, respectively).

A fourth infestation was attempted on three rabbits. From the first rabbit no ticks dropped and 2 partially ticks were pulled off the host on day 22. The mouth parts of these ticks were

Table 1

Effect of successive infestations of rabbits on engorgement weights (g) of female A. variegatum

Infestation no.	Rabbits						Group means	
							Treated	Controls
(Day)	1	2	3	4	5	6	(6)	(3)
1 (0)	1.10	1.91	2.54	2.93	1.64	2.29	2.07±0.66	
2 (80)	1.57	0.50	2.20	0.75	0.82	0.82	1.11^{a}0.64	2.68^{b}±0.63
3 (98)	0.60	0.89	1.08	0.34	0.38	0.61	0.65^{a}±0.46	2.46^{b}±0.14

- Means in a row followed by the same letter do not differ significantly

- Reduction in $\bar{X}$ with infestations significant $P<0.01$.

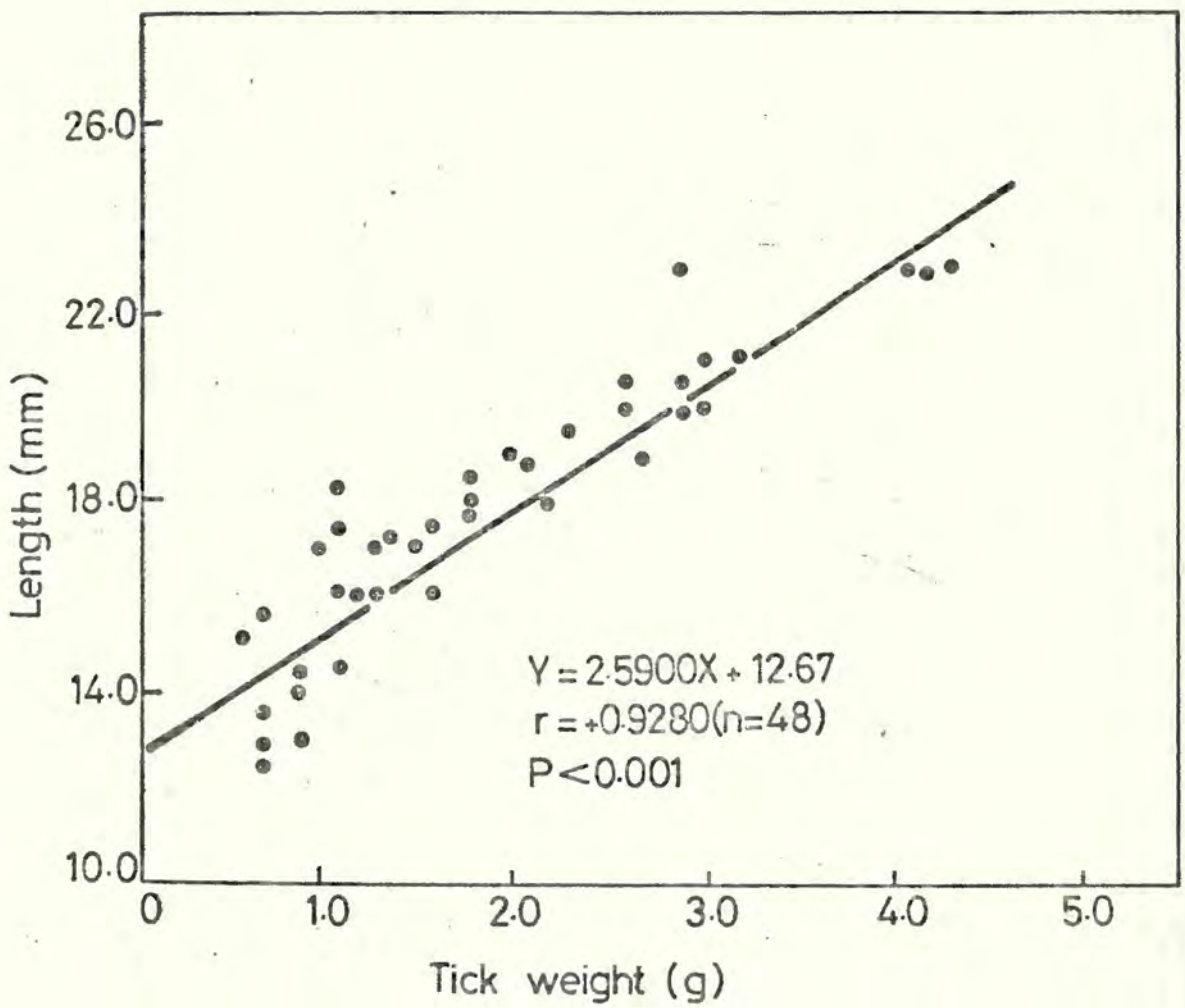
Fig. 15

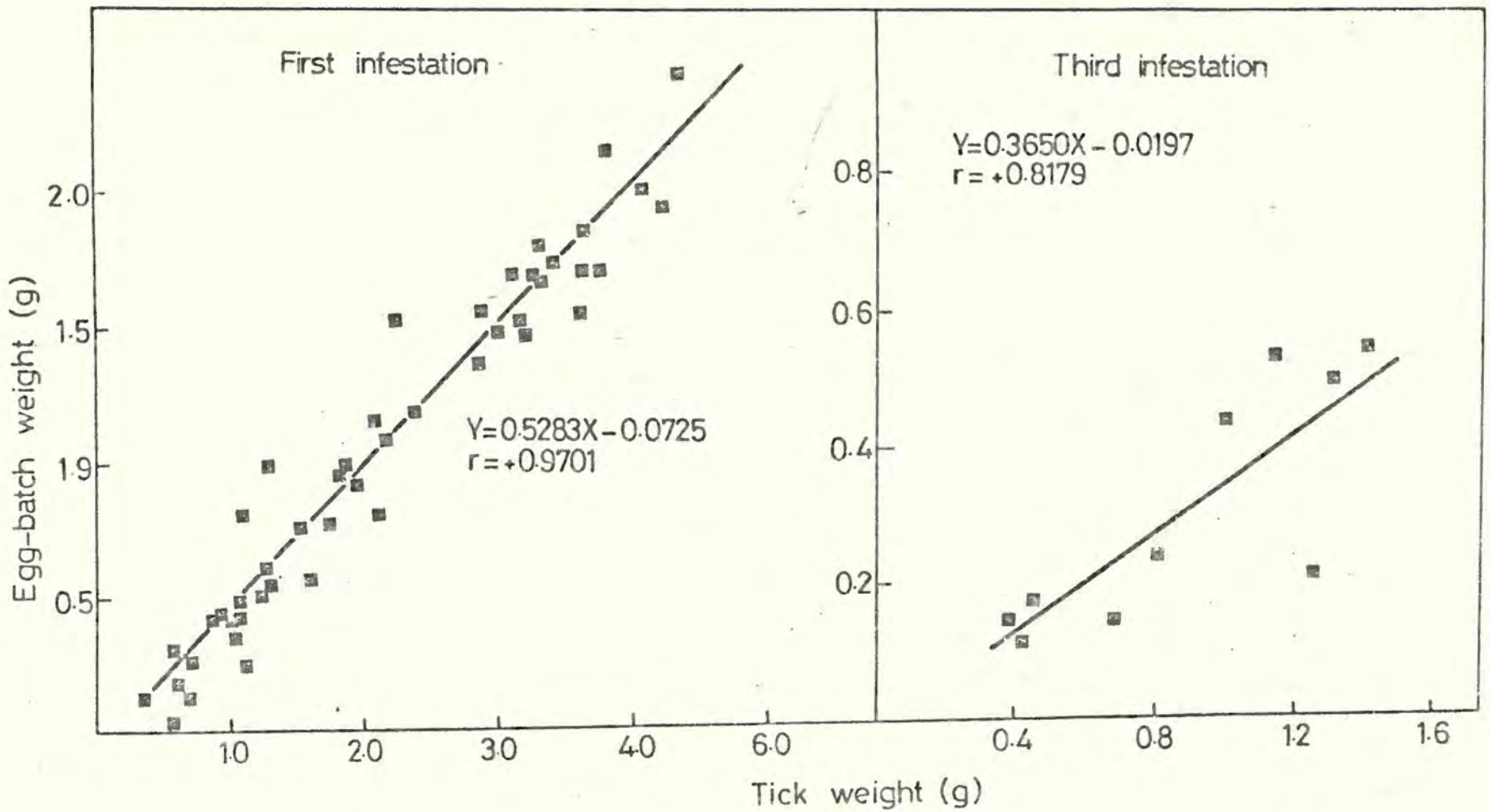
Feeding performance of A. variegatum nymphs (<10mg) detached from resistant rabbits and refed (within 2-5h) on tick-naive ones

Solid bars: weights of ticks which dropped from resistant rabbits on days 6 and 7 (mean weight 3.3±1.9mg).
Open bars: the same ticks after they had re-attached and fed to repletion (mean weight 39.9±12.6 mg) on tick-naive rabbits

Fig. 3

Regression of length versus weight of engorged female A. variegatum fed on tick-naive rabbits




First infestation
Y=0.5283X-0.0725
r=+0.9701
Third infestation
Y=0.3650X-0.0197
r=+0.8179
Egg-batch weight (g)
Tick weight (g)
0.5
1.9
1.5
2.0
0.2
0.4
0.6
0.8
1.0
2.0
3.0
4.0
6.0
0.4
0.8
1.2
1.6

totally blocked by host scar tissue (Fig. 5). From the second rabbit 6 ticks dropped with an average weight of 0.692 g. The heaviest tick, which weighed 1.319, g was greenish-black in colour and died immediately after dropping. Only one tick dropped from the third rabbit. Its weight was 1.509 g and it also had the same greenish-black colour. It too, died immediately after detachment. Four partially fed ticks were found shrivelled up and had died while still attached on the host (Fig. 6).

Although there was a high correlation between the egg-batch weight and the engorged weight of females which dropped in the first, second and third infestations, by the third infestation the slope of the regression decreased (Fig. 7). This indicated that after the third infestation the ticks were converting their blood meal into eggs less efficiently than after the first infestation. The egg conversion factor, calculated as egg-batch weight/tick weight, was reduced from 49% in the first infestation to 42% in the second and to 34% by the third infestation. This difference was not significant using the d-test(Bailey, 1974). The feeding performance of the female ticks is summarised in Table 2. Only 25.6% of the ticks that dropped during the third infestation were able to lay eggs.

1.2 Nymphs

1.2.1 Feeding performance

In the first infestation 72.5% of the nymphs engorged and had an average weight of 45.2 $\pm$ 1.7 mg after 7.9 $\pm$ 1.4 days. It was

Fig. 5. Partially fed female A. variegatum, picked off the resistant rabbit, 22 days post attachment. The mouth parts are masked with the host scar tissue.

Fig. 6. Partially fed female A. variegatum which had died and shrivelled while still attached on the host.

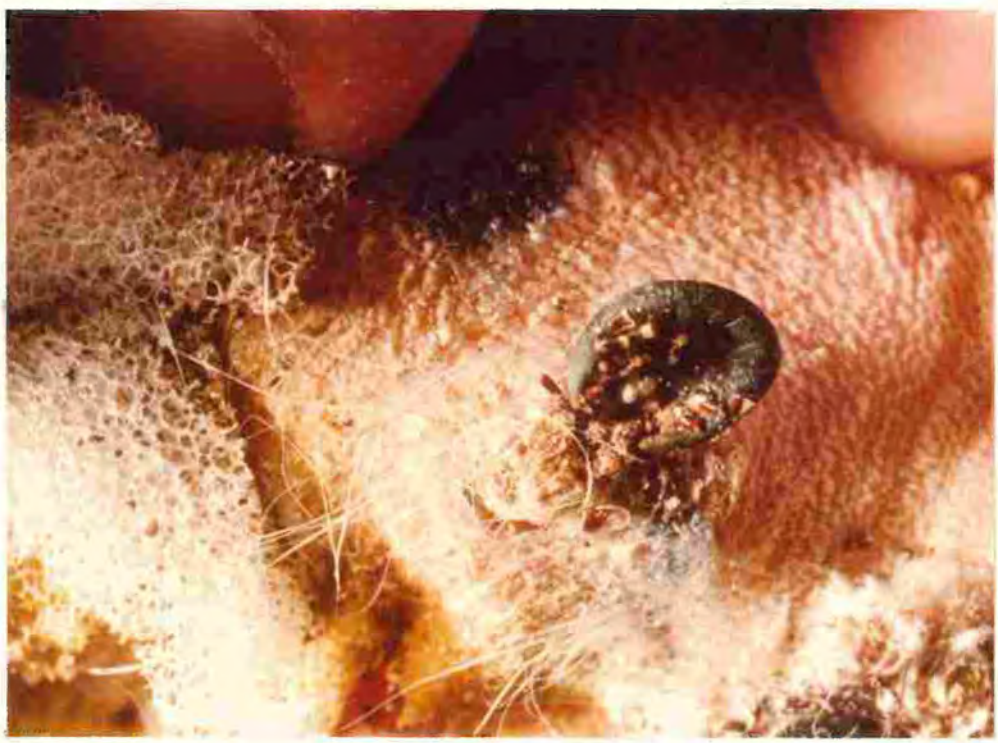


Fig. 4

Regression of egg-batch weight versus tick weight in A. variegatum females fed on rabbits; results for the first and third infestations are shown

Fig. 7

Linear regression analysis of egg-batch weight and weight of female A. variegatum fed repeatedly on rabbits during 3 repeated infestations

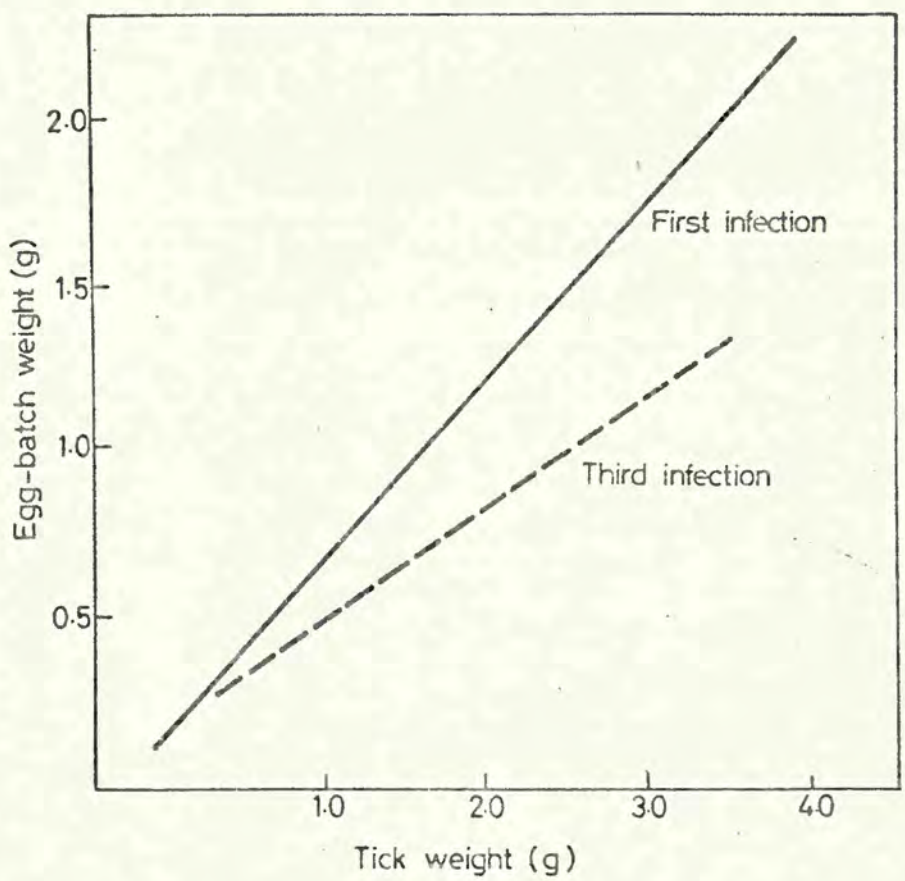


Table 2

Means + standard deviations of engorged tick weight (g), engorged egg-batch weight (g) and conversion factor* of A. variegatum females dropped over 3 successive infestations.

Parameter	Infestation number		
	1	2	3
Tick weight	2.148	1.261	0.880
Egg-batch weight	1.063	0.535	0.302
% females laying	100.0	98.2	52.6
C.f.*	0.49	0.42	0.34
No. females observed	40	21	10

C.f.* = $\frac{\text{egg-batch weight}}{\text{tick weight}}$

found that the majority of nymphs which weighed less than 20.0 mg for detachment did not develop into adults. Of the few which succeeded in moulting, the resultant adults were small, with a reduced survival period, and they also fed poorly (section 1.2.2). Therefore, it was decided that in the case of nymphs the term "engorged", in this thesis, would be used only for ticks which weigh 20.0 mg or more after detachment.

Figure 8 shows the performance of nymphs fed repeatedly on 6 rabbits plus tick- naive controls. There was a steady drop in the percentage of nymphs engorging over successive infestations. There was no significant change in the percentage of nymphs engorging on all the control rabbits . All differences found between the resistant group and the controls in each infestation were significant ($p < 0.01$) , except for the second infestation where $P < 0.05$.

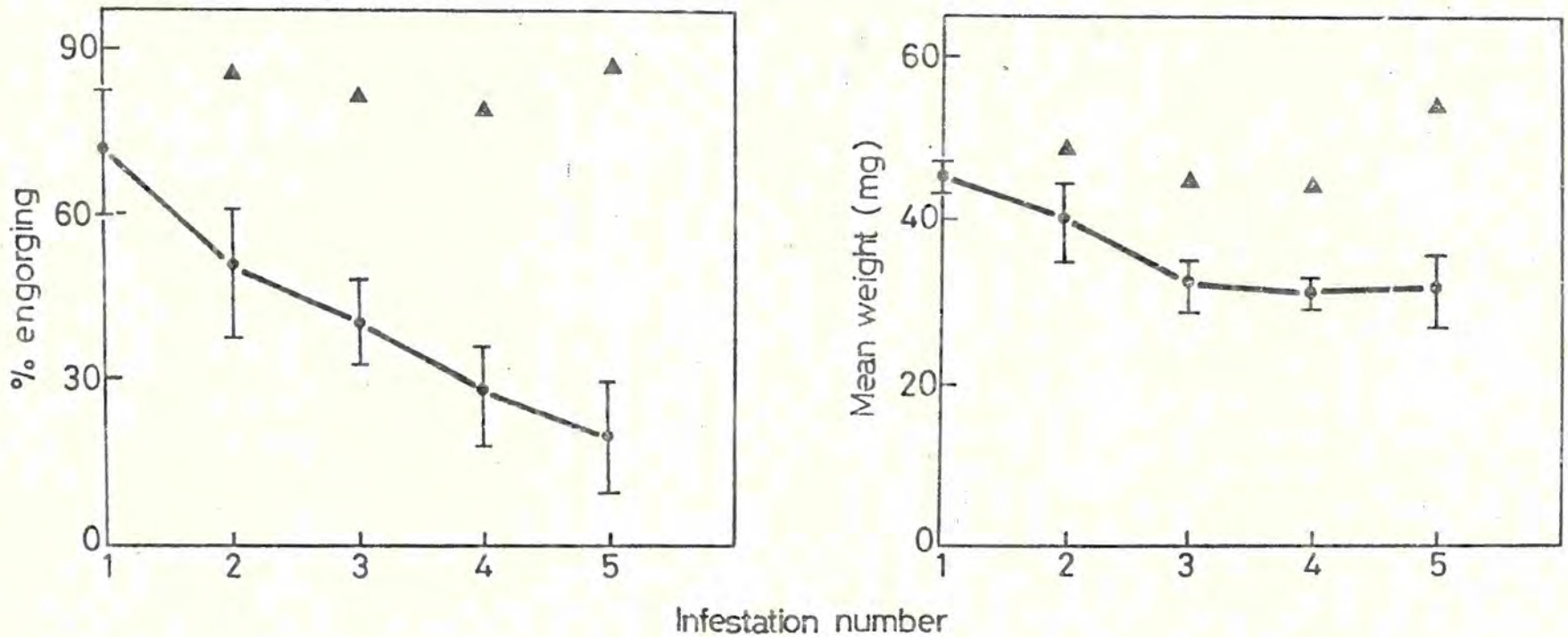
The mean engorged weights were also reduced ($P < 0.05$) compared with the controls. From the third to the fifth infestation the mean weight did not change significantly (32.5, 30.9, 31.5 mg). In all treatments, two-way analysis of variance showed no significant difference within groups for either of the resistant group or the controls.

1.2.2 Development of nymphs

The majority of male ticks which moulted from nymphs that had engorged on resistant animals during the third infestation, showed a pattern of scutal pigmentation which

Fig. 8

Performance of A. variegatum nymphs fed successively on 6 rabbits
The bars are ± 1 S.D.



was atypical of A. variegatum. There were two lateral spots on the scutum (fig. 9). This pattern was similar to that of A. lepidum (Fig. 10). The 4-8% of the small ticks which dropped from tick-naive rabbits moulted into small adults but did not show this changed pattern of scutal ornamentation.

All attempts to produce progeny from these abnormal males by feeding them with females on either susceptible or resistant rabbits were unsuccessful. Three feeding regimens were carried out with 10 males and 10 females on 3 different rabbits. The small abnormal males were mated with small females from the same batch which had engorged on resistant rabbits. The mean engorged weight was 0.347 $\pm$ 0.140 g (n = 5). When the males were mated with normal size females (which engorged on tick-naive rabbits), only two females dropped weighing 1.120 and 1.520 g. Here, the attachment of females beside the prefed males was poor; some females dettached after only 2 days and one weighing (67.8 mg) was dead . In the third feeding regimen, normal males (fed as nymphs on tick-susceptible rabbits) were mated with small females (from resistant rabbits). The mean engorged weight was 0.221 $\pm$ 0.215 g (n = 7) and 3 partially fed ones dropped were dead on detachment . All the engorged females , however either failed to lay eggs or , for the few that oviposited, the eggs did not hatch.

Fig. 9. Amblyomma variegatum males; A : typical specimen from the colony. B : Abnormally ornamented; moulted from a nymph which had engorged on a rabbit resistant to nymphs of the same species. Note here the additional two lateral spots (arrows) and also note the difference in tick size.

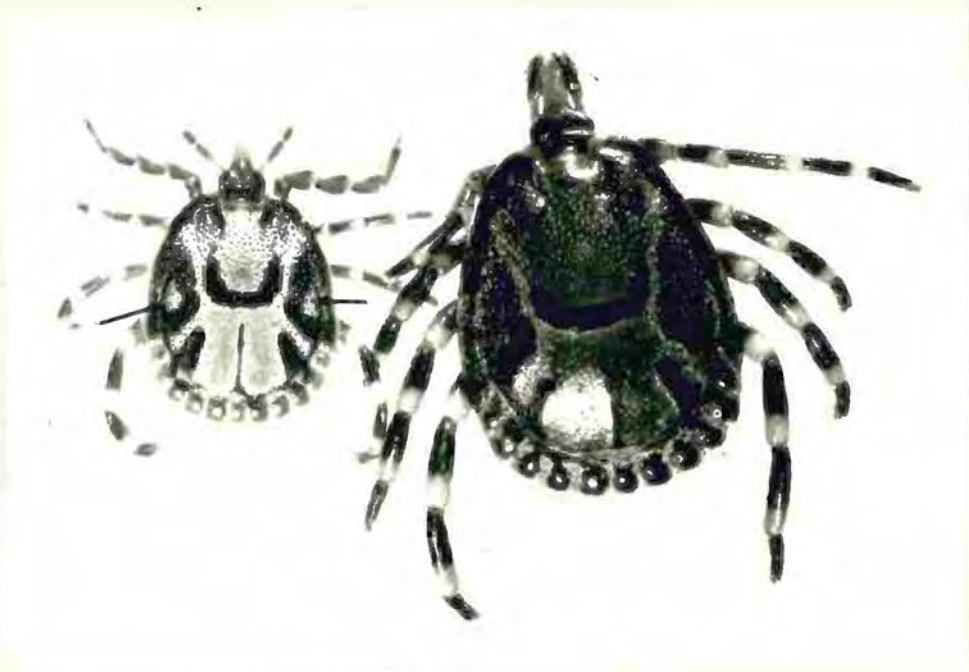
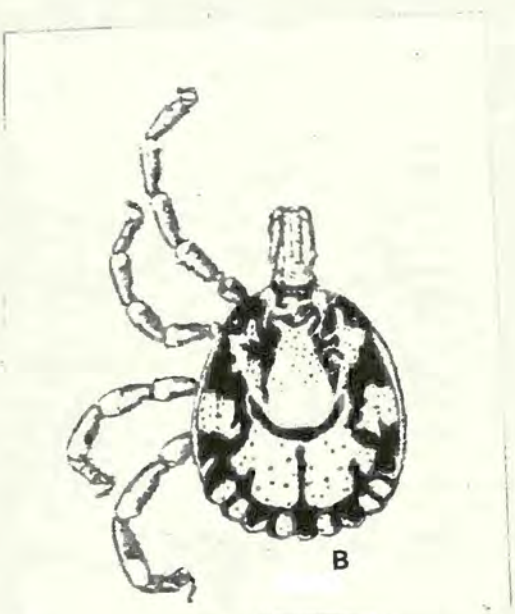


Fig. 10. A : Abnormally ornamented A. variegatum male (described in Figure 9) while B : is A. lepidum male (Hoogstraal, 1956). Note the similar pigmented spot at each scutal midlength that crosses the lateral groove.



1.3 Laval Feeding

Since the number of larvae used for repeated infestations was high (3000/rabbit), only 500-1500 of the engorged larvae which dropped from each were counted and weighed. Therefore, the only parameter considered was the engorged weight. In the first infestation 1500 larvae were weighed from each of the 6 rabbits; the mean weight of 10 larvae was 28.5 $\pm$ 1.2 mg and they took 7.9 $\pm$ 1.4 days to engorge and drop.

Two way analysis of variance showed that there was no significant change in the mean engorged weight with successive infestations. Figure 11 shows the feeding performance of larvae on rabbits infested repeatedly. There was however , a significant reduction in the mean weight of ticks dropped in the third to the six infestations , compared to the first infestation. This level of reduction in weight remained constant at 22.8%.

2. CROSS RESISTANCE EXPERIMENTS

Rabbits which had become resistant by repeated feedings of A. variegatum adults were challenged with larvae and nymphs (Table 3). The nymphs that engorged weighed less ($P < 0.01$) than those from the controls. When compared with the percentage of nymphs engorging on the controls, there was also a reduction ($P < 0.01$) amounting to 30% for both

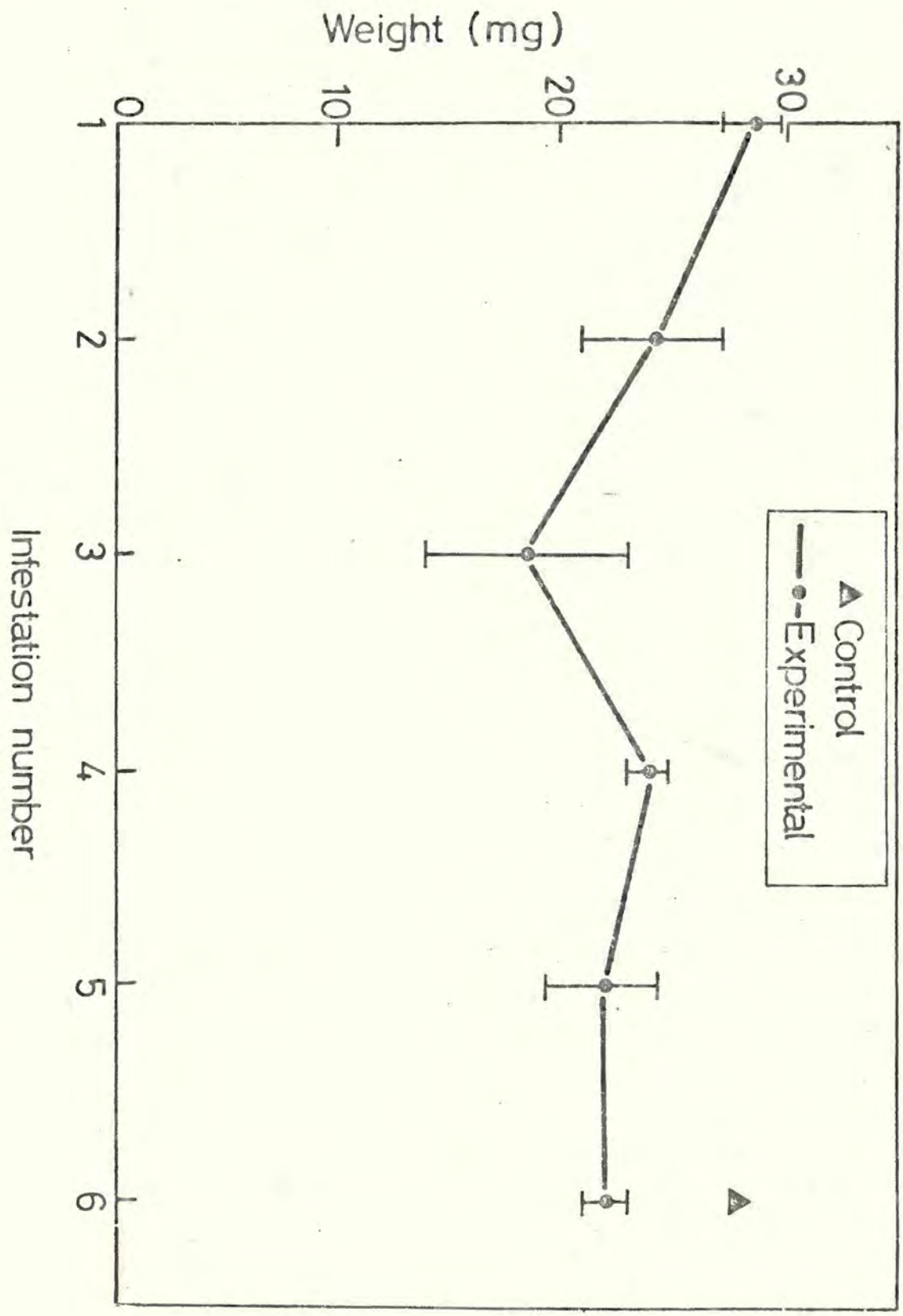

Weight (mg)
0
10
20
30
1
2
3
4
5
6
Infestation number
▲ Control
Experimental

Table 3

Results of challenging rabbits resistant to *A. variegatum* adults, and tick-naive control rabbits with nymphs and larvae of the same tick species simultaneously.

Rabbit no.	nymphs(100/right ear)		larvae (500/left ear)	
	no. engorged	weight(mg) mean ± SD	no engorged	weight(mg) mean ± SD
1	65	40.9±12.5	200	12.3±3.7
2	74	41.8±12.7	75	17.9±3.8
3	50	32.9± 8.8	195	14.5±4.0
4	61	38.0±12.1	450	18.3±4.8
5	57	37.6±11.0	60	16.9±2.2
X±SD	61.4±9.0[a]	38.2± 3.5[a]	196.0±156.3[a]	15.9±2.5[a]
Controls				
1	81	57.6±13.6	470	29.8±1.6
2	92	53.7±13.1	450	27.2±1.8
3	89	52.4±14.8	445	30.4±1.9
X±SD	87.3±5.7[b]	54.6±2.7[b]	450.0±13.2[b*]	29.1±1.7[b]

Means in columns followed by a different letter differ significantly ($P<0.01$) except b* ($P<0.05$).

parameters. The rabbits also showed significant level of resistance to the feeding of the larvae. The number engorging and their mean weight were reduced by 57.1% and 47.0%, respectively($P < 0.05$ and $P < 0.01$).

The second group of rabbits which were made resistant to nymphs of A. variegatum were also challenged with the tick immatures (Table 4). When infested with additional nymphs they supported the engorgement of fewer ($P < 0.01$) ticks than the controls. This reduction amounted to 77.5%. The mean weight was also reduced by 42.3%, which was significant ($P < 0.01$). There was a reduction ($P < 0.01$) in the percentage of larvae (69.2%) that engorged on these resistant rabbits and also in their mean engorged weight (42.6%).

The group of rabbits which were made resistant to A. variegatum by infesting them with the immature stages of this tick were challenged with nymphs of both species (Table 5). The results obtained by feeding additional nymphs of A. variegatum on resistant rabbits supported the fact that the rabbits were resistant since a lower percentage engorged and dropped ($P < 0.01$). Their mean weight was also reduced compared with those from tick-naive control rabbits. However, the feeding of R. appendiculatus was not affected and the results for the above parameters were similar to those fed on the controls. Interestingly, one rabbit in the resistant group (no. 8) showed a considerable degree of resistance to R. appendiculatus (23.1% reduction in engorged

Table 4

Results of challenging rabbits resistant to A.variegatum nymphs, and tick-naive control rabbits with nymphs and larvae of the same tick species simultaneously.

Rabbit no	nymphs (100/right ear)		larvae(100/left ear)	
	no. engorged	weight(mg) mean ± SD	no. engorged	weight(mg) mean ± SD
7	31	30.6± 8.0	70	17.3±2.7
8	13	34.4±10.6	70	14.2±3.9
9	7	27.5± 4.0	110	17.4±2.6
10	33	28.1± 5.5	210	16.8±4.1
11	14	37.1±11.5	220	17.9±4.6
X±SD	19.6^{a}±11.6	31.5^{a}± 4.1	136.0^{a}±7.0	16.7^{a}±1.3
Controls (3)				
X±SD	87.3^{b}±5.7	54.6^{b}± 2.7	445.0^{b}±13.2	29.1^{b}±1.7

Means in columns followed by a different letter differ significantly ($P<0.01$).

Table 5

Mean feeding performance of A. variegatum and R. appendiculatus nymphs on rabbits previously made resistant to A. variegatum by four successive infestations with nymphs. Tick-naive rabbits were also infested.

Rabbit no.	A. variegatum (100/right ear)		R. appendiculatus (100/left ear)	
	no. engorged	weight(mg) $\bar{X} \pm SD$	no. engorged	weight(mg) $\bar{X} \pm SD$
7	75	37.0±10.6	100	45.9
8	86	42.4±17.1	75	36.3±5.5
9	44	35.9±13.9	83	47.6±5.5
10	67	34.3±10.4	76	46.5±9.1
11	58	33.9±10.1	88	42.5±2.4
$\bar{X}$±SD	66.0±16.0[a]	36.7±3.4[a]	84.4±10.2[a]	43.6±4.5[a]
Controls				
1	92	55.2±14.6	62	41.6±7.3
2	98	51.6±13.5	83	51.3±6.6
3	82	48.1±12.2	98	48.6±5.0
X+SD	90.7±8[b]	51.6±3.6[b]	81.6±18.0[a]	47.2±5.0[a]

Means in columns followed by a different letter differ significantly.

weight) compared to a mean reduction of 3.6% for the rest of the group. At the same time, this rabbit showed a lower resistance level to A. variegatum than the other animals in the group (8.5% reduction in mean weight compared to 31.8%).

The group of rabbits (5) which was exposed to 3 weekly infestation with R. appendiculatus larvae was challenged later with adults (Table 6). Although 83% of the adults fed and dropped, the mean engorgement weight was reduced by 53% when compared to those engorged on controls. This resistant group was also challenged with nymphs of R. appendiculatus (Table 7) and the mean engorged weight was reduced by 59.0% compared to the controls.

The group of rabbits which showed a high degree of resistance to feeding by different stages of R. appendiculatus was challenged with A. variegatum nymphs (Table 8). Simultaneous feeding by R. appendiculatus nymphs supported the fact that these animals were indeed resistant to this instar as the mean engorged weight was reduced by 64.4%. There was a reduction ($P < 0.05$) both in the percentage of A. variegatum nymphs engorging and in their mean weight as compared to those which engorged on the controls amounting to 27.7% and 18.9%, respectively.

Table 6

Mean feeding performance of 20 females *R.appendiculatus* on rabbits made resistant by 3-weekly infestations with 500 larvae of the same tick species, and on tick-naive control rabbits.

Rabbit no.	no. engorged	weight (mg) ($\bar{X} \pm SD$)
1	20	174.3±102.5
2	20	144.4± 70.6
3	10	138.5±120.3
4	13	148.2±116.2
5	20	140.5±125.6
Mean ± SD	16.6±4.8	149.2± 14.5
Control 1	20	285.8± 71.7
Control 2	20	343.4± 78.8
Mean ± SD	20	314.6± 28.8

Table 7

Mean feeding performance of 300 nymphs R. appendiculatus on rabbits previously made resistant by 3 weekly infestations with 500 larvae followed by one adult feed of the same tick species. Three tick-naive rabbits were also infested.

Rabbit No	No. engorged	Weight (mg) $\bar{X} \pm SD$
1	176	19.2 ± 4.4
2	125	19.7 ± 4.8
3	195	23.1 ± 6.4
4	165	17.8 ± 6.0
5	185	17.5 ± 3.9
$\bar{X} \pm SD$	169.0±27.0	19.5 ± 2.2
Controls		
1	285	46.5 ± 3.0
2	267	50.7 ± 6.1
3	258	44.0 ± 2.5
$\bar{X} \pm SD$	270.0±13.7	47.1 ± 3.4

Table 8

Mean feeding performance of R. appendiculatus and A.variegatum nymphs on rabbits previously made resistant to R. appendiculatus by the feeding of larvae, nymphs and adults of the same tick species. Tick-naive control rabbits were also infested.

Rabbits no.	R. appendiculatus (100/left ear)		A. variegatum (100/right ear)	
	no. engorged	weight(mg) $\bar{X} \pm SD$	no. engorged	weight(mg) $\bar{X} \pm SD$
1	90	15.7±2.4	75	40.6±24.4
2	81	20.2±3.4	70	38.4±11.6
3	70	16.3±3.8	59	43.3±14.1
4	81	15.3±4.2	50	43.6±13.3
5	80	15.7±2.2	74	44.3±14.6
$\bar{X}$±SD	79.8±7.4	16.6[a]±2.0	65.6[a]±10.8	42.0[a]±2.4
Controls				
1	67	41.6± 7.3	92	55.2±14.6
2	98	51.3± 6.6	98	51.6±13.6
3	87	48.6±10.2	82	48.1±12.2
$\bar{X} \pm SD$	84.0±15.7	47.2[b]±5.0	90.7[b] ±8.I	51.6[b]±3.6

Means in columns followed by a different letter differ

3. GROWTH OF A. VARIEGATUM NYMPHS ON TICK-NAIVE AND TICK-RESISTANT RABBITS

3.1 Growth of A. variegatum Nymphs on Tick-Naive Rabbits

Figure 12 shows the proposed feeding categories of A. variegatum nymphs fed on tick-naive rabbits while Table 9 shows the percentage of ticks which fell within these categories. There was no significant difference in the mean lengths of unfed nymphs (1.90 ± 0.11mm) and nymphs fed for 1 day, 2 days or 3 days (2.13±0.17mm). They were therefore, put into feeding category N1 (unfed). The minimum length for N1 was taken to be the mean length of the unfed stage minus 2 standard deviations i.e. 1.70 mm and the maximum was taken as the mean length of ticks picked off on day 3 plus 1 S.D. i.e. 2.3 mm. One hundred percent of the unfed ticks and the ticks fed for 1 day or 2 days , and 82% of the ticks fed for 3 days, fell within the limits defined for category N1. The second feeding category (N2) had a lower limit of 2.4 mm . The upper limit was the mean length of ticks picked off on day 4 (2.4 ± 0.3) plus 2 S.D. i.e. 3.0 mm. Fifty-one percent of the ticks picked off on day 4 , and 48% of those on day 5 fell within this range. The third feeding category (N3) ranged between a length of 3.1 mm to the mean length of the fully engorged ticks (5.66 ± 0.58) minus 2 S.D. i.e. 4.5 mm. Fifty-two percent of the nymphs picked off on day 5 and 56% of those on days 6, 7 and 8 fell within the range defined

Fig. 12

Growth of A. variegatum nymphs on susceptible rabbits

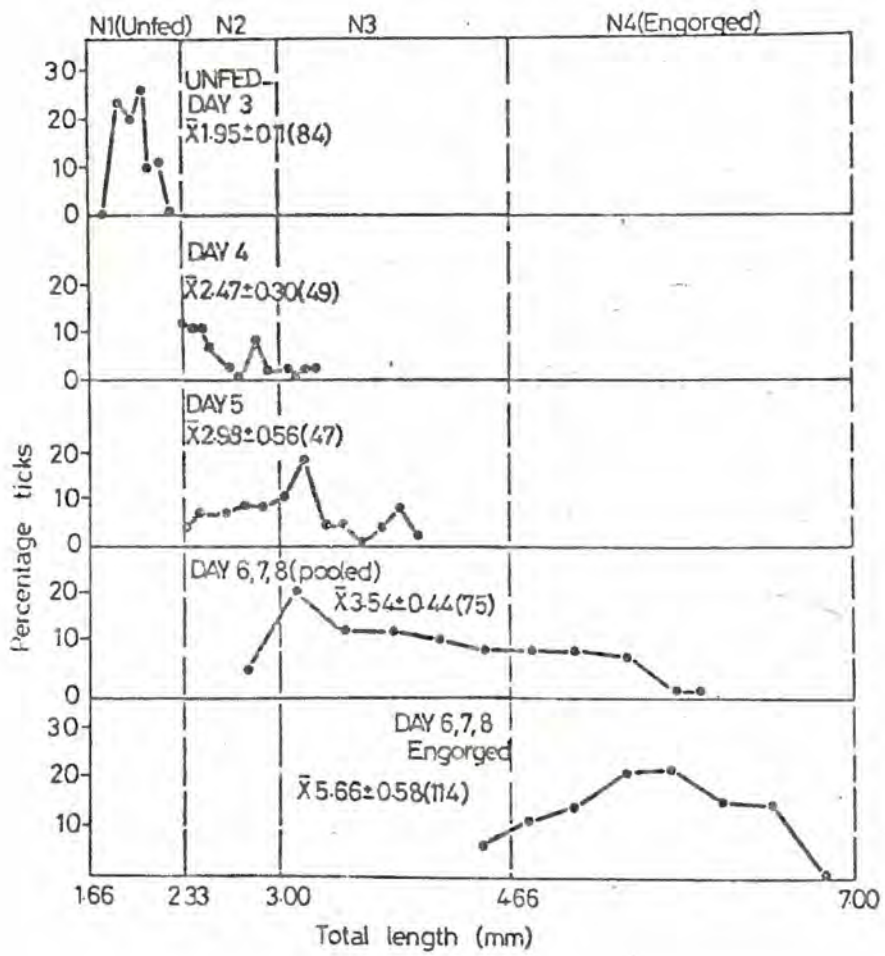


Table 9

Proposed feeding categories for A. variegatum nymphs picked off tick-naive rabbits and the percentage of ticks within each category.

Category	size range (mm)	% of ticks within each category							
		unfed	day 1	day 2	day 3	day 4	day5	day6,7,8	engorged
N1	1.7-2.3	100	100	100	82.0	41.0	2.1		
N2	2.4-3.0				8.0	51.0	46.0	4.4	
N3	3.1-4.5					8.0	51.9	56.0	4.0
N4	≥ 4.6							39.6	96.0

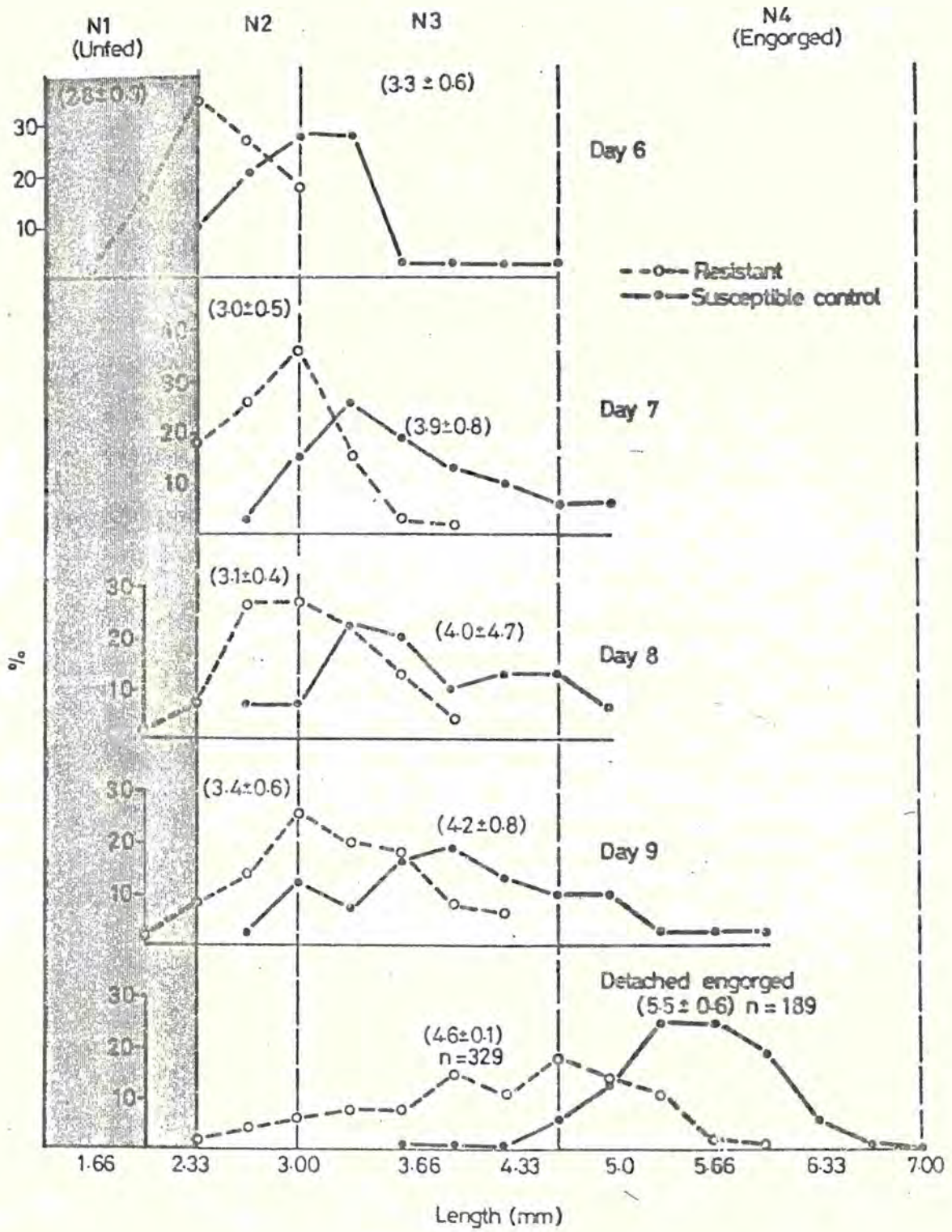
for category N3. Nymphs that detached after full engorgement were placed in a separate category (N4 , engorged) with a length at 4.6 mm or greater. Ninty-six percent of the nymphs that dropped after engorgement fell within this category.

3.2 Growth of A. variegatum Nymphs on Resistant Rabbits

Ticks feeding on resistant rabbits were easily picked off the host, whereas those feeding on tick-naive controls were firmly attached and had to be forcibly removed. When ticks from the resistant rabbits were removed, the mouth-parts were usually blocked with host scar tissue. There were noticeable feeding cavities or necrotic areas were visible and there was no bleeding. However, ticks taken from the controls came away with clean mouth-parts leaving distinct feeding canal in the skin, which was accompanied by copious bleeding from the pre-formed haematomas.

3.2.1 Changes in tick length

Data obtained from the three tick-naive control rabbits in this experiment (Fig. 13) supported the results previously obtained (section 3.1). Thus, the mean length of nymphs picked off the hosts on days 6, 7 and 8 was 3.7 $\pm$ 0.4 mm compared to 3.5 $\pm$ 0.4 mm. In the present experiment 92% of the fully engorged ticks measured 4.6 mm compared to the 96% in the previous experiment (section 3.1) which had the same length. Therefore, the comparisons between the resistant group and the tick-naive control rabbits in this experiment, were valid.


N1
(Unfed)
N2
N3
N4
(Engorged)
(2·8±0·3)
(3·3 ± 0·6)
Day 6
Resistant
Susceptible control
(3·0±0·5)
Day 7
(3·9±0·8)
(3·1±0·4)
Day 8
(4·0±4·7)
(3·4±0·6)
Day 9
(4·2±0·8)
Detached engorged
(5·5 ± 0·6) n = 189
(4·6±0·1)
n = 329
%
1·66
2·33
3·00
3·66
4·33
5·0
5·66
6·33
7·00
Length (mm)

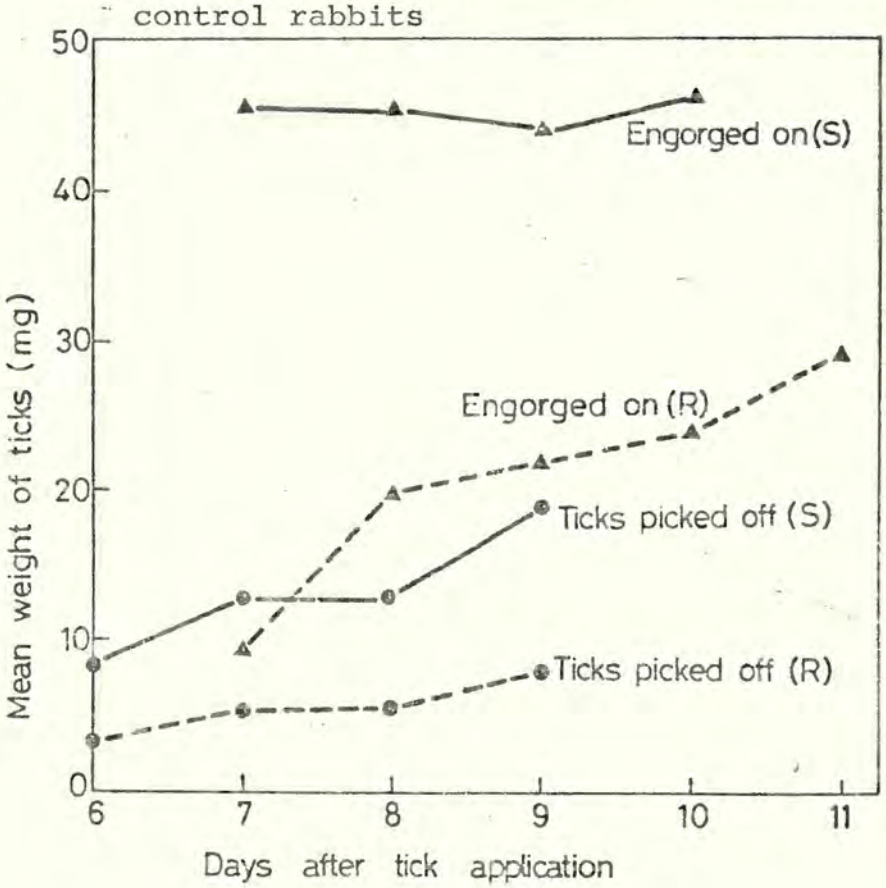
Forty-two percent of the ticks picked off the control rabbits on day 6 fell in category N3 whereas no ticks from the resistant group reached this category and 40% of them fell in N2. On day 7 of feeding, 82% of the ticks from the control rabbits fitted into N3, compared to only 20% from the resistant group. There was dynamic growth on days 8 and 9 of nymphs feeding on the control rabbits . There was no growth during the same period by ticks which in categories N1, N2 and N3 from resistant rabbits. Ninety-two percent of detached, engorged nymphs from the control rabbits fitted into N4 compared to 28% from the resistant group. Sixty percent of the ticks which dropped from the resistant rabbits fitted into category N3 while 12% still fitted into N2.

3.2.2 Changes in tick weight

The mean weight of ticks pulled off daily and of those which dropped engorged from the 2 groups of rabbits are shown in Fig. 14. The weights reflected differences similar to those in length obtained in section 3.2.1. The weights of ticks which were pulled off on days 6-9 from the control rabbits were higher than those obtained from rabbits in the resistant group. This difference was also reflected in weights of nymphs which dropped after engorgement. The mean engorged weight of ticks which dropped from the control rabbits was 43.8 mg compared to 19.2 mg for those from the resistant rabbits.

Fig. 14

Daily mean weights of A. variegatum nymphs which were picked off the host while feeding and those which dropped fully engorged, from two groups of rabbits; one group (6) made resistant(R) to A. variegatumnymphs and one group(3) tick-naive(5) control rabbits



3.3 Re-feeding of Ticks in Categories N2 and N3 Dropped from Resistant Rabbits transferred to Susceptible Rabbits

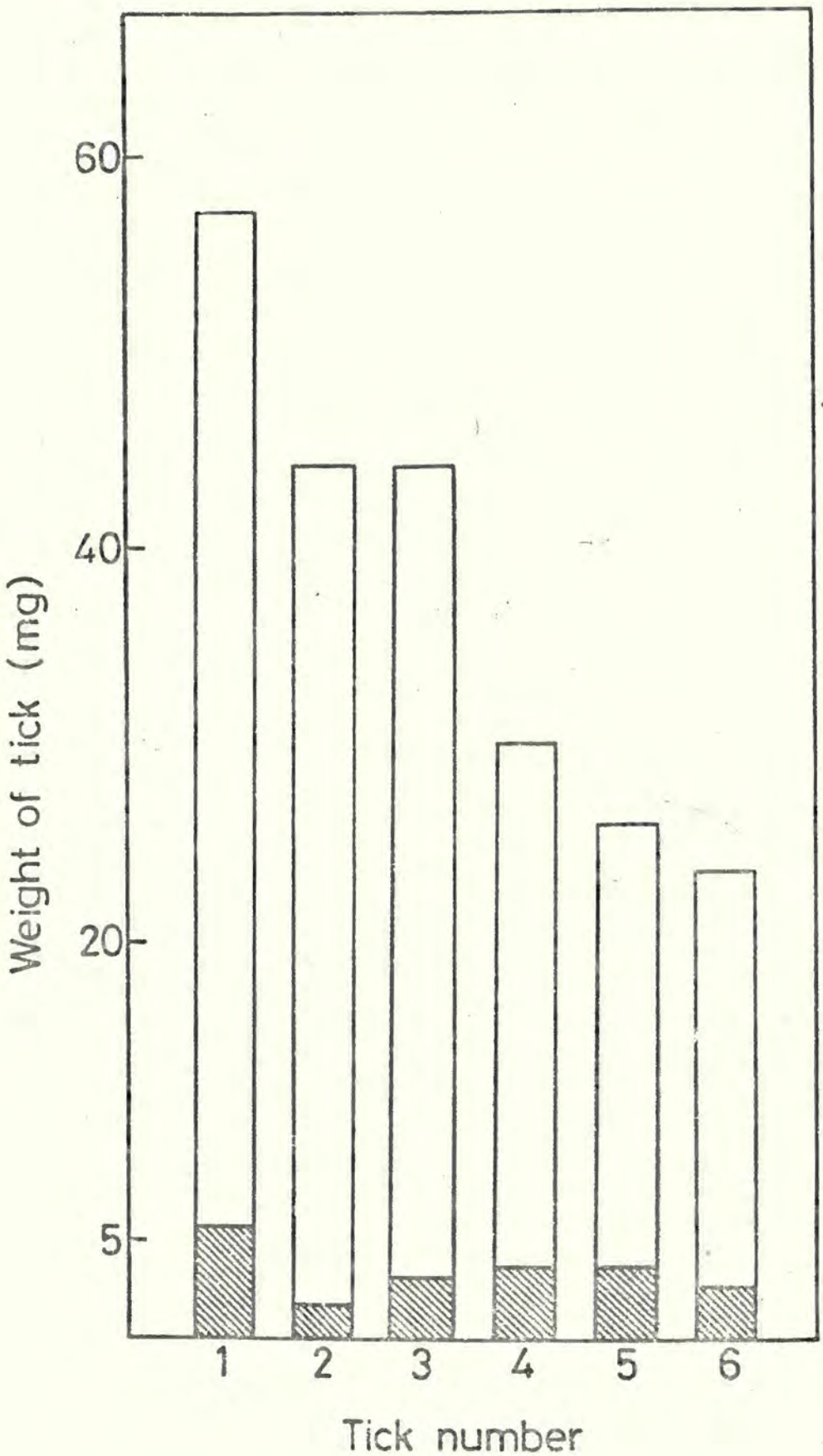
Ticks of feeding categories N2 and N3 which detached from resistant rabbits were weighed and divided into two groups; those which weighed less than 10 mg and others which weighed between 10 - 20 mg. Within 2-5 hours of dropping they were put onto tick-naive rabbits and allowed to reattach and feed. None of the ticks which weighed 10-20 mg reattached or fed. Six of the ten ticks which had weighed less than 10 mg (3.3 $\pm$ 1.4 mg) reattached and fed for 1-3 days before dropping off the host (Fig. 15), by which time their mean engorged weight had increased to 39.9 $\pm$ 12.6 mg.

4. TICK PARALYSIS

Two of the 18 rabbits were diagnosed to be suffering from tick paralysis during the course of various A. variegatum infestations.

The first case:

After the first nymphal feeding terminated, one of the rabbits showed nervous symptoms which could be described as turning disease or circling disease. The animal's head was tilted slightly to the left. The prognosis was favourable. The rabbit was given intensive care, good food and 4 daily injections of streptomycin/penicilin (Pfizer) treatment as the parotid lymphnodes were swollen. The condition improved and the rabbit became normal in the course of 2 weeks.


60
40
20
5
Weight of tick (mg)
1
2
3
4
5
6
Tick number

After the rabbit became normal, a second infestation was carried out to see the effect of tick re-feeding. One hundred nymphs were applied on each ear restrained in ear bags. On day 5 the rabbit started to develop the same nervous symptoms again, including circling movements. The neck also started to tilt to the left side. The condition seemed to be more acute by day 7. At this stage the attached ticks, which were all partially engorged, were pulled off the host. After removal of the ticks the rabbit was subjected to good nutritional regimen and health care as described above. After 2 weeks it became normal again. The head pointed upright and the nervous symptoms disappeared.

Three weeks after the second infestation a third feeding was attempted with 200 nymphs. The ticks were confined in a capsule fixed on the back of the rabbit. The feeding site was intended to be away from the head region. By day 9 when the ticks were dropping engorged, the symptoms appeared again. The ticks were allowed to feed to repletion. By day 13 the symptoms became progressive and clear. The head and neck also tilted to one side. When the rabbit tried to feed its movements were uncoordinated which led to a complete loss of control over its body. Following this, the rabbit rotated from right to left trying to maintain itself in an upright position. The condition became very severe and the rabbit was not able to feed although several attempts were made. The continuous rotation, falling and failure to maintain itself upright prevented the rabbit from feeding and drinking. It became weak and anaemic. Subsequently, it was decided to destroy the rabbit.

The second case:

Another rabbit developed similar paralysis after the adults of A. variegatum completed their feeding. The head and neck also tilted to the right side. The condition did not improve and became chronic. Unlike the first case, the second rabbit was capable of taking food and water with the tilted neck. Although the animal was in good condition it was also sacrificed. Photographs of the above two rabbits are shown in Fig. 16.

Fig. 16. Two rabbits with paralysis as a result of feeding A. variegatum adults (left) and nymphs (right) on them. Note the tilting of head and neck to one side. The feeding capsule had been removed from the back of the rabbit on the right.

DISCUSSION

There was great variation in the engorged weights of adult A. variegatum fed on the ears of tick-naive rabbits. This was partly overcome by feeding them in capsules fixed on the backs of rabbits. This method gave results that were better for comparisons. Female ticks engorged and dropped with greater weights and there were no significant differences between ticks from tick-naive controls. Although the ears are not suitable feeding sites for adult, they are, however, suitable for feeding immatures.

Rabbits became resistant to repeated feedings by different stages of A. variegatum. They acquired significant levels of resistance by the second infestation with adults and nymphs and by the third infestation with larvae. Resistance to adults and nymphs was greater than to larvae. This was manifested by 73.6% reduction in the mean engorgement weight of adults by the third infestation, 70% and 42.3% reduction in the numbers engorging and the mean weight of the nymphs by the 5th infestation, respectively, and 22.8% reduction in the mean weight of engorged larvae by the 6th infestation. Resistance in rabbits to adults was manifested by reduced engorged weights of female ticks with a lower proportion able to lay eggs and a lower conversion efficiency. Riek (1962), Hewetson (1968) and Wagland (1978) observed that ticks dropped from pure B. taurus breeds (susceptible cattle) were significantly heavier and laid a

heavier batch of eggs than ticks from pure B. inducus (resistant) cattle. Latif (1984a) found that the resultant larval infestation on the pasture from ticks engorged on crossbred cattle was 36.5% greater than from the local Zebu cattle. These results are consistent with the present finding that ticks engorged on control rabbits not only had heavier weights and were all able to lay eggs, but were also able to convert their blood-meal into eggs more efficiently.

The drop in egg conversion factor in the presence of resistance suggests the involvement of a toxic factor, in addition to poor feeding, which affects the ability of ticks to produce eggs. This finding is in agreement with that obtained with I. ricinus (Bowessidjaou et al., 1977). Resistance in rabbits was also manifested by inhibition of tick feeding which was observed in three forms; i) ticks were found dead in situ ii) ticks died immediately after detachment (iii) ticks that fed partially and remained attached in this partial feeding stage. When ticks in group (iii) feeding were removed forcibly their mouthparts were found to be totally blocked in host scar tissue.

Some partially fed nymphs, of categories N1 and N2, which dropped from resistant rabbits and were put onto tick-naive rabbits, reattached and fed to repletion. This gives further evidence of a factor(s) in the resistant animals which somehow interrupts the feeding of ticks leads to their premature detachment. This factor(s) is not present in the susceptible hosts.

All these responses of rabbits to ticks were apparently immunological (the involvement of humoral factors in resistance to ticks is shown in Chapter 2). It was found that 72% of nymphs which fed and dropped from resistant rabbits fitted in feeding categories N2 and N3 and only 28% fell in N4 (engorged). In this case resistance in rabbits was manifested against ticks of N2 and N3 causing their premature detachment.

So far, there is evidence for at least 2 types of responses : the physical removal of the ectoparasite and an inhibition of feeding (Willadsen, 1980). Both of these manifestations may be as a result of the same immunological response. Inhibition of tick feeding which has been observed with A. variegatum adults in the above three forms may be due to the long feeding period of this stage, and hence longer association with the immune hosts. However, rejection could be associated with shorter feeding periods of the immatures. Many of the A. americanum adults which did not engorge fully on resistant cattle died and appeared to shrivel, while still attached on the host, at various stages of engorgement (Strother et al., 1974). Similarly, cattle on exposure to I. holocyclus can acquire an immunity that results in removal of the ticks by grooming, death of the ticks in situ and reduction in their engorged weights (Doube and Kemp, 1975). Death of the larvae on immune animals coincides with the onset of excretion by I. holocyclus of faeces containing blood (Bagnall, 1978). Bagnall (1978), detected basophils and their granules in the gut of affected larvae. This supports the hypothesis that

ingestion of these cells might exert a lethal effect on ticks. The effects of immunity on B. microplus larvae on cattle varied, with some ticks drowning in serous exudate (Riek, 1962), though, this is rare (Roberts, 1971).

Engorged weights of ticks varied much less than the numbers engorging after resistance had been acquired (Fig. 4). Therefore, the percentage engorging was a useful parameter for comparison and may be more pertinent to the analysis of resistance in field populations of ticks. Levels of tick resistance of Australian cattle were assessed by artificial infestations and counting the numbers of survivals (Wharton and Utech, 1970; Utech et al., 1978b) or by analysing the age-structure of parasitic populations of each instar (Sutherst et al., 1979) rather than taking weights.

Literature concerning cross-immunity of a sensitized host to other tick species is scanty (Trager, 1939a; Heller-Haupt et al., 1981; McTier et al., 1981; De Castro et al., 1985). The only detailed study was that of McTier et al. (1981) while the others treated the subject superficially. In the present study cross-immunity within and between A. variegatum and R. appendiculatus has been studied in detail. In this chapter the feeding performance of ticks has been described and in the subsequent chapters the involvement of antibody mediated response along with the demonstration of skin cellular reactions are also described.

High levels of resistance were found to the three stages of A. veriegatum and R. appendiculatus. Although rabbits sensitized to A. variegatum larvae showed a considerably lower level of resistance to this instar (28.2% reduction in mean weight) their feeding was more disturbed when fed on rabbits resistant to either adults or nymphs. Rabbits resistant to A. variegatum adults were also protected against nymphs.

Rabbits sensitized to R. appendiculatus larvae showed a high degree of resistance to feeding of adults of the same species. When this group of rabbits was challenged with nymphs they also showed significant level of cross-protection.

The high level of cross-immunity between the life stages of ticks demonstrated in resistant rabbits in this study might give an explanation for some observations made for cattle in the field. It has been reported that some animals showed greater resistance than others to all instars and species of ticks and that animals which carried more adults stages of a species also carried more of its immature stages (Sutherst et al., 1979; Kaiser et al., 1982; Latif, 1984a,b). Cattle exposed to female I. holocyclus rapidly acquired a significant degree of resistance to the adults and this resistance was also manifested against the larvae and nymphs (Doube, 1975; Bagnall, 1978).

Rabbits sensitized to R. appendiculatus showed appreciable levels of resistance to A. variegatum nymphs ($P<0.05$). This

resulted in 27.7% and 19.0 percent reduction in number of ticks engorging and in their mean weights, respectively. This degree of protection was less when compared to feeding as a result of a homologous challenge (70% and 42.3%). However, R. appendiculatus appeared unaffected in its ability to engorge on A. variegatum resistant hosts. One rabbit in this group showed considerable resistance to a heterologous challenge (23.1% reduction in mean engorgement weight as compared to 3.6% of the whole group). However, its immune effect for a homologous challenge was lost (8.5% reduction in mean weight compared to 31.8% of the whole group). It was also observed that A. variegatum resistant rabbits challenged with R. appendiculatus had a reduced immune protection to A. variegatum. Feeding by R. appendiculatus might have caused the observed immunosuppression in these animals which led to better feeding of A. variegatum ; although it remained significantly lower from ticks fed on control rabbits. Immunosuppression in guinea pigs has been reported for D. andersoni by Wikel et al. (1978). Allen (1973) and Wikel and Allen (1976b) also found that if methotrexate or cyclophosphamide (both are immunosuppressive chemicals) were administered to immune guinea pigs before an infestation with ticks, then the expression of immunity was largerly blocked.

The results reported in this chapter on cross-immunity of R. appendiculatus resistant rabbits to A. variegatum do not agree with the results obtained by Heller-Haupt et al., (1981) and De Castro et al. (1985). These authors could not demonstrate any

cross-protection between R. appendiculatus resistant hosts to A. variegatum infestation in either rabbits or cattle. However, appreciable cross-immunity has been reported for two Dermacentor species (Trager, 1939a; McTier et al., 1981). Moreover, A. americanum resistant guinea-pigs were reported to be cross-resistant to D. variabilis, but not D. andersoni (McTier et al 1981). Surprisingly, in these experiments, the cross-protection to D. variabilis was even stronger than the resistance to A. americanum.

It has been observed that the size of the adults is positively correlated to the weight of engorged nymphs from which they moulted. All nymphs which were fully engorged on tick susceptible rabbits, and those which did not fit in category N4 (engorged), moulted into adults. Amongst these, the male ticks had typical taxonomic features of the species as described by Hoogstraal (1956). The 'Muguga' tick colony been maintained on susceptible rabbits for many years and the ticks have retained their taxonomic identity. Of the nymphs which engorged and dropped from rabbits resistant to A. variegatum, only 28% fitted into N4 (engorged). The majority of these light ticks developed into adults which had an atypical pattern of scutal ornamentation i.e., 2 lateral spots on the scutum. These lateral spots, which are similar to those present on A. lepidum, are characteristic for A. lepidum to distinguish them from all other Amblyomma species (Hoostraal, 1956). All the efforts to produce a generation from

these abnormally ornamented ticks were unsuccessful. The engorgement weights of such females (0.2284g) were far less than the average weight of ticks fed on susceptible rabbits (2.0700 g). The atypical ticks deposited small numbers of eggs but no larvae hatched from them. Obviously, such ticks do not pose problems in the field.

Since this abnormal feature had not appeared previously in the 'Muguga' tick colony, it can be stated that the occurrence of such ticks was not due to inbreeding, or to extended culture on abnormal hosts. Therefore, there is a factor(s) in the resistant rabbits which probably affected the ticks in such a way as to change the pattern of scutal ornamentation. This factor(s) might have a specific effect on the ticks since 70% of moulted adults were similarly affected i.e. presence of 2 lateral spots. Furthermore, ticks which dropped from susceptible rabbits, but had reduced engorged weights, did not show such effects. This gives further evidence of the effect of immunity in rabbits to ticks. Walker (1959) observed in some A. variegatum specimens an additional coppery coloured spot which was limited to the marginal groove, whereas in the present study the spot crossed the lateral groove. If tick collections made by Walker (1959) were from cattle it would be evidence of natural occurrence of the phenomenon. It has also been reported that ticks changed their feeding behaviour as a result of acquired resistance to ticks by the host . The two-host ticks H. rufipes and H. impressum fed on

immuned hosts showed ashift to 3-host feeding behaviour. This was manifested by an increased rate of detachment of engorged larvae and a decline in numbers of engorged nymphs, although individuals which were fed on non-immune hosts behaved normally (Dittrich, 1980; Rahman, 1984).

Hard ticks of the genera Ixodes, Rhipicephalus, Hyalomma, Rhipicentor, Haemaphysalis, Amblyomma and Dermacentor have been reported to cause tick paralysis in animals in various parts of the world (Nelson et al., 1975). Paralysis in rabbits due to feeding of adults and nymphs of A. variegatum has also been observed in this study. Usually the symptoms of paralysis started just before detachment of the ticks; ticks were not toxic until the 4th day of feeding (Nelson et al, 1975). This possibly depends upon the feeding behaviour of the ticks. The peak production of toxins coincides with the period of intense feeding and salivation (Kemp et al., 1982). The symptoms observed in rabbits in this study were different from those caused by Dermacentor or Ixodes in which domestic pets, livestock, wildlife, and man may be affected by an ascending, flaccid, muscular paralysis. This usually culminates in acute respiratory distress, aspiration pneumonia and death in the absence of proper treatment (Stone and Wright, 1979). Differences in signs from different species do not necessarily imply different toxins (Nelson et al, 1975). Differential diagnosis between tick toxicosis, botulism, intercurrent infections and neuropathies of obscure etiology pose problems (Nelson et al., 1975).

The evidence of paralysis in rabbits in the present study was circumstantial. The removal of feeding ticks at the time when symptoms started to appear led to the removal of the symptoms and the animals returned to normal. This was the only diagnostic method used to reach a conclusion. The rabbit was obviously not able to acquire resistance to the toxins injected by the tick, since it re-developed the paralysis each of the three times it was infested. Paralytic animals made rapid and complete recovery following removal of feeding ticks (Baeza, 1979; Jessup, 1979; Botzler et al., 1980; Lane et al., 1984). The question of acquired immunity to tick toxins is complicated by both positive and negative results with various species of ticks on various hosts, and by wide variation in toxicity of the tick challenge (Nelson et al., 1975). After four infestations of calves with I. holocyclus, 2 calves became paralysed but neither died (Doube, 1975).

In this study paralysis was observed in the 2 groups of rabbits infested with A. variegatum adults and nymphs, but not in the group infested repeatedly with larvae. It seems that a minority of rabbits are susceptible to paralysis since only 2 out of 18 rabbits developed the syndrome. All the stages of I. ricinus caused paralsysis and females were found to be responsible for most cases reported in the field (Doube, 1975). There was no difference in toxicity between the laboratory bred or field collected female ticks (Doube, 1975). It is remarkable that some tick species of D. andersoni induced paralysis in some ecological sites but not in others (Nelson et al. 1975.

CHAPTER 2

Elucidation of humoral antibodies in immune resistance to A. variegatum and R. appendiculatus in rabbits.

In the previous chapter it was demonstrated (i) that rabbits acquired significant levels of resistance by repeated feeding of A. variegatum and R. appendiculatus (ii) that there existed intra-specific resistance between the different stages of ticks (iii) that there was an appreciable level of inter-specific resistance in rabbits resistant to R. appendiculatus against A. variegatum but not vice versa. These phenomenan necessiated further immunological studies to explain the possible mechanisms of both intra-and inter-specific resistance. Therefore, this chapter is concerned with finding out if resistance to the two tick species is accompanied by the production of antibodies by the host and, if so, to investigate common antigens in the different stages of each species and in the two genera. Thus, immunodiffusion tests were carried to fulfil these objectives.

Results

Antisera obtained from the groups of rabbits resistant to adult A. variegatum reacted with protein extracts from six different tick tissue sources. The antisera reacted

with at least three antigens from the salivary glands (Fig. 17). Precipitin lines were also obtained from reactions with protein extracts prepared from the internal organs and the ovaries. However, there was no visible precipitin reaction with proteins extracted from the midgut (Fig. 17). With this particular antiserum the reactions against the larval and nymphal homogenates were very faint. Antisera from other rabbits in the same group also recognized two antigens (Fig. 17). Since there was no detectable reaction with the midgut extract, an attempt was made to see if the reacting antigens from the salivary gland extracts had immunological identity with those from the internal organs (which consisted predominantly of midgut). The precipitin lines reacted with complete immunological identity (Fig. 18). It is worth mentioning that although the six rabbits reacted differently against the tested antigens, the sera of all rabbits in this group contained precipitating antibodies against salivary gland extracts (Fig. 19).

The sera from the second group of rabbits (which had been rendered resistant to A. variegatum nymphs) reacted with homologous antigens to form multiple precipitin lines (Fig. 20). One of the precipitin lines indicated complete immunological identity between the reacting antigens from larval and nymphal homogenates (Fig. 21).

Apparently, sera from the third group of rabbits (which had been exposed to three repeated infestations of A. variegatum larvae) seemed to contain low levels of circulating antibodies, as indicated by very weak reactions against antigen extracts from different developmental stages and tissues of A. variegatum. This was true when the sera

collected after the fourth infestation was also tested. Results froman immunodiffusion test using serum from one of the rabbits which gave relatively intense precipitin lines with larval extracts is shown in Figure 22; at least two visible precipitin lines were formed.

Sera from all the rabbits which had been exposed to three different infestations of different stages of R. appendiculatus, recognized antigens prepared from the larval stages and from the salivary glands of the same tick species. Testing the sera from all five rabbits against homologous salivary gland extracts in an ouchterlony immunodiffusion test, resulted in 5-7 precipitin lines (Fig. 23). However, the same sera did not contain detectable antibodies against the midgut antigens (Fig. 24).

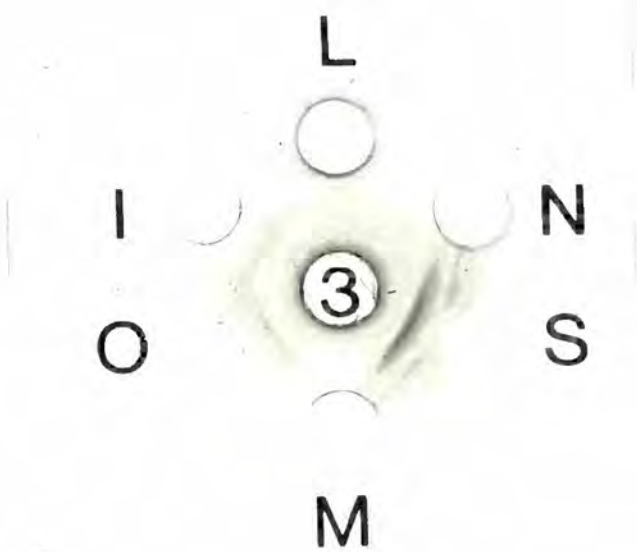
It was observed that in immunodiffusion tests using salivary glands antigens from unfed adult ticks that the reaction with homologous antisera gave much fainter precipitin lines than against similar antigens from partially fed ticks (results not shown). This presumably reflects the developmental pattern of salivary gland antigens in the two species.

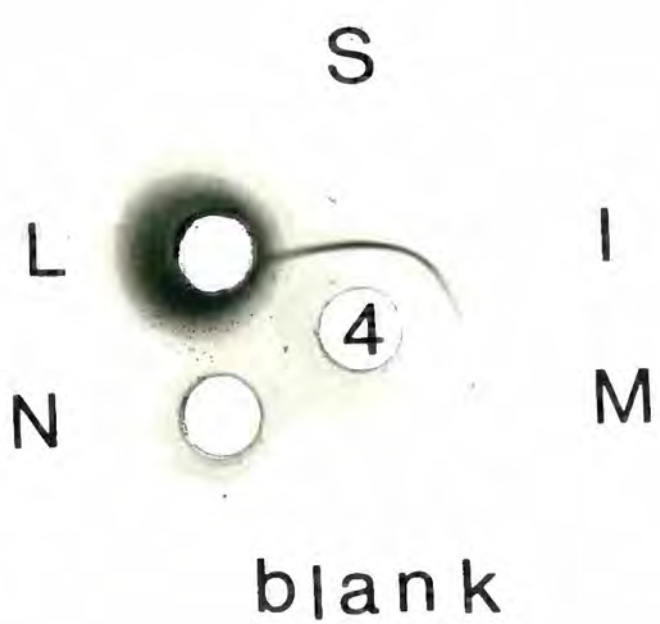
In another experiment, salivary glands extracts from either R. appendiculatus or A. variegatum were reacted with sera from selected rabbits which had received three successive infestations of either species. While sera from either groups of rabbits reacted with extracts of salivary glands from the same tick species that infested

the rabbits (homologous reactions), there were no visible cross-reactions (heterologous reactions) as demonstrated in Figure 25. When a similar experiment was repeated with additional tissue antigens for cross-reactivity, lines resulted only from homologous reactions (Fig. 26).

Fig. 17. Immunodiffusion test to demonstrate the reaction of different developmental stages and tissues with antiserum from a rabbit (no.3; central well) which had been exposed to three repeated infestations with adult A. variegatum. The peripheral wells contain, L- larval homogenate, N - nymphal homogenate, S- adult salivary gland extract, M - midgut extract, O - ovarian extracts, and I - internal organs extracts from partially engorged A. variegatum.

Fig. 18. Immunodiffusion pattern in agar gel; central well contains antiserum from a rabbit (no.4) which had three repeated infestations with adult A. variegatum. The peripheral wells contain antigens prepared from the same tick species; refer to Figure 17 for explanation of symbols.

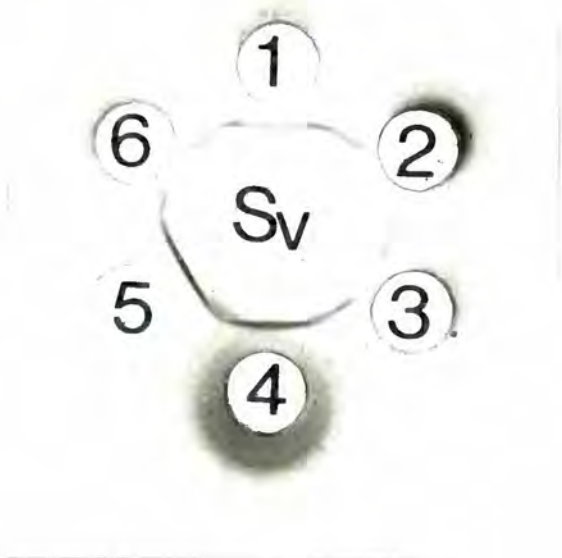




blank

Fig. 19. Ouchterlony test showing the reactions of antisera from six rabbits (peripheral wells, 1-6) which had three successive infestations with adults A. variegatum, against Sv - Salivary gland extract (central well) prepared from partially fed ticks of the same species.

Fig. 20. Immunodiffusion reaction with antiserum, from a rabbit (no.8, central well) which had three repeated infestations with nymphs A. variegatum, against different extracts prepared from the same species; explanation of symbols in peripheral wells as for Figure 17.


1
2
3
4
5
6
Sv

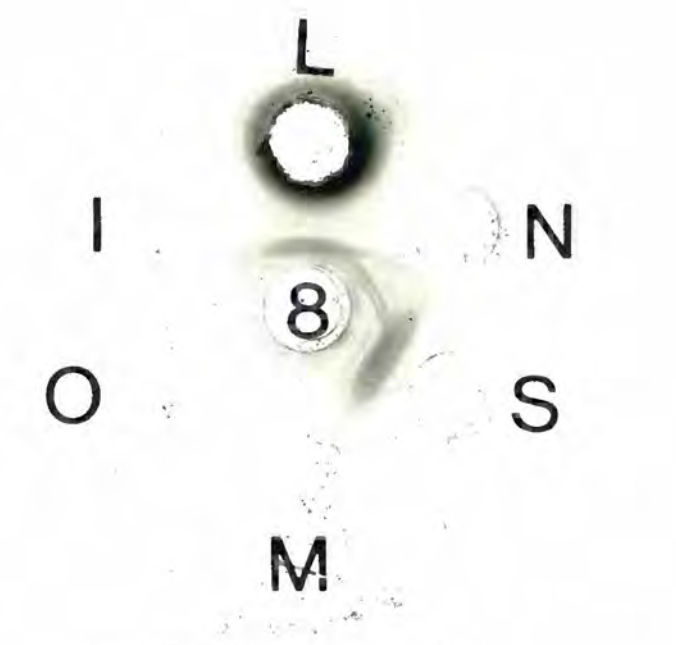
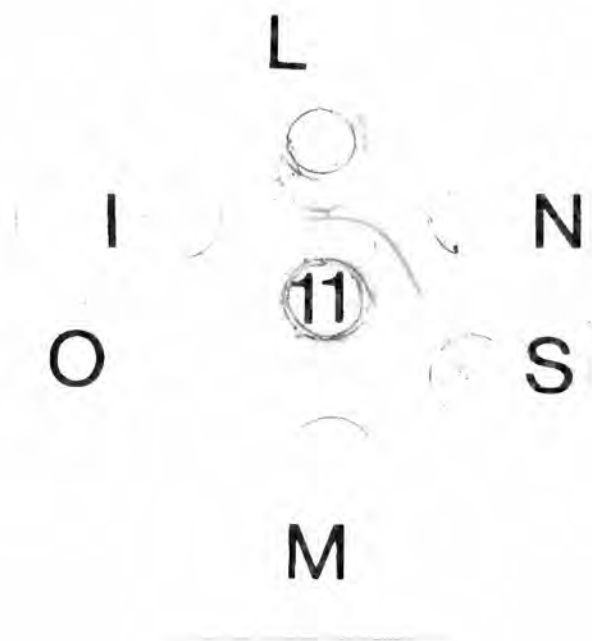

L
I
N
8
O
S
M

Fig. 21. Ouchterlony immunodiffusion showing immunological identity reaction of L - larval homogenate and N - nymphal homogenate of A. variegatum against antiserum from a rabbit (no. 11, central well) which had been exposed to three nymphal infestations with the same tick species. For explanation of other symbols in the peripheral wells refer to Figure 17.

Fig. 22. Immunodiffusion test to demonstrate the reaction of different instars and tissue extracts from the tick A. variegatum against antiserum from a rabbit (no. 16, central well) which had been exposed to three successive infestations with larvae of the same species; symbols in the peripheral wells as in Figure 17.


L
I
N
11
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S
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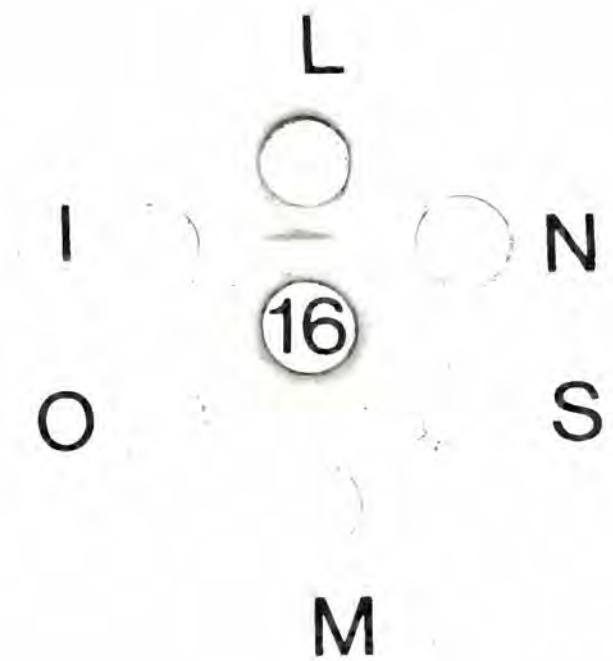
L
I
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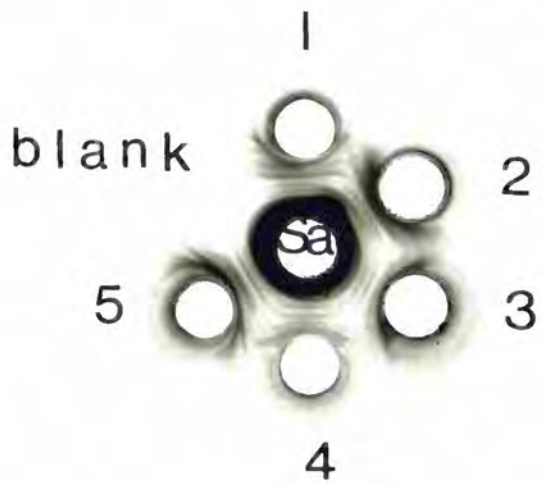
16
O
S
M

Fig. 23. Ouchterlony immunodiffusion showing the reaction of antisera (peripheral wells 1-5) from the group of rabbits which had been exposed to different infestation by R. appendiculatus against Sa - salivary glands extracts prepared from partially engorged ticks of the same species. The peripheral wells were reloaded with the various sera after 24 hours.

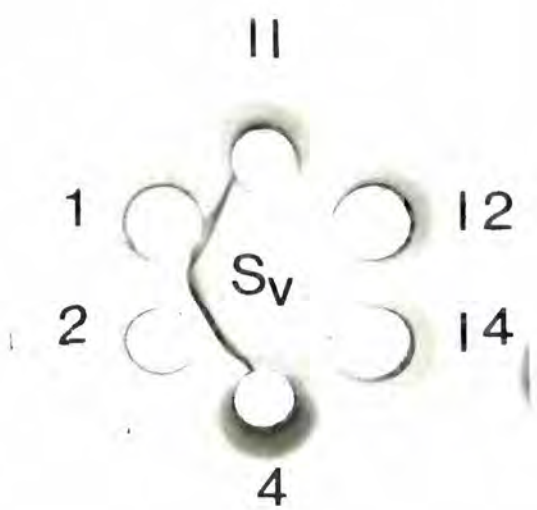
Fig. 24. Immunodiffusion test to demonstrate the reaction of antiserum, from a rabbit (no. 5, central well) which had been exposed to the three instars of R. appendiculatus, against Sa - salivary gland and Ma - midgut extracts prepared from partially fed adults of the same species.

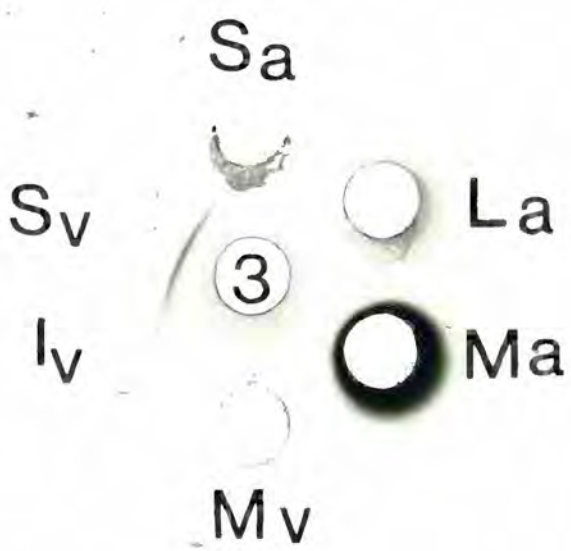

1
blank
2
Sa
5
3
4


Sa
5
Sa
5
Ma
Sa
R- app

Fig. 25. Immunodiffusion pattern of Sv - salivary gland extracts (central well), from partially engorged adults A. variegatum, reacting with antisera from rabbits (no. 1, 2, 4, peripheral wells) which had three repeated infestations with adults A. variegatum and antisera from another group (no. 11, 12, 14, peripheral wells) which had been exposed to infestations with the three instars of R. appendiculatus.

Fig. 26. Ouchterlony immunodiffusion showing immunological reaction of antiserum from a rabbit (no. 3, central well), which had three successive infestations with adults A. variegatum, against different tissue extracts in the peripheral wells; Sa - salivary gland, La - larval homogenate, Ma - midgut extracts from R. appendiculatus while Sv - salivary gland extract, Iv - whole internal organs homogenate and Mv - midgut extract prepared from R. appendiculatus.


11
1
12
Sv
2
14
4


Sa
Sv
La
3
Iv
Ma
Mv

DISCUSSION

Antibodies have been demonstrated in the serum of all the animals within the four groups of rabbits which had been exposed to natural tick feeding in three successive tick infestations. Of the four groups of rabbits, three had been fed upon by either larval, nymphal or adult stages of A. variegatum and the fourth group had been infested with all stages of R. appendiculatus. There are two pieces of circumstancial evidence which suggest that these antibodies might have a protective function: firstly, such reacting antibodies were absent in sera from tick-naive animals and, secondly, their concentration in sera from rabbits infested with larvae was low, as indicated by weak precipitin lines in immunodiffusion tests. Thus, tick-naive animals allowed a greater number of ticks to feed and drop with higher engorgement weights than did rabbits which had been exposed to six infestations of A. variegatum larvae. However, the latter group showed a lower level of resistance (expressed in terms of engorgement weights) compared to animals made resistant with repeated infestations of either nymphs or adults. While testing antisera from immunized animals by immunodiffusion, Allen and Humphreys (1979) observed that strong multiple bands formed with sera from animals which expressed a high level of immunity while single bands were obtained from a calf which gave the least impressive results. Similar results were also obtained by Roberts and Kerr (1976) when passive transfer of plasma from highly resistant animals significantly protected susceptible recipients. On the other hand, transfer of plasma from non-immune or poorly immune

animals did not confer resistance. However, Willadsen et al. (1978) found that the antibody titres from sensitized animals did not necessarily correlate with the degree of protective immunity. This was also demonstrated by Cunningham (1981) who immunized rabbits with protein extracts of eggs and unfed ticks to obtain high antibody titres. However, on challenging these animals with ticks, no adverse effects on them were observed. Such information provides little to explain the processes involved in host animals with naturally acquired resistance (Nelson et al., 1977).

The immunodiffusion tests demonstrated intra-specific cross-reactivity. Thus, sera from rabbits infested with adults reacted to give intense precipitin lines against adult, nymphal and larval extracts just like the reactions obtained with sera from rabbits fed upon by nymphs, against antigens from adults, larvae and nymphs. While the sera from rabbits infested with larvae also reacted with the same three antigens, only weak precipitin lines were produced . These results indicate the presence of common antigens within the three tick stages. The larval and nymphal extracts have at least one identical antigen in common.

The various sera did not react with the midgut extracts. Protein extracts from whole internal organs produced precipitin lines of complete identity with antigens from salivary glands indicating that probably the antigens common to the three stages of A. variegatum originated from the salivary glands. The present findings are

consistent with those of Brown and Askenase (1983) who found that passive transfer of immune serum from hosts resistant to various life stages (larvae, nymphs, adults) of A. americanum to tick-naive recipient animals conferred immune protection in the latter against larval challenge. The feeding of A. maculatum nymphs on rabbits immunized with an extract derived from homogenized adults induced an immediate hypersensitivity type reaction (Arthus reaction) within 8-12 hours (McGowan et al., 1980). These results suggested that both nymphs and adults shared common immunogens which probably originated from the salivary gland secretions. These findings confirm the results reported in Chapter 1 on the feeding performance of ticks in cross-resistance experiments i.e. rabbits resistant to the adult stage also showed significant resistance to the tick immatures, and rabbits resistant to nymphs also showed significant protection against the feeding of larvae. The weak immunological reactions with sera from rabbits infested with larvae was also reflected in low levels of resistance to tick challenge in the same rabbits.

Rabbits which acquired resistance by repeated feeding of R. appendiculatus expressed a significant degree of resistance when challenged with A. variegatum, but not vice versa (Chapter 1). However, immunodiffusion tests did not reveal detectable inter-specific cross-reactivity. A similar cross-resistance phenomenon was reported for A. americanum and R. sanguineus (Brown and Askenase, 1983), although immune serum from R. sanguineus resistant animals failed to confer resistance against A. americanum (Brown et al., 1982). These

results indicated that the immune responses to heterologous challenge may be entirely cell-mediated. In fact, Brown and Askenase (1983) suggested that such responses were T-cell mediated. In the current study (Chapter 3) the R. appendiculatus/A. variegatum situation was clarified; when an immediate hypersensitivity response was observed at A. variegatum nymphal attachment sites on rabbits resistant to R. appendiculatus (lesions were characterized by vesicle formation and intense eosinophilic infiltration and degranulation).

Some speculations can be made with respect to immunodiffusion results where reactions between serum from resistant animals and midgut extracts are not detectable. It is possible that the midgut associated antigens do not enter the host during the feeding process. These results are in agreement with the findings of Ackerman et al. (1980) who failed to demonstrate immunoprecipitation reactions between sera fromrabbits or rats repeatedly challenged with D. variabilis or immunized with midgut antigens. These findings lead to an important observation that in vaccination trials against ticks using salivary gland antigens, there is expected to be continuous boosting through daily natural challenge by feeding ticks, thus resulting in high levels of immunity and protection. Labarthe et al. (1985) immunized calves with B. microplus salivary gland extracts and the immune serum recognized both the immunogen and whole larval extract. Challenging these calves with live tick larvae enhanced the formation of antibodies against the larval extract and against tick saliva. On the other hand, immunization using the midgut antigens should theoretically have an immediate effect on ticks in the primary challenge i.e. inhibition of

feeding, impairment of egg development, egg hatching or moulting. If not, this procedure will have no further immunological activation or boosting through natural tick feeding due to lack of common antigens passed via the tick saliva into the host. The evidence for this is provided by Ackerman et al. (1980) who found that resistance induced with midgut extracts did not generate immuno-allergic responses.

The weak precipitin bands obtained with different antisera against salivary glands from unfed ticks compared to the strong multiple bands with salivary glands from partially fed ticks, can be explained. The salivary glands of feeding ticks increase in size and in total protein content as feeding progresses (McSwain, 1982), which probably coincides with the period of maximum protein synthesis 6 days after attachment. The salivary glands tend to degenerate in fully engorged ticks. Immunizing animals using extracts from tissues of unfed ticks was ineffective in achieving protection against feeding ticks (Allen and Humphreys, 1979). These findings have practical implications for the development of a vaccine.

Little information is available regarding the type of antibody involved in eliciting resistance and its effect on ticks. Gel diffusion studies with immune serum from rabbits repeatedly exposed to H. longicornis revealed the presence of specific IgG antibodies (Fujisaki, 1978) but the role played by this class of immunoglubolins in resistance was not described. Brown et al (1982) reported that IgG antibodies are involved in cutaneous basophil responses. The

requirement of basophils in immune resistance has already been established (discussed in Chapter 3). The immediate hypersensitivity reactions which have been equated with resistance are mediated by a homocytotropic antibody probably IgE (Boese, 1974; Willadsen, 1980). Antibodies ingested in the tick blood meal can react with target antigens associated with the digestive tract to alter its digestive and absorptive properties by cell destruction (Floyd, Pers. Comm.).

In summary, antibodies to natural feeding of ticks have been demonstrated in four groups of rabbits repeatedly infested with different stages of either A. variegatum or R. appendiculatus. There is high cross-reactivity between the three stages within the tick species. However, there is no demonstrable reaction between the two genera. Therefore the cross-resistance observed between the different species of ticks (Chapter 1) is probably cell-mediated.

CHAPTER 3

Host skin cellular reactions in response to feeding of A. variegatum and R. appendiculatus

The results obtained in Chapter 1 have shown that the rabbits which were made resistant by repeated feedings of R. appendiculatus indicated a considerable resistance to A. variegatum but not vice versa. This phenomenon necessitated further studies on the host parasite relationships of these two tick species in order to gain an insight into possible cross-resistance mechanisms. The involvement of humoral factors was already discussed in Chapter 2. The objective of this part of the work was to study the variation in the cellular reactions, in the skin of a host 48 hours after simultaneous infestation with two tick species i.e. A. variegatum and R. appendiculatus. The histopathology of the tick bite was studied in three groups of rabbits. These were i) tick-naive rabbits ii) rabbits which were resistant to R. appendiculatus iii) rabbits made resistant to A. variegatum. The preparation of these rabbits has been described in the general materials and methods (Section 2.1.).

RESULTS

1. Macroscopic reactions

The gross signs of the inflammation in the skin of rabbits resistant to R. appendiculatus and A. variegatum, resulting from homologous infestation initially showed erythema limited to the

bite of an attached tick or an abandoned feeding site. This was followed by the development of a diffused erythematous papula with marked oedema. The ears were swollen and a serous exudate from non-specific areas was observed, accompanied by sloughing of tissues 48 hours post attachment (Fig. 27). The reaction with A. variegatum nymphs fed on the rabbits resistant to R. appendiculatus showed diffuse erythematous papulae but no serous exudation or sloughing was evident (Fig. 27). These signs of inflammation were insignificant in the control rabbits with both tick species and also with R. appendiculatus nymphs fed on rabbits resistant to A. variegatum (Fig. 28).

2. Histopathology of the bite reaction in tick-naive rabbits.

2.1. The host response to R. appendiculatus

This tick has short mouthparts which barely penetrated the epidermis. A very large external supporting cement cone formed around the mouthparts (Fig. 29). The host blood vessels were affected by the tick and showed dilation and engorgement with blood. Red blood cells were mostly recognized within the vessels. This feature typified most of the sections examined (Fig. 30). However, there was no cellular infiltration around the vessels. The lesion developed directly within the papillary layer of the dermis. A simple cavity (i.e. a well demarcated space beneath the mouth-parts) formed under the attachment sites of some ticks whilst in others no tissue changes were apparent beneath the mouthparts. The average number of inflammatory

Fig. 27. Secondary feeding of R. appendiculatus nymphs on the right (R) ear (exudation and sloughing of the skin) of a rabbit previously exposed to three repeated infestations with nymphs of the same species. Simultaneous feeding with A. variegatum nymphs is on the left (L) ear (erythematous swellings around the mouth parts). Photograph taken about 48 h after tick attachment.

Fig. 28. Secondary feeding of A. variegatum nymphs on the left (L) ear (lesions marked by exudation and necrosis) of rabbit previously exposed to three repeated infestations with nymphs of the same species. Simultaneous feeding with R. appendiculatus nymphs is on the right (R) ear. Photograph taken about 48 h post attachment.

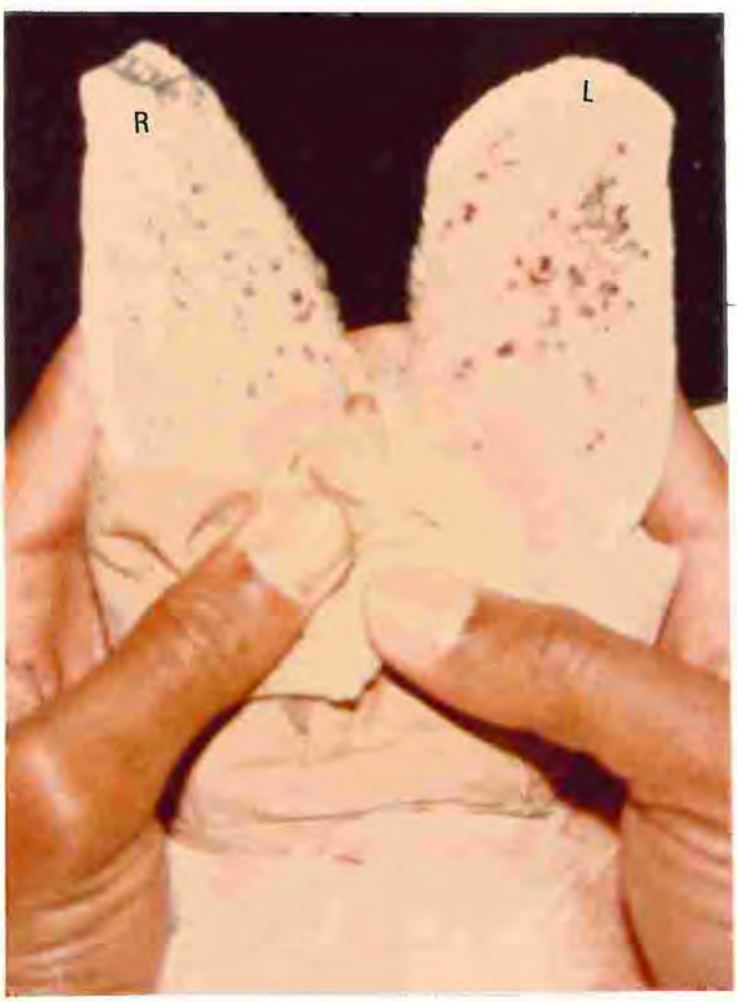


Fig. 29. Attachment site of R. appendiculatus nymph on a tick-naive rabbit. The cavity (Ca) is obvious within the epidermis (Ep) and dermis (D) beneath the mouthparts (MP). The hair follicles (HF) are intact and the feeding lesion is negligible. Total magnification : 700 X.

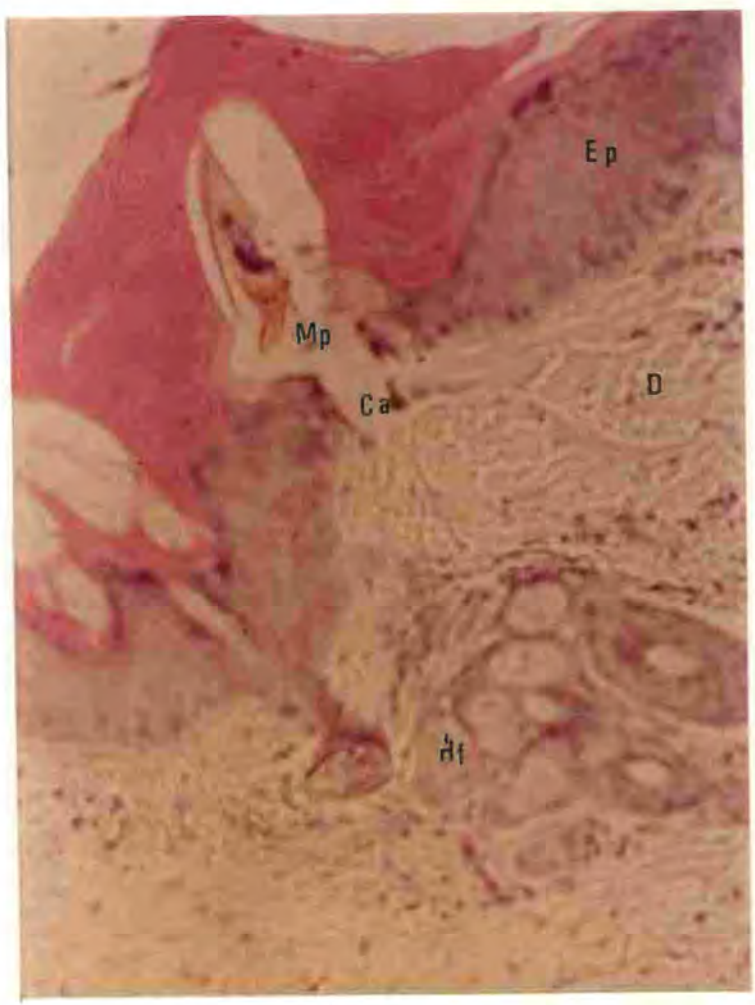


Fig. 30. Feeding of R. appendiculatus on a tick-naive rabbit. Blood vessels (arrows) are engorged with blood and RBC mostly recognized. For explanation of other symbols refer to Figure 29. Total magnification : 840 X

cells per unit area of the lesion was small (4.8/0.01mm^2, Table 10). The predominant cells within these were mononuclear cells (56.2%), followed by neutrophils (39.6%) and very few eosinophils (4.2%). However, some of the lesions showed insignificant reactions. The overlying epidermis and hair follicles adjacent to the lesion were intact and undamaged.

2.2 The host response to A. variegatum

This tick has long mouth-parts and a thin casing cement (perirostral cement) formed around the chelicerae and hypostome which were fully inserted into the dermis (Fig. 31). A small external supporting cement cone was formed around the hypostome. Presumably due to the deep penetration, the size of the lesion was large and extended into the reticular dermis (Fig. 32). This extensive lesion typified all sections examined. The average number of inflammatory cells per unit area of the lesion was 50.5/0.01 mm^2 (Table 10) was about 10 times that in the lesions induced by the feeding of R. appendiculatus. The predominant cells within the infiltrate were neutrophils (73.3%), followed by mononuclear cells (25.1%) and very few eosinophils (1.6%). The neutrophils were present below the attachment sites while the mononuclear cells were at the periphery of the lesion. The epidermis and hair follicle epithelium were intact and signs of hyperplasia were not evident. Only a small part of the epidermis was lost around the inserted mouth-parts. Tick saliva, or regurgitated material, secreted into the feeding site near the ear cartilage affected it as shown by

Table 10

Cellular responses and reactions, about 48 h post attachment, in skin of tick-naive rabbits at attachment sites of A. variegatum nymphs on the right ear and R. appendiculatus nymphs on the left ear*.

Responses	A. variegatum		R. appendiculatus	
Cellular infiltrate	no./0.01mm^2	%	no./0.01mm^2	%
Neutrophils	37.0	73.3	1.9	39.6
Eosinophils	.8	1.6	0.2	4.2
Mononuclear	12.7	25.1	2.7	56.2
Total	50.5		4.8	
Reactions				
Vesicles		-		-
Epidermal hyperplasia (mm)		(0.04)		(0.03)
Hair follicles hyperplasia		-		-
Fibroblast		-		-

* These are mean cell counts per unit area of the lesion based on 4 biopsies, five sections from each biopsy and 5-8 fields per section.

(-) indicates negligible reactions.

Fig. 31. Longitudinal section of the mouth parts at the attachment site of A: variegatum nymph. The external (epidermal) cement (EC), perirostral cement (PC), mouth parts (MP, hypostomal teeth (HT), epidermis (EP) and dermis (D) are all apparent. Total magnification : 1400 X.

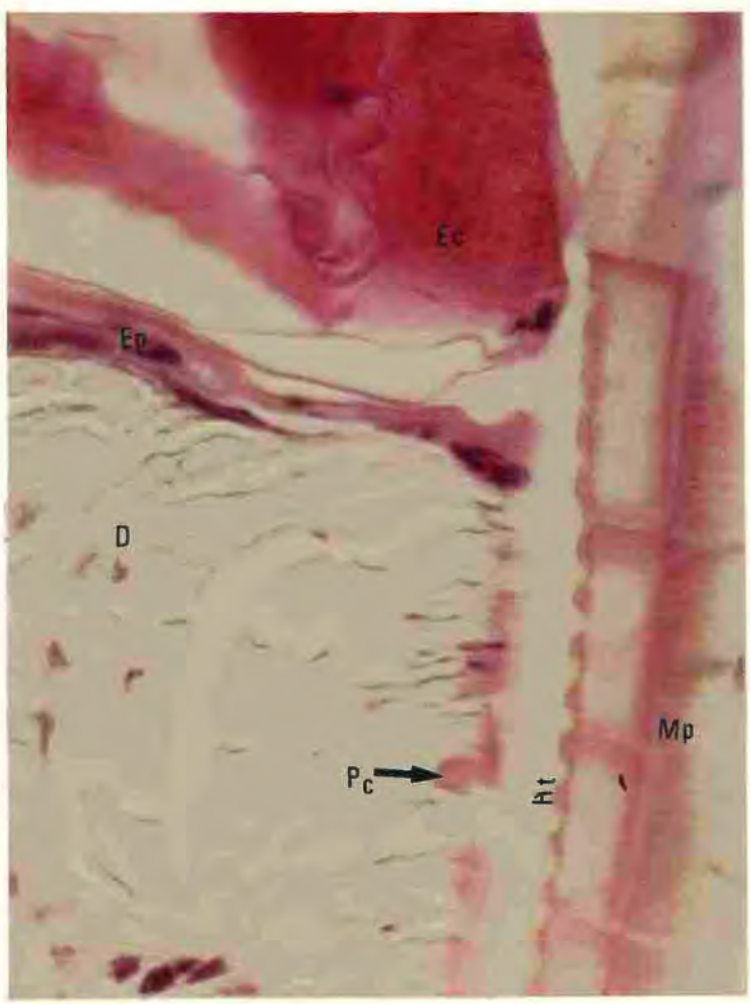
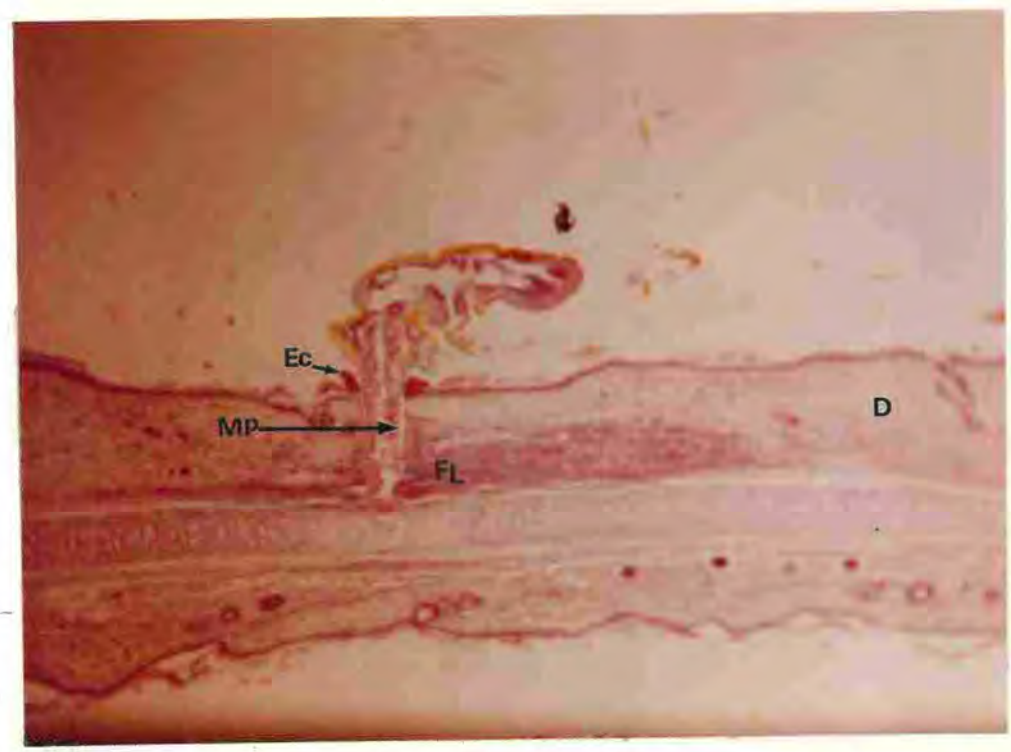


Fig. 32. Attachment site of A. variegatum on a tick-naive rabbit. The mouth parts (MP) are inserted well into the dermis (D). The feeding lesion (FL) is extensive but not compact and the epidermis does not show any evidence of hyperplasia. The external cement (EC) is apparent. Total magnification : 120 X.



the disarranged, abnormally shaped and lysed cartilage cells (Fig. 33).

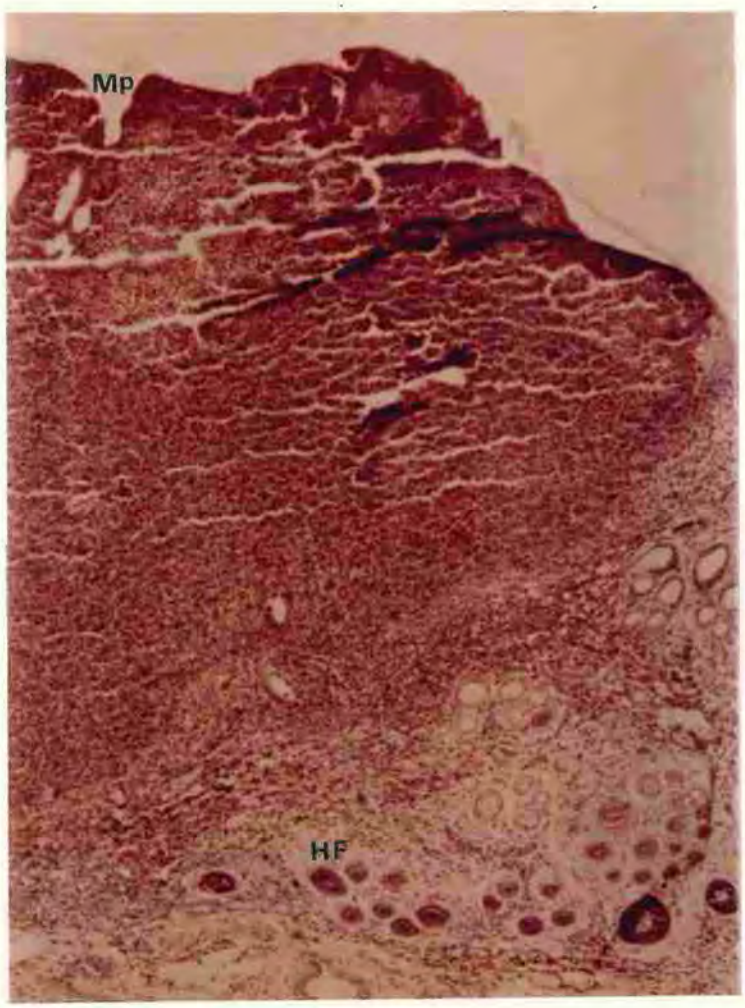
3. Histopathology of acquired resistance to R. appendiculatus

3.1. The host response to R. appendiculatus

The host reaction was characterized by the development of clearly demarcated and compact lesions (Fig. 34). Intra-epidermal nodules (vesicles), which contained necrotic epithelial cells and degenerated leucocytes, were formed (Figs. 35 and 36). The follicles, surrounding the lesions, became hyperplastic and showed sub-epidermal growth which appeared to encapsulate the feeding lesion. The sweat glands, sebaceous glands and hair follicles showed necrotic changes and were masked by heavy cellular infiltration in the lesion. The lesion was further compounded by widespread epithelial cell degeneration and pycnosis. The epidermis was lacking around the feeding lesion. There was a change from the normal blue-stained appearance of the tissues to a marked eosinophilic reddening (indicative of hyalinization) which characterised the feeding lesion (Fig. 37) and tended to regress towards the periphery. The eosinophils showed evidence of breakdown and disintegration with their granules lying in the tissues. Eosinophils with distinct cytoplasm and nuclei (Fig. 38) were found towards the periphery of the vesicle while neutrophils and mononuclear cells were found particularly around the feeding lesion. Eosinophilic infiltration occurred around the deeper blood vessels (Fig. 39). Fibroblasts were recognized adjacent to the feeding lesion.

Fig. 33. Feeding site of A. variegatum nymphs in the skin of a tick-naive rabbit showing the lysis of ear-cartilage cells (arrows). Total magnification : 1800 X

Fig. 34. Cellular infiltration and tissue reaction in the skin of a rabbit resistant to R. appendiculatus in response to homologous nymphal feeding. The lesion is compact and shows marked necrosis, the epidermis is lacking and also note the disappearance of hair follicles (HF) and associated glands in the lesion. MP - mouth parts. Total magnification : 640 X.



Figs. 35, 36. Development of intra-epidermal vesicles in the tick attachment sites on a resistant rabbit to R. appendiculatus. The feeding of both species produced the same kind of lesions. In both figures the well defined vesicles (arrows) contain degenerated leucocytes and necrotic epithelial cells. Note also the epidermal (EP) hyperplasia and the intense leucocytic infiltration in the dermis (D). Figure 35 contains series of small vesicles. Total magnification : 640 X.

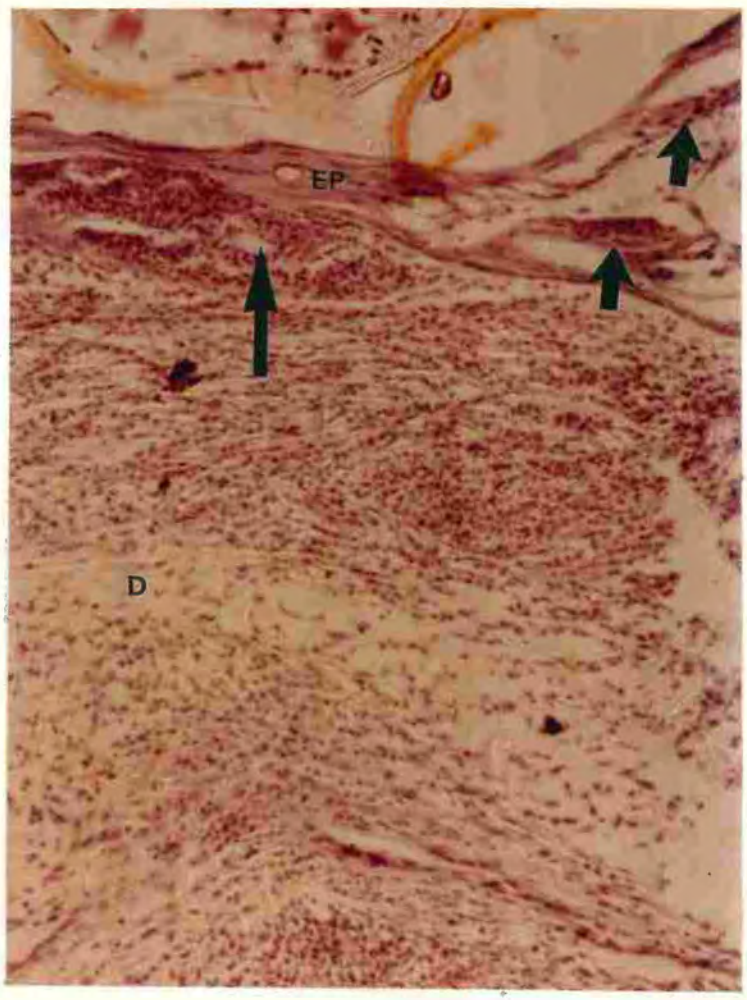


Fig. 35

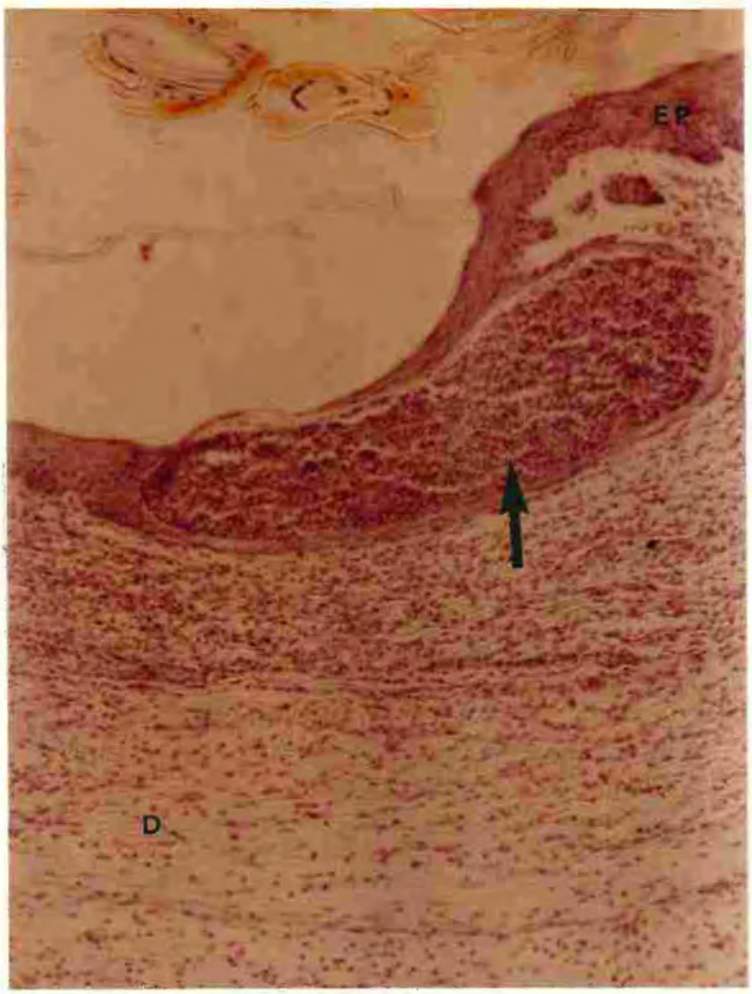


Fig. 36

Figs. 37, 38. Feeding lesion in the skin of a rabbit resistant to R. appendiculatus in response to homologous feeding with nymphs. A marked eosinophilic reddish colour in the middle (M) of the feeding lesion is indicative of hyalinization. This tended to regress towards the periphery of the lesion. Intact eosinophils (arrow) can be identified at the periphery, see also Fig. 38. Total magnification : 1000 X and 3000 X respectively.

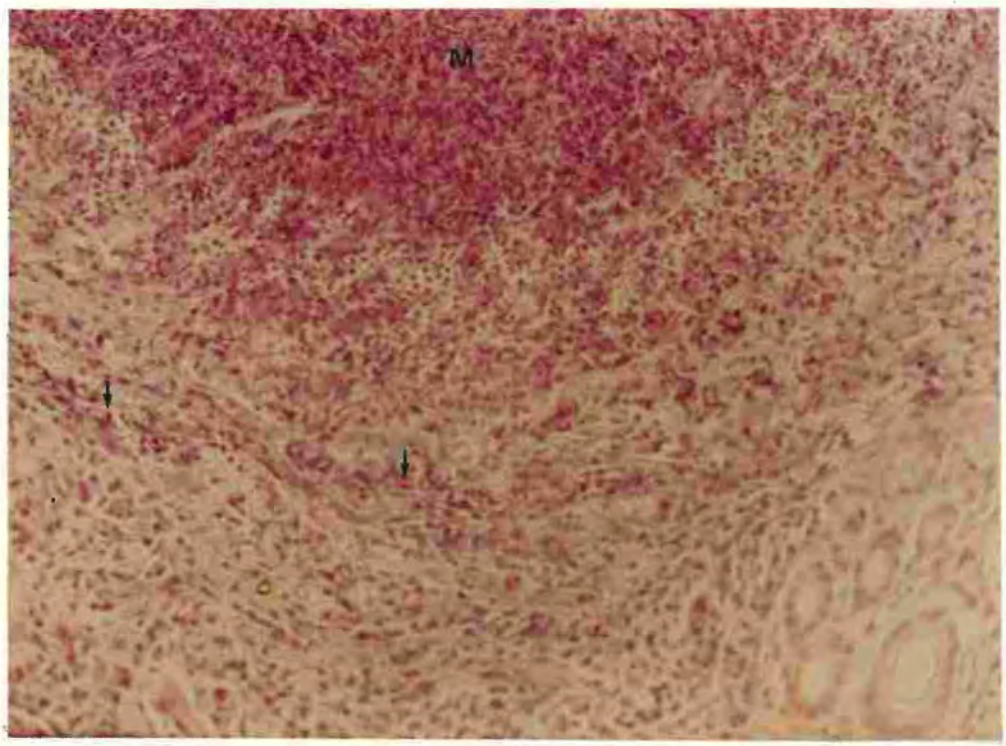


Fig. 37

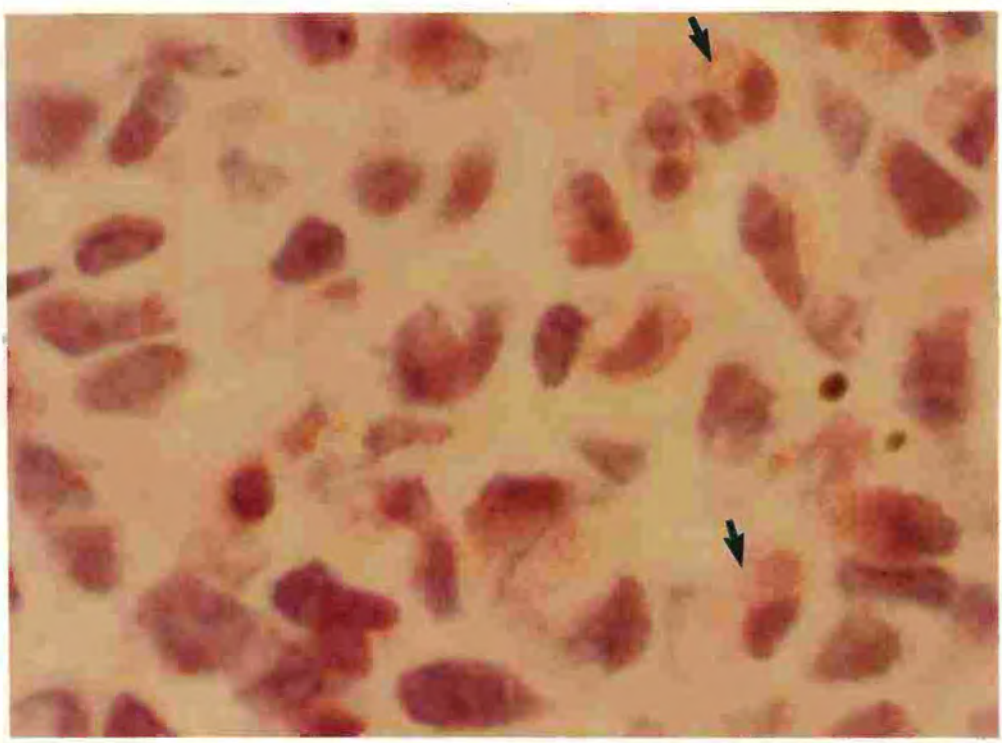
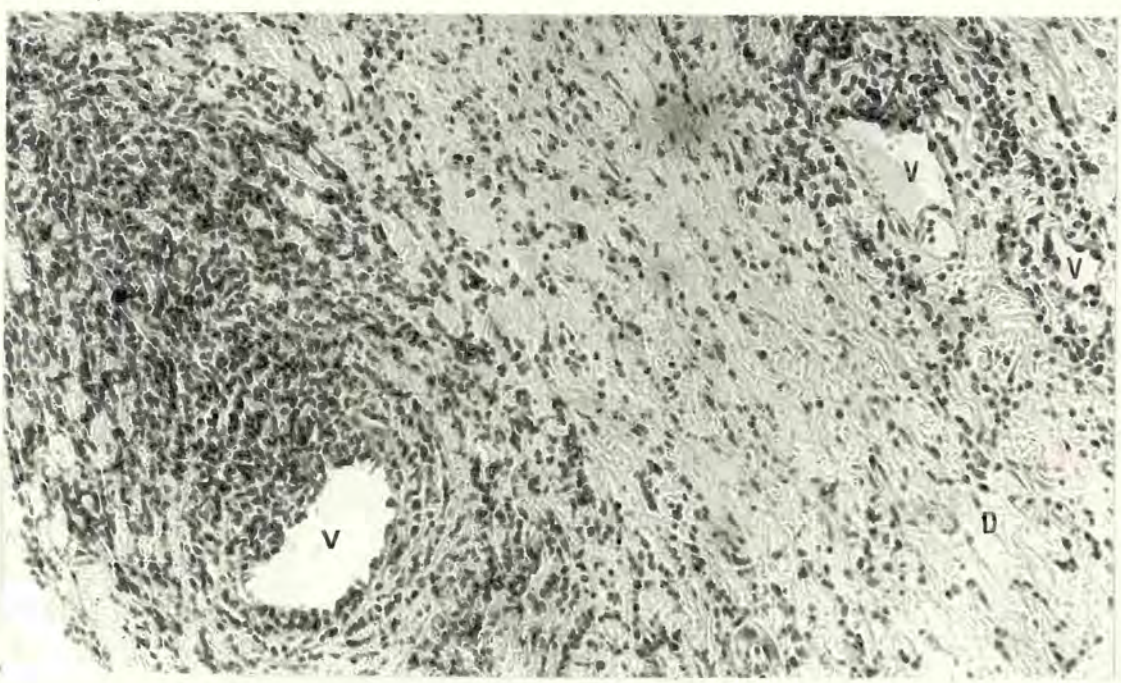


Fig. 38

Fig. 39. Leucocytic infiltration in the dermis (D) around the deeper blood vessels (V) ; mobilization is toward the mouth parts in response to the homologous feeding of the tick on a resistant rabbit to R. appendiculatus. Total magnification : 1800 X



The average number of inflammatory cells per unit area of the lesion (Table 11) was 22.4/0.01mm^2, with eosinophils being the predominant cell type (52.2%), followed by neutrophils (24.1%) and mononuclear cells (23.9%). So few basophils were seen that they did not warrant quantification and mast cells were not readily detected either.

3.2 The host response to A. variegatum

The feeding of this tick on rabbits resistant to R. appendiculatus induced similar skin reactions to those described for homologous challenge (Section 3.1). Thus, large vesicles and compact feeding lesions were formed (Fig. 40). There was epidermal and hair follicle hyperplasia, epithelial cell disintegration and leucocyctic degeneration. The average number of inflammatory cells per unit area of the lesion was 31.4/0.001mm^2, which was greater than that in R. appendiculatus attachment sites (Table 11). However, the predominant cells were neutrophils (51.9%) followed by eosinophils (28.3%) and mononuclear cells (19.8%). The number and percentage of neutrophils present in the lesion was significantly ($P<0.01$) higher than those produced by the feeding of R. appendiculatus on the other ear. This was not the situation with the mononuclear cells, where the number and percentage of the infiltrate did not differ. On the other hand it was the percentage, but not the number, of eosinophils which differed significantly ($P<0.01$) between the two attachment sites.

Table 11

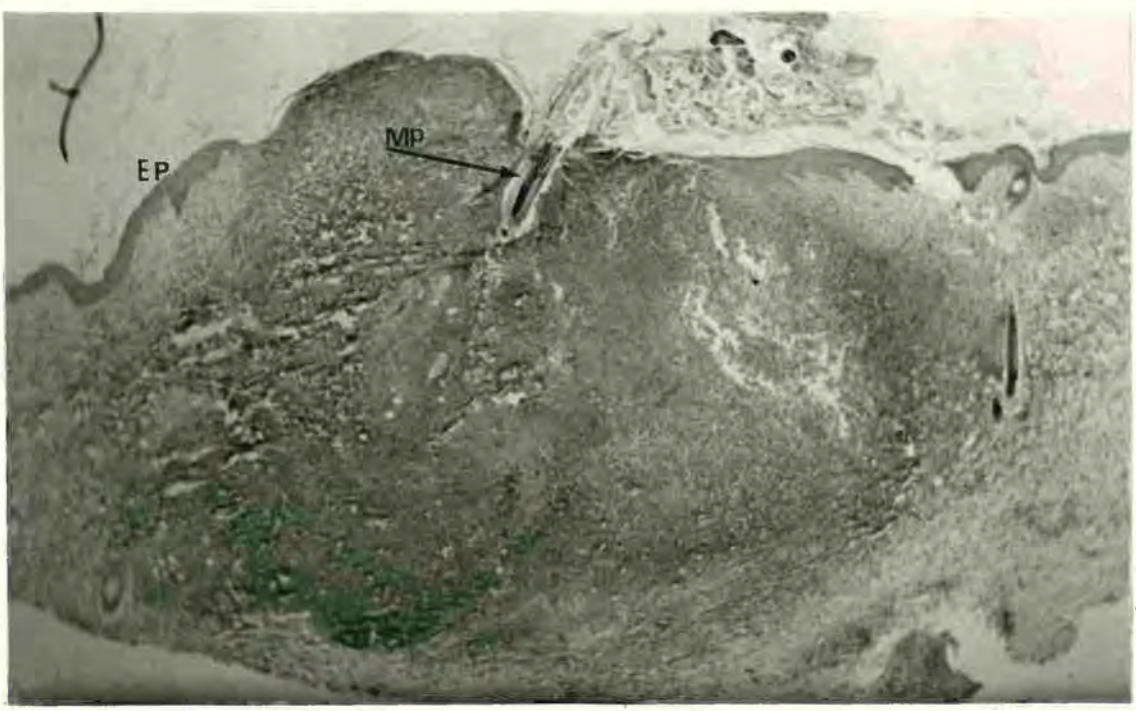
Cellular responses and reactions, about 48 h post attachment, in skin of rabbits resistant to R. appendiculatus at attachment sites of A. variegatum nymphs on the right ear and R. appendiculatus nymphs feeding sites on the left ear*.

Responses	A. variegatum		R. appendiculatus	
Cellular infiltrate	no./0.01mm^2	%	no./0.01mm^2	%
Neutrophils	17.3	51.9	6.1	24.0
Eosinophils	7.7	28.3	10.6	52.2
Mononuclear	6.4	19.8	6.0	23.9
Total	31.4		22.4	
Reactions				
Vesicles	+		+	
Epidermal hyperplesia (mm)	+ 0.07		+ 0.06	
Hair follicles hyperplesia	+		+	
Fibroblast	+		+	

* These are, mean cell counts per unit area of the lesion based on four biopsies, five sections from each biopsy and 5-8 fields per section.

(+,-) indicates intense or negligible reactions respectively.

Fig. 40. The feeding site of A. variegatum nymphs in the skin of a rabbit resistant to R. appendiculatus. Note the epidermal hyperplasia, the epidermis (EP) is lacking around the mouth parts (MP) as well as the disappearance of hair follicles and associated structures. Total magnification : 1000 X.



4. Histopathology of acquired resistance to A. variegatum

The host response to the homologous feeding produced skin reactions at attachment sites similar to those described for the tick when fed on rabbits resistant to R. appendiculatus (Section 3.2). The results of skin reactions from the feeding of the two tick species on rabbits resistant to A. variegatum are shown in Table 12. The lesion was characterized also by the predominance of neutrophils (56.6% of the infiltrate), followed by eosinophils (29%) and mononuclear cells (15.3%). These rabbits however, did not seem to respond to R. appendiculatus feeding on the other ear. There was slight thickening of the epidermis but not of the hair follicles. The feeding lesion was small, with very few inflammatory cells per unit area of the lesion ($5.3/0.01mm^2$) infiltrating the attachment sites. Most of the sections showed negligible cellular changes. This situation was similar to that obtained by feeding this species on tick-naive rabbits (Section 2.1), with the exception that eosinophils were the predominant cells (49.1%) followed by mononuclear cells (35.8%) and very few neutrophils (15.1%).

DISCUSSION

The histopathological studies on the attachment sites of nymphs of A. variegatum and R. appendiculatus, 48 hours post attachment, have revealed differences between the three groups of rabbits which were related to the nature of their resistance. There was some variation in cellular infiltration between separate lesions, within animals of the same group, particularly within the two groups of resistant rabbits.

Table 12

Cellular responses and reactions, about 48 h post attachment, in skin of rabbits resistant to A. variegatum at attachment sites of A. variegatum nymphs on the right ear and R. appendiculatus nymphs on the left ear*.

Responses	A. variegatum		R. appendiculatus	
Cellular infiltrate	no./0.01mm^2	%	no./0.01mm^2	%
Neutrophils	18.2	56.7	0.8	15.1
Eosinophils	9.3	29.0	2.6	49.1
Mononuclear	4.6	15.3	1.9	35.8
Total	32.1		5.3	
Reactions				
Vesicles	+		-	
Epidermal hypersplasia	+ (0.06)		- (0.04)	
Hair follicles hyperplasia	+		-	
Fibroblast	+		-	

* These are mean cell counts per unit area of the lesion based on 4 biopsies, five sections from each biopsy and 5-8 fields per section.

(+,-) indicates intense or negligible reactions respectively.

This could result from the difference in the duration of tick attachment. Thus there were successful nymphs which attached readily, and unsuccessful nymphs which attached and detached several times. The evidence for this was more than 20 abandoned feeding sites, noticable by hyperaemic swellings, detected on the ears of some resistant animals. Since the cellular reaction in the tick feeding sites is dynamic (i.e. the longer the attachment, the stronger the reaction) these differences were to be expected. It was found that the larvae of B. microplus feed on resistant cattle less efficiently and move more often than on tick-susceptible hosts (Kemp et al., 1976) and high tick mortalities occur within the first 24 hours of larval application (Koudstaal et al., 1978). This problem was overcome by examination of more biopsies of tick attachment.

Moorhouse and Tatchell (1966) and Tatchell and Moorhouse (1968) have described in detail the feeding process of B. microplus. Tick attachment is accomplished by the secretion of a cement substance in which the mouthparts are embedded and which adheres firmly to the host skin. A secondary cement secretion preceeds the final engorgement of the tick. Both types of cement were secreted within 48 hours in this study.

The external cement material deposited by R. appendiculatus nymphs, with short mouthparts, was far more abundant than that deposited by A. variegatum nymphs. The long mouthparts of the latter species were firmly embedded in the dermis and probably needed no further cement support. The cement is an inert material (i.e. immunologically

inactive) and does not provoke the development of host parasite factors inimical to the tick (Moorhouse and Tatchell, 1966). The active salivary gland agents and/or the regurgitated materials are antigenic and set in motion host responses which are normally detrimental, but sometimes of use to the tick (Kemp et al., 1982).

The dilation and engorgement of skin blood capillaries adjacent to tick attachment in tick-naive rabbits was a common occurrence and not necessarily associated with host immune response and can be attributed to the action of tick saliva (Kemp et al., 1982). The simple cavity formed in most of feeding lesions has also been described before (Tatchell and Moorhouse, 1968). It is due to the host neutrophils which penetrate the lesions (Tatchell and Moorhouse, 1970). However, it is not an inevitable effect and some ticks completed engorgement without the formation of such a cavity (Tatchell and Moorhouse, 1968).

The reaction at attachment sites in tick-naive rabbits differed with the two tick species. A. variegatum feeding lesions were extensive and the total number of inflammatory cells was about 10 times greater than the feeding lesions of R. appendiculatus on the other ear. The predominant cell type with the former tick species was the neutrophil (73.3% of the infiltrate) while mononuclear cells predominated at the attachment sites of R. appendiculatus. Both attachment sites showed very few eosinophils (4.2% and 1.6%, respectively). Brown et al. (1980) observed similar neutrophilia (87% of the infiltrate in the cavity)

at the attachment sites of A. americanum larvae after 48 hours on previously unexposed guinea pigs, and very few eosinophils (2.6% of the infiltrate). Working with the same type of hosts, but with a different tick species, Brown et al. (1984) found a different cellular pattern. Thus, analysis of female feeding sites of I. holocyclus at 12, 24, 48, 72 hours post attachment revealed the dominance of mononuclear cells in the infiltrate; eosinophils were essentially absent, comprising only 1-3%. The primary infestation was characterized also by the apparent absence of eosinophilic infiltrations around blood vessels, absence of epidermal vesicles and no hair follicle hyperplasia. Berenberg et al. (1972) showed that salivary gland extracts of D. variabilis produce chemotatic activity for neutrophils on incubation with dog, human or mouse serum with purified C5. This non-immunological reaction would explain the local influx of neutrophils in the skin of tick-naive rabbits.

The group of rabbits which were made resistant to R. appendiculatus responded vigorously to the homologous challenge as well as to the heterologous challenge. Both attachments a showed similar type of reaction. However, the R. appendiculatus attachments were characterized by eosinophilia, while those of A. variegatum showed neutrophilia, although there was no significant difference in the number of these cells between the two lesions. In both attachments, the total number of inflammatory cells andtissue reactions increased in magnitude compared to lesions from tick-naive rabbits. Thus, the degree of eosinophilic concentration at the sites of attachment, the eosinophil infiltration around deeper blood vessels and the incidence

of epidermal vesiculation were all markedly increased. The tick-naive rabbits showed neither mobilization of eosinophils nor epidermal changes in their skin lesions. In some biopsies from resistant rabbits the cellular counts, especially for eosinophils, could not be quantified properly as they had already reached the stage of degeneration and degranulation; hence an under estimation was inevitable. A stronger reaction was observed with the feeding of A. americanum on previously unexposed guinea pigs at 10 days post-attachment, but the number of neutrophils counted after 48 hours was greater, because of the tremendous volume of cells present at 10 days which made counting impossible (Brown and Knapp, 1980). The biopsies could as well be done as early as 3 hours post attachment of the ticks, since eosinophils predominate in the cellular infiltrate of resistant animals (Schleger et al., 1976).

Attraction of eosinophils to the attachment sites in the resistant rabbits in response to homologous challenge coincided with the findings of Schleger et al., (1976) and Allen et al., (1977) on cattle; Brossard and Fivas (1982) and Rubaire-Akiki and Mutinga (1980) on rabbits, Brown and Knapp (1981) and Brown et al. (1983) on guinea pigs. The virtual lack of eosinophil infiltration at attachment sites during the primary infestation on tick-naive rabbits emphasizes the immunological basis of the reaction. An increased eosinophilia is characteristic of immediate or anaphylactic response syndromes to bites of haematophagous arthropods (Larrivee et al., 1964 cited by Tatchell and Moorhouse, 1968). They could be attracted to tick feeding lesions by a chemotatic factor of anaphylaxis (Kay and Austin, 1971;

Kay et al., 1971) from degenerating mast cells and basophils, or by antigen antibody complex (Kay and Austin, 1972). A wide range of enzymes is released from disrupted eosinophil granules which degrade invading micro-organisms (Wassom and Gleich, 1979) but they may also cause local tissue damage as evidenced by epidermal vesicles in highly resistant hosts, and so cause irritation (Sinclair, 1973). Irritation might stimulate grooming which is a factor in the resistance of cattle to B. microplus (Snowball, 1956; Riek, 1962; Bennett, 1969). Not only would moving larvae be made susceptible to grooming but an epidermal vesicle under the attachment site would facilitate removal of already attached ticks from the host.

The ability of the host to produce epidermal vesicles beneath attached ticks has also been reported by many workers (Allen, 1973; Allen et al., 1977; Wikel and Allen,1982; Brown et al., 1984). It was also described as epidermal in growth to wall off the feeding lesion (Trager 1939b; Rubaire-Akiki and Mutinga, 1980). However, Tatchell and Moorhouse (1968) could not demonstrate any pathological lesions or epidermal proliferation in the skin of cattle of different levels of resistance to B. microplus. In fact, they found the definitive feeding lesions to be similar in all hosts examined (Moorhouse and Tatchell, 1969). Hyalinization, which characterized the appearance of the vesicles and compact feeding lesions of resistant rabbits, usually involves the connective tissues and basement membranes and indicates the presence of proteins (Thomson, 1978). By the use of immunofluorescence, IgG and complement component C3 were observed at tick attchment sites in vesicles and along the dermo-epidermal junction

(Allen et al., 1977). Therefore the eosinophilic appearance indicates the local involvement of humoral factors at the attachment site. The presence of antibodies in the resistant rabbits has already been confirmed by immunodiffusion tests (Chapter 2).

The immediate hypersensitivity to tick infestation, which has been related with resistance above, resulted in an abnormal susceptibility in European breeds of cattle (Tatchell and Moorhouse, 1968; Tatchell, 1969; Moorhouse and Tatchell, 1969). These two workers noted an early intense infiltration of eosinophils into the area of the mouthparts, which was typical of an immediate hypersensitivity response, sensitized cattle as well as in previously unexposed cattle. They also found little difference in extent or intensity of reaction before and after acquisition of resistance. Therefore, they suggested that hypersensitivity should be thought of as the extreme in a spectrum of sensitization which produced resistance in some animals and susceptibility in others. The current study does not coincide with that hypothesis, particularly the early infiltration of eosinophils in susceptible hosts. However, the hosts and the ticks were different and therefore each may have a different host-parasite interaction. Ultimately the results from laboratory animals should be tested on the natural host species.

In contrast to tick feeding sites on guinea pigs (Allen, 1973; Bagnall, 1978; Brown and Askenase, 1981) and cattle (Allen et al., 1977; Brown et al., 1984), basophils were insignificant in the present study. They also formed a smaller proportion of the cellular

infiltrate (4-9%) on a H. a. anatolicum challenge infestation on rabbits (Gill and Walker, 1985). The comparatively weaker basophil response in rabbits could be host species-specific. Basophils are also found to be more demonstrable in sections of epon-embedded skin but they are not revealed by more traditional histological methods. Furthermore, they are first detected in the skin at days 5-7 from initiation of a first infestation in guinea pigs (Wikel and Allen, 1982; Brown et al., 1984) and a basophil rich-skin reaction occurs 48-72 hours after initiation of a second infestation. Therefore the timing of biopsies for basophils in the present study was different from the aforementioned work.

Amblyomma variegatum nymphs fed on one ear of tick-naive rabbits elicited a stronger cellular reaction than R. appendiculatus nymphs fed on the other ear. With the latter species the reaction was minimal and not apparent 48 hours post attachment. The hypothesis of Noval (1978) is therefore disproved by the results of the present study. That is, ticks with long mouthparts do not provoke host body responses, and thus resistance is not acquired, whereas ticks with short mouthparts do induce resistance in the host. Recently, Gill and Walker (1985) have also shown that rabbits can acquire a significant level of resistance to H. a. anatolicum and the host cells involved in the resistance response were mast cells, basophils and eosinophils. In fact, deeply inserted mouthparts enable the tick to reach more highly vascularised areas and hence more damage is to be expected, compared to the superficial epidermal feeding of ticks with short mouth parts .

However, the feeding of both types of tick on resistant hosts elicits significant reactions which are not related to the length of the mouthparts but largely depend on the amount of antigen injected and the presence of any antibodies already formed by host.

In summary, rabbits which were made resistant to A. variegatum and R. appendiculatus by repeated infestation of nymphs showed epidermal vesiculation and significant mobilization of eosinophils at tick feeding sites, which is not apparent with tick-naive rabbits. Eosinophils were considered as the major effector of resistance. The feeding of A. variegatum nymphs on rabbits resistant to R. appendiculatus produced large accumulations of eosinophils and resulted in vesicle formation, a situation which probably affects the tick feeding performance. This would partly substantiate the results from the cross-resistance experiments (Chapter 1). On the other hand, the slightcellular and tissue involvements at sites of R. appendiculatus attachments in the skin of rabbits resistant to A. variegatum is probably not deterimental to tick feeding. This situation also confirms the results from cross-resistance experiments (Chapter 1) in which R. appendiculatus nymphs fed successfully to normal sizes on rabbits resistant to A. variegatum nymphs.

CHAPTER 4

OTHER OBSERVATIONS

1. Feeding of A. variegatum on cattle previously exposed to R. appenduculatus

The studies on the host relationships of A. variegatum were originally planned to be carried out on both rabbits and cattle. However, it was not possible to continue with the work on cattle due to drought conditions which caused a severe shortage of cattle food during the first year of the study. This section shows the results from the preliminary experiments with cattle kept indoors. The objective was to investigate cross-resistance between A. variegatum and R. appendiculatus. It was also intended to see if it was possible to render these cattle resistant to A. variegatum by feeding adults and nymphs of this species on them.

MATERIALS AND METHODS

Cross Resistance Tests:

Six B. taurus cattle ($1^1/2$ - 2 years old) were used. They had been previously exposed to different infestations with adults and nymphs of R. appendiculatus. One hundred nymphs of R. appendiculatus were applied to one ear of each calf and 100 nymphs of A. variegatum to the other ear. Two tick-naive rabbits were used as controls and each was infested with 100 nymphs of each species separately on the ears.

Repeated feeding with A. variegatum:

Four of the six cattle were infested repeatedly with nymphs and adults of A. variegatum. Thus, three weeks after the completion of the 100 nymphal tests, each animal was infested with 400 nymphs applied on one ear. Five tick-naive control rabbits were also infested with 100 nymphs on both ears. Seven weeks later, 50 males and 50 females were fed on each calf. The ticks were applied using the tailored bag method. The animals, four weeks after the completion of the adults feed, were finally challenged with 200 nymphs applied on one ear of each calf; one hundred nymphs were also applied to the ears of the 5 tick-naive control rabbits.

The engorged female ticks were weighed and measured and the egg-batches produced by 30 females were weighed individually.

RESULTS

Cross-resistance between R. appendiculatus and A. variegatum:

One of the control rabbits died after the feed of R. appendiculatus was completed but the A. variegatum feed was still in progress. Therefore, feeding performance was judged by the mean data obtained from 5 tick-naive rabbits which were infested with the two tick species later. The feeding performance of both tick species on cattle and rabbits is shown in Table 13. The number of R. appendiculatus engorged and dropped from all cattle was reduced by 38.1% as

Table 13

Mean results of test feeds of 100 nymphs each of R.appendiculatus and A. variegatum on cattle previously exposed to R. appendiculatus, and on tick-naive control rabbits. The engorged ticks were weighed individually.

Animal no.	R. appendiculatus		A. variegatum	
	no. engorged	weight(mg) $\bar{X} \pm SD$	no. engorged	weight(mg) $\bar{X} \pm SD$
124	85	4.5±3.0	61	35.0±11.0
209	66	4.2±1.5	55	31.0±11.0
241	37	3.7±1.9	66	28.3±10.6
247	65	3.9±2.5	52	33.2[illegible]
250	59	4.4±1.4	70	32.9±[illegible]
267	42	2.7±2.2	21	24.5± 7.8
$\bar{X}$±SD	59.0±17.5	3.9±0.7	54.2±12.6	30.8± 3.8
rabbits				
$\bar{X}$±SD	86.5±13.1	9.3±0.9	82.8± 6.5	48.3± 4.3

judged by the results from control rabbits. There was also a reduction of 58.1% in the mean engorgement weight. At the same time there were reductions of 36.2% and 34.5% respectively for A. variegatum nymphs.

Repeated Feeding of A. variegatum

Results from the three nymphal infestations are shown in Table 14. There was a reduction in the percentage of nymphs engorging in the second and third feeds, compared with the first feed, by 46.5% and 28.2% respectively. However, the mean engorgement weights were slightly increased in the second and third feeds. If the results were judged by data from control rabbits, there were also reductions of 36.2%, 62.3% and 52.5% in the numbers of detached fully engorged nymphs for the three infestations. Similarly, the engorged weights were reduced by 34.5%, 25.1% and 12.7%.

The feeding performance of A. variegatum females is shown in Table 15. All cattle dropped similar numbers of engorged female ticks and with similar engorgement weights. The pre-oviposition period was 9.3 ± 1.1 days (n = 58). The conversion factor of female weights into eggs, calculated as before (chapter 1) was 0.44 ± 0.08 (n = 29). Regression of length on weight for engorged females was calculated (Fig. 41). These two parameters were highly correlated; the correlation coefficient was + 0.8830 (P 0.001). There was no significant difference between regressions of egg batch weight and total engorgement weight for females

Table 14

Mean feeding performance of *A. variegatum* nymphs on cattle already made resistant to *R. appendiculatus*; details of infestations given in text; percentages engorged in brackets.

Animal	1st feed (100NN)		2nd feed (400 NN)		3rd feed (200 NN)	
	no. engorged	weight(mg) $\bar{X} \pm SD$	no. engorged	weight(mg) $\bar{X} \pm SD$	no. engorged	weight(mg) $\bar{X} \pm SD$
124	61	35.0±11.0	132(33)	35.4±5.4	106(53)	40.0±13.3
241	66	28.3±10.6	80(20)	32.8±6.0	78(39)	42.0±12.5
247	52	33.2±12.3	212(53)	34.2±4.8	76(38)	40.7±10.9
267	21	24.5± 7.8	40(40)	32.6±8.7	50(50)	43.6± 9.6
209	55	31.0±11.0	-	-	-	-
250	70	32.9±13.8	-	-	-	-
$\bar{X}$±SD	54.2±12.6	30.8± 3.8	116.0±74.3	33.8±4.8	77.5±22.9	41.6± 1.6
			(29.0±18.6)		(38.8± 9.9)	
rabbits (5)						
$\bar{X}$±SD	82.8±6.5	48.3± 4.3	(77.0± 4.3)	45.1±0.8	(81.2± 5.9)	47.6± 4.4

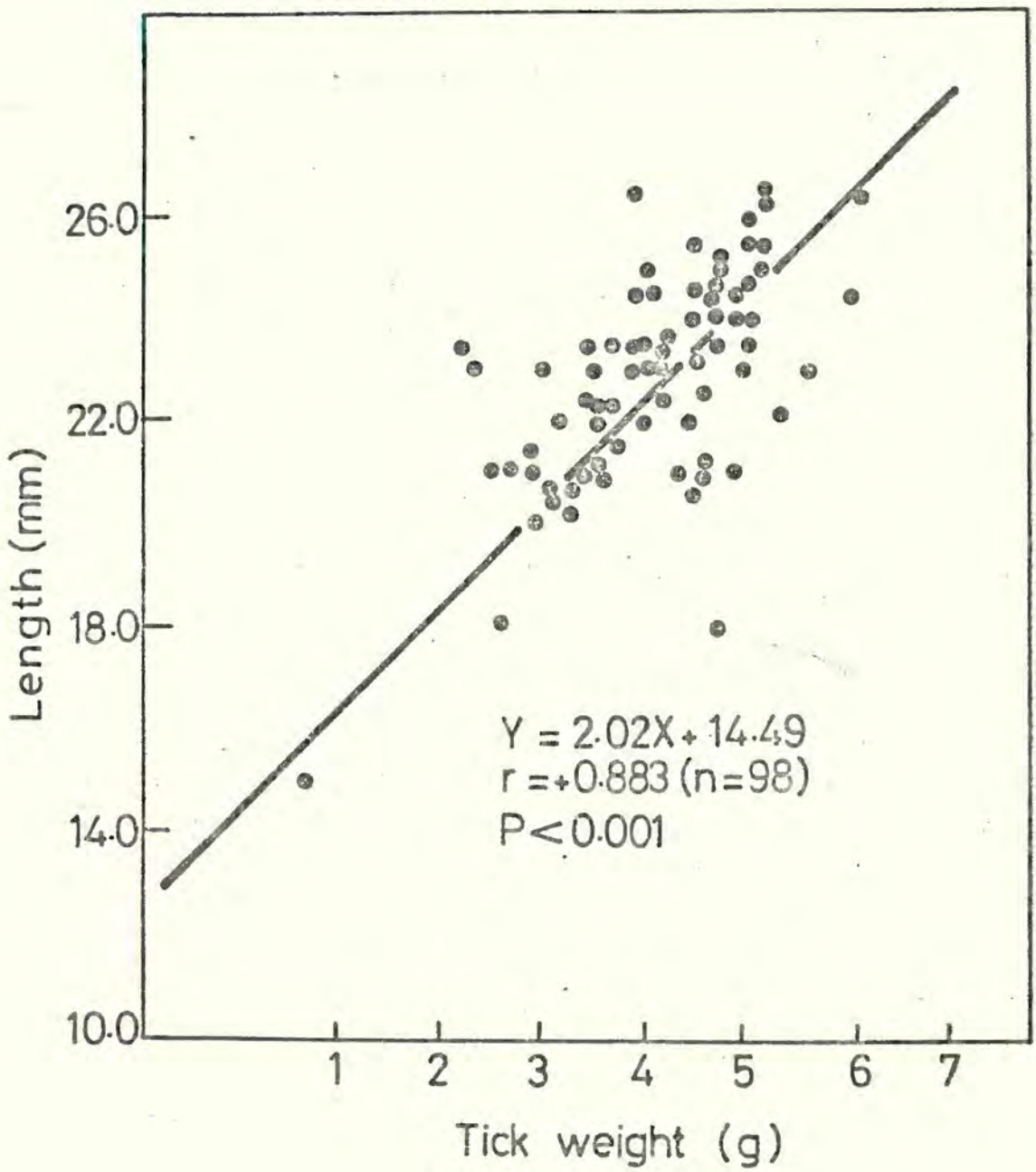
Table 15

Mean feeding performance of 50 female A. variegatum on cattle previously exposed to R. appendiculatus

Animal no	no.^{oo} attached	%	no. engorged	%	weight(g) $\bar{X} \pm SD$	length(mm) $\bar{X} \pm SD$
124	48	96	30	60	4.4±0.9	23.1±1.8
241	31	62	30	60	3.9±0.9	22.3±2.1
250	48	96	33	66	4.0±1.0	22.5±2.2
267	44	88	34	68	4.1±0.9	23.1±1.9
$\bar{X}$±SD	42.8 ± 8.1	85.5 ±16.1	31.8 ± 2.1	63.5 ± 4.1	4.1 ±0.2	22.8±0.4

Fig. 41

Regression of length versus weight of engorged female A. variegatum fed on cattle resistant to R. appendiculatus



that fed on rabbits and on cattle. The data were, therefore, combined and the results are shown in Fig. 42. These two parameters were highly correlated and the correlation coefficient was + 0.9500 (P 0.001).

DISCUSSION

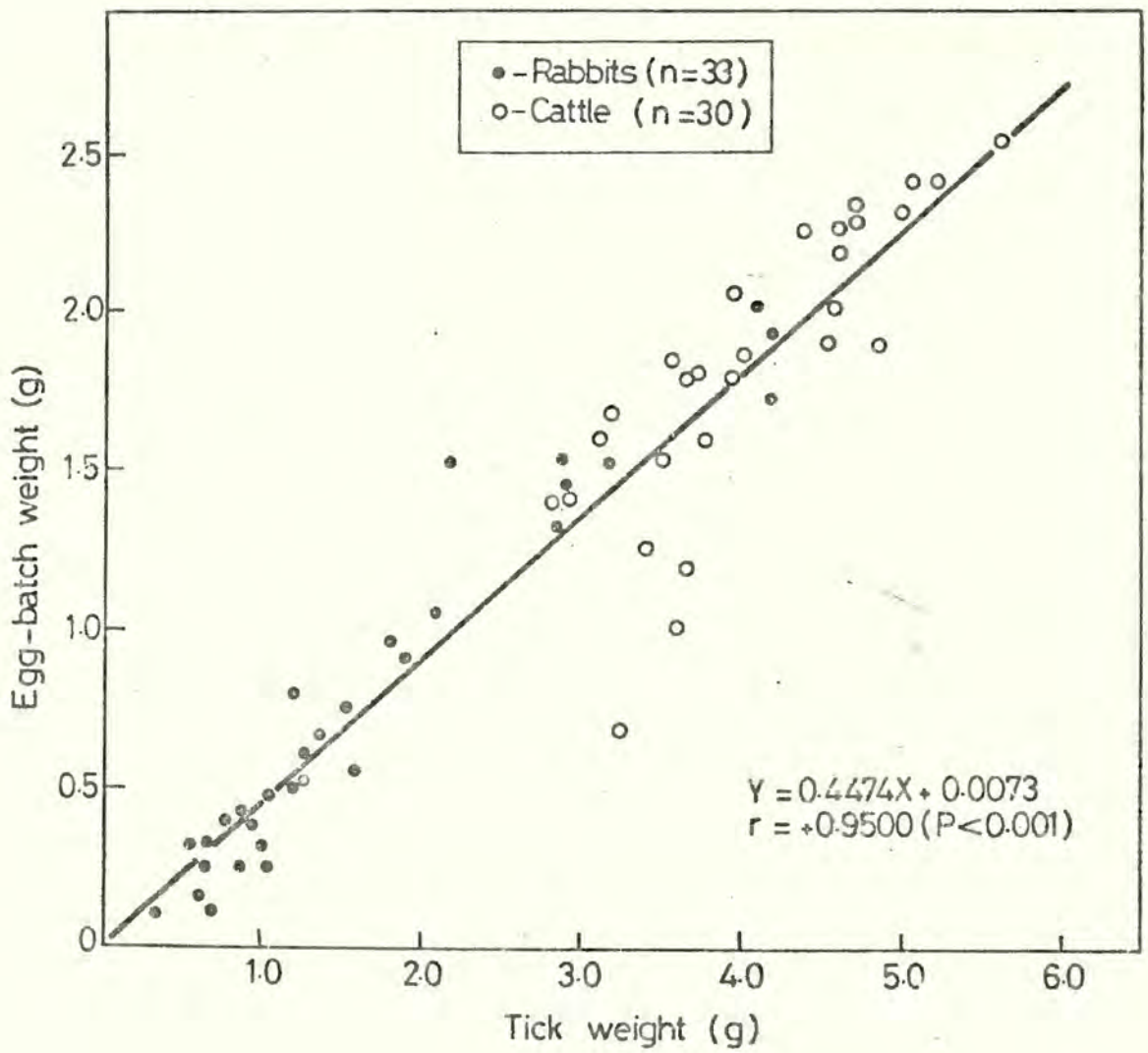
The tailored-bag method of feeding A. variegatum adults on cattle was found to be satisfactory. Females of A. variegatum engorging on cattle dropped with heavier weights than those from rabbits. Mean weights of 4.1 g were obtained from cattle fed ticks compared with an average of 2.4 g from rabbits. These results, therefore, indicated that rabbits are poor hosts for A. variegatum adults compared with cattle, but both hosts are equally suitable for feeding the immatures.

The following relationships were found to be highly correlated (P 0.001) for ticks engorged on either rabbits or cattle: the length and weight at engorgement, the engorged weight in females and the resultant egg batch weight. Furthermore, the conversion factor of engorged weight into eggs was also the same for ticks dropped from either host. Accordingly, the use of the rabbit as a model for the tick host relationship was valid.

Cattle became resistant to R. appendiculatus after repeated infestations. These cattle also showed considerable resistance to A. variegatum nymphs. These findings substantiated the results obtained from rabbits (chapter 1) i.e. animals resistant to R. appendiculatus

Fig. 42

Regression of egg-batch weight versus total weight of engorged female A. variegatum fed on tick-naive rabbits and cattle



also showed cross-resistance to A. variegatum. However, data from cattle susceptible to ticks would be required to confirm these findings fully.

Cattle already resistant to R. appendiculatus when fed three times with A. variegatum nymphs and once with adults showed reductions in the mean percentage of nymphs engorging (Table 14). However, the engorged weights of the nymphs were not uniform and in fact, increased during the experiment. These mean weights were, in all cases, less than the weights of the ticks engorged on control rabbits. The percentage of ticks engorging per animal is, therefore, more appropriate for comparing resistance levels than the mean weight. This also agrees with the results obtained from rabbits (chapter 1).

It was evident that the first feed with A. variegatum nymphs resulted in an increase in the resistance level of cattle (Table 14). However, more feeding with adults did not boost resistance, since the percentage engorging and the mean weight of nymphs in the third feed were not reduced as compared to the second feed.

2. ABNORMAL DEVELOPMNET IN A. VARIEGATUM

Abnormalities in ticks have been described by many authors. Brumpt (1922; 1934 cited by Hoogstraal, 1956) described gynandromorphic specimens of A. variegatum and Rechav (1977) described similar abnormalities for A. hebraeum. This phenomenon, in which half of the tick has male characters while the other half shows female characters has been reported to occur with greater frequency in the genus Amblyomma than in other genera. Dias (1953, cited by Rechav, 1977) reported that 43% of the cases occurred in this genus.

Hybridization of ticks has also been reported to affect the appearance of the progeny. Thus, the males obtained from an interspecific hybridization of Hyalomma excavatum and Hyalomma marginatum were very pleiomorphic and showed external morphological features of both species (Cwilich and Hadani, 1963). Certain morphological characters of the hybrid obtained from mating D. variabilis females with D. andersoni males appeared intermediate, while others resembled one parent species more than the other (Oliver et al., 1972). Moreover, several of the F1 progeny resulting from a cross between A. americanum females and A. maculatum males exhibited various malformations; missing or curly legs, nymphal-adult intermediates and gynandromorphism (Gladney and Dawkins, 1973).

Other cases of abnormal development were reported among engorged female B. annulatus by Sakla et al. (1980). The abnormal features

included incomplete or partial twinning of the posterior area (bilobed), trifurcation of the large disfigured tibial segment and the absence of legs on one side. In the present study abnormal individuals from A. variegatum also occurred as described below.

2.1 Origin of the Abnormal Individuals

Engorged females A. variegatum dropped from tick-naive rabbits were usually kept individually in tubes for oviposition. Eggs from several batches were then pooled and the subsequent larvae were used for maintaining the colony. The abnormal ticks arose from this colony. However, it was not possible to know whether they originated from one or several females.

2.2 External Features

2.2.1 Engorged larvae

A total of 16 engorged larvae with abnormal external features were observed out of 5000. Two characteristics found to be common to all were partial twinning of the posterior region of the body (with two exceptions) and the presence of two anal grooves (Fig. 43). The white appearance of the Malpighian tubules leading to each anal pore could be seen clearly on the ventral side. The normal three pairs of legs were present in all larvae, but some showed an additional pair positioned posterior to the anal grooves.

2.2.2 Nymphs and adults

The normal external features which were retained by nymphs and adults of the abnormal strain were the mouth-parts, four pairs of legs and one pair of laterally positioned spiracular plates. A common feature of the abnormal nymphs and adults was a bilobed posterior region and the presence of two anal grooves. In the adults two genital grooves were present in males as well as females. Depending on the number of legs and spiracular plates present, the abnormal ticks were grouped into three (Table 16). Ticks in the first group (Figs. 44, 45, 46) had four pairs of legs and one additional large spiracular plate at the posterior margin of the body. The second group (Figs. 47, 48) had five pairs of legs with the additional pair originating from the posterior region of the body. There was an additional pair of spiracular plates each of which was situated in the posterior margin of a lobe. In this group there was also a chitinized outgrowth situated anterior to the base of the 4th pair of legs. Ticks in the third group (Figs. 49, 50) had six pairs of legs. The two additional pairs were at the posterior margin of the body. There was an additional pair of spiracular plates as described for ticks in the second group. It has been observed that nymphs from each group developed into adults which then retained the same features of the group.

2.3 Morphology of Internal Organs

Partially fed males and females of group one were dissected to examine the gross morphology of the internal organs.

2.3.1 Digestive, excretory and salivary glands

There was only one midgut which connected to the exterior by a bifurcated hindgut. Each branch of the hindgut (rectum) led to a separate enlarged rectal sac before opening to an anal pore (Fig. 51). Both rectal sacs were filled with a semisolid sticky secretion. The branches of the alimentary canal on either side (Fig. 52) were equal in size and length as shown by the uniform distension of the midgut, diverticulae, hindguts and rectal sacs.

There were two pairs of Malpighian tubules, each one being connected separately to a rectal sac. Both tubules were white in appearance. This was very clear in the fully engorged immatures where the white-coloured tubules terminated in separate anal grooves.

There were only two salivary glands, as in normal ticks.

2.3.2 Reproductive organs

There were two ovaries which opened separately into one vagina. These were associated with two pairs of tubular accessory glands. In male ticks there were two pairs of testes. Each pair was connected to a separate pair of accessory glands. Both organs were well developed (Fig.53).

2.4 Feeding and Development of the Abnormal Ticks

All of the 16 engorged larvae developed into nymphs. These nymphs were then fed on one ear of a tick-naive rabbit whilst 100 normal nymphs were applied on the other ear. Eleven abnormal nymphs engorged successfully and dropped with an average weight of 49.9 $\pm$ 13.6 mg compared to 52.4 $\pm$ 14.8 mg for the normal nymphs. However, the length of the engorged abnormal nymphs (4.3 $\pm$ 0.4 mm) was far less than the width (6.2 $\pm$ 0.6 mm).

From the eleven nymphs six females and four males moulted into adults. The abnormal males were fed on one rabbit while 10 normal males were applied on another rabbit. Six days later three abnormal females were also applied on the same site with males on each of the two rabbits. Both males and females attached well. One male and one female was pulled off on day 6 for dissection (section 3). Another two males and three females were lost due to the removal of capsule by the rabbit. A female from the abnormal individuals, which attached for 3 weeks with an abnormal male, did not engorge. Three of the normal males feeding on the other rabbit were removed and put with the attached abnormal female to see if that could enhance its engorgement. The males attached readily beside the feeding female and the abnormal male was removed. After 10 more days (i.e. day 31) this female still failed to engorge, but detached. Another female which was mated with normal males did engorge and dropped weighing 1.0 g. Interestingly, this female was observed to be mated by two males. Each one was attached to a genital pore and the female was grasping both with its legs. However, eggs from this only ovipositing female did not hatch.

Fig. 43. Engorged larvae of A. variegatum :
(a) ventral view of normal larva; (b) - (f) abnormal individuals. (i) Ventral view showing two anal grooves (A). Note also the posterior partial bifurcation in the abnormal larvae.

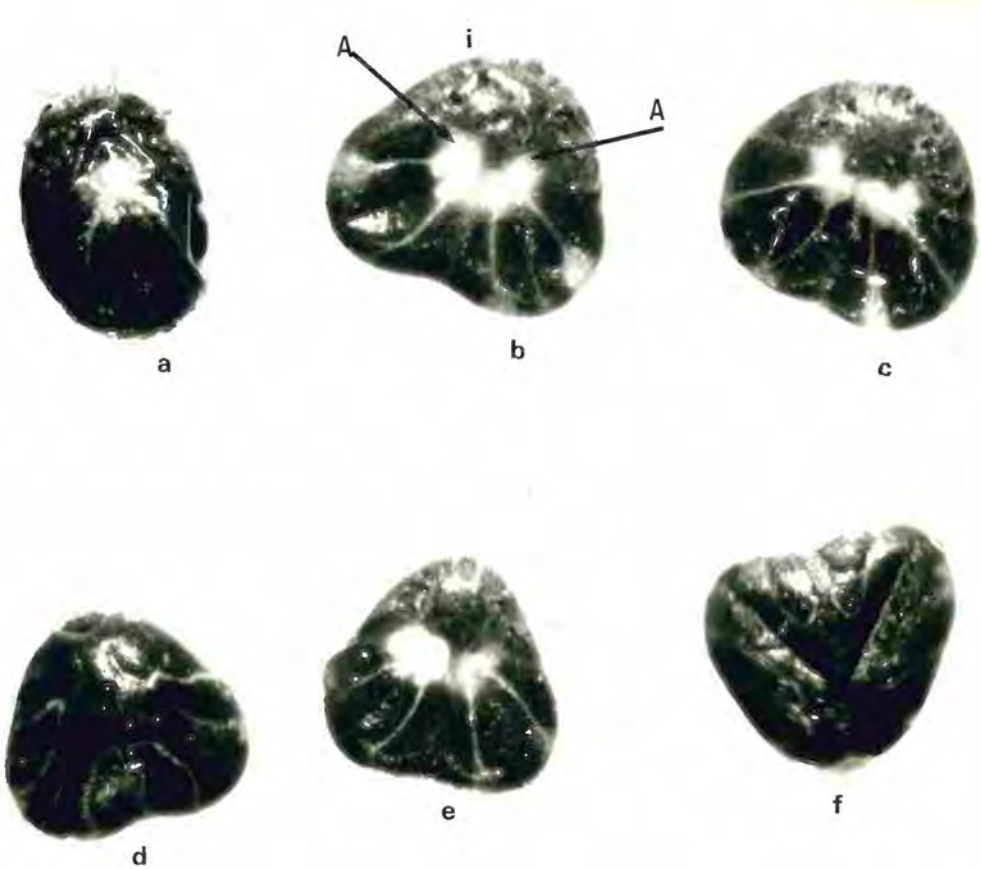


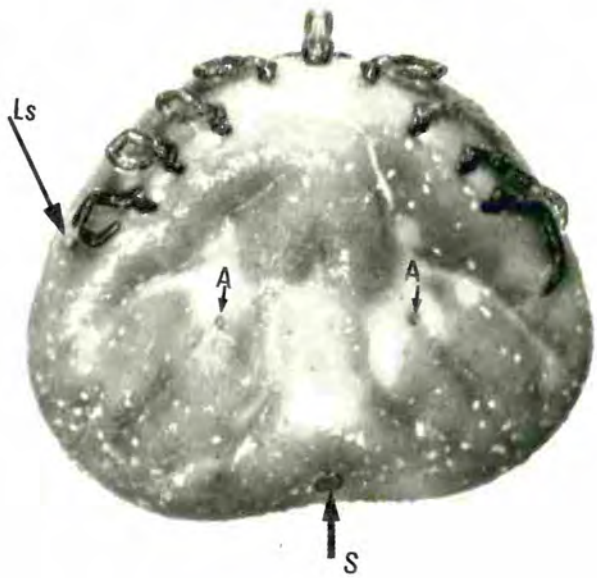
Table 16

Abnormal external features which characterized three groups of aberrant A. variegatum adults.

Group No.	No. abnormal adults	Genital grooves	Anal grooves	Spiracular plates (post.)	Pairs of legs
1	8	2	2	1	4
2	2	2	2	2	5
3	1	2	2	2	6

Fig. 44. Engorged nymph of type 1 : (A) anal grooves; (S) posterior spiracular plates; (LS) lateral spiracular plates (normal).

Fig. 45. Unfed female of type 1: (G) genital grooves; A; S; LS (see figure 44).


Ls
A
A
S

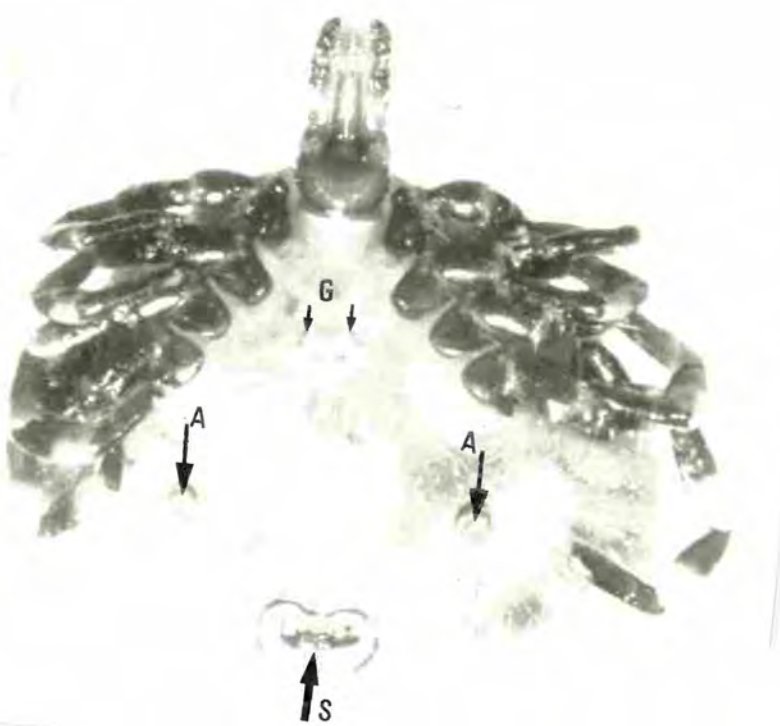
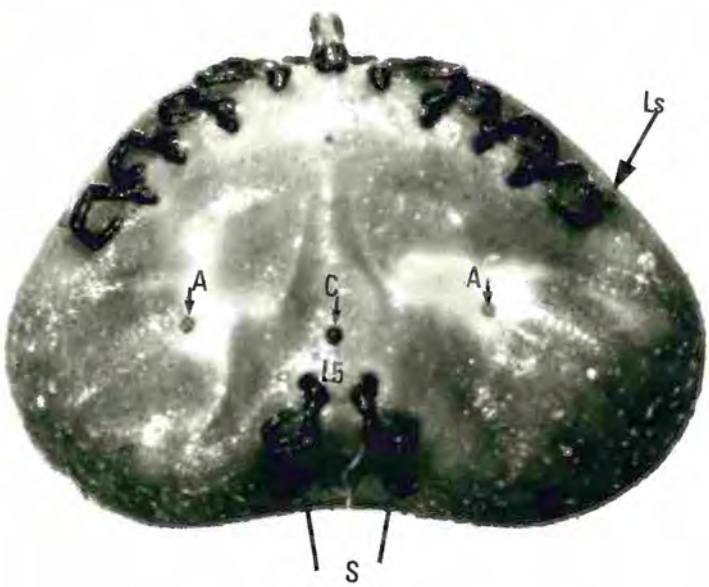
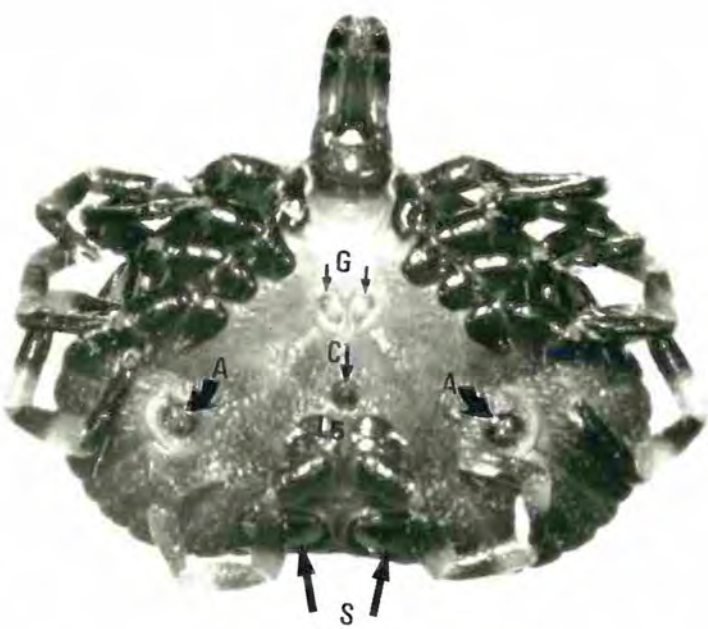
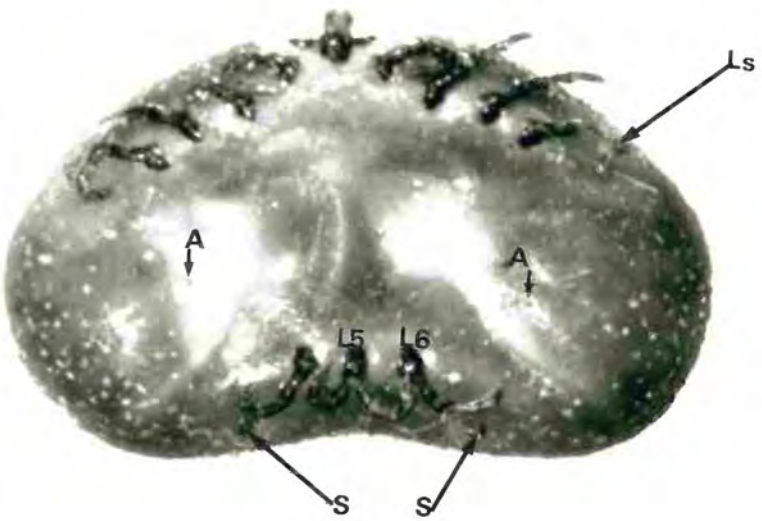

G
A
A
S

Fig. 46. Unfed male of type 1: For symbols refer to figure 44.


Ls
A
C
A
L5
S


G
C
A
A
L5
S


Ls
A
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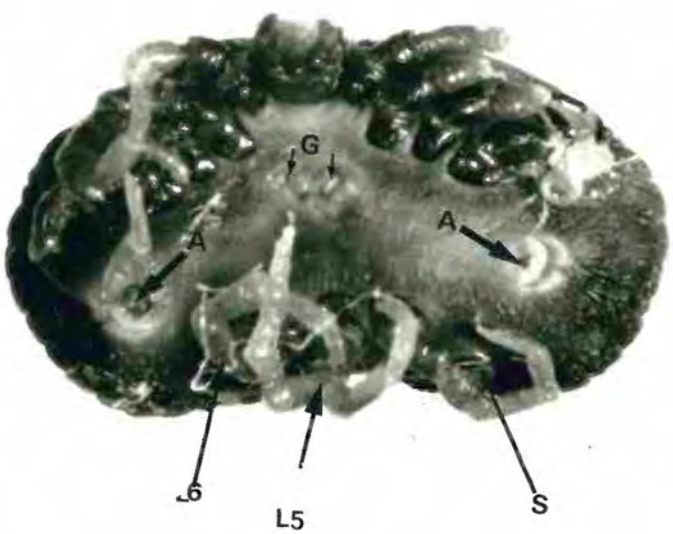
L5
L6
S
S


G
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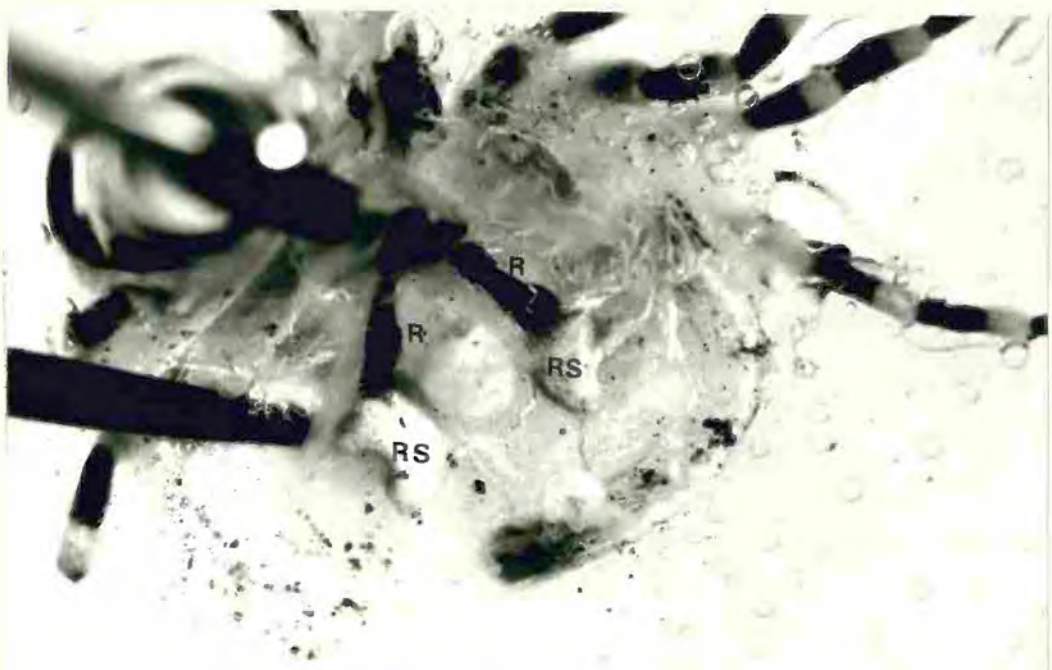

Fig. 51. Partially fed female of type 1 dissected to show the bifurcation of the midgut into two rectums (R) while each rectum opens separately into a rectal sac (RS).

Fig. 52. Partially fed female of type 1 dissected to show the uniformly distended midgut diverticulae.

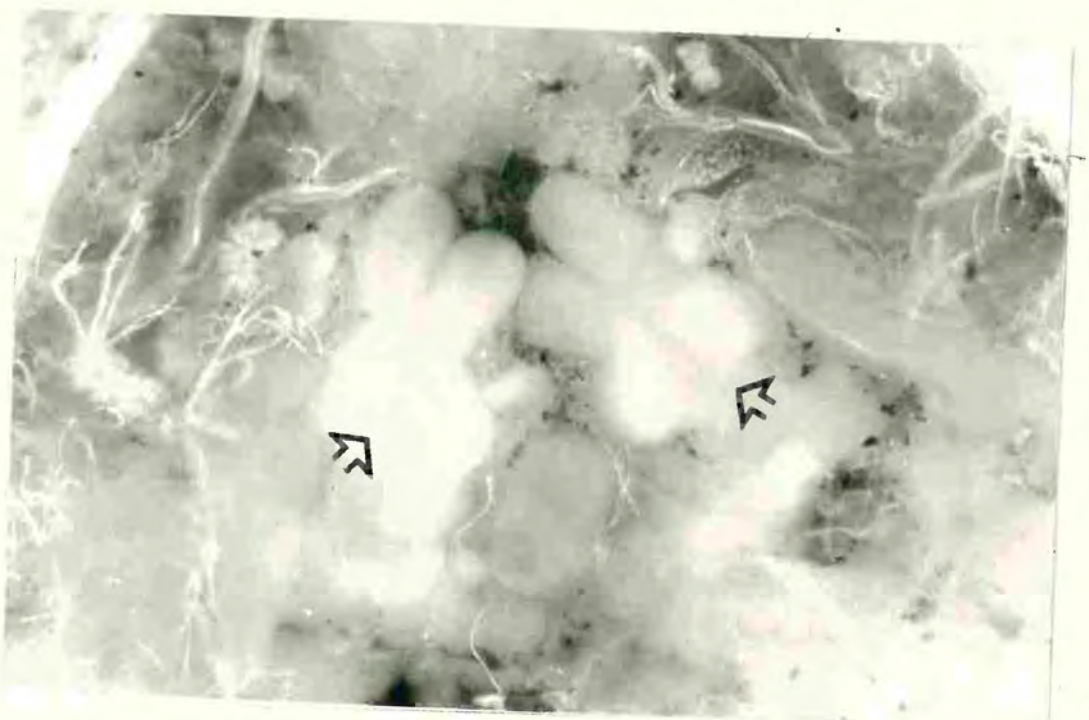

Fig. 53. Partially fed male of type 1 dissected to show the two pairs of accessory reproductive glands (shown by arrows), each being associated with one pair of testes and a genital opening.

CHAPTER 5

General discussion and conclusions

Since the rabbits used in this study had no contact with ticks, other than by experimental infestation, so the immune reactions and manifestations must have arisen naturally as a result of host-parasite interactions. All the host responses were stimulated by the natural feeding mechanisms of ticks and no immunization of any sort was attempted. The host-parasite relationships of A. variegatum and R. appendiculatus in rabbits have been described and confirm the already established phenomenon that rabbits are able to acquire a significant degree of resistance by repeated infestations (Willadsen, 1980). In the current study, animals expressed their immunity in different ways, depending on the feeding instar.

Resistance in rabbits to the adult tick was manifested by reduced engorged weight, and fewer ticks that were able to lay eggs. The egg conversion factor was also reduced. Similarly, they allowed significantly less immatures to complete their engorgement and these also dropped with reduced engorged weights. The feeding of the ticks was also greatly inhibited. Thus, some ticks were observed to die and shrivel while still on the host; others died immediately after detachment ; whilst a third group fed partially but were unable to complete their blood meal or detach as the mouth-parts were masked

by host scar tissue. It was also found that 72% of nymphs fed on resistant animals dropped prematurely with body sizes in feeding categories N2 and N3 and only 28% were able to complete their blood meal and reach N4 (engorged).

Immunity in rabbits also interfered with vital physiological processes in the tick and possibly inhibited some factors or hormones which are responsible for the scutal pigmentation patterns. Thus, two additional spots were regularly observed on male A. variegatum which had fed as nymphs on resistant animals. This abnormal character is, however, typical of A. lepidum. Newson (personal comm.) saw it only rarely in survey material of A. variegatum from cattle which were not dipped, and had presumably developed resistance to this species.

Although the presence of antibodies to ticks has been demonstrated in all serum samples from resistant rabbits, their classes and specific effects on the tick have not been studied. However, there are two pieces of circumstantial evidence which suggest that these antibodies are protective. Firstly, they were not demonstrated in the sera of tick-naive rabbits. Secondly, sera obtained after larval infestation gave weak immunodiffusion reactions against different tick antigens, but in this case resistance against ticks was poorly manifested. There is other evidence of the involvement of antibody in acquired resistance to ticks. Thus, intravenous transfer of immune serum from guine-pigs twice-infested with A. americanum to tick-naive animals conferred a significant level of immunity (Brown et al., 1982). Similar results have also been obtained by other workers

against B. microplus (Roberts and Kerr, 1976), A. americanum and R. sanguineus (Brown, 1981; Brown and Askenase, 1982; Brown et al., 1982b; Askenase et al. 1982). On the other hand, some attempts with D. andersoni were unsuccessful (Wikel and Allen, 1976a) and I. holocyclus (Bagnall and Rothwell, 1974). Wikel and Allen (1976b) gave indirect evidence for the requirements of antibody in immunity when they successfully blocked the immune responses in tick resistant animals by injecting specific immunosuppressants.

Rabbits rendered resistant to A. variegatum and R. appendiculatus through repeated tick infestations also showed characteristic tick feeding lesions at the attachment sites which were different from lesions in tick-naive rabbits. Thus, the degree of eosinophilic concentration, the eosinophilic perivascular cuffing and the incidence of epidermal vesiculation, were all significantly increased in resistant animals. Attraction of eosinophils, which were considered as the main effector cells in resistance, is consistent with the findings of Schleger et al. (1976) and Allen et al. (1977) on cattle; Brossard and Fivas (1982), Rubaire-Akiki and Mutinga (1980) and Gill and Walker (1985) on rabbits, and Brown and Knapp (1981) on guinea pigs. The virtual absence of these cells at attachment sites with primary infestation (1.6% - 4.2% of the infiltrate) emphasizes the immunological basis of the reaction.

The work by Brown and Askenase (1983) revealed the cooperation between basophils and eosinophils in the immune response to tick

infestation. Basophil degranulation may promote immunity by rapidly releasing a cell-associated histamine which may contribute to resistance. Basophil degranulation may also promote immunity by recruiting eosinophils to tick feeding sites; the major basic protein of the eosinophil granule damages several parasites in vitro (Butterworth et al., 1979; Wassom et al., 1979; Kierszenbaum et al., 1981). Eosinophils have also been implicated as effectors of resistance in a variety of host-parasite systems in vivo (Mahmoud et al., 1975; Grove et al., 1977; Capron et al., 1981). These studies suggest a protective role for eosinophils in immunity to ticks; hosts depleted of eosinophils by anti-guinea pig eosinophil serum had impaired resistance to ticks, despite normal numbers of basophils at feeding sites (Brown et al., 1982).

The extensive tissue reactions and damage in the skin of animals previously exposed to ticks, as a defence mechanism, is sometimes complicated by secondary bacterial infestations, thus creating an unpleasant situation for the host. This was observed in rabbits during the third adult infestation where the skin became indurated and necrotic with white cheesy material oozing from the sites of tick attachments. Tatchell (1981) related the loss of teat function in cows (due to tissue cirrhosis) directly to A. lepidum infestations. In Sudan 53% of replacement heifers in Um Banein Dairy Farm, lost 1-3 quarters of the udder due to A. lepidum infestation (Paine, 1983).

Considerable work has been done on the histology of tick attachment sites, but comparative studies with a single host, whether

immune or tick-naive, with a variety of tick species are lacking. Under natural conditions, animals are usually exposed to infestation with more than one tick species. It is, therefore, interesting to consider the responses in the skin of exposed animals at the attachment sites of various tick species. The histology of the attachment sites of I. holocyclus and B. microplus on cattle, with different tick instars and separate experiments, were found to be different (Allen et al., 1977; Tatchell and Moorhouse, 1968). In the present study, histological comparisons were made with nymphs fed on a group of tick-naive rabbits and on a group resistant to A. variegatum and another to R. appendiculatus. The cellular reaction at the attachment sites in a tick-naive host varied with the species. Thus the cellular infiltrate in the feeding lesion of the former species was about 10 times greater than that in the lesion of the latter species. Rabbits resistant to R. appendiculatus when challenged with the nymphs of both species (each fed on a separate ear) demonstrated similar tissue reactions and cellular infiltration in the two ears. However, rabbits resistant to A. variegatum showed intensive reaction with homologous challenge and minimal (sometimes negligible) reaction with the heterologous challenge. These findings from both resistant groups substantiate the results obtained during the cross-resistance experiments i.e. R. appendiculatus nymphs fed successfully to normal body sizes on rabbits resistant to A. variegatum but not vice versa. However, sera from both groups did not demonstrate inter-specific

cross-reactivity in immunodiffusion tests. Similar results have also been obtained by Brown and Askenase (1982), in that guinea pigs resistant to R. sanguineus exhibited a weaker, but significant, resistant response against active challenge by A. americanum, yet immune serum against R. sanguineus was ineffective in protecting the hosts against A. americanum. The failure to demonstrate antibodies may indicate that the immune resistance response to the heterologous challenge was entirely cell-mediated. In fact this assumption was true with R. appendiculatus resistant rabbits and their response to A. variegatum challenge. However, Dhadialla (pers. comm.) found that sera from rabbits immunized with R. appendiculatus midgut extract demonstrated precipitin lines against the homologous midgut antigens as well as against A. variegatum midgut antigens. Therefore, more sensitive methods like the Western blotting technique (Towbin et al., 1979) should be employed to identify the possible shared antigens between these two tick species.

The immunodiffusion tests demonstrated intra-species cross-reactivity. Thus, the anti-adult and anti-nymphal infestation sera both recognized the adult, nymphal and larval antigens. These results indicate the presence of shared antigens among the three tick stages. These findings also substantiate the results obtained during the cross-resistance experiments. Rabbits which had acquired resistance by repeated infestations of adult A. variegatum showed significant protection against the immature stages, while rabbits made resistant by repeated feeding of nymphs showed significant resistance against the larvae. These findings agree with some observations

obtained from cattle under natural tick challenge by Kaiser et al. (1983) and Latif (1984a,b) who found significant correlations between the ranking of cattle in terms of counts of ticks within and between species.

The present results, however, contradict those of Norval (1978) on A. hebraeum in which rabbits and sheep were unable to acquire resistance to the larvae and nymphs, even after repeated feeds. Norval (1975) hypothesised that ticks which have long, deeply penetrating mouth-parts (e.g. Amblyomma and Hyalomma) generally are less susceptible to host resistance mechanisms than those attaching more superficially with short mouth-parts (e.g. Rhipicephalus and Boophilus). In the current study rabbits became resistant by repeated feedings of the different stages of A. variegatum. However, resistance to adults and nymphs was stronger than to larvae. Antibody was also demonstrated in sera of all rabbits in response to tick feeding. Furthermore, rabbits which were made resistant to A.variegatum showed skin cellular responses at sites of tick attachment which were indicative of an immediate hypersensitivity reaction, a situation which was not seen with tick-naive rabbits. All these immunologically mediated responses confirm the ability of rabbits to acquire a significant level of resistance to A. variegatum.

Amblyomma variegatum nymphs (with long mouth-parts) that fed on one ear of tick-naive rabbits elicited a stronger cellular response at their attachment sites than R. appendiculatus nymphs (with short mouth-parts) fed on the other ear. The latter species elicited

a minimal reaction which was not apparent 48 hours post attachment. On tick resistant rabbits both species induced intense host reactions, irrespective of the length of the mouthparts. There are, therefore, no grounds to support the hypothesis of Norval (1975). In fact, there were many contradictions in the work of Norval (1978). Firstly, he observed localized swelling with considerable irritation in the ears of some rabbits, which gave exceptionally low tick yields. Secondly, he found in more than one rabbit that the tick yield increased with the onset of illness. Thirdly, the engorged weights decreased again once the rabbits had recovered. All these observations indicate that the rabbits had immuno-responded to tick infestation, a fact which he did not describe. The number of experimental animals he used (2 sheep only) was also not adequate. The results of the current study agree with those of many workers who were able to demonstrate resistance in experimental and domestic animals using ticks with long mouth-parts e.g. A. americanum (Strother et al., 1974; Brown et al., 1984; George et al. 1985), A. variegatum and A. hebraeum (Heller-Haupt et al., 1980) and H. a. anatolicum (Latif, 1984a,b; Gill and Walker, 1985).

There are some indicators for biological control of A. variegatum emerging from the present study. Firstly, the tick feeding performance on hosts previously exposed to R. appendiculatus was significantly reduced, and the results from cattle confirm those obtained with rabbits. Secondly, animals

resistant to A. variegatum yielded engorged nymphs of low weights, the majority of which moulted into adults which had an atypical pattern of ornamentation. However, the most important point is that these adults had a poor feeding performance and such ticks would pose less of a problem in the field. Thirdly, ticks carrying abnormal features, and most probably infertile, have been identified but the frequency of such abnormal characters within the natural population is not known. Therefore, an integrated control programme incorporating immunization with immunogens prepared from R. appendiculatus, could be successful for controlling both A. variegatum and R. appendiculatus.

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