IMMUNE RESPONSE OF CATTLE TO BOOPHILUS ANNULATUS (ACARI : IXODOIDEA)

By

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THESIS

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Department of Parasitology
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DECLARATION

I hereby declare that this thesis entitled "Immune Response of Cattle to Boophilus annulatus (Acari :Ixodoidea)" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

Mannuthy,
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CERTIFICATE

Certified that this thesis entitled "Immune Response of Cattle to Boophilus annulatus (Acari: Ixodoidea)" is a record of research work done independently by Sri. R. Sreekrishnan under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Introduction

INTRODUCTION

Parasites constitute the most important pathogenic agents affecting health and production in domestic animals. Among these parasites the position of ticks is second to none. Their importance is so significant that research on different aspects relating them gained much attention throughout the world today.

The climatic conditions in tropical countries, including India, are most congenial for the propagation of ticks. They have got a very wide range of hosts starting from reptiles, birds and small mammals like rodents to large domestic and wild animals including elephants. Because of this very vast host range, it is not easy to wipe off ticks from nature and their infestation on domestic animals will continue to be a problem.

Ticks of animals are found in all States of India. In Kerala, nine species of hard ticks (Family - Ixodidae) are so far recorded from domestic animals. They are:

1. Boophilus annulatus
2. Rhipicephalus sanguineus
3. R. haemaphysaloides
4 R. turanicus
5. Haemaphysalis bispinosa

6. H. turturis
7. H. spinigera
8. Hyalomma anatolicum
9. Amblyomma integrum

Eventhough, all these ticks can infect cattle, only six of them were recorded on cattle in Kerala. They were B. annulatus, H.bispinosa, R. haemaphysaloides, R. sanguineus, A. integrum and Hyalomma anatolicum in the order of prevalence (Rajamohanan, 1982).

Boophilus annulatus is the most important cattle tick in Kerala. It is very difficult to find cattle, which free range on hilly areas of the state, free from this tick. This tick has been recorded from all parts of Kerala in all seasons, eventhough in summer season, the prevalence is very low. Their role in inducing pathogenic effect and reducing production in cattle are well documented. The females of B. annulatus are potential blood suckers, one female removing as much as 0.5-2.0 ml of blood from the body of the host. The fact that the number of ticks found on the body of free range cattle may run to thousands is an indication of the magnitude of loss in production and health of cattle in the state. The loss in the quality of hide due to the injury and allergic reaction caused by the ticks is also very significant. A variety of pathogens like viruses, rickettsiae, bacteria and protozoa are transmitted by ticks, and in Kerala

B. annulatus is identified as the most important vector for Babesiosis in cattle (Rajamohanan, 1982).

Control of ticks continue to be a problem throughout the world. A clear knowledge of the biology of various ticks and the introduction of newer acaricidal agents have helped a lot in formulating satisfactory control measures against ticks. But the use of acaricides has its own drawbacks also. Apart from producing toxic effect on the host, they subsequently remain as residual toxin in foods and environment. Apart from that, many acaricides have now been proved to be less effective against ticks because of the resistance to them developed by ticks. This has resulted in a re-thinking about the use of toxic acaricides and development of alternate methods for the control of ticks, especially utilizing the immune mechanisms inherent in the host.

It is known that hosts acquire resistance to tick infestation following exposure to ticks. This phenomenon has been studied in detail in different tick-host systems by various workers. The response of the host to various tick antigens have also been studied and all these have created a thinking among scientists that satisfactory methods could be evolved for immunization of animals against tick infestation.

The present study was carried out on B. annulatus - cattle system with the following objectives.

1. to study the changes in the biology of the tick induced by acquired resistance of the host due to repeat exposure.

2. to detect the degree of development of antibody against tick antigen in both artificially and naturally immunized hosts.

3. to study the cellular reaction around the tick-feeding sites as a result of a possible immune reaction.

4. to explore the possibility of evolving a practical method for immunizing the cattle against B. annulatus so that a satisfactory level of control could be achieved with the minimum hazard of acaricide residue in the environment.

Review of Literature

REVIEW OF LITERATURE

Willadsen (1980) and Tatchell (1987) reviewed the mechanisms of host resistance to ticks.

2.1. Effects of Repeated Infestation of Hosts by Ticks

2.1.1. In laboratory animals

Trager (1939 a) was the first to observe acquired resistance to ticks in animals. In an experiment with guinea-pigs, he found that successive batches of the larvae of the American dog tick, Dermacentor variabilis, when permitted to feed subsequently on the same host, showed a reduction in the number of engorged larvae. There was reduction in the number of engorged larvae in the second and later feedings when compared with the first feeding, or they even failed to engorge. He concluded from the experiment that a solid immunity developed in the hosts following repeated infestations with ticks can prevent larvae from engorging and reduce the quantity of blood consumed by nymphs and adults. He further observed that rabbits also develop an immunity following single infestation with D. variabilis, which effectively prevents subsequent batches of larvae from engorging. His experiments showed that deer mice also become resistant to larvae of D. variabilis after two or three infestations.

Similar reports on the tick-host system involving rabbits and guinea-pigs as hosts were made for Dermacentor variabilis (Trager, 1939 a), Hyalomma anatolicum excavatum (Kohler et al., 1967), Rhipicephalus sanguineus (Garin and Grabarev, 1972), R. appendiculatus (Branagan, 1974), Ixodes ricinus (Bowessidjaou et al., 1977) and H. marginatum rufipes (Rahman, 1984). But Fujisaki (1978) found that in the case of Haemaphysalis longicornis infestation in rabbits, although the engorged weights were reduced, the number of ticks engorging, length of feeding times and percentage of hatchability of eggs were all unaltered.

The effects of re-infestation with Dermacentor andersoni in guinea-pigs were studied by Allen (1973) and Wikel and Allen (1976 a). They observed that there was a significant reduction in the number of engoged larvae after second infestation.

Similarly, the effect of re-infestation of guinea-pigs with Ixodes holocyclus was observed by Bagnall and Rothwell (1974) and Bagnall (1975). They found that in second infestation, the engorged larval percentage was 1-2 as compared to 40 per cent in the initial exposure.

Later, Norval (1978) observed that repeated infestation of rabbits and sheep with larvae and numphs of Amblyomma hebraeum failed to bring about any progressive decline in either the tick

yield or the engorged weight of fed ticks. He was of the opinion that rabbits and sheep were unable to acquire resistance against larvae and nymphs of A hebraeum.

Brown and Knapp (1981) made studies on Amblyomma americanum - guinea-pig system. They found a significant increase in mortality rate of the larval ticks in second and third feedings when compared to initial feeding. In the second and third nymphal feedings also, the mortality was significantly high. There was also irregularities in moulting periods, some of them even failed to moult.

Dipeolu and Harunah (1984) observed that rabbits repeatedly exposed to larvae, nymphs and adults of Amblyomma variegatum developed resistance to infestation with the adult ticks. They found that rabbits could develop ressistance to Boophilus decoloratus also, upon repeated exposure.

Alani and Herbert (1987) observed that sheep infested repeatedly with adults of Haemaphysalis punctata and rabbits infested with the larvae and nymphs of H. punctata and Ixodes ricinus developed resistance to these ticks. The resistance was manifested by delayed engorgement, reduction in engorged weight and tick yield. Fecundity of adult female ticks and viability and hatchability of eggs also were reduced significantly.

Induction of resistance in rabbits through repeated infestation of the tick Rhipicephalus haemaphysaloides was observed by Samantaray et al., (1988). After 10 repeated infestations, they could find that the mean engorgement period, mean weight of engorged female ticks and total number of eggs laid varied significantly. The evidence of resistance in rabbits to subsequent tick attachment increased progressively after each subsequent tick infestation. But such resistance was observed to be waning out once the rabbits were kept away from ticks for a longer period of 79 days.

2.1.2. In Cattle

2.1.2.1. Boophilus microplus-cattle system.

Riek (1962) found that fecundity, fertility and engorgement were affected in a Boophilus microplus-cattle system in re-infestation. He observed that the expression of host resistance even resulted in some of the larvae getting drowned in serous exudate from the host reaction.

Roberts (1968a) observed that the main expression of resistance in Bos taurus to B. microplus was the rejection of larvae in the first 24 h of parasitic life-cycle. Other new instars were also similarly affected. According to him, a

previously unexposed host acquire resistance and start to reject the ticks by about 8 days after infestation.

Hewetson and Nolan (1968) and Roberts (1968b) reported that Bos taurus or Bos taurus x Bos indicus cross-breds did not manifest any sign of immunity against B. microplus after repeat infestation. However, Hewetson (1971) observed prolongation of tick feeding time, reduction of egg laying and viability in B. microplus after repeat infestation in Bos indicus, due to an acquired resistance.

The growth and behaviour of larvae of B. microplus on British breed cattle with different resistance level to ticks were studied in Australia by Kemp et al., (1976). They observed that the larval growth rate was slower for the first 3 days in highly resistant animals. Using larvae labelled with ^{32}P it was found that attachement times were shorter, and more time was spent wandering on highly resistant animals during the first 16 hours. On the second day, attachments had stabilised, but more detachments still occurred from highly resistant animals.

A high rate of loss of larvae of B. microplus on hosts with high resistance was observed by Koudstaal et al., (1978). They found that in British breed cattle of high resistance, the loss

of larvae due to grooming ranged from 9 to 54 per cent during the first 24 hours of infestation. Animals of low resistance did not lose a significant number of larvae as a result of grooming.

Wagland (1978) found that the weights of fully engorged female ticks were reduced on resistant Bos indicus cattle by 30 per cent. Wagland (1979) further observed a slightly different pattern for the rejection of ticks in Bos indicus from those seen in Bos taurus, where he found a gradual reduction in number of various instars throughout the life-cycle.

Amin-Babjee and Riek (1986) observed reduction in the tick yield and engorged weight in calves when re-infested with B. microuplus larvae. This effect was gradually increased during the course of a six-infestation period. However, they could not find any reduction in the egg production, viability and hatchability during the whole period.

Miranpuri (1989) studied the effect of repeated pure and mixed infestations of B. microplus and Hyalomma anatolicum in cross-bred calves. There was significant decline in the engorged yield of female B. microplus from the first to seventeenth exposure. With H. anatolicum also the same observation was recorded. The animals exposed to repeated infestation with both the ticks, reacted in different ways, proving that host responses

elicited to one species do not provide cross resistance to the other species.

2.1.2.2. Other tick cattle systems.

Strother et al., (1974) reported that in cattle infested with Amblyomma americanum, many of the ticks which did not engorge fully died on the host at various stages and levels of engorgement. According to Doube and Kemp (1975), exposure to Ixodes holocyclus conferred a type of immunity to cattle characterized by the removal of ticks by grooming, death of the ticks in situ, or reduction in the engorged weights. Sutherst et al., (1979) reported that cattle acquired resistance against all the different instars of Haemaphysalis longicornis.

Observations on repeated sensitization of Friesland x Hereford cattle with Rhipicephalus appendiculatus were made by Fivaz et al., (1984). They found that repeated sensitization resulted in reduced tick yield at each instar level. Latif (1984) reported from Sudan that cattle acquired resistance to nymphs of Hyalomma anatolicum anatolicum and Rhipicephalus evertsi, the significant effects being marked reduction in yield of engorged ticks and engorged weight.

George et al., (1985) reported similar observations with Amblyomma americanum infestation on cattle. According to them pure bred and crossbred Bos indicus calves acquired resistance after the first exposure. This resistance was manifested by significant reductions in engorged female tick yield, mean engorged weight of female ticks and the mean weight of egg masses.

De Castro et al., (1985) compared the tick yield between previously infested and non-infested cattle for 24 weeks. They exposed cattle once a week to adult Rhipicephalus appendiculatus. The engorged female yield from sensitized cattle were fewer in number and lighter in weight. The engorgement time was also less when compared to those fed on the control animals.

2.2. Host antibody response to ticks

Willadsen (1980) reviewed the research on host antibody response to ticks.

2.2.1. In laboratory animals

Trager (1939a) was able to transfer partial immunity to Dermacentor variabilis with specific antiserum in guinea-pigs.

Complement-fixing antibodies were later reported by Trager (1939b). Precipitating and Complement-fixing antibodies have been reported in rabbits infested with Hyalomma anatolicum excavatum and Rhipicephalus sanguineus by Kohler et al., (1967). Later Weiland and Emokpare (1968) also made a similar report. Bagnall and Rothwell (1974), however, failed to transfer immunity to Ixodes holocyclus with antiserum in guinea-pigs.

Similarly, Wikel and Allen (1976a) found that transfer of serum from immune donors to recipient guinea-pigs at a rate of 1.5 ml per 100 g body weight did not confer immunity against Dermacentor andersoni. Wikel and Allen (1976b) further reported that administration of cyclophosphamide parenterally to immune hosts in sufficient doses to block the B-cells prior to infestation with ticks was largely successful in blocking the development of immunity. Wikel and Allen (1977) found that depletion of complement with cobra venom factor drastically affected immunity.

Brossard (1977) injected serum from immune rabbits to fresh experimental rabbits at a rate of about 0.25 ml per 100 g body weight four hours before an initial infestation with Ixodes ricinus ticks, and again at 24 hours later. The engorged weight of the female ticks fed on these hosts were significantly less than those fed on the controls. On an experiment using the

salivary gland antigen of Ixodes ricinus, Bowessidjaou et al. (1977) noted that corresponding antibody appeared towards the end of a first infestation and reached high titres on a second, without any increase thereafter. However, he observed that the hosts became progressively more immune.

The development of an antibody of the class IgG to Haemaphysalis longicornis in rabbits was observed by Fujisaki (1978).

Wikel (1979) reported that C4 deficient guinea-pigs with a total deficiency in the classical pathway of complement activation, but with an intact alternate pathway, acquired tick resistance.

Brown and Askenase (1983) also opined that guinea-pigs sensitized by tick-feeding acquire resistance to a challenge feeding. They considered it to be mediated by sensitized T-lymphocytes and Immune serum factors. IgG1 antibodies were the major serum factors. Cross reactivity of the immune response occured following heterologous tick-feeding challenge with ticks of three different genera (Amblyomma, Dermacentor and Rhipicephalus). Brown and Askenase (1985) further demonstrated that Fc receptors of immunoglobulin on host cells such as mast

cells and basophils are required for antibody-mediated immune rejection of Amblyomma americanum ticks from guinea-pigs.

Whelen, Richardson and Wikel (1984) were able to identify immunogenic polypeptides in the sera of tick-infested guinea-pig with the use of immunoblotting.

Minoura et al., (1985) also detected a rabbit IgG in the haemolymph of an argasid tick, Ornithodoros moubata, which had engorged on rabbits. A delayed hypersensitivity reaction in the skin of infected rabbits subsequent to injection of salivary antigens from Ixodes ricinus was observed by Girardin and Brossard (1985).

Mongi et al., (1986) employed immunodiffusion technique using the extracts derived from adult Rhipicephalus appendiculatus and antisera from rabbits immunized with those extracts.

Ben-Yakir et al., (1986) employed ELISA to quantify the host IgG fractions in the haemolymph of Dermacentor variabilis and Amblyomma americanum.

Whelan et al., (1986) employed Dot-ELISA to assess the antibody response of guinea-pigs to repeated Dermacentor andersoni infestations. They did not observe a direct correlation between resistance and antibody titre.

Papatheodorou and Brossard (1987) reported that serum C3 level of rabbits infested 3 times with Ixodes ricinus females reached a peak 6 days after the beginning of each infestation, the highest being after third infestation. They found that the C3 level in the midgut of ticks also followed the same pattern.

Using ELISA, with salivary gland antigen of Rhipicephalus appendiculatus, Njau and Nyindo (1987 a) were able to detect antibodies to both homologous and heterologous (using R. evertsi evertsi) tick challenges. Njau and Nyindo (1987 b) further reported that repeated infestation for four times with Rhipicephalus appendiculatus produced anti-tick antibody development in rabbits which was detected using ELISA. The peak response was after the third infestation.

Precipitating antibodies were demonstrated in rabbits experimentally immunized with extracts of Rhipicephalus sanguineus by Puttalakshmamma et al., (1988 a). By employing zinc sulphate turbidity test, they found that the peak level of immunoglobulins were attained during the second infestation.

2.2.2. In Cattle

2.2.2.1. Boophilus microplus - cattle system.

Brossard (1976) found that serum gamma globulin concentration significantly increased in cattle following infestation with B. microplus. Indirect immunofluorescent studies showed the presence of specific and non-specific antibodies to the salivary gland antigen of adult female ticks.

Roberts and Kerr (1976) passively transferred immunity to cattle by using large volumes of plasma derived from cattle highly immune to B. microplus. It resulted in a significant reduction of tick yield. Willadsen et al., (1978) measured specific antibodies to a purified tick antigen by indirect haemagglutination, but found that its concentration did not correlate with the levels of immunity.

Tracey-Patte (1979) observed that activity of an enzyme from B. microplus which is secreted into the host's skin within 1 hour of attachment can be removed by a host previously exposed to the tick. This type of removal does not occur in unexposed host. This phenomenon was considered to be antibody-dependent.

Later, Tracey-Patte et al., (1987) detected bovine IgG1 and albumin in haemolymph of B. microplus ticks fed on normal as well as immunized cattle. The amount of IgG1 in ticks fed on

vaccinated cattle was about 4-5 times that of those fed on normal cattle, and the amount of albumin was about 150 times more.

2.2.2.2. Other tick-cattle systems.

Allen and Humphreys (1979) observed that calves developed antibodies to Dermacentor andersoni following inoculation of various antigens prepared from the tissues of the tick. They demonstrated this with the help of immunodiffusion test.

Rosenberg (1984) showed that cattle expressed peak serum antibody titre to the salivary gland antigen of Hyalomma anatolicum excavatum and Rhipicephalus appendiculatus following 4th infestation with the adult tick within a period of 4 months. ELISA and immediate hypersensitivity tests were carried out, which gave positive results.

Whelen et al., (1984) showed dot-ELISA test to be highly successful in detecting serum antibodies in cattle to salivary gland antigens of ticks. They found that Bos taurus calves and cows exposed to 4 repeat infestations with Dermacentor andersoni, and Bos indicus calves exposed 3 times to Amblyomma americanum developed significant antibody response. The antibody developed in both the groups were cross-reactive to the D. andersoni and A. americanum salivary gland antigens.

Binta et al., (1984) were able to detect homocytotropic antibodies against R. appendiculatus in the sera of tick-resistant cattle. The transfer of these failed to sensitize the skin of heterologous species of hosts.

2.3. Tissue Response to Tick-feeding

2.3.1. In Laboratory animals and small ruminants

Allen (1973) observed hyperplasia of epidermis and marked infiltration by basophils on the skin of guinea-pigs resistant to Dermacentor andersoni.

McLaren et al., (1983) made electron microscopic observations on the feeding lesions of the larvae of R. appendiculatus on guinea-pigs. The observations revealed that in actively sensitized guinea-pigs and those received immune serum the primary lesions were characterized by domination of neutrophils and eosinophils, while the secondary lesions were dominated by basophils.

Mbemba (1983), working with adult R. appendiculatus infestation of sheep, reported increased thickening of the epidermis correlating with the number of infestations. He reported the occurrence of haemorrhages into the tissues from the third exposure onwards.

Johnston and Brown (1985), described the cellular response in the feeding lesion of an argasid tick, Ornithodoros parkeri. They observed marked tissue basophilic infiltration only in infestation on a previously exposed guinea-pig. In such hosts, increased eosinophilic reaction was also observed.

Gill and Walker (1985) quantitatively analysed the sequential changes of cellular reaction in the Hyalomma anatolicum anatolicum - feeding sites on rabbits. The primary infestation was characterized by an abundance of neutrophils and mononuclear cells. Eosinophils were not abundant in numbers, and showed a gradual reduction proportionate to the engorgement time, and the basophil number was low. But in tertiary infestation, there was significant increase in the numbers of basophils and eosinophils and marked early degranulation of mast cells and basophils. The basophil and mononuclear cell number increased as the feeding advanced with a decrease in the eosinophil population.

Abdul-Amir and Gray (1987) studied the resistance of sheep to Ixodes ricinus. They observed that three repeated infestations of ewes with adult I. ricinus ticks caused a tissue lesion predominated by neutrophils. This was followed by basophilic infiltration and degranulation accompanied by

mononuclear cells. Mononuclear cells later dominated the infiltrate. Eosinophils were in abudance, especially during tertiary infestation. Degranulation of mast cells and basophils also was most rapid in the tertiary infestation.

2.3.2. In cattle

Tatchell and Moorhouse (1968) described the sequential changes at the sites of bite of B. microplus on cattle. The lesion consisted of a cavity containing neutrophils, some lymphocytes and erythrocytes in an area of heavily infiltrated collagen. A typical immediate hypersensitivity reaction marked with an early intense infiltration of eosinophils into the area of the mouth parts was observed on sensitized European cattle.

Kemp and Bourne (1980) concluded that the earlier detachment of B. microplus larvae from highly resistant cattle is largely mediated through histamine release and associated tissue changes at the site.

Amblyomma americanum - feeding sites on cattle were studied in detail for cellular response by Brown, Barker and Askenase (1984). The lesion in tick-naive hosts consisted mainly of mononuclear cells initially. Granulocytes began to appear after 24 hours and was abundant by 48 hours. Basophils were the most

abundant granulocytes. Secondary and tertiary hosts expressed significant basophil response as early as six hours post-tick infestation. The cutaneous basophil levels showed a two-fold increase in tertiary hosts compared to the secondary hosts. Neutrophils and Eosinophils were also notable in the lesion in secondary hosts, while in tertiary hosts, they were markedly reduced. Mast cell levels were not significant in both the type of lesions.

Schillinger (1985) reported that infiltration of eosinophils following the early breakdown of mast cells adjacent to tick-mouth parts were not significant on Amblyamma variegatum-feeding sites on cattle skin. The eosinophilic infiltration followed a decreasing pattern upon repeated infestation. Repeated infestation with Hyalomma anatolicum excavatum caused epidermal vesicles. They contained neutrophils and eosinophils, and, after third infestation, erythrocytes also. Mast cell degranulation leading to an increased eosinophilic infiltration occured early in the infestation.

Gill (1986) observed that the cellular changes at the feeding site of H.anatolicum anatolicum in cattle were similar to the pattern in rabbits as described by Gill and Walker (1985), excepting that the ecsinophil numbers were not much reduced in the teritary infestation as in rabbits. However, there was a

progressive decrease in the detectable number of mast cells in the post-teritary infestation period.

2.4. Tick Antigens and Artifical Immunization

2.4.1. In laboratory animals

Trager (1939b) used extracts of cephalic glands, salivary glands and digestive tract of partially engorged adult females and salivary glands of unfed adult female of Dermacentor variabilis as antigens. He obtained partial immunity in guinea-pigs with an intradermal injection of these extracts.

Garin and Grabarev (1972) reported success in immunizing rabbits with subcutaneous injections of Rhipicephalus sanguineus salivary glands.

Bagnall (1975) reported that subcutaneous injection of 1.4 mg of larval extract of Ixodes holocyclus produced immunity in guinea-pigs.

Allen and Humphreys (1979) made a major advance in the immunization trials when they immunized guinea-pigs against Dermacentor andersoni with extracts of either midgut and reproductive organs (antigen I) or all internal organs (antigen II) of the tick. Both groups of immunized hosts showed

significant results. Those given antigen I, after experimental exposure, yielded ticks which laid fewer eggs than those fed on controls. The percentage of hatchability of these eggs was zero. Those given antigen II did not allow ticks to engorge and no eggs were laid. The antigens I and II were prepeared from female ticks that fed for 5 days on guinea-pigs.

McGowan et al., (1980) observed the development of antibodies in rabbits following the inoculation of a homogenized whole-tick extract of adult Amblyomma maculatum. The homogenized tick extract contained 22-24 proteins. Skin responses were marked in treated animals compared to the controls. Significant reduction in engorged weights were observed in female ticks fed on immunized hosts. The weight of egg masses produced by female ticks that fed on immunized hosts both as nymphs and adults were also significantly less than those fed only as nymphs.

Ackerman et al., (1980) observed that rats developed resistance to adult D. variabilis following inoculation with the mid gut antigen derived from the ticks. The resistance was manifested by delayed attachment, reduced engorged weights, prolonged pre-oviposition period, irregular pattern of egg laying and reduced hatchability of eggs. Such effects were not observed when the whole-tick extract was used as the antigen.

Wikel (1981) reported that salivary gland antigen derived from partially engorged female D. andersoni ticks induced resistance in guinea-pigs. This was evident irrespective of the use of an adjuvant along with antigen or the differences in the route of administration. The immunity was expressed by a significant reduction in the number and weight of engorged larvae.

Brown, Shapiro and Askenase (1984) observed that salivary gland extracts derived from A. americanum induced resistance to the tick in guinea-pig. Using SDS-PAGE, they identified the particular antigen responsible for immune response, as having a molecular weight of 20,000.

Wikel (1985) observed that guinea-pigs developed significant degree of resistance to adult A. americanum following subcutaneous administation of cells derived from primary tissue culture of developing larvae. The resistance was expressed as a reduction in the engorged weights of females, reduced oviposition and mortality of ticks in situ. He noted that these cells were also able to induce a significant degree of cross resistance to infestation with D. andersoni.

A larval extract of Rhipicephalus appendiculatus was found to be immunogenic in rabbits on inoculation with or without

Freund's Complete Adjuvant (FCA) by Binta et al., (1985). The response was manifested as increased engorgement time for the ticks fed on inoculated rabbits and lower hatchability of eggs from such females.

Studies by Brown and Askenase (1986) supported the early finding of Brown, Shapiro and Askenase (1984) that the salivary gland antigen of A. americanum is a protein of molecular weight of about 20,000. They found this substance in the crude salivary gland extracts and cement extracts. This was recognized by polyclonal IgG1 molecules of the serum of sensitized guinea-pigs.

Serum from resistant guinea-pig was successfully employed to detect major tick antigens derived from R. appendiculatus by Shapiro et al., (1986). Twelve different antigens with molecular weights ranging from 16,000 to 1,20,000 were identified. Salivary glands were the major sources of these antigens. Many of these antigens were shared by the tick cement also Gut extract was predominated by antigen of molecular weight of 31,000. Larval and nymphal tick extracts lacked many of the antigens present in adult ticks.

Gill et al., (1986) observed that nine proteins in the saliva and 17 in the salivary gland extracts from females of H. a. anatolicum reacted with sera of hypersensitized rabbits. They

identified the majority of antigens as glycoproteins with molecular weight between 14,400 and 1,30,000. One antigen of molecular weight 1,30,000 showed acid phosphatase activity. Another of molecular weight 96,000 showed both non-specific esterase and aminopeptidase activity.

Wikel and Whelen (1986) reported on the attempts to identify, characterize and isolate tick immunogens using protein immunoblotting.

Srivastava et al., (1987) used whole-tick extract from fully engorged ticks (antigen I) and from partially fed females (antigern II) for immunization of rabbits against Boophilus microplus. Each rabbit was given the antigens at the rate of 2.88 mg protein per kg body weight intradermally. Significant reduction occurred in larval attachment rate, number of engorged larvae, nymphs and adults. Engorged weight of female ticks fed on rabbits immunized with antigen II was less than those fed on rabbits immunized with antigen I.

Puttalakshmamma et al., (1988b) evaluated different extracts of Rhipicephalus sanguineus ticks for immunizing property. According to them, egg extract induced failure in larval moulting, larval extract induced reduction in the number of nymphs recovered, nymphal extract induced reduction in

hatchability of eggs, and the adult extract induced reduction in engorged weight and egg mass weight.

Banerjee and Manohar (1990) administered primary culture cells of embryonic H.a. anatolicum to rabbits which were previously unexposed to ticks. After challenge with unfed male and female H.a. anatolicum, they observed a significant increase in the engorgement period of female tick and also a significant decrease in the mean egg mass weight.

2.4.2. In cattle

Brossard (1976) inoculated two day-old calves with antigen derived from 100 salivary glands from partially engorged adult female B. microplus subcutaneously. Challenge infestation of these calves at 2 and five months of age resulted in lowered engorged weights of ticks than those obtained from the controls.

Allen and Humphreys (1979) immunized calves with the antigen I against D. andersoni in the same way as they immunized guinea-pigs. The calves weighing between 180 and 280 lb were given 67 mg of antigen in Freund's Incomplete Adjuvant. The ticks yielded from such calves showed a reduction in engorged weights, egg production and hatchability of eggs.

Wilkinson and Allen (1983) could not get any significant response to challenge with D. andersoni in cattle previously inoculated with an extract of D. varibilis suspended in aluminium hydroxide adjuvant.

Binta and Cunningham (1984) observed immediate hypersensitivity reaction in the skin of sensitized cattle when inoculated intradermally with an extract from the larvae of R. appendiculatus, which intensified with the age of cattle and duration of previous exposure to ticks.

Labarthe et al., (1985) reported occurrence of cross-reacting antigens in the salivary substances of B. microplus and Stomoxys calcitrans.

Johnston et al., (1986) reported the presence of protective antigens both in the supernatent and the pellet derived from extract of B. microplus adult females. They observed 70-90% reduction in tick population on vaccinated cattle compared with the unvaccinated group.

Kemp et al., (1986) observed that upto 60 per cent of the female B. microplus ticks and also the males which fed on vaccined cattle, suffered damage to their gut. Such females either failed to engorge, or if they did, many died before egg

laying. There was no hypersensitivity reaction at the site of tick-infestation.

Agbede and Kemp (1986) also reported that the primary site of damage in B. microplus ticks which fed on vaccinated cattle was the gut. The host leucocytes which reached the haemolymph had destroyed the tick muscle and the Malpighian tubules of the female ticks. In male ticks, in addition, the accessory gland of the reporductive organ also was damaged. The salivary glands were not affected.

Jackson and Opdebeek (1989) used membrane antigens extracted from the mid gut of B. microplus to immunize sheep and cattle. The antibody levels were measured by ELISA.Cattle vaccinated with either 500 μg antigen in two doses or 50 or 500 μg in three doses had significant antibody response and were equally protected, against challenge with 40000 ticks compared to control cattle. In another experiment they found that cattle vaccinated with 2.95 mg of the midgut antigen divided into twelve doses over six months had antibody levels that reached a plateau after the receipt of 1.2 mg antigen. They exhibited a protection level of 96 per cent against challenge with B. microplus.

Materials and Methods

MATERIALS AND METHODS

3.1. The Tick Species

3.1.1. Tick Collection and identification

Fully engorged adult female Boophilus annulatus ticks were collected from the body of calves stationed at various farms of the Kerala Agricultural University. A few male ticks also were collected for the specific identification of species. The collected ticks were placed in a wide-mouthed glass bottle, the mouth of which was secured with a thin muslin cloth before being brought to the laboratory. Identification was done by using the keys given by Sharif (1928) and Arthur (1960).

3.1.2. Colonization of ticks in laboratory

The live engorged female ticks were placed individually in clean glass vials plugged with cotton wool. The vials were then transferred to a dessicator containing 15 per cent potassium hydroxide solution so as to maintain a relative humidity of 85 per cent and kept at room temperature. The ticks were allowed to oviposit under these conditions, following which the eggs laid were transferred separately to fresh glass vials with the help of a camel hair brush. One hundred mg of egg mass was weighed correctly and the number of eggs in this counted under binocular dissection microscope. Correctly weighed egg masses were

transferred to clean glass vials, their mouths covered with a layer of muslin cloth, and stored in the dessicator for hatching. After hatching, the larvae were kept in the same vials without disturbance till further use. The dessicator was kept open for 5-10 minutes everyday for the purpose of aeration.

3.2. Experimental Animals

3.2.1. For repeated infestation studies

Five cross-bred tick-naive female calves aged two months were selected from the University Livestock Farm, Mannuthy, and used as experimental animals. They were stationed at the farm itself in individual pens and maintained on routine diet. The calves were subjected to thorough clinical examination and dewormed with albendazole (Valbazen-Eskayef) prior to the start of the experiment. This was repeated at monthly intervals to ensure the health of the calves.

3.2.2. For immunization trials

Two cross-bred male calves of about 80-90 kg body weight and around seven months of age were purchased from the farmers. They were stationed in the experimental animal shed in individual pens and provided with green grass and commercially available concentrate feed (Godrej). They were dewormed with albendazole once in three weeks starting from one week prior to immunization.

One cross-bred female calf of about four months of age was used as control. It was also stationed under identical conditions.

3.3. Experimental programme

Five cross-bred female calves constituted the experimental group for trials with repeated infestations (Group I). Two cross-bred male calves were used as experimental group for immunization trials (Group II). One cross-bred female calf formed the control for immunization trial (C). All the animals received the same number of seed-ticks at every time of infestation. The calves of group I alone received three infestations each, the second and third infestation being given three weeks after they became tick-free following the previous infestation.

3.4. Immunological studies

3.4.1. Infestation of experimental calves with ticks

Larval ticks for experimental infestation were obtained as mentioned in 3.1.2. The number of seed-ticks in each glass vial were calculated before they were released on the body of the

animals. These larval ticks were maintained at identical conditions for a pre-release period of seven days to make them starve and to facilitate easy attachment on the hosts. On the 8th day the seed ticks were activated by warming the glass vials containing them to 37°C. They were then released on to the host's body over the back region at the rate of about 3600 larvae per animal. The hosts were not groomed or washed during the infestation period and were stationed indoors for the complete period of study.

3.4.2. Attachment and engorgement of ticks

The number of female ticks successfully attached to the hosts were assessed for each host by retrieving the engorged females from the body of the hosts after 21 days of larval release, and also from the floor of the pen where they have dropped off from the body of the hosts. They were counted and the percentage of attachment was calculated.

3.4.3. Engorged body weight of ticks

The fully engorged adult female ticks recovered from the experimental and control hosts were weighed individually using a monopan balance and the weights recorded.

3.4.4. Egg mass weight

The previously weighed engorged female ticks were placed in individual vials to oviposit, and the egg masses were collected into seperate glass vials with the help of camel hair brush. The weight of the egg masses laid by individual female ticks were then recorded using a monopan balance.

3.4.5. Egg number

The egg masses from individual ticks were separately transferred to petridishes and the eggs were counted using a binocular dissection microscope with the help of a camel hair brush. The number of eggs laid by each individual female tick was thus recorded. The numbers of eggs in 100 mg of egg mass was also counted separately to standardise the egg number and weight ratio.

3.4.6. Hatchability of eggs

A representative sample of 100 eggs from individual ticks were used for this study. They were placed in separate glass vials in the dessicator at ambient temperature and 85 per cent of R.H. for hatching. The seed-ticks hatched out of 100 eggs were counted after arresting their movement by chilling.

3.5. Preparation of antigen

For the preparation fo tick antigen, the method described by Srivastava et al., (1987) was followed with slight modifications.

Whole-tick tissue extract was used as antigen. It was prepared from partially engorged female ticks. One hundred partially engorged female ticks with a mean weight of 98.00 ± 3.00 mg were selected. Their external surfaces were thoroughly cleaned with a camel hair brush. This was followed by washing three times with 70 per cent alcohol to get rid of micro-organisms from the body surface.

The ticks were then individually transferred to a pre-seterilized glass mortar. Homogenization was done with the addition of small quantities of chilled phosphate buffered saline (P.B.S.- pH 7.2). During homogenization, small quantities of saline were added to obtain a final concentration of roughly around one gram of tick tissue per 10 ml of saline. The homogenate was then centrifuged at 1600 g at 28°C for 15 minutes and then the supernatent transferred to another tube for centrifugation. This was done three times and the final supernatent was filtered with Whatman No. 1 filter paper. The

sediment was again homogenized in saline and processed as described above.

The filtered whole-tick tissue extracts were supplemented with penicillin at the rate of 4000 IU/ml and streptomycin at the rate of 40 mg/ml. They were then stored in sterile rubber-stoppered glass vials at 0°C till further use. Prior to their use, the final protein concentration in the tick extract was estimated by Lowry (Folin-Ciocalteu) method as described by Garvey et al., (1977). The concentration was estimated to be at 4mg/ml.

3.6. Immunization

The antigen prepared as above, was emulsified with an equal volume of Freund's Complete Adjuvant (FCA). 20ml of this emulsion (containing 40mg of protein) was administered on day '0' to each calf of group II. They received a booster dose of 10 ml of tick extract without adjuvant on the day seven. The control calf was given 10 ml of FCA alone on the day '0'. All the injections were given subcutaneously. Seven days after the booster dose, the animals were exposed to infestation with the seed-ticks. This was considered as the day '0' of infestation.

3.7. In vitro immunological test

3.7.1. Collection of serum

Serum samples were collected from all experimental animals belonging to group I and II at fortnighly intervals starting from the day '0' of infestation. The pre-infestation serum was collected on day '0' and the post-infestation sera on days 14 and 28. A drop of 1:10,000 Thiomersal was added to each serum sample to prevent bacterial contamination and the samples were stored at 0°C till further use.

3.7.2. Immunodiffusion test

The double gel diffusion technique of Ouchterlony was employed for the detection of serum antibodies against B. annulatus.

Preparation of the gel

0.8 g of agarose (Sisco Research Lab, Bombay) was dissolved in 100 ml of PBS (pH 7.2) by boiling in a waterbath for 45 minutes. 0.5 ml of phenol was added to it to prevent bacterial contamination.

Procedure of test

The test was run on coated microscopic glass slides. The slides were coated with a film of about 1.5 ml of hot molten 2% agar (DIFCO) and dried, before adding 3.5ml of molten agarose medium. The medium was allowed to solidify in room temperature following which the slides were transferred to a petridish and kept at refrigeration temperature for 20 minutes. The slides were then taken out and wells with 4 mm diameter were punched out allowing a distance of 4 mm between the wells using a template.

The sera collected from the experimental calves were run against the prepared antigen. The central well was charged with the antigen and the peripheral wells with the sera of experimental calves. The slides were incubated in a humid chamber at room temperature for 48 hours. In the case of serum samples of calves belonging to group I, the serum wells were re-charged at 24 hours.

3.8. Histopathology

Pieces of skin with a size of about 1 sq. cm. were collected from the site of tick-bite on the host. The skin pieces were collected with the tick in situ, and then, the body of the tick was snipped off close to the epidermis with a sharp pair of scissors, so as to retain the mouth parts in situ. The tissue pieces were then transferred to 10 per cent formalin for fixation.

The skin tissues were processed for histopathology and paraffin embedded sections were cut at 5-7 μ thickness. The sections were stained by Haematoxylin-Eosin method as detailed by Sheehan and Hrapchak (1980).

3.9. Statistical analysis:

The data obtained from the calves of group I pertaining to the effects of repeated infestation were analysed statistically. The five parameters, namely the number of engorged females, mean engorged weights, mean egg mass weight, mean egg number and percentage of hatchability, were subjected to analysis of variance (ANOVA) (Snedecor and Cohran, 1967). The data of mean percentages of hatchability were subjected to arcsine transformation prior to analysis of variance. The data obtained from calves of group II were analysed by Students' 't' test.

Results

RESULTS

4.1. Effects of Repeated Infestations

The effects of repeated infestation of calves with Boophilus annulatus were studied with the help of five parameters relating to the biology of the ticks, viz., the number of engorged females, mean engorged weight, mean egg mass weight, mean egg number and mean percentage hatchability. Other parameters including the antibody response and the histopathology of the tick-feeding site were also studied.

4.1.1. Number of engorged female ticks

The number of engorged female ticks obtained from first infestation were found to range from 39 to 141 and in the second infestation, from 23 to 114. The third infestation recorded the minimum number of ticks as 4 against a maximum number of 37 (Tables I to III).

The percentage of engorgement of ticks on each animal was calculated for each infestation. The mean engorgement percentage also was found to follow a decreasing pattern from the primary (2.68%) to the tertiary (0.81%) infestations (Fig.1).

Table I. Feeding and Reproductive parameters of ticks following first infestation

Animal Number	Number of engorged female ticks	Engorged weight(mg) Mean±SE*	Egg mass weight(mg) Mean±SE*	Egg number Mean±SE*	Hatchability (%) Mean±SE*
GI 1	141	148.0± 5.71	53.87± 2.38	1487± 101.05	97.0± 0.98
GI 2	39	132.5± 5.40	59.6± 3.32	1710± 35.29	67.5± 4.70
GI 3	78	119.2± 7.27	47.8± 2.39	1321± 33.05	86.0± 1.17
GI 4	94	136.0± 2.78	47.5 2.14	1194± 25.26	99.5± 3.16
GI 5	131	161.4± 4.05	54.26± 3.09	1589± 31.13	89.5± 1.39

*SE - Standard error

Table II. Feeding and Reproductive parameters of ticks following second infestation

Animal Number	Number of engorged female ticks	Engorged weight(mg) Mean±SE*	Egg mass weight(mg) Mean±SE*	Egg number Mean±SE*	Hatchability (%) Mean±SE*
GI 1	58	101.1±10.91	61.2±4.65	1604±44.98	83.4±5.33
GI 2	23	113.0±19.84	58.8±2.23	1545±41.54	87.6±2.27
GI 3	47	74.8±3.51	32.6±2.23	1346±118.73	89.0±2.40
GI 4	98	100.8±3.16	56.2±8.13	1591±187.53	92.6±3.04
GI 5	114	149.7±3.92	62.0±2.09	1632±38.92	89.2±2.14

* SE - Standard error

Table III. Feeding and Reproductive parameters of ticks following third infestation

Animal Number	Number of engorged female ticks	Engorged weight(mg) Mean±SE*	Egg mass weight(mg) Mean±SE*	Egg number Mean±SE*	Hatchability (%) Mean±SE*
GI 1	36	141.6±3.81	50.6±5.99	1526±113.23	73.6±12.58
GI 2	4	115.0±9.19	44.6±4.83	885±86.50	91.0±1.77
GI 3	35	147.8±10.37	53.4±6.93	1616±118.57	62.0±16.15
GI 4	34	150.6±8.39	42.2±7.67	1542±170.69	63.4±15.28
GI 5	37	143.6±4.65	46.0±4.41	1346±133.56	81.8±5.21

* SE - Standard error

Table IV. ANOVA table for the number of engorged female ticks

Source	DF	SS	MS	F
Between infestations	2	11443.6	5721.8	5.20*
Within infestations	12	13206	1100.5	
Total	14	24649.6		

CD = 45.7175

Treatment	T_1	T_2	T_3
Mean $\pm$ Standard error **	$96.6^{a}\pm 18.47$	$68.0^{ab}\pm 16.71$	$29.20^{b}\pm 6.32$

* $P < 0.05$

** Means with common letters do not differ significantly.

CD Critical difference at 5% level.

Table V. ANOVA table for the mean engorged weights

Source	DF	SS	MS	F
Between infestations	2	3347.746	1673.873	4.18*
Within infestations	12	4804.004	400.33	
Total	14	8151.75		

CD = 27.5738

Treatment	T_1	T_2	T_3
Mean $\pm$ Standard error **	$139.42^{a} \pm 7.16$	$107.88^{b} \pm 12.18$	$139.72^{ac} \pm 6.38$

* $P < 0.05$

** Means with common letters do not differ significantly.

CD Critical difference at 5% level.

Table VI. ANOVA table for the mean egg mass weights

Source	DF	SS	MS	F
Between infestations	2	126.96	63.48	0.97 (N.S)
Within infestations	12	787.04	65.59	
Total	14	914.00		

Treatment	T_1	T_2	T_3
Mean $\pm$ Standard error	52.61$\pm$2.26	54.16$\pm$5.48	47.36$\pm$2.04

N.S. : Non-Significant.

Table VII. ANOVA table for the mean egg number

Source	DF	SS	MS	F
Between infestations	2	64513	32256.5	0.68 (N.S)
Within infestations	12	572096	47674.67	
Total	14	636609		

Treatment	T_1	T_2	T_3
Mean $\pm$ Standard error	1460.2$\pm$92.18	1543.6$\pm$51.36	1383$\pm$132.17

N.S. : Non-Significant.

Table VIII. ANOVA table for the mean percentage hatchability *

Source	DF	SS	MS	F
Between infestations	2	405.92	202.96	2.74 (N.S)
Within infestations	12	889.34	74.11	
Total	14	1295.26		

Treatment	T_1	T_2	T_3
Mean $\pm$ Standard error	87.90$\pm$5.65	88.37$\pm$1.48	74.36$\pm$5.50

N.S. : Non-Significant.

* Percentages were subjected to arcsine transformation prior to ANOVA

Table IX. Feeding and Reproductive parameters of ticks following immunization with tick antigen

Animal Number	Number of engorged female ticks	Engorged weight(mg) Mean±SE*	Egg mass weight(mg) Mean±SE*	Egg number Mean±SE*	Hatchability (%) Mean±SE*
GII 1	123	143.4±7.85	51.86±4.95	1185±105.48	51.46±6.97
GII 2	37	104.0±7.99	47.06±4.04	909±91.13	48.6±5.42
C	210	194.2±3.75	56.4±2.31	1705.13±43.96	90.4±1.25

* SE - Standard error

Table X. Immunization trial-analysis of mean engorged weight

	Control	Experimental	't'
Mean (mg)	194.20	123.70	
			7.23 **
S.E (mg)	3.75	6.61	

** The means differ significantly ($P < 0.01$)

Table XI. Immunization trial-analysis of mean egg mass weight

	Control	Experimental	't'
Mean (mg)	56.4	49.47	
			1.45 (N.S.)
S.E (mg)	2.31	3.17	

N.S. There is no significant difference between the mean egg mass weight.

Table XII. Immunization trial-analysis of mean egg number

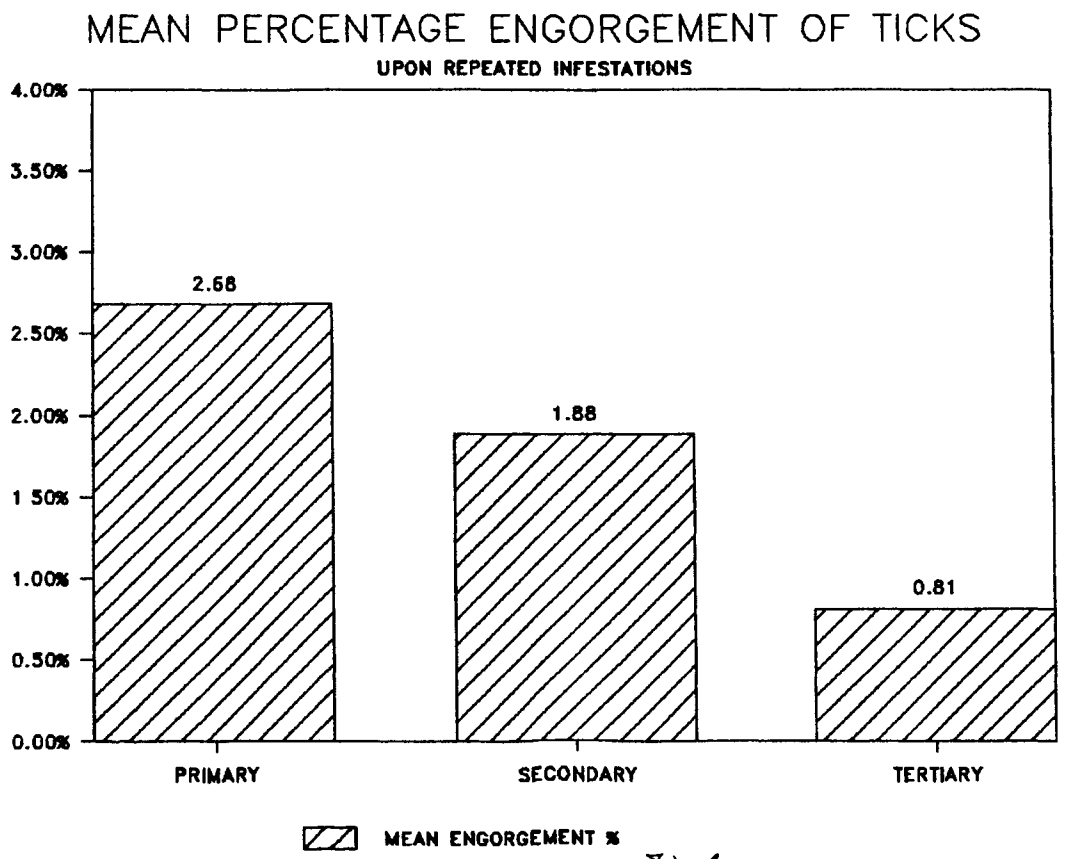
	Control	Experimental	't'
Mean (mg)	1705.13	1047.16	
			6.07 **
S.E (mg)	43.96	73.13	

** The mean egg number of control and experimental groups differ significantly ($P < 0.01$)

Table XIII. Immunization trial-analysis of mean percentage hatchability

	Control	Experimental	't'
Mean (%)	90.40	50.03	
			6.47 **
S.E (%)	1.25	4.34	

** The mean percentage of hatchability differ significantly ($P<0.01$) between the control and experimental groups.


MEAN PERCENTAGE ENGORGEMENT OF TICKS
UPON REPEATED INFESTATIONS
4.00%
3.50%
3.00%
2.50%
2.00%
1 50%
1.00%
0.50%
0.00%
2.68
1.88
0.81
PRIMARY
SECONDARY
TERTIARY
MEAN ENGORGEMENT %

The number of engorged female ticks with regard to repeated infestations were analysed by analysis of variance to find out whether the differences obtained above were significant. The mean number of engorged female ticks in the first exposure was 96.6±18.47. In the second exposure it was 68.0±16.71 and subsequently reduced to 29.20±6.32 in the third exposure. The critical difference obtained was 45.7125. This indicated that the number of engorged female ticks present in the first exposure and the third exposure differed significantly ($P < 0.05$) since the difference in the mean values between these exposures exceeded the critical difference value. Eventhough there was a significant difference in the number of engorged female ticks between the first exposure and the third exposure, there was no significant difference between the first and the second exposures, nor between second and third exposures (Table IV).

4.1.2. Mean engorged weight of ticks

The ticks obtained from first infestation recorded a minimum and maximum engorged weight of 119.2±7.27 mg and 161.4±4.05 mg respectively. Following the second infestation, the corresponding values were 74.8±3.51 mg and 149.7±3.92 mg respectively. In the third infestation the minimum engorged weight was found to be 115.0±9.19 mg and the maximum, 150.6±8.39 mg (Table I to III). Apparently much difference was not observed between the infestations with regard to the mean engorged weight of female ticks.

Eventhough there was no appreciable difference in the parameters observed, analysis of variance was done to find out whether there is any statistically significant difference. The mean engorged weight in the first infestation was 139.42±7.16 mg. In the second infestation it reduced to 107.88±12.18 mg and in the third infestation, varied to 139.72±6.38 mg. The critical difference obtained in analysis of variance was 27.5738. When the mean engorged weight was considered, there was significant difference between the first exposure and the second exposure and between the second and third exposures ($P<0.05$). But there was no significant difference between the first and third exaposures (Table V).

4.1.3. Mean egg mass weight

The mean weight of egg masses laid by the engorged female ticks following first infestation recorded a minimum of 47.5±2.14 mg and a maximum of 59.6±3.32 mg. Following second infestation the minimum of the mean egg mass weight recorded was 32.6±2.23 mg against a maximum of 62.0±2.0 mg. In the third infestation, the corresponding values were 42.2±7.67 mg and 53.4±6.93 mg respectively (Table I to III). There appeared to be a difference between these values, particularly between those recorded in first and second infestations.

The egg mass weights were subjected to analysis of variance to find out whether the apparent difference between the infestations in this regard had any significance. The mean egg mass weight in the first infestation was 52.61±2.26 mg, in the second infestation, it was 54.16±5.48 mg and in the third infestation 47.36±2.04 mg. The F value in analysis of variance was 0.97 which was not significant, suggesting that there is no significant difference in the mean egg mass weight of the first infestation and subsequent infestations (table VI).

4.1.4. Mean egg number

The minimum value for the mean egg number following first infestation was found to be 1194±25.26 and the maximum 1710±35.29. In the second infestation, the minimum and maximum values were 1346±118.73 and 1632±38.92 respectively. The lowest value of egg number recorded in third infestation was 885±86.5 and the highest value 1616±118.57 (table I to III). There was no apparent difference between the infestations with regard to the mean egg numbers.

Analysis of variance was employed with the mean value of egg number for each infestation to find out whether there was any significant difference between the infestations. The mean egg number of first infestation was 1460.2±92.18 while in the second infestation it was 1543.6±51.36 and in the third, 1383±132.17.

The F value was 0.68 which was non-significant at 5 per cent level. This indicated that there was no significant difference between the first exposure and the subsequent exposures regarding mean egg number (Table VII).

4.1.5. Mean percentage hatchability

The first infestation recorded the mean percentage hatchability at a minimum of 67.5±4.70 and a maximum 99.5±3.16. In the second and third exposures, these values were 83.4±5.33 and 92.6±3.04 and 62.0±16.15 and 91.0±1.77 respectively (Table I to III). These results did not show any apparent difference in hatchability between infestations.

The arcsine transformed values of percentages were used for analysis of variance to reduce the error while doing the analysis. The mean percetage of hatchability for the first infestation was 87.90±5.65 and that for the second 88.37±1.48, while the third infestation had a mean of 74.36±5.50. The F value obtained was 2.74 at 5 per cent level. This indicated that with regard to the percentage hatchability of eggs laid by engorged female ticks, there was no significant difference between the first and the subsequent infestations (Table VIII).

4.1.6. Detection of antibody response

The agarose gel preparation on the slide did not record any visible changes in between the wells containing the experimental serum of '0' day and the antigen after keeping for immnodiffusion for 48 hours. There was a precipitin line formed between the positive control serum and the antigen. Using the sera collected on 14th and 28th days also, no other changes were observed, but found identical to that obtained with '0' day serum (Fig.2).

4.1.7. Histopathology

The biopsy material collected from the site of bite on the skin of calves with primary infestation were subjected to histopathological examination. There was a low degree of diffuse infiltration with neutrophils and eosinophils. Focal degenerative changes were observed with progressive necrosis. Slight degenerative changes were seen at the hair follicles. Hyalinisation and slight fibrous tissue reaction were present in the dermis (Fig.6).

More pronounced reaction was observed in skin biopsy material in the second infestation. In this case, the prominent cells in the tissue reaction were lymphocytes and to a certain extent, the macrophages and eosinophils. The sections showed distinct perivascular lymphocytic infiltration. There was

Fig. 2

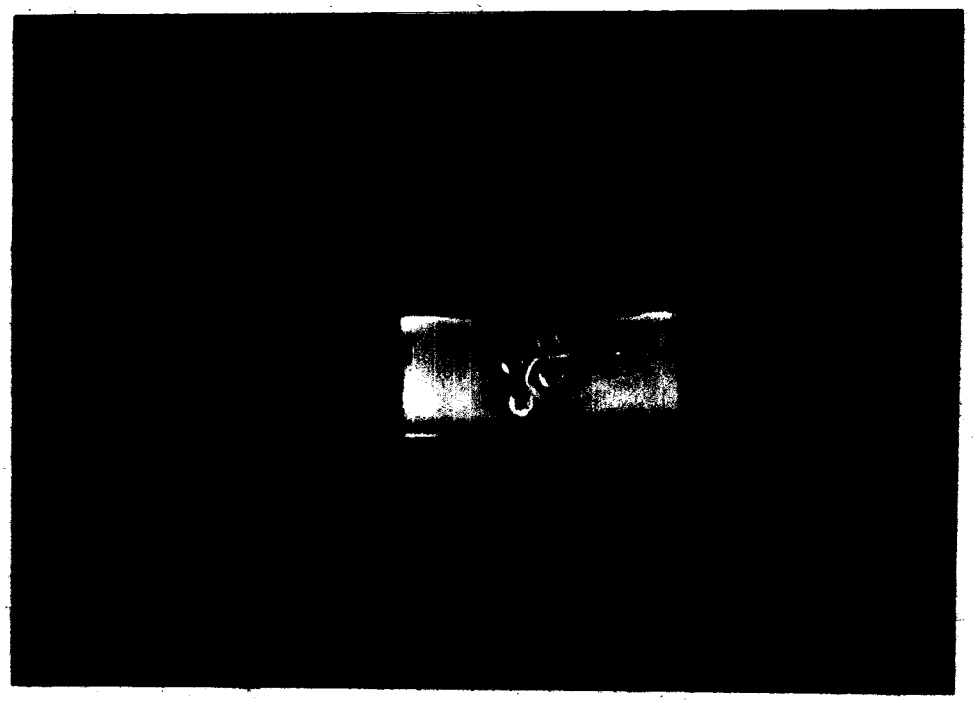


Fig. 3

Fig. 4

Fig. 5

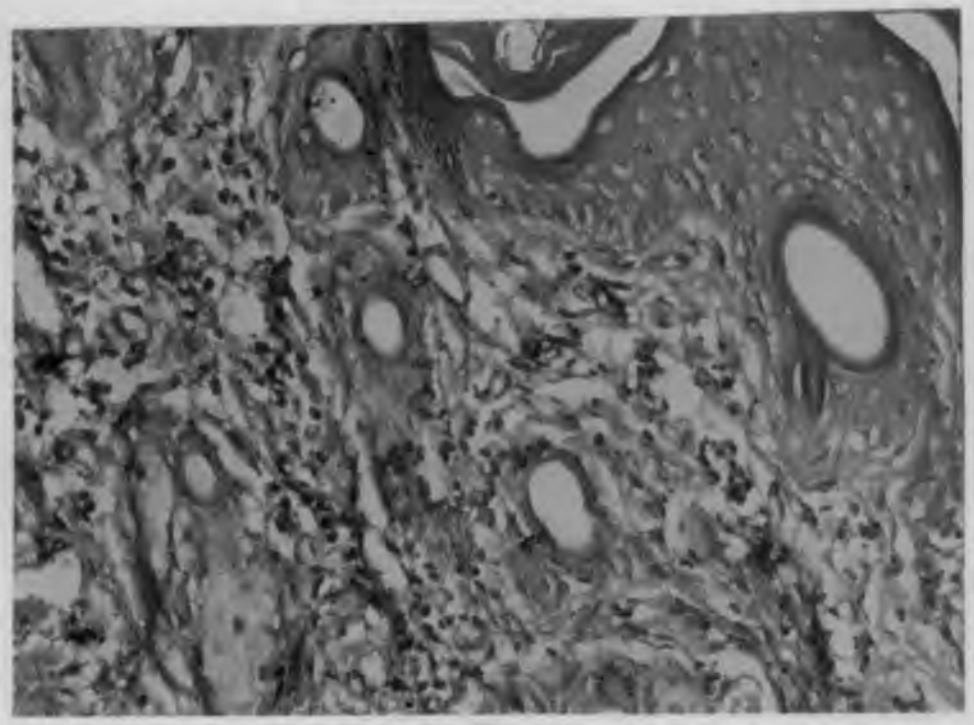


Fig. 6

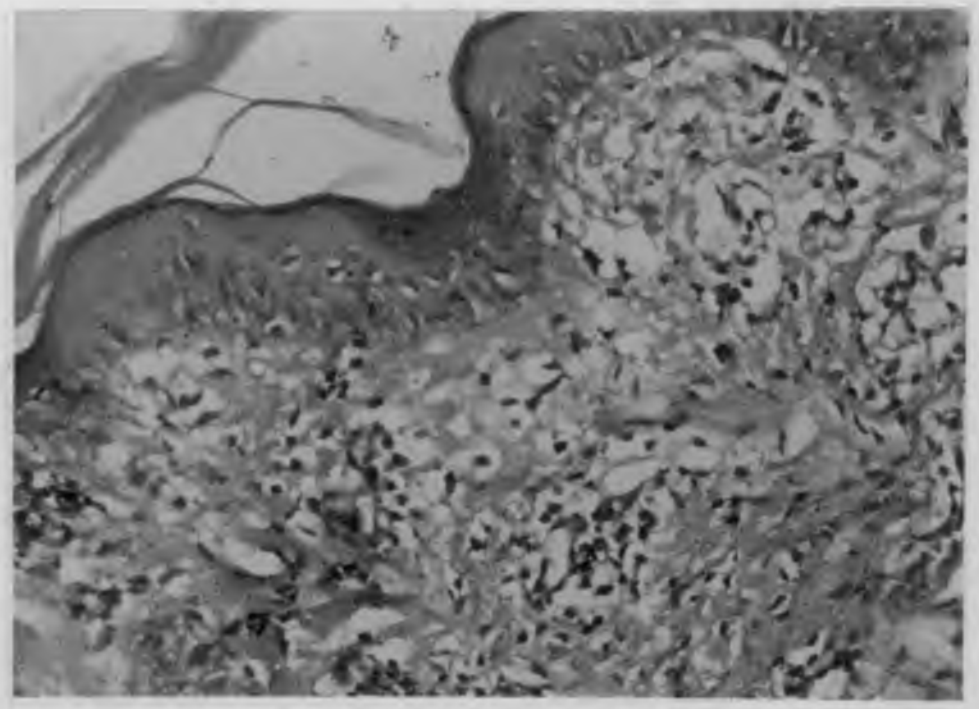


Fig. 7

Fig. 8

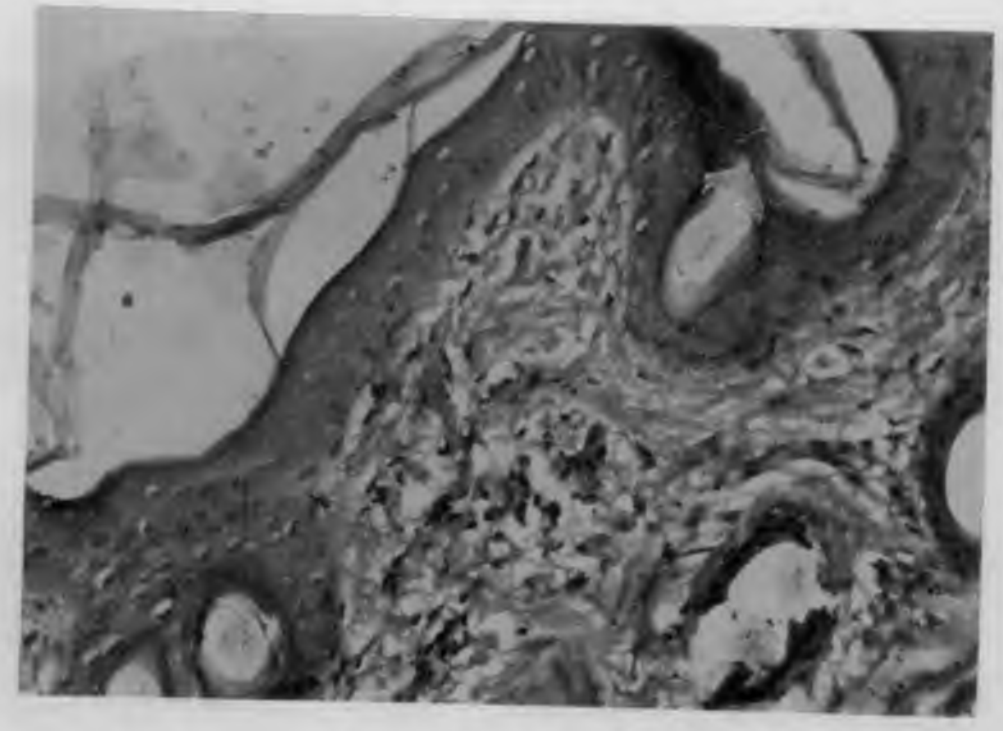


Fig. 9

nodular accumulation of lymphocytes and macrophages around the blood vessels. The epidermal layer showed slight hyperkeratosis. Acanthosis also was observed. There was also slight dermal fibrosis and moderate diffuse infiltration with eosinophils (Fig.7).

In third infestation, the skin biopsy revealed diffuse infiltration of lymphocytes and neutrophils with predominance of the former. A few eosinophils also were present. The dermis showed a moderate degree of fibrosis. Degenerative changes were observed in hair follicles. Slight hyperkeratosis was observed on certain areas of the epidermis. Focal areas of necrosis also were evident (Fig.8).

4.2. Effects of immunization

The effects of immunization of the calves with the tick antigen were studied with the help of the five parameters related to the biology of the tick, the antibody response and histopathology of skin as mentioned in 4.1.

4.2.1. Number of engorged female ticks

The number of engorged female ticks obtained from the control calf was 210. Of the experimental calves, G II 1 yielded

123 ticks, while G II 2 yielded only 37 ticks (Table IX). The number of engorged female ticks obtained from the immunized calves was lower than that obtained from the control calf.

4.2.2. Mean engorged weight of ticks

The mean engorged weight of ticks obtained from the experimental animal G II 1 was 143.4±7.85 mg and that obtained from G II 2 was 104.0±7.99 mg. The ticks yielded from the control calf gave a mean weight of 194.2±3.75 mg. This indicated an apparent difference between the engorged weight of ticks from immunized and control calves (Table IX).

Student's 't' test was used to test the significance of the differences between the immunized calves and control calf regarding the mean engorged weight of ticks. The mean engorged weight of ticks obtained from immunized calves was 123.70±6.61 mg while that of ticks obtained from control calf was 194.20±3.75 mg. The 't' value obtained was 7.23. This indicated that the mean engorged weight of ticks obtained from immunized calves and those from control calf differed significantly at 1 per cent level (Table X).

4.2.3. Mean egg mass weight

The mean weight of egg masses laid by ticks fed on GII 1 was 51.86 ± 4.95 mg. Those fed on GII 2 had a mean egg mass weight of 47.06 ± 4.04 mg as against 56.4 ± 2.31 mg recorded by ticks fed on the control calf (Table IX).

The mean egg mass weight was subjected to Student's 't' test to test the significance of the difference between the two groups. The mean egg mass weight of the ticks fed on immunized calves was 49.47 ± 3.17 mg and those of ticks fed on control calf was 56.4 ± 2.31 mg. The 't' value obtained was 1.45. This indicated that the mean weight of egg masses laid by ticks fed on immunized calves and those fed on control calf did not differ significantly (Table XI).

4.2.4. Mean egg number

The mean number of eggs laid by ticks fed on G II 1 was 1185 ± 105.48 and those fed on G II 2 was 909 ± 91.13. The ticks fed on control calf gave a mean egg number of 1705.13 ± 43.96 (Table IX).

Student's 't' test was employed to compare the mean egg number between the two groups, ie., 1047.16 ± 73.13 of immunized calves and 1705.13 ± 43.96 of control calf. The 't' value

obtained was 6.07, which indicated that at 1 per cent level, the mean egg number differed significantly between the ticks fed on immunized calves and those ticks fed on control (Table XII).

4.2.5. Mean percentage hatchability

The mean percentage hatchability of eggs of ticks from the immunized calf G II 1 was 51.46 ± 6.97 and those from G II 2 48.6 ± 5.42, while those fed on control calf gave a value of 90.4 ± 1.25. (Table IX). This obviously showed a marked difference between the two groups.

The Student's 't' test was employed to test whether the difference observed above between the immunized and control calves was significant. The mean hatchability percentage of eggs laid by ticks obtained from immunized calves was 50.03 ± 4.34 as against 90.4 ± 1.25 recorded by control calf. The 't' value obtained was 6.47, indicating that the mean percentage hatchability recorded for eggs laid by ticks fed on immunized calves was significantly lower than that of eggs from ticks fed on the non-immunized control (Table XIII).

4.2.6. Detection of antibody response

Sera of immunized calves gave strong precipitin lines against the tick antigen when immunodiffusion test was conducted.

Positive results were ontained using sera of '0' day, 14th day and 28th day post-infestation. The precipitin lines, in all three cases were identical (Fig. 3,4&5).

4.2.7. Histopathology

The sections of skin from the site of tick-bite on immunized animals showed scattered perivascular lymphocytic infiltration. Very few eosinophils were seen distributed in the dermis. A few macrophages were also present (Fig. 9). The cellular reaction was more pronounced than in the case of primary tick infestation, but less severe when compared to the second and third infestations.

Discussion

DISCUSSION

5.1. Effect of repeated infestation

The results show that there is significant immune response in cattle against tick infestation at primary, secondary and subsequent levels of infestations. But the degree of development of resistance differed in relation to the different parameters of performance of ticks.

The tick yields on repeated exposures were significant enough to prove that immunity is developed against ticks in repeated infestation. This was in confirmity with the results obtained by Trager (1939 a), Allen (1973) and Wikel and Allen (1976) in respect of Dermacentor andersoni - Guinea-pig system. Similar results were obtained by Amin-Babjee and Riek (1986) and Miranpuri (1989) with B. microplus - cattle system.

Barring very few reports, there had only been observations of a gradual reduction in the yield of engorged female ticks on repeated infestations in various tick-host systems. This indicate that the resistance is acquired slowly and repeated exposure to the tick antigen is necessary for consolidating the acquired resistance. In the present study also the reduction in tick yield was gradual. There was a reduction in the second infestation, when compared with the first, but not of a significant degree. The corresponding reduction in the third infestation was not

significantly different from that of the second, but significantly lower than that of the first infestation. These results are in confirmity with those of Miranpuri (1989).

5.1.2. Effect on the engorged weight of ticks

Reduction in engorged weight of ticks on repeated infestation was significant in the second exposure. Even though there is no significant reduction in the third infestation, the experiment proved that acquired resistance will be developed in repeat infestations and will be manifested by different phenomena. Riek (1962) could observe a similar phenomenon with B. microplus - cattle system. Similar results were also obtained by Doube and Kemp (1975), Fujisaki (1978), Latif (1984) and George et al., (1985).

Variation in the degree of immunity at different levels of exposure as observed in the present study was also reported by different workers. Brown et al., (1984) was of opinion that this phenomenon is due to faulty experimental design and an unpredictable tick-behaviour. But in the opinion of Wikel and Whelen (1986) there is a possible tick-induced host immunosuppression. But Samantaray et al., (1988) observed that a waning of the resistance was evident in the host confined to a tick-free atmosphere based on the study on Rhipicephalus haemaphysaloides - rabbit system.

5.1.3. Effect on egg mass weight

In the present study no significant difference could be obtained as far as the egg mass weight from ticks on repeat exposure was considered. But several workers, Riek (1962), Hewetson (1971), George et al., (1985) and Alani and Herbert (1987) could obtain a result showing a positive correlation between the host resistance and egg output by ticks. However, Brown et al., (1984) and Amin-Babjee and Riek (1986) could not observe this phenomenon, which is in confirmity with the present study.

5.1.4. Effect on the egg number

A variation in the egg number after repeat infestations was reported by Riek (1962), Alani and Herbert (1987), and Samantaray et al., (1988). But in the present study no significant variation could be obtained in this line. Brown et al., (1984) and Amin-Babjee and Riek (1986) are also of opinion that the egg number need not be influenced by acquired resistance of the host, as observed in the present study.

5.1.5. Effect on hatchability

The relation between hatchability of tick eggs and acquired host resistance is a factor not of any significant relevance as observed by Fujisaki (1978) and Amin-Babjee and Riek (1986).

Even though Alani and Herbert (1987) observed a reduction in hatchability, no significant difference could be observed in the present study in this line.

5.2. Antibody response

It appears that there is no previous report on the use of immunodiffusion test to detect antibody response of hosts in natural infestations with ticks. This may probably be due to the fact that the natural tick infestations can induce only a very low level of antibodies. In the present study no significant level of antibodies could be observed against natural tick infestations. However, Brossard (1976) could find an increase in serum gamma globulin fractions in cattle following repeated infestations with B. microplus. Since this is a non-specific observation, no attempt was made in the present study in this line.

5.3. Host tissue reaction to tick-bite

The present observations reveal that there is a definite inflammatory reaction at the site of attachment, which is progressive from an acute neutrophilic reaction to a chronic lymphocytic one with repeated infestations. The significant reduction in neutrophilic infiltration progressive with repeat infestations is suggestive of a transformation to chronic tissue reaction.

Brown et al., (1984) were of opinion that in B. microplus infestation an eosinophil-mediated cutaneous resistance is produced whereas in Amblyomma infestations a basophil-mediated resistance is produced. They attributed this difference to species-specific salivary substances, breed differences and to difference in staining procedures. Mbemba (1983), Gill and Walker (1985), Gill (1986) and Abdul-Amir and Gray (1987) also obtained similar results indicating to a predominance of mononuclear cells in the latter stages. However, the present study seems to be the only one with regard to the tissue reaction at the latter phase of tick-attachment when they have produced a possibly significant reaction.

5.4. Artificial Immunization

In the present study artificial immunization against Boophilus annulatus in cattle was tried using a crude antigen obtained from partially engorged female ticks. No published report is available on the use of B. annulatus antigen in such a study. The results obtained clearly indicate that immunity could be induced in the hosts against the ticks using the tick tissue as antigen. The different parameters used to measure the development of immunity were number of engorged female ticks, engorged weight, egg mass weight, egg number and percentage hatchability, tissue reaction at the site of bite and the concentration of antibodies as evidenced by immunodiffusion test.

From the observations made, excepting egg mass weight, all other parameters exhibited a significant result. Among the positive results obtained, the immunodiffusion test was the most significant one suggesting that the artificial immunization using the tick antigen can easily provoke the production of specific immunoglobulins. The artificially immunized calves gave a picture different from that of the naturally sensitized hosts.

The whole-tick extract is an amalgam of a wide variety of antigens, each of which may evoke a different type of antibody response in the host. When trying a satisfactory immunization process, it is always preferable to use a specific antigen which possesses the maximum antigenicity. Efforts are on to find such an antigen in tick-host systems, since a long time. Salivary gland antigens were thought to be the best source till 1979 when Allen and Humphreys proposed that the internal organs of Dermacentor andersoni ticks, preferably midgut and reproductive organs are more immunogenic than the other organs. Ackerman et al., (1980) also opined that midgut extracts were more immunogenic when compared to whole-tick extracts of D. variabilis. The fact that the tick-mouth part which always comes into contact with the skin while it feeds may not be antigenic, was pointed out by Willadsen (1987), while reviewing the subject. He proposed that a 'concealed' antigen only would be the choice for effecting immunization. Identification of the right antigen is necessary for producing an immunizing agent. From the present

study it is inferred that an effective immunity could be induced using tick antigens. The use of more specific antigens like the salivary gland antigens and gut or reproductive organs would be appropriate especially the gut antigen. The gut tissue is more in terms of quantity and can be obtained more easily than the salivary gland antigen. More investigations need to be carried out in this direction so as to produce a vaccine which can evoke protective immunity in cattle against B. annulatus. The possibility of evolving such a control measure certainly looks bright.

Summary

SUMMARY

A study was conducted to assess the development of immunity against infestation with the tick, Boophilus annulatus in cattle. An attempt was also made to assess the immunogenicity of the whole-tick extract derived from partially engorged adult female B. annulatus ticks with a view to evolve a possible control measure.

Five cross-bred female calves of two months of age which were not exposed to ticks previously were used for the trials with repeated infestations and were designated as Group I. Two cross-bred male calves of 7 months of age were used as experimental group for immunization trial and designated as Group II. One cross-bred female calf of 4 months of age formed the control (C) for the immunization trial. All the animals were infested with around 3600 larvae. In the group I, three repeat infestations were given, the second and third infestations after a lapse of three weeks of becoming tick-free. The group II received the larvae two weeks after the first inoculation with the tick-extract @ 0.5 mg per kg body weight, along with Freund's Complete Adjuvant (FCA). The control received only FCA. A booster dose was given to group II at the same dose rate without FCA on the 7th day following first inoculation.

Five parameters with respect to the biology of the ticks were considered, namely, the number of engorged female ticks, the

Artificial immunization using tick extracts resulted in the reduction of engorged tick yield, engorged weight, egg mass weight, egg number and percentage hatchability of eggs of ticks fed on immunized animals when compared to the control. However, reduction in egg mass weight was found to be non-significant. Immunodiffusion revealed indentical reaction between the antigen and the sera of immunized calves at all stages. The reaction was identical on 14th day and 42nd day following inoculation. This suggested a steady maintenance of antibody level throughout the infestation period. Immunization however, did not result in a marked tissue reaction as in repeated infestations in immunized animals. Lymphocytes were the predominant cells with less of macrophages and very few eosinophils.

The results of the present study indicate that a slight to moderate degree of resistance was developed in calves against infestation with B. annulatus following repeated infestation, as well as inoculation of tick extract. This resistance was partially protective in nature. The possibility of immunizing cattle against B. annulatus infestation appeared bright in the light of the results obtained.

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IMMUNE RESPONSE OF CATTLE TO BOOPHILUS ANNULATUS (ACARI : IXODOIDEA)

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was undertaken to assess the nature and degree of development of acquired resistance in calves against the cattle tick Boophilus annulatus. Observations were made mainly on the development of natural resistance against repeat infestations and the effect of artificial immunization using the tick antigen. Tissue reactions induced by the ticks at the site of bite were also studied. The results indicated the development of resistance in the host, which was gradual and varying in degrees. Immunodiffusion test failed to reveal the presence of tick antibodies in the sera of calves after repeated infestations, indicating that easily demonstrable quantities of antibodies are not developed even after repeated infestations. The cellular reactions at the site of tick-bite revealed the development of a resistant reaction at the site of bite. Studies made on artificial immunization using the whole-tick extract gave varying results. However, the immunodiffusion test using sera of immunized calves revealed that there is a steady maintenance of antibody level throughout the infestation period.

Thus, the results obtained in the present study indicate that antibodies are developed against the tick Boophilus annulatus in calves on natural infestations and using the tick antigen, and that there is a clear possibility of immunizing calves against B. annulatus artificially.