ADAPTATION OF FIELD STRAINS OF *RHIPICEPHALUS APPENDICULATUS*, NEUMANN TO HOST RESISTANCE TO TICK INFESTATION

By

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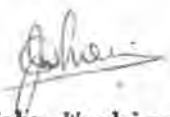
A thesis submitted in fulfilment for the Degree of Master of Science in the University of Nairobi

1986

Declarations

This thesis is my original work and has not been presented for a degree in any other University

Signed: 

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Acknowledgements

I wish to first of all thank Professor T.R. Odhiambo on behalf of ICIPE for allowing me and making it possible for me to carry out this work at ICIPE. I wish also to express my thanks to the former Livestock Ticks Research Programme Leader, the late Dr. M.P. Cunningham, and the current Programme Leader, Dr. P.B. Capstick, for their support and for the useful comments they made throughout the study. Hearty thanks go to my two supervisors, Dr. G.R. Karuhize and Dr. R.M. Newson for their guidance during the study.

I wish also to thank Mr. D.K. Punyua and Dr. J.J. de Castro for their comments during the course of study. Many thanks also go to Messrs.J. Mugane, J. Ndungu, F. Thuo, M. Kimondo, J. Mbuthi and M. Gitau for their technical assistance. Last, but not least, my thanks go to Miss Veronicah Nderito for kindly typing the thesis.

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Summary

The possibility of adaptation to host resistance by field strains of R. appendiculatus was investigated by comparing the feeding and breeding performance of two field strains with a laboratory strain (Muguga) which has been bred and maintained for about 30 years on susceptible rabbits. Results have shown that the laboratory strain has smaller eggs and smaller unfed larvae, nymphs and adults than the field strains. When fed on susceptible rabbits the laboratory strain females laid eggs with a mean weight of 41 ± 1 µg while those of the field strains were 47 ± 1 µg and 46 ± µg respectively. The sizes of unfed larvae, nymphs and adults showed similar differences.

Eggs and larvae of laboratory and field strains from females fed on cattle and rabbits were also compared. In both laboratory and field strains, eggs and larvae from ticks fed on cattle hosts were larger than those from rabbits. Since cattle are the main hosts of R. appendiculatus, it is possible that the use of rabbit hosts has exerted selection pressure for smaller size on the laboratory strain of ticks.

When fed successively on the same hosts, field strain larvae and nymphs remained significantly larger than those of the laboratory strain. Laboratory and field strain females fed to similar engorged weights on susceptible rabbits, but during the

2nd and 3rd infestations on the same hosts, the field strains yielded females twice as heavy as the laboratory strain females. The proportion of ticks surviving the feed decreased with subsequent infestations for both the laboratory and field strains. But although there was no significant difference in the proportions of nymphs and adults, the proportion of laboratory strain larvae that fed successfully decreased to a significantly lower level over the 4 feeds than that of the field strains. When proportions of larvae, nymphs and adults were combined, it was observed that a slightly higher proportion of the laboratory strain fed on susceptible rabbits than the field strains. But on previously exposed rabbits the situation was reversed. A comparison of reproduction efficiency showed that the laboratory strain females reproduced better on susceptible hosts, while the field strains reproduced significantly better on previously exposed hosts.

When hosts previously exposed to ticks were challenged with laboratory and field strains, it was observed that cross-protection was low. Field strains, in particular, fed significantly better on hosts previously exposed to the laboratory strain. Cross-protection between the field strains, however, was found to be high. Observations made on cattle hosts showed that similar results to those reported above for rabbits could be expected on cattle.

These results indicate that the laboratory strain has a higher reproductive ability on susceptible hosts than the field strains. The field strains, on the other hand, have a higher reproductive ability on previously exposed hosts. This reflects adaptation to the host environment that the strains have been exposed to. Caution is therefore needed when interpreting results on host resistance against ticks obtained with ticks bred in captivity for a long time. The absence of high cross-protection is another aspect to consider in tick control by host resistance.

CHAPTER I

INTRODUCTION

1.1. Economic importance of Rhipicephalus appendiculatus

The ixodid tick Rhipicephalus appendiculatus, Neumann 1901 is commonly known as the African brown ear tick because the adult ticks feed mainly on the ears of cattle. It is a 3-host tick and all instars do feed on cattle. Though it is essentially a cattle tick, R. appendiculatus is also commonly found feeding on sheep, goats and on many wild bovids especially the African buffalo, Syncerus caffer, and the eland, Taurotragus oryx (Yeoman and Walker, 1967). Rhipicephalus appendiculatus is the vector of the haemoprotozoan Theileria parva parva, Theiler which causes the deadly East Coast fever (ECF) and Theileria parva lawrencei which causes corridor disease in cattle. Mortality in cattle infected with T. parva parva can be up to 100% under laboratory conditions and more than 70% in endemic areas. Furthermore a single tick with only one salivary gland acinus infected is capable of causing the death of a cow (Lewis, 1950; Young, 1981). It is therefore understandable that ECF is the most dreaded cattle disease among East African farmers. The fact that an ECF infection can be caused by a single infected tick means that tick control ought to be 100% effective in order to prevent ECF (Cunningham, 1981).

Rhipicephalus appendiculatus also transmits Theileria taurotragi which may also be involved in cattle theileriosis. It also transmits the virus of Nairobi sheep disease and the rickettsia causing tick-bite fever in man. When present in large numbers, R. appendiculatus can cause tick toxicosis and may also predispose cattle to bacterial infections through the feeding lesions. Cattle that contract ECF and recover become immune to the disease, but may also act as an ECF reservoir through a carrier state (Young et al., 1981). Wildlife may also serve as a disease reservoir, as is the case with corridor disease which is primarily a buffalo theileriosis (Irvin et al., 1981)

Rhipicephalus appendiculatus is widely distributed in East, Central and Southern Africa (Hoogstraal, 1956). In Kenya, it is mostly found in the south-western corner, but occurs in all the provinces except the dry North Eastern Province. It is found all the way from sea level to altitudes of over 2,000m wherever there are suitable habitats and a rainfall of over 500 millimetres (Walker, 1974). The distribution of ECF and corridor diseases closely follow that of the vector tick, and therefore cover the whole of the high-producing cattle areas of Kenya.

1.2. Control of R. appendiculatus

In view of the economic importance R. appendiculatus highlighted above, it is necessary to control it. Control of R. appendiculatus and all other economically important tick species

has long been carried out by use of chemical acaricides. This involves application at close, regular, intervals throughout the year and results in high running costs and the development of acaricide resistance in ticks (Cunningham, 1981; Keating, 1983). Chemical acaricides are also toxic to livestock and man, and some such as DDT, are known to accumulate in vertebrate muscles, thereby affecting the quality of meat and milk. The situation makes it imperative to search for alternative control measures in order to reduce the intensity of acaricide usage.

Alternatives that have been considered to the use of acaricides include pasture spelling, sterile-male technique, natural tick parasites and predators, and the use of host resistance to tick infestation. Pasture spelling has been shown to have some success in the control of Boophilus microplus and Boophilus annulatus (Cunningham, 1981). Boophilus species are one-host ticks so that unfed nymphs and adults do not leave the host. The larvae are the only free-living instar on the pasture and their survival is usually less than five months (Wilkinson, 1964). Pasture spelling has therefore been recommended for use in integrated management of B. microplus in Australia (Sutherst et al., 1979). This method would not, however, be practicable in the case of a 3-host tick such as R. appendiculatus whose adults can survive for up to two years (Young et al., 1983; Newson et al., 1984; Chiera and Punyua, in preparation). The sterile-male technique and the use of parasites and predators have not been shown to be capable of controlling ticks.

Host resistance prevents ticks from feeding adequately due to factors arising from immunologically induced changes in the host animal (Wakelin, 1978). It is a defence mechanism of the host against parasitic attack. In Australia, the control of B. microplus now depends largely on the use of cattle with improved ability to develop resistance, in an integrated method of control. Research into methods of artificially immunizing animals against ticks is also being carried out (Allen and Humphries 1979; Mongi, 1980; Johnston et al., 1986). It is hoped that an immunizing agent or agents will eventually be found to protect livestock against ticks and thus also tick-borne diseases. However, such an immunizing agent would have to transcend variation between tick strains to be effective.

1.3. Objective of this study

Most of the information available concerning host resistance against R. appendiculatus has been obtained using a laboratory strain which has been bred and maintained on susceptible rabbits for about 30 years. There is good evidence from such information that R. appendiculatus cannot maintain itself if fed entirely on highly resistant hosts (Chiera et al., 1985b; Newson et al., unpublished). This suggested that the laboratory strain of R. appendiculatus is not behaving as might be expected under natural conditions, since it is an obvious fact that ticks are still abundant on undipped livestock and also on wildlife, despite the fact that host resistance might be expected to be present in most

of the hosts under field challenge. It was against this background therefore, that it was deemed necessary to compare the feeding and breeding performance of laboratory and field strains of R. appendiculatus with respect to host resistance.

This study has therefore examined the feeding and breeding performance of laboratory and field strains on both susceptible and previously exposed hosts. The hypothesis being tested was that the field strains having been exposed mainly to resistant hosts (hosts previously exposed to ticks) are better adapted to host resistance than the laboratory strain. That is, field strains would have a higher reproductive potential on resistant hosts than the laboratory strain. The laboratory strain, on the other hand, having been exposed only to susceptible hosts for a long time would be expected to be well adapted to susceptible hosts. In addition, the comparison between the laboratory and field strains would indicate how much reliance can be placed on data obtained with the laboratory strain. At the same time information would be obtained as to whether strain differences would present serious difficulties in the search for an immunizing agent.

CHAPTER 2

LITERATURE REVIEW

Host resistance or immunity to parasites is the capacity of the host to protect itself against parasites (Gaafar, 1972). This ability of the host to defend itself has been known since the turn of the century. Minchella (1985) has referred to it as the 'ultimate strategy in the continuum of host responses against parasitic attack'. Host resistance is difficult to define in precise terms. It may be considered to be absolute in cases where the parasites are eliminated, or relative in cases where only the reproductive potential of the parasites is reduced (Balashov, 1972; Hildemann, 1973; Wakelin, 1978).

Blood-sucking arthropod ectoparasites fall into two broad categories, depending on the speed at which they feed before leaving the host (Chandler and Read, 1961; Tatchell, 1969). The first group includes those that feed quicky and leave the host immediately. The second group includes those that feed much more slowly and spend several days on the host before leaving it. As a general rule, argasid ticks belong to the first group and ixodid ticks to the second group. Ixodids therefore risk sensitising the hosts immune system, and thereby being prevented from completing their feed. Furthermore, Brown (1985) suggests that even the feeding, fertility and survival of certain fast feeding insects

could be interfered with by systemically occurring factors, caused by previous feeding.

When ticks feed on a tick-naive host they are nearly all capable of engorging and attaining maximum size. However, a host with a history of previous infestations may mount an immunological response against the ticks which can cause total rejection of some ticks and retard the feeding of others. Retarded feeding results in reduced egg production in female ticks (Trager, 1939; Riek, 1962; Balashov, 1972; Allen, 1973; Wakelin, 1978; Randolph, 1979; Chiera et al., 1985a).

The question, therefore, is whether the parasites survive on resistant hosts because the host responses are weak or whether parasites successfully withstand host responses. It is known, for instance, that survival of the parasites improves during lactation of the host, and that unresponsive, but otherwise normal, members of the host population may also play an important role in the survival of the parasite population (Wakelin, 1976). In this connection Gladney et al. (1973) reported more ticks on steers under field challenge that were losing weight than on those that were gaining weight. Survival of the parasites may also vary on different sites of the same host (Trager, 1939; Wakelin, 1984; Mackenzie, 1984), between sexes (Wharton et al., 1970), or even on the same host but at different times (Riek, 1962).
Immunosuppression of host resistance induced by artificial antigens has been reported (Wikel and Allen, 1980), but this condition may

vary with the antigens used, animals and sequence of immunization (Pross and Eidinger, 1974). It has also been suggested that variability in host resistance can be caused by such factors as prior experience, behavioural factors and genetic factors (McCallum and Anderson, 1984).

Other specific mechanisms for avoiding host responses are known or have been suggested, particularly for endoparasites (Dineen, 1963; Vickerman, 1974; Wakelin, 1976; Minchella, 1985). Though the exact way they do it is not known, schistosomes are the only group of parasites known to acquire or copy non-antigenic host proteins in order not to provoke a host response. Trypanosomes, on the other hand, develop fresh surface coats of glycoproteins as soon as the host develops antibodies to the previous coat. It is also known that low numbers of parasites over long periods of time may provide insufficient stimulus to provoke a protective response in the host. The helminth worm Nippostrongylus brasiliensis adapts itself in the host using such means (Ogilvie, 1974; Wakelin, 1976). It is not yet known how acetyl- cholinesterase isoenzymes are involved, but adapted worms show isoenzyme patterns not present in non-adapted worms.

In the case of host resistance to tick infestation, there are no known instances of adaptation by the parasite. No evidence of adaptation to host resistance was found in the cattle tick B. microplus (Wilkinson, 1962; Stewart et al., 1982) However, Tatchell (1969) contends that a parasite must become adapted to

host resistance if it is to survive. It must be realized that the phenomenon of host resistance is mutually beneficial to both the parasite and the host. It would not be in the best interests of the parasite if the host succumbed to parasitic attack. In the absence of host resistance, tick numbers would increase to such high levels that they could kill the host, thereby endangering their own survival. The best association is not necessarily the one in which the parasite does least damage to the host (Anderson and May, 1982). Thus host resistance helps to keep the parasite population at a level that the host can sustain (Dineen, 1963; Tatchell, 1969; Balashov, 1972; Anderson and May, 1978; Terry, 1984). The ability to develop high levels of host resistance may also be associated with undesirable characters, so that the most highly resistant host is not necessarily the best adapted one (Minchella and LoVerde, 1983). Since the ability to develop host resistance is heritable (Wharton et al., 1970; Minchella, 1985), the development of the host-parasite association towards homeostasis is a likely result.

Ixodid tick feeding and repletion take time to complete. R. appendiculatus larvae take at least three days attached to their hosts, nymphs take at least four days while adult females take more than a week to engorge and drop off the host. It is not known, however, how long the host takes to develop resistance, though it is likely to be shorter than the time required for each instar to complete feeding (Tatchell and Moorhouse, 1968; Wagland, 1978). Thus, for instance, Boese (1974) showed that ticks applied seven

days after a primary infestation were affected by host resistance. Moreover, Gillett (1967) has suggested that individual parasites which are fast feeders or those capable of delaying the onset of host reaction are likely to produce more progeny.

The types of reactions occurring on the skin of host animals after repeated infestations with R. appendiculatus are given by Branagan (1974), who also suggested that the speed of engorgement could be influenced by systemic factors. One category of host response known as immediate hypersensitivity involves the release of histamine, and more is released in highly resistant animals than in others (Riek, 1962; Balashov, 1972). This increased histamine level, however, may aid the feeding of ticks (Tatchell and Moorhouse, 1968). Mast cells and basophils have also been implicated in tick resistance by the host (Allen, 1973; Matsuda et al., 1985). Host resistance prevents ticks from feeding on the host, while the environment of the host skin kills them (Roberts, 1971). Most larval mortality on resistant hosts occurs in the first 24 hours and is caused by dehydration of the larvae which are prevented from attaching and starting to feed. But it is not known whether other factors, particularly from the blood, are involved in larval mortality.

The feeding and breeding performance of laboratory and field strains of ticks on susceptible and resistant hosts have been studied elsewhere. Stewart et al. (1982) compared a laboratory strain of B. microplus maintained in captivity for many years with

a recently isolated field composite strain on susceptible and resistant hosts. They found differences in weight of engorged ticks, weight of eggs produced and hatchability of the eggs. Hunt and Drummond (1983) also carried out a similar comparison with strains of the lone star tick, Amblyomma americanum, on susceptible hosts. They compared a strain maintained in the laboratory for 15 years with a field strain from which it was originally isolated. They, too, found differences in the duration of engorgement, pre-oviposition and oviposition periods, proportion of the female weight converted into eggs and in the hatchability of the eggs. Similar differences are likely to be found in R. appendiculatus.

CHAPTER 3

MATERIALS AND METHODS

3.1. Ticks

3.1.1. Source of ticks

Laboratory strain (LS): This is the strain of R. appendiculatus maintained at the Kenya Agricultural Research Institute, Muguga. This strain originated from the field but has since been bred and maintained on susceptible rabbits for over 30 years. At the beginning of the current experiments, ten male and ten female ticks were picked at random from the R. appendiculatus culture and fed on a susceptible rabbit. The eggs of the engorged females were then mixed and left to hatch. A line of the laboratory strain was thus established for use in these experiments.

Field strain (FS1): This strain came from Narok District in Kenya. Thirty-one engorged females of R. appendiculatus were picked off cattle, brought to the laboratory and maintained under similar conditions to the laboratory strain to lay eggs. The eggs were then mixed and left to hatch under the same conditions. These ticks together with their progeny were used for the experiments. In Narok where the females were collected, cattle are grazed communally over a large area and mingle with other domestic animals as well as wildlife. Assuming that cattle pick up ticks at random, these ticks were representative of the true tick population.

Before the females collected from the field were allowed to lay eggs, their scutal lengths were measured for future reference.

Field strain (FS2): This strain was collected from the Nanyuki area of Kenya. Thirty-three engorged females of R. appendiculatus were collected off cattle on a ranch. In this area cattle and wildlife serve as hosts for the ticks. Scutal lengths of these females were taken before they laid eggs.

Field strain (FS3): This strain was collected from Narok District, about 100 km from where FS1 strain was collected. This strain was not used for comparisons with the laboratory strain, since initial observations showed that it was similar to FS1 strain.

3.1.2. Maintenance and handling of the ticks

Engorged females for egg production were placed in a desiccator over saturated potassium chloride solution which gives a relative humidity (r.h.) of 85% (Winston and Bates, 1960). The desiccator was kept in an incubator at 28°C. When the weight of the eggs laid was required, the females were inspected every day until oviposition started. The eggs were then removed and weighed on the tenth day after the start of oviposition on a Sartorius balance. Any eggs laid thereafter were weighed on the 18th day. Most of the females finished laying by the 10th day and no laying was observed after 18 days. After weighing the eggs, small aliquots from each batch were taken and mixed within strains. All

the eggs were then kept to hatch at 85% r.h. and 28°C.

Unfed larvae, nymphs and adults were kept in a room with temperature varying between 17-23°C, and relative humidity maintained above 80% by a humidifier (Defensor Model 505). Some unfed ticks were also kept in Kilner jars over saturated potassium sulphate solution (relative humidity about 96%) in the tick room. Engorged larvae and nymphs were kept in a desiccator at 85% r.h. and 28°C to moult. After moulting they were transferred to the tick room. All larvae and nymphs were used within three months of hatching or moulting. Adults were used within about four months of moulting.

Larvae were counted by use of either of two procedures. A vial containing active larvae was placed on a bottle cap surrounded by water in a petri dish. The water prevented larvae from straying. Small groups of larvae were then allowed to climb onto strips of transparent paper on which they were counted and transferred into a small vial partially immersed in ice. The larvae immediately became immobilized by cold and were kept there for less than five minutes while the counting was going on. Test larvae left immobilized in this way for about two hours showed no ill effects later. Alternatively, small groups of larvae were picked off the vial by means of a strip of paper and placed on a white bench. The larvae on the bench were then picked up by a vacuum pump through a Pasteur pipette and counted into a small tube plugged with cotton wool at the far end. The small tube containing

counted larvae was then removed and sealed with cotton wool. These procedures allowed larvae to be counted with a high degree of accuracy. Nymphs and adults were counted by picking them up using a light pair of forceps and placing them in a vial.

Length measurements were made with the use of a camera lucida attachment on a Wild M-5 dissecting microscope. The image of the measuring scale was superimposed on the tick, and measurements could be made to an accuracy of 0.02 mm or 0.04 mm depending on the magnification. Engorged larvae and engorged nymphs were weighed as a group after collecting them daily. Engorged females were weighed individually.

3.2. Hosts

New Zealand white rabbits and Friesian (Bos taurus) cattle were used for the experiments. The rabbits were obtained from two sources but for any single experiment, all the rabbits were from the same source, of the same age and were picked at random when allocating them to experimental groups. All rabbits were assumed to be fully susceptible to ticks, since previous contact with ticks could be ruled out. The rabbits were maintained on commercial pellets and water, to which the coccidiostat Furazone was added.

Cattle were reared in stalls from birth and varied in age between one year and one and a half years when they were used for these experiments. They were fed on commercial concentrates, hay

and water. Just before they were infested with ticks, they were subjected to a skin test (for a separate experiment) by injection of larval homogenate prepared from the laboratory strain.

Comparisons of feeding performance were carried out on batches of five rabbits or three cattle for each experimental group. The ticks were fed on rabbits and cattle ears using the methods described by Bailey (1960) and Irvin et al. (1973), restrained in cloth sleeves secured with the adhesive tape Leukoplast.

3.3. Assessment of feeding performance of freshly obtained field strain larvae

The ability of freshly acquired FS larvae to feed on susceptible rabbits and on rabbits previously exposed to the LS larvae was studied in comparison with the LS larvae. Previously exposed rabbits had each fed larvae from two egg batches (approximately 10,000 larvae).

One hundred LS larvae were applied on one ear of each of previously exposed and on susceptible rabbits. A similar number of the FS larvae were then applied on the other ear. The mean weights of the engorged larvae and the percentage engorging were recorded.

3.4. Assessment of reproduction efficiency of engorged females

Unfed adults were then fed on susceptible rabbits, previously exposed rabbits, susceptible cattle and previously exposed cattle in order to assess reproduction efficiency. Ten males and 10 females were applied on each ear of previously exposed rabbits, 20 males and 20 females on each ear of susceptible rabbits and 25 males and 25 females on each ear of a cow. Engorged females were kept at 85% r.h. and 28°C to lay eggs. The eggs from each female were then weighed.

Ten small samples of eggs from females of each strain fed on susceptible rabbits and on susceptible cattle were weighed and eggs counted to determine mean egg weights. The lengths of larvae from the same egg masses were also taken for comparison.

3.5. Assessment of effect of successive infestations of the host on tick feeding

The following procedure was used to study the effect of successive infestations of the same host with larvae, nymphs or adults.

3.5.1. Successive infestations with larvae

Two hundred and fifty LS larvae were applied to feed on each

ear of five susceptible rabbits. This procedure was repeated concurrently with each of the field strains on another five susceptible rabbits. The engorged larvae started to drop on the third day and they were collected, counted and weighed. This was done every morning until feeding was finished, usually on the fifth day.

A new infestation similar to the first one was applied on the same rabbit at the end of each week. Four such successive infestations were carried out using the same strain. A comparison was then made between the LS and FS on the basis of the weight of the engorged larvae and the percentage engorging.

The rabbits were left free of ticks for about one week and then challenged with larvae and nymphs of LS and FS. The challenge consisted of 100 larvae and 50 nymphs of the LS applied to one ear of each rabbit, and 100 larvae and 50 nymphs of the FS applied on the other ear. Mean engorged weights and percentages feeding were compared.

The engorged nymphs were left to moult at 85% and 28^{o}C and scutal lengths of the adults measured. This allowed a second comparison to be made on the size of the ticks. Our previous work (Chiera and Newson, unpublished) had shown that pre-male and pre-female nymphs differ in size and that the effect of host resistance on their size is dissimilar. For these reasons, comparison of the strains using unfed adult scutal lengths instead

of weights of engorged nymphs from which they moulted, was preferred.

Comparisons of the LS ticks and the ticks of each of the FS were done at different times and with different batches of ticks.

3.5.2. Successive infestations with nymphs

Twenty five LS nymphs were applied on each ear of five susceptible rabbits. This was repeated concurrently with each of the FS on another five susceptible rabbits. The rabbits were checked for engorged nymphs on the third day and thereafter once every day. The engorged nymphs were counted and weighed. A similar infestation was applied on the same rabbits one day after the last engorged nymph had dropped off. Four such successive infestations were carried out on the same rabbits using the same strain.

The rabbits were then left free of ticks for at least one week. A challenge infestation like the one described above for larvae was then carried out. The scutal lengths of adults resulting from successive infestations and the challenge infestation were also taken.

3.5.3. Successive infestations with adults

Two males and two females of the LS were applied on each ear

of five susceptible rabbits. This was repeated concurrently with each of the FS on another five susceptible rabbits. Checking for engorged females started on day five and each engorged female that dropped was listed and weighed. This continued until all the females had dropped, after which the males were removed from the hosts. One day after the last female had dropped, a similar new infestation was applied on the same rabbits. Three such successive infestations were applied. The engorged females were kept to lay eggs and the eggs were weighed.

The rabbits were then left free of ticks for at least one week. A challenge infestation was then applied. This consisted of 100 larvae, 50 nymphs and 10 adults (5 males plus 5 females) of the LS on one ear and similar numbers of the FS ticks on the other ear. Scutal lengths of the adults moulting from engorged nymphs were taken.

Successive infestations of the LS and FS2 adult ticks were also carried out on 6 Friesian cattle (B. taurus). Fifty LS adult ticks (male:female = 25:25) were applied on each ear of 3 cattle picked at random. This was repeated concurrently with FS2 ticks on the other 3 cattle. A similar infestation was applied on the same cattle two weeks after the first infestation. The third and final infestation included ticks of the LS and FS2 on the same hosts, thereby serving as a challenge infestation as well. At each infestation a new set of two susceptible rabbits were infested with adult ticks of both strains. Four previously exposed rabbits were

also included in the final infestation in order to provide a direct comparison between cattle and rabbits.

For the challenge infestation, 200 larvae, 50 nymphs and 50 adults of the LS were applied on one ear of each cow, previously exposed rabbits and susceptible rabbits. Similar numbers of FS2 ticks were applied on the other ear of each of the above hosts.

3.6. Assessment of cross-protection between field strains

Twenty-five FS1 nymphs were applied on each ear of two susceptible rabbits. This was repeated concurrently with FS2 nymphs using another two susceptible rabbits. Engorged nymphs were counted and weighed. A second similar infestation was applied on the same rabbits after the second infestation. The rabbits were then left free of ticks for at least one week, then challenged with larvae and nymphs of both strains. Cross protection was then assessed on the basis of the differences in engorged weights and percentages engorging on homologous and heterologous rabbit hosts.

Further information on cross-protection beween field strains was obtained by application at the same time of FS1 and FS2 larvae on rabbits previously exposed to approximately 10,000 FS2 strain larvae each.

3.7. Statistical treatment

In order to assess the differences between LS and FS, the following tests were carried out. A comparison of any two means was done by either a t-test or by one-way analysis of variance. More than two means were compared by Duncan's Studentized Range Test. Two-way analysis of variance was used to compare performance of strains during successive infestations. Percentages were converted to arcsin $\sqrt{p}$ for statistical treatment. Comparison of regressions was done by analysis of variance for y after correcting for the regression (Mather, 1973). Means for egg weights, female engorged weights and tick length measurements were based on the number of individual ticks involved, while the means for the number or percentage of ticks engorging and engorged weights of larvae and nymphs were based on the means from individual rabbits and the number of rabbits involved.

CHAPTER 4

RESULTS

4.1. Size of field strain ticks in relation to host resistance

Rhipicephalus appendiculatus females collected in the field from three different areas of Kenya were similar in size, as judged by mean scutal lengths (Table 1). A plot of the regression of unfed adult scutal length against the engorged weight of the preceding nymph (Fig. 1) showed a linear relationship for males and a logarithmic one for females. The range of nymph engorged weights was obtained by feeding nymphs on a wide range of previously exposed rabbits and susceptible rabbits. The data showing scutal lengths of adults fed on susceptible rabbits (Tables 13 and 14), together with the data contained in Fig. 1 and Table 1, show that the field-collected females must have been moulted from small engorged nymphs, which suggests that the nymphs had fed on a host population showing a fair amount of host resistance.

4.2. The feeding and breeding performance of ticks on susceptible and on previously exposed hosts

4.2.1. Size of unfed ticks and engorged ticks

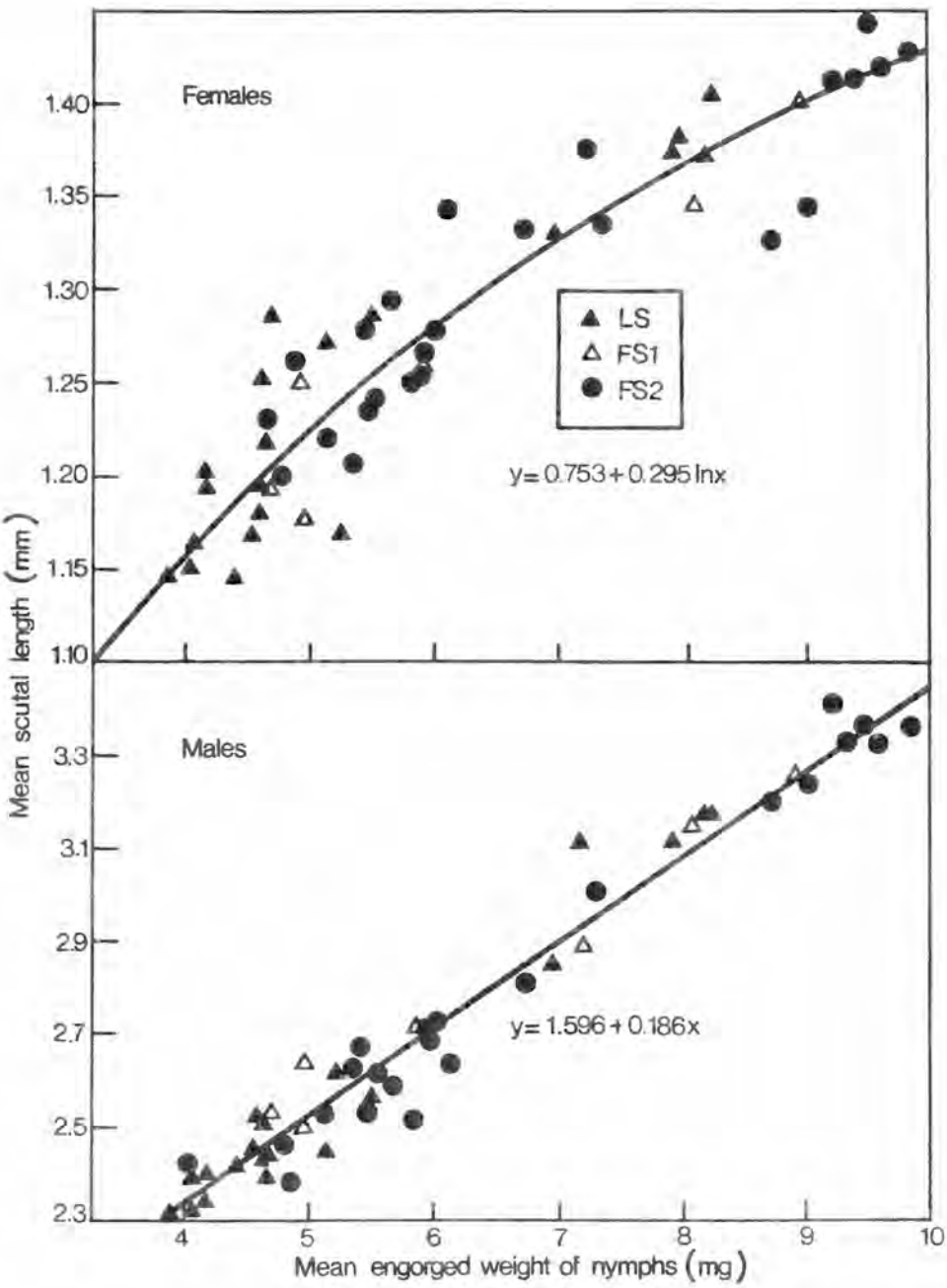
When larvae, nymphs and adults were fed on susceptible

TABLE 1. Mean scutal lengths of *R. appendiculatus* female samples collected in the field, with estimated mean weights of the engorged nymphs from which they moulted

Strain	Number of females	Scutal length ± S.E. (mm)	Estimated wt of nymphs (mg)
FS1	31	1.25 ± 0.02	5.3
FS2	33	1.24 ± 0.01	5.3
FS3	69	1.21 ± 0.01	4.7

FIGURE 1. Regressions of scutal lengths of R. appendiculatus males and females against engorged weights of nymphs from which they moulted

FIGURE 1


Females
Males
LS
FS1
FS2
y= 0.753 + 0.295 lnx
y= 1.596 + 0.186x
Mean scutal length (mm)
Mean engorged weight of nymphs (mg)
1.40
1.35
1.30
1.25
1.20
1.15
1.10
3.3
3.1
2.9
2.7
2.5
2.3
4
5
6
7
8
9
10

rabbits and thereafter the sizes of LS and FS ticks compared, the following observations were made. The mean egg weight (Table 2), the mean length of unfed larvae (Table 3), and the mean scutal length of the unfed nymphs (Table 4) were found to be significantly smaller ($P<0.01$) in the LS than in the FS. On the other hand, the two field strains were similar with respect to these parameters. Moreover, the mean scutal lengths of the unfed adults (Table 5) differed significantly ($P<0.01$) in all the three strains, the LS having the smallest adults, followed by FS1, while FS2 had the largest adults.

Table 6 shows that the mean egg weights of samples of females engorged on cattle hosts were slightly higher than those of samples from females engorged on rabbits during a primary infestation. Furthermore, when the lengths of larvae hatching from the same samples of eggs were compared (Table 7), the larvae resulting from females fed on cattle were, on average, significantly larger than larvae from females fed on rabbits. This was true for both the LS and FS2 larvae. The data also indicated that for both the LS and FS2 larvae, those from females fed on susceptible rabbits were significantly larger than those from females fed on previously exposed rabbits. The reverse was the case for cattle hosts.

The mean engorged weights of larvae (Table 11) and nymphs (Table 12) engorging on susceptible rabbits were correlated with the unfed size of the instar concerned. The scutal lengths of the adults moulting from these nymphs (Tables 13 and 14) showed that FS

TABLE 2. Mean egg weights from females of *R. appendiculatus* strains fed on susceptible rabbits

Strain	Number of samples	Mean ± S.E. (µg)
LS	10	41 ± 1[a]*
FS1	10	47 ± 1[b]
FS2	10	46 ± 1[b]

*Means not having a common letter are significantly different ($P<0.01$).

FIGURE 4

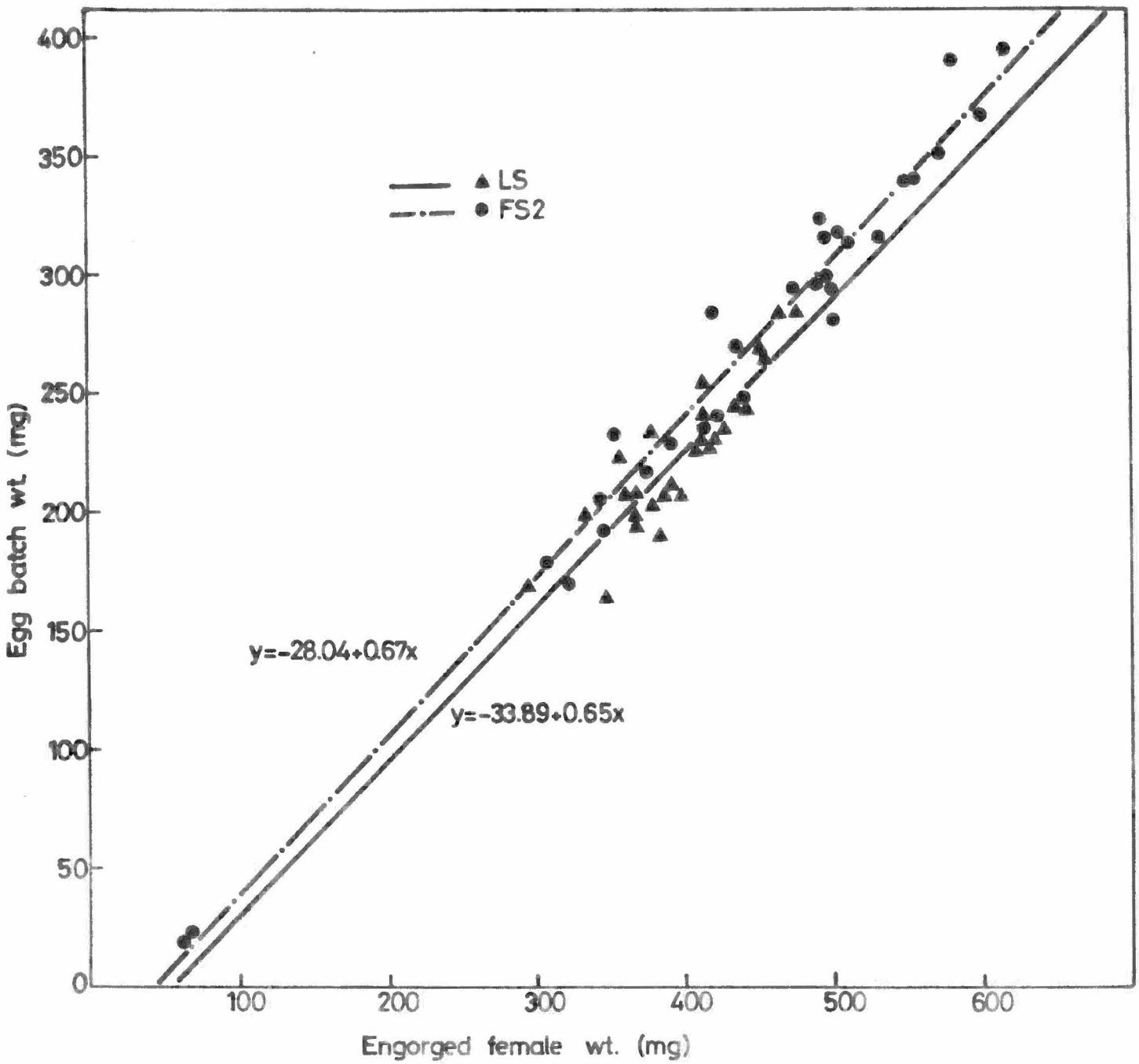

400
350
300
250
200
150
100
50
0
Egg batch wt (mg)
▲ LS
● FS2
y=-28.04+0.67x
y=-33.89+0.65x
100
200
300
400
500
600
Engorged female wt. (mg)

FIGURE 4 Regression of egg batch weight against engorged R. appendiculatus female weight: comparison between LS and FS2 when fed on rabbit

FIGURE 3

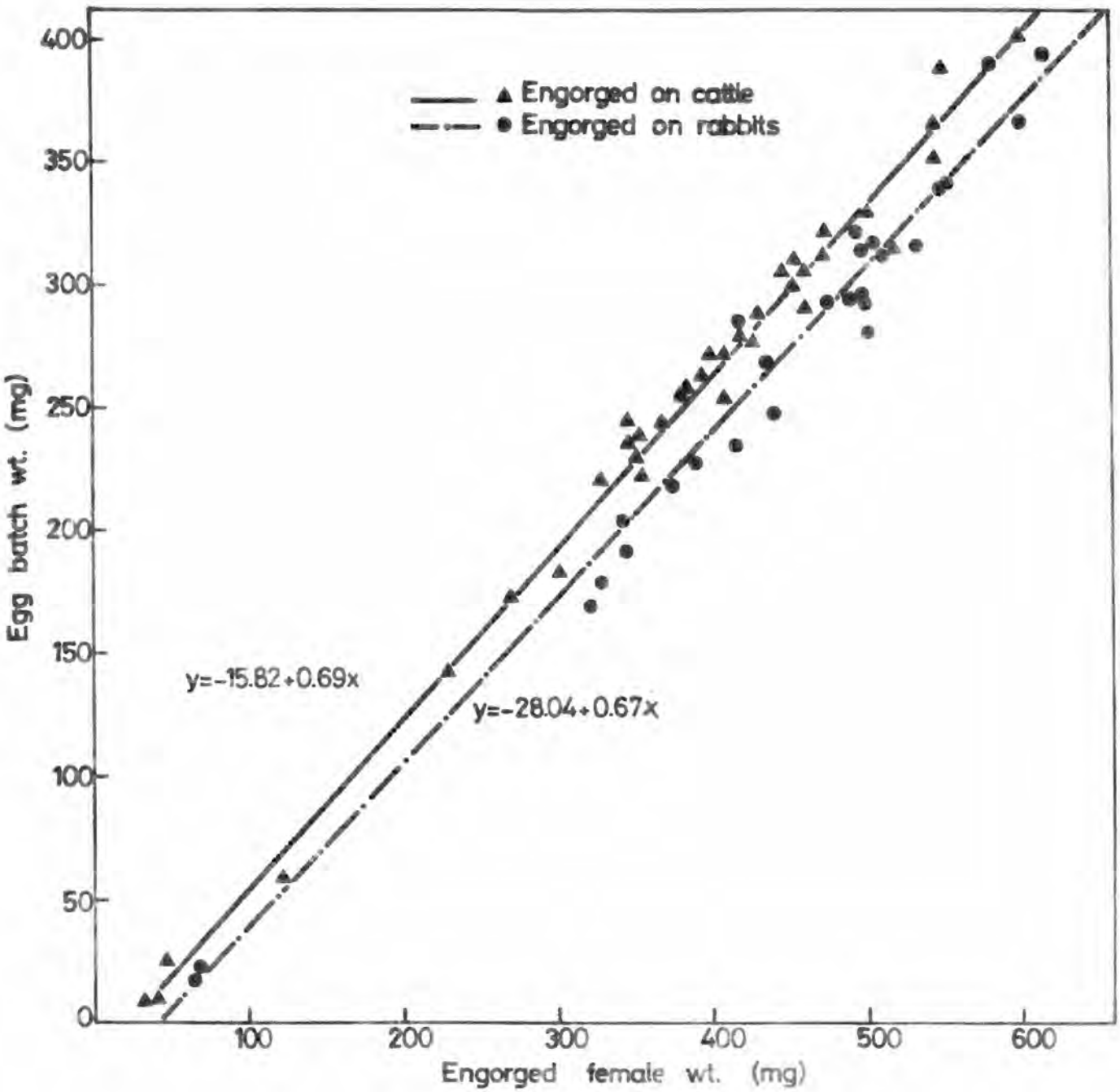

▲ Engorged on cattle
● Engorged on rabbits
y=-15.82+0.69x
y=-28.04+0.67x
Egg batch wt. (mg)
Engorged female wt. (mg)
0
50
100
150
200
250
300
350
400
100
200
300
400
500
600

FIGURE 3. Regression of egg batch weight against engorged R. appendiculatus female weight: comparison between rabbit and cattle hosts feeding FS2

FIGURE 2

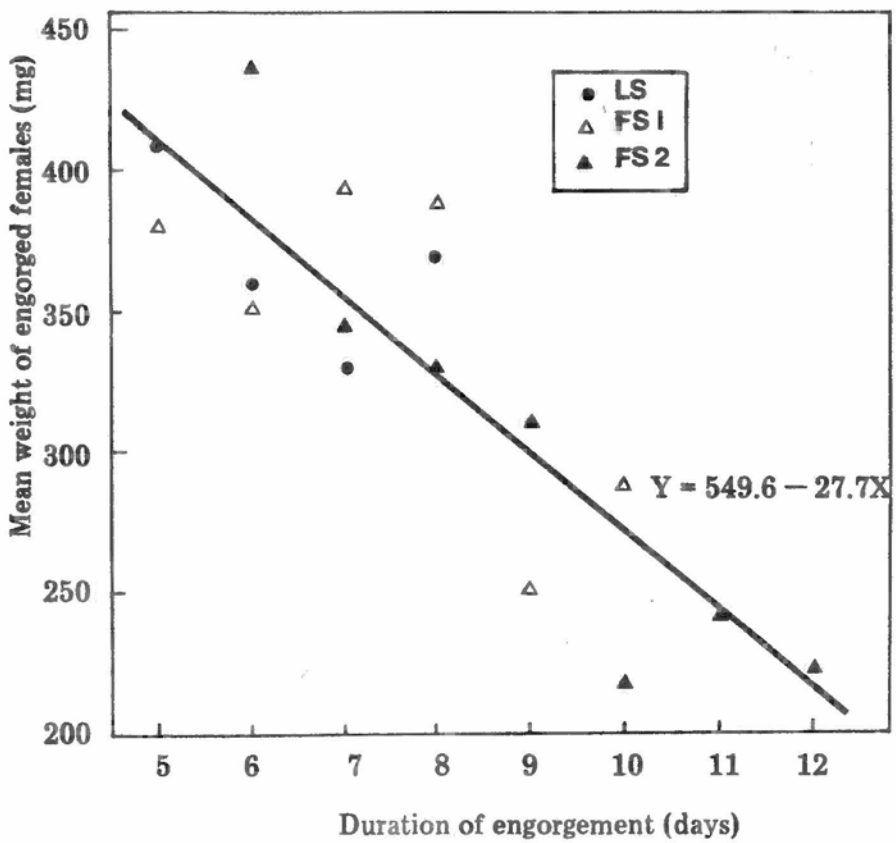

450
400
350
300
250
200
Mean weight of engorged females (mg)
LS
FS I
FS 2
Y = 549.6 − 27.7X
5
6
7
8
9
10
11
12
Duration of engorgement (days)

FIGURE 2. Regression of mean daily engorged weight of R. appendiculatus females against duration of feeding

fed on susceptible rabbits, but only slightly so on cattle. The mean engorged weights during the primary infestation with adult ticks, suggested that the cattle were not fully susceptible to the LS. When fed on previously exposed rabbits and cattle, however, REI of FS2 was found to be significantly higher ($P<0.05$) than that of the LS.

The mean egg weight of each strain and the egg batch weight of each female were used to calculate the estimated number of eggs per female for each strain. The LS produced significantly more eggs per female than the FS when fed on susceptible rabbits, but significantly fewer eggs per female than FS when fed on previously exposed rabbits and cattle. The LS produced more eggs per female when fed on susceptible hosts due to the fact that it produced a similar mean egg batch weight as FS (Table 8), while at the same time its eggs were significantly lighter (Table 2).

4.3. Effects of successive infestations of the host on tick feeding

4.3.1 Size of engorged ticks

LS larvae had smaller engorged weights than FS larvae when both were fed on susceptible rabbits (first infestation, Table 11). Analysis of variance (Appendix 5) showed that the weights of all the strains were significantly reduced during successive infestations and that the engorged weights of LS remained significantly lower than those of FS.

produced larger adults than the LS. However, engorged weights of females fed on susceptible rabbits (Table 8 and 15) showed that, despite the fact that FS unfed females were significantly larger than the LS, their engorged weights were similar.

The mean engorged weights of the females referred to above were plotted against the duration of engorgement (Figure 2) and showed an inverse relationship with a highly significant slope, indicating that fast feeding females on susceptible rabbits reached higher weights than slower feeders. On the average, LS females were the fastest feeders, followed by FS1 whilst FS2 females were the slowest. It was also observed towards the end of the infestation, particularly for FS2 ticks, that the skin reactions characteristic of host resistance were present. The exact time when the reactions appeared was not recorded, however.

A similar relationship between engorged weight and duration of engorgement was not observed for larvae and nymphs fed on susceptible rabbits.

4.2.2. Reproduction efficiency on susceptible and on previously exposed hosts.

When adult ticks resulting from larvae and nymphs fed on susceptible rabbits were themselves fed on susceptible and on previously exposed rabbits and cattle, the following observations were made. The data in Figures 3 and 4 and in Tables 8-10 showed

that the egg batch weight and the engorged weight of females are linearly correlated. There was no significant difference in the regression coefficients for ticks fed on susceptible rabbits or cattle, or even between strains. But there were significant differences ($P<0.001$) in the positions of the regression lines as shown by analysis of variance for y after correction (Appendices 1-4). As a result FS2 produced a greater weight of eggs per given weight of engorged female tick than the LS, when fed on either susceptible rabbits or cattle. In addition both LS and FS produced a greater weight of eggs per given weight of engorged female when fed on cattle hosts than on rabbit hosts. FS2 strain produced 7-11% greater weight of eggs when fed on cattle than when fed on rabbits, while the LS produced 10-14% greater weight of eggs on cattle. On the other hand FS2 produced 5-7% and 1-3% greater weight of eggs than LS when fed on rabbits and cattle respectively.

The index of conversion efficiency (CEI), simply defined as the proportion of the engorged weight of the female converted into eggs (Hunt and Drummond, 1983) was similar for LS and FS when fed on susceptible rabbits and cattle. On previously exposed rabbits and cattle, however, CEI of FS2 was significantly higher than that of LS. FS1 was omitted from some of the comparisons to reduce the amount of work involved. The mean egg weight for each strain, the engorged weight and the egg batch weight of each female were used to calculate the index of reproduction efficiency (REI), which is the number of eggs produced per gramme of engorged female weight. REI of LS was found to be significantly higher than that of FS when

TABLE 7. Mean length ± S.E. (μ) of *R. appendiculatus* larvae hatching from eggs of females fed on rabbits and cattle with or without previous tick infestation (number of larvae = 60)

Strain	Host	With previous infestation	Without previous infestation
LS	Rabbit	550 ± 3^{a}*	564 ± 3^{b}
	Cow	584 ± 3^{c}	577 ± 4^{c}
FS2	Rabbit	576 ± 3^{c}	596 ± 2^{d}
	Cow	618 ± 2^{e}	604 ± 3^{d}

*Values not having a common letter are significantly different ($P<0.05$).

TABLE 5. Mean scutal lengths of unfed adult ticks resulting from larvae and nymphs of R. appendiculatus strains fed on susceptible rabbits

Strain	Number of ticks	Mean ± S.E. (mm)
	Males	
LS1	40	2.90 ± 0.03[a*]
FS1	40	3.09 ± 0.04[b]
FS2	80	3.33 ± 0.02[c]
	Females	
LS	40	1.34 ± 0.01[a]
FS1	40	1.39 ± 0.01[b]
FS2	80	1.42 ± 0.01[c]

*Means for each sex not having a common letter are significantly different ($P<0.01$).

TABLE 6. Mean egg weights of R. appendiculatus from females fed on rabbits and cattle during a primary infestation

Strain	Host	Number of samples	Egg wt. ± S.E. (µg)
LS	Rabbit	10	40 ± 1[a]*
	Cow	10	42 ± 1[a]
FS2	Rabbit	10	47 ± 1[b]
	Cow	10	50 ± 1[b]

*Values in this column not having a common letter are significantly different ($P<0.05$).

TABLE 3. Mean length of unfed larvae from females of R. appendiculatus strains fed on susceptible rabbits

Strain	Number of larvae	Mean ± S.E. (μ)
LS	50	566 ± 3[a]*
FS1	55	603 ± 3[b]
FS2	64	609 ± 2[b]

*Means not having a common letter are significantly different ($P<0.01$).

TABLE 4. Mean scutal lengths of unfed nymphs moulted from larvae of R. appendiculatus strains fed on susceptible rabbits

Strain	Number of nymphs	Mean ± S.E. (μ)
LS	60	473 ± 3[a*]
FS1	60	498 ± 3[b]
FS2	60	500 ± 2[b]

*Means not having a common letter are significantly different ($P<0.01$).

TABLE 8. Reproduction efficiency of R. appendiculatus females fed on susceptible rabbits (CEI = Index of Conversion Efficiency; REI = Index of Reproduction Efficiency)

Parameter	Strain		
	LS	FS1	FS2
Number of females	35	37	68
Mean engorged wt ± S.E.(mg)	354 ± 11^{b}*	351 ± 13^{b}	300 ± 16^{a}
Mean egg batch wt ± S.E. (mg)	212 ± 6^{b}	219 ± 10^{b}	163 ± 11^{a}
CEI ± S.E.	0.60 ± 0.01^{b}	0.61 ± 0.01^{b}	0.50 ± 0.01^{a}
REI ± S.E.(x 10^{-4})	1.46 ± 0.02^{c}	1.31 ± 0.03^{b}	1.10 ± 0.03^{a}
Estimated no. of eggs/female ± S.E. (x 10^{-3})	5.18 ± 0.16^{c}	4.58 ± 0.17^{b}	3.54 ± 0.18^{a}

*Values in each row not having a common letter are significantly different ($P<0.05$).

TABLE 9. Reproduction efficiency of *R. appendiculatus* females fed on previously exposed rabbits (CEI = Index of Conversion Efficiency; REI = Index of Reproduction Efficiency)

Parameter	Strain	
	LS	FS2
Number of females	16	14
Mean engorged wt ± S.E.(mg)	75 ± 14[a]*	197 ± 35[b]
Mean egg batch wt ± S.E.(mg)	31 ± 9[a]	114 ± 24[b]
CEI ± S.E.	0.32 ± 0.04[a]	0.48 ± 0.04[b]
REI ± S.E($\times 10^{-4}$)	0.79 ± 0.01[a]	1.02 ± 0.07[b]
Estimated no. of eggs/female ± S.E. ($\times 10^{-3}$)	0.77 ± 0.23[a]	2.41 ± 0.50[b]

*Values in each row not having a common letter are significantly different ($P<0.01$).

TABLE 10. Reproduction efficiency of R. appendiculatus females on cattle (CEI = Index of Conversion Efficiency; REI = Index of Reproduction Efficiency)

Parameter	1st Infestation		3rd Infestation	
	LS	FS2	LS	FS2
Number of females	43*	68	36	55
Mean engorged wt ± S.E(mg)	266 ± 15^{b}**	393 ± 15^{d}	204 ± 20^{a}	323 ± 15^{c}
Mean egg batch wt ± S.E.(mg)	162 ± 11^{b}	255 ± 11^{c}	92 ± 14^{a}	183 ± 11^{b}
CEI ± S.E.	0.57 ± 0.02^{bc}	0.63 ± 0.01^{c}	0.37 ± 0.04^{a}	0.54 ± 0.03^{b}
REI ± S.E.(x 10^{-4})	1.37 ± 0.5^{d}	1.26 ± 0.02^{cd}	0.88 ± 0.10^{a}	1.08 ± 0.05^{b}
Estimated no. of eggs/female ± S.E. (x 10^{-3})	3.9 ± 0.3^{b}	5.1 ± 0.2^{c}	2.2 ± 0.3^{a}	3.7 ± 0.2^{b}

* Many ticks were either squashed or lost from each host, so no comparison of numbers fed can be made.

** Means in each row not having a common letter are significantly different ($P<0.05$).

TABLE 11. Mean weight ± S.E. (mg) of R. appendiculatus larvae engorging when five rabbits were infested with 500 larvae each in succession

Strains	Infestation number			
	1	2	3	4
LS (a)	0.52^{b*} ± 0.01	0.45^{a} ± 0.02	0.40^{a} ± 0.02	0.40^{a} ± 0.02
FS1	0.56^{b} ± 0.02	0.54^{b} ± 0.02	0.48^{a} ± 0.02	0.48^{a} ± 0.02
LS (b)	0.52^{b} ± 0.01	0.41^{a} ± 0.01	0.41^{a} ± 0.01	0.40^{a} ± 0.01
FS2	0.60^{b} ± 0.01	0.48^{a} ± 0.01	0.48^{a} ± 0.02	0.45^{a} ± 0.01

* Values in each row not having a common letter are significantly different ($P<0.05$).

TABLE 12. Mean weight ± S.E. (mg) of R. appendiculatus nymphs engorging when rabbits were infested with 50 nymphs each in succession

	Strain	Infestation number			
		1	2	3	4
(a)	LS	9.1 ± 0.02^{b*}	4.1 ± 0.4^{a}	3.6 ± 0.3^{a}	3.8 ± 0.4^{a}
	FS1	9.8 ± 0.2^{c}	6.2 ± 0.5^{b}	4.6 ± 0.4^{a}	4.6 ± 0.5^{a}
(b)	LS	7.8 ± 0.2^{b}	4.8 ± 0.2^{a}	4.4 ± 0.2^{a}	4.5 ± 0.2^{a}
	FS2	9.5 ± 0.1^{b}	6.5 ± 0.3^{a}	5.8 ± 0.2^{a}	5.0 ± 0.4^{a}

*Values in each row not having a common letter are significantly different ($P<0.05$).

The mean engorged weights of the nymphs showed a similar picture (Table 12; Appendix 6), though the weight of the engorged nymphs for each strain was reduced by a larger factor than that of the larvae during the infestations. The scutal lengths of the unfed adults into which the nymphs moulted also gave a similar picture (Tables 13 and 14; Appendices 7 and 9). The data indicated that, on average, the size of the LS ticks was reduced considerably by host resistance during the second infestation, and changed but little during subsequent infestations. In contrast, the size of the field strain ticks was reduced comparatively less during the second infestation and went steadily down with subsequent infestations. The analysis of variance confirmed this reduction of size with subsequent infestations, in addition to the fact that the size of the LS ticks remained significantly lower than that of the FS ticks.

The scutal lengths of the second, third and fourth infestations in Tables 13 were then converted into percentages of that at first infestation, to make a comparison between the size of males and females possible. Analysis of variance (Appendix 8) showed a highly significant difference ($P<0.001$) between male and female percentages. This confirmed the fact that the size of the male tick is affected more by host resistance than that of the female tick.

During successive infestations of adult ticks on rabbits, the mean engorged weights of the females were significantly reduced in

TABLE 13. Mean scutal length ± S.E. (mm) of adult R. appendiculatus moulted from nymphs engorging when five rabbits were infested with 50 nymphs each in succession

Strain	Sex	Infestation number			
		1	2	3	4
LS	Males	3.08^{b} ± 0.03	2.44^{a} ± 0.02	2.43^{a} ± 0.03	2.42^{a} ± 0.02
	Females	1.37^{c} ± 0.01	1.25^{b} ± 0.01	1.19^{a} ± 0.01	1.16^{a} ± 0.01
FS2	Males	3.35^{d} ± 0.02	2.79^{c} ± 0.04	2.67^{b} ± 0.03	2.54^{a} ± 0.03
	Females	1.42^{d} ± 0.01	1.32^{c} ± 0.01	1.27^{b} ± 0.01	1.20^{a} ± 0.01

*Values in each row not having a common letter are significantly different ($P<0.05$).

TABLE 14. Mean scutal lengths ± S.E. (mm) of adult R. appendiculatus moulted from nymphs engorging when rabbits were infested with 50 nymphs each in succession

Strain	Sex	Infestation number			
		1	2	3	4
LS	Males	2.91^{b*} ±0.02	2.17^{a} ±0.07	2.11^{a} ±0.02	2.09^{a} ±0.03
LS	Females	1.30^{b} ±0.01	1.07^{a} ±0.02	1.05^{a} ±0.02	1.06^{a} ±0.02
FS1	Males	3.03^{c} ±0.02	2.48^{b} ±0.03	2.31^{a} ±0.03	2.26^{a} ±0.03
FS1	Females	1.33^{d} ±0.01	1.21^{c} ±0.01	1.16^{b} ±0.01	1.12^{a} ±0.02

*Values in each row not having a common letter are significantly different ($P<0.05$).

TABLE 15. Mean weight ± S.E. (mg) of R. appendiculatus females engorging when five rabbits were infested with 8 adults each in succession

	Strain	Infestation number		
		1	2	3
(a)	LS	394 ± 23^{b*}	100 ± 25^{a}	74 ± 31^{a}
	FS1	404 ± 28^{b}	208 ± 36^{a}	160 ± 32^{a}
(b)	LS	363 ± 24^{b}	120 ± 19^{a}	113 ± 24^{a}
	FS2	393 ± 33^{b}	273 ± 21^{a}	195 ± 33^{a}

*Values in each row not having a common letter are significantly different ($P<0.05$).

all three strains during the second infestation (Table 15). There was only a moderate reduction of weight during the third infestation. Although there was no significant difference between the mean engorged weight of the LS females and FS females during the first infestation (i.e. on susceptible rabbits), the weights of the LS and FS were significantly different during the second and third infestations on the same rabbits, as revealed by analysis of variance (Appendix 10). During the second and third infestations, the mean engorged weights of FS females were, on average, double those of the LS females. This difference was maintained when two further infestations were applied on rabbits feeding the LS and FS2 females. The fourth infestation yielded a mean engorged weight of 74 mg for the LS females and 144 mg for the FS2 females. The fifth infestation yielded 61 mg and 126 mg, respectively.

Successive infestations of 100 adult ticks each were applied on cattle alongside infestations of 80 adult ticks on susceptible rabbits. During the first infestation, LS yielded a fairly low mean engorged weight on cattle (Table 16), which changed only slightly during the next two infestations. FS2, however, yielded a fairly good mean weight during the first infestation, comparable to those obtained on susceptible rabbits. During the next two infestations the mean engorged weight of FS2 females was significantly reduced, but remained significantly higher than that of LS.

TABLE 16. Mean engorged weight ± S.E. (mg) and number of R. appendiculatus females from three cattle infested with adult ticks in succession and from susceptible rabbits

Strain	Infestation number 1		2		3	
	no.*	wt.	no.	wt	no.	wt.
			Cattle			
LS	45	259^{a}** ±16	27	203^{a} ±19	25	207^{a} ±24
FS2	92	394^{d} ±12	52	318^{b} ±14	28	326^{bc} ±28
			Susceptible rabbits			
LS	40	398^{de} ±12	29	395^{de} ±11	36	371^{cd} ±17
FS2	31	442^{ef} ±23	35	491^{f} ±16	36	368^{cd} ±26

*Many ticks were either squashed or lost from each host so comparison of numbers cannot be made.

**Weight values not having a common letter are significantly different ($P<0.05$).

TABLE 17. Mean percentages (± S.E.) of *R. appendiculatus* larvae engorging when five rabbits were infested with 500 larvae each in succession

Strain	Infestation number			
	1	2	3	4
(a) LS	$90 \pm 1^{c*}$	68 ± 7^{b}	42 ± 6^{a}	33 ± 4^{a}
FS1	87 ± 3^{b}	88 ± 1^{b}	59 ± 6^{a}	61 ± 9^{a}
(b) LS	86 ± 3^{c}	55 ± 9^{b}	30 ± 8^{a}	36 ± 6^{a}
FS2	90 ± 1^{c}	84 ± 3^{b}	43 ± 2^{a}	47 ± 3^{a}

* Values in each row not having a common letter are significantly different ($P<0.05$).

4.3.2. Proportion of ticks engorging

When susceptible rabbits were infested with 500 larvae each in succession the proportion that managed to engorge went down with succeeding infestations for both LS and FS (Table 17). This was clearly shown by the analysis of variance (Appendix 11), which also showed that there was a highly significant difference ($P<0.001$) between the LS and FS larvae. Although there was very little difference in the proportions of larvae engorging on susceptible rabbits, significantly fewer LS larvae engorged on previously exposed rabbits compared to FS larvae.

When the percentages of larvae engorging during successive infestations were plotted against the mean engorged weights (Figure 5), the following observations were made. The data of the LS indicated that there was a direct relationship between mean engorged weights and percentages engorging. As the mean engorged weight became reduced so also did the mean percentages of engorged larvae. The data for the field strains, however, particularly for FS2, seemed to suggest a different relationship. In spite of the fact that reduction of the engorged weight was highest during the second infestation, there was very little reduction in the percentage of the FS larvae engorging. The third infestation seemed to produce the reverse effect, suggesting that different factors were involved in each case.

When rabbits were successively infested with 50 nymphs each,

FIGURE 5. Relationship between the percentage of R. appendiculatus larvae engorging and mean weight during 4 infestations of 500 larvae each in succession on rabbits

FIGURE 5

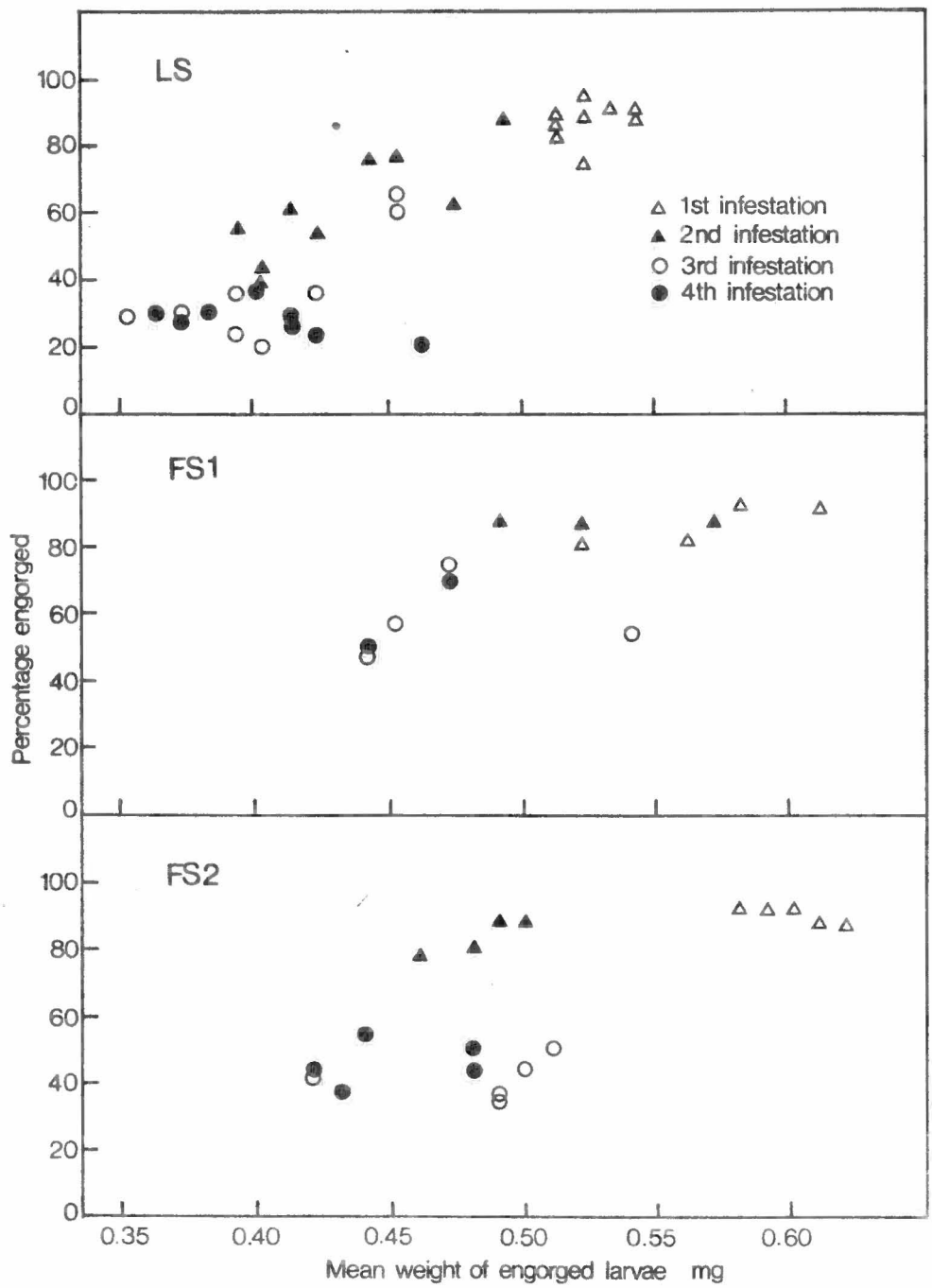

LS
FS1
FS2
1st infestation
2nd infestation
3rd infestation
4th infestation
Percentage engorged
0
20
40
60
80
100
0.35
0.40
0.45
0.50
0.55
0.60
Mean weight of engorged larvae mg

TABLE 18. Mean percentages (± S.E.) of *R. appendiculatus* nymphs engorging when five rabbits were infested with 50 nymphs each in succession

Strain	Infestation number			
	1	2	3	4
(a) LS	96 ± 2^{b}*	81 ± 3^{a}	76 ± 10^{a}	73 ± 3^{a}
FS1	90 ± 5^{a}	89 ± 2^{a}	84 ± 5^{a}	88 ± 3^{a}
(b) LS	98 ± 2^{b}	87 ± 3^{ab}	90 ± 2^{ab}	84 ± 4^{a}
FS2	90 ± 4^{ab}	92 ± 1^{b}	86 ± 3^{ab}	81 ± 4^{a}

*Values in each row not having a common letter are significantly different ($P<0.05$).

TABLE 19. Mean number (± S.E.) of *R. appendiculatus* females engorging when five rabbits were infested with 8 adults each in succession (sex ratio = 4:4)

Strain	Infestation number		
	1	2	3
(a) LS	4.0 ± 0.0^{b}*	3.8 ± 0.2^{b}	2.4 ± 0.7^{a}
FS1	3.8 ± 0.2^{a}	2.8 ± 0.5^{a}	3.6 ± 0.2^{a}
(b) LS	3.6 ± 0.2^{a}	3.8 ± 0.2^{a}	2.6 ± 0.7^{a}
FS2	2.8 ± 0.6^{a}	3.8 ± 0.2^{a}	3.8 ± 0.2^{a}

*Values in each row not having a common letter are significantly different ($P<0.05$).

there was only a slight drop in percentage engorging (Table 18). Similarly, there was little, if any, change in the proportion of adults engorging when three successive infestations of 8 adults each were applied on each rabbit (Table 19).

The proportions of larvae, nymphs and females engorging on rabbits with no previous infestations, on rabbits with one previous infestation and on rabbits with two previous infestations were combined to derive an estimate of the proportions in each case that would eventually end up as engorged females. The data (Table 20) showed that slightly more engorged females would be yielded by the LS than FS if fed on rabbits with no previous infestations. However, on previously exposed rabbits, the yield of engorged females would be higher for FS than for LS.

4.3.3. Mortality during moulting and hatching

Some mortality occurred during the moulting of larvae fed on rabbits (Table 21). It was slightly higher on rabbits previously exposed to ticks than on susceptible rabbits. There was however no difference between LS and FS1. Mortality during the moulting of nymphs was negligible. No further data was collected on mortality during moulting.

Hatching of eggs from females engorging on susceptible and on previously exposed hosts was assessed (Table 22 and 23). There was no difference between the hatchability of eggs of females fed on

TABLE 20. Estimated proportions of R. appendiculatus larvae that eventually became engorged females after larvae, nymphs and adults were fed on rabbits with various previous infestations (from data of Tables 17-19)

Strain	Number of previous infestations		
	0	1	2
(a) LS	0.86	0.52	0.19
FS1	0.74	0.55	0.45
(b) LS	0.76	0.45	0.18
FS2	0.57	0.73	0.35

TABLE 21. Mean percentage mortality (± S.E.) during moulting of R. appendiculatus larvae following succcessive infestations on five rabbits

Strain	Infestation number			
	1	2	3	4
LS	2.5 ± 0.9^{a}*	6.9 ± 1.9^{ab}	9.1 ± 2.1^{b}	6.1 ± 1.7^{ab}
FS1	3.1 ± 0.7^{a}	6.3 ± 3.2^{a}	9.2 ± 4.1^{a}	4.7 ± 0.9^{a}

*Values in each row not having a common letter are significantly different ($P<0.01$).

TABLE 22. Percentage of eggs hatching from R. appendiculatus females used in primary infestations on susceptible cattle and susceptible rabbits

Strain	Hosts	Number of egg batches	Mean % hatching ± S.E.
LS	Rabbits	40	78 ± 3^{a}
	Cattle	41	86 ± 4^{ab}
FS2	Rabbits	31	85 ± 5^{ab}
	Cattle	44	93 ± 3^{b}

*Means not having a common letter are significantly different ($P<0.05$).

TABLE 23. Percentage of eggs hatching from R. appendiculatus females used in a challenge infestation

Hosts	Strain previously infested with	Challenging strain	Number of egg batches	Mean % hatching ± S.E.
Rabbits	LS	LS	15	31 ± 11[a*]
		FS2	16	93 ± 5[b]
	FS2	LS	17	75 ± 6[b]
		FS2	12	66 ± 11[b]
	None	LS	36	79 ± 4[b]
		FS2	36	76 ± 5[b]
Cattle	LS	LS	19	87 ± 6[b]
		FS2	27	87 ± 5[b]
	FS2	LS	10	78 ± 10[b]
		FS2	27	77 ± 5[b]

*Means not having a common letter are significantly different ($P<0.05$).

susceptible hosts and previously exposed hosts. There was no difference in hatchability between the eggs of females fed on cattle and those of females fed on rabbits. The low hatchability of eggs recorded for LS females engorging on rabbits previously exposed to the same strain may have been due to inclusion of unmated females, since it was recorded mainly in the smallest egg batches. Hatchability of LS eggs and FS eggs was similar.

4.4. Feeding and breeding performance on homologous and heterologous hosts

4.4.1. Weight and proportion of engorging ticks

When the feeding performance of larvae obtained from freshly acquired FS was compared to that of the LS larvae on susceptible and on previously exposed rabbits, the following observations were made. Both the engorged weight and the proportion of the LS engorging on rabbits previously exposed to the LS larvae were significantly lower than those of the larvae of the two field strains (Table 24). On susceptible rabbits however, the LS larvae fed equally well. Further challenge feeds on rabbits and cattle previously exposed to the LS and FS gave more information regarding homologous and heterologous hosts. Since weight of engorged ticks proved to be more consistent, and therefore more reliable, than the proportion engorging, the observations were mainly based on engorged weights.

The data in general indicated that FS fed significantly

TABLE 24. Mean percentage and weight of R. appendiculatus engorging on rabbits previously infested with approximately 10,000 laboratory strain larvae per rabbit and on susceptible rabbits

Type of hosts	Strain fed	% engorged ± S.E.	Mean wt ± S.E. (mg)
(a) Previously exposed	LS	54 ± 2^{a}*	0.37 ± 0.01^{a}
	FS1	87 ± 3^{c}	0.50 ± 0.01^{b}
Susceptible	LS	85 ± 4^{c}	0.51 ± 0.01^{b}
	FS1	75 ± 1^{b}	0.54 ± 0.02^{b}
(b) Previously exposed	LS	66 ± 1^{a}	0.43 ± 0.01^{a}
	FS2	93 ± 2^{b}	0.49 ± 0.01^{b}

*Values in each column in each of (a) and (b) not having a common letter are significantly different ($P<0.05$).

TABLE 25. Mean percentage and weight of field strain larvae engorging on rabbits previously exposed to approximately 10,000 FS2 larvae

Strain	% engorged ± S.E.	Mean wt ± S.E. (mg)
FS1	82 ± 5[a*]	0.46 ± 0.02[a]
FS2	84 ± 2[a]	0.46 ± 0.01[a]

*Values in each column were statistically similar ($P \geq 0.05$)

TABLE 26. Mean percentage and engorged weight of challenge ticks engorging when applied after 4 infestations of 500 R. appendiculatus larvae each per rabbit

Strain previously infested with	Challenging Instar	Strain	% engorged ± S.E.	Mean wt ± S.E. (mg)
	LS	Larvae	LS	34 ± 11[a]*	0.40 ± 0.03[a]
			FS1	77 ± 7[b]	0.53 ± 0.01[b]
	FS1		LS	19 ± 2[a]	0.40 ± 0.01[a]
			FS1	41 ± 19[ab]	0.42 ± 0.05[a]
(a)					
	LS	Nymphs	LS	91 ± 2[a]	5.5 ± 0.2[a]
			FS1	79 ± 9[a]	7.2 ± 0.5[b]
	FS1		LS	90 ± 0[a]	6.2 ± 0.0[ab]
			FS1	84 ± 12[a]	5.9 ± 0.1[a]
	LS	Larvae	LS	59 ± 8[a]	0.40 ± 0.02[a]
			FS2	88 ± 1[b]	0.48 ± 0.01[b]
	FS2		LS	65 ± 2[a]	0.42 ± 0.02[a]
			FS2	62 ± 5[a]	0.44 ± 0.01[a]
(b)					
	LS	Nymphs	LS	68 ± 3[a]	4.6 ± 0.4[a]
			FS2	96 ± 2[c]	8.8 ± 0.3[c]
	FS2		LS	59 ± 8[a]	5.0 ± 0.4[ab]
			FS2	83 ± 3[b]	5.6 ± 0.3[b]

*Values for each instar in each column of (a) and (b) not having a common letter are significantly different ($P<0.05$).

TABLE 27. Mean percentage and engorged weight of challenge ticks engorged when applied after 4 infestations of 50 R. appendiculatus nymphs each per rabbit

Strain previously infested with	Challenging Instar	Strain	% engorged ± S.E.	Mean wt ± S.E.(mg)
	LS	Larvae	LS	46 ± 6^{a}	0.34 ± 0.00^{a}*
			FS1	48 ± 8^{a}	0.42 ± 0.04^{b}
	FS1		LS	60 ± 7^{a}	0.37 ± 0.01^{a}
			FS1	71 ± 5^{a}	0.42 ± 0.02^{b}
(a)					
	LS	Nymphs	LS	87 ± 4^{a}	4.0 ± 0.2^{a}
			FS1	86 ± 4^{a}	5.0 ± 0.4^{a}
	FS1		LS	91 ± 4^{a}	4.6 ± 0.5^{a}
			FS1	94 ± 2^{a}	4.6 ± 0.4^{a}
	LS	Larvae	LS	47 ± 8^{a}	0.35 ± 0.02^{a}
			FS2	66 ± 6^{a}	0.46 ± 0.01^{c}
	FS2		LS	38 ± 9^{a}	0.42 ± 0.01^{b}
			FS2	38 ± 12^{a}	0.40 ± 0.01^{b}
(b)					
	LS	Nymphs	LS	83 ± 2^{a}	4.1 ± 0.1^{a}
			FS2	92 ± 2^{b}	6.3 ± 0.2^{c}
	FS2		LS	79 ± 2^{a}	5.0 ± 0.1^{b}
			FS2	84 ± 2^{a}	5.1 ± 0.1^{b}

*Values for each instar in each column in each of (a) and (b) not having a common letter are significantly different ($P<0.05$).

TABLE 28. Mean percentage and engorged weight of challenge ticks engorging, when applied after 2 infestations of 50 R. appendiculatus nymphs each per rabbit

Strain previously infested with	Challenging Instar	Challenging Strain	% Engorged ± S.E.	Mean wt ± S.E. (mg)
FS1	Larvae	FS1	60 ± ^{a}*	0.42 ± 0.02^{a}
		FS2	68 ± 8^{a}	0.45 ± 0.01^{a}
FS2		FS1	46 ± 19^{a}	0.44 ± 0.02^{a}
		FS2	52 ± 9^{a}	0.42 ± 0.02^{a}
FS1	Nymphs	FS1	80 ± 0^{a}	4.8 ± 0.1^{a}
		FS2	94 ± 2^{b}	6.9 ± 0.2^{b}
FS2		FS1	95 ± 1^{b}	5.5 ± 0.4^{ab}
		FS2	98 ± 0^{b}	4.8 ± 0.1^{a}

*Values for each instar in each column not having a common letter are significantly different ($P<0.05$).

TABLE 29. Mean number and engorged weight of challenge ticks engorging when applied after 3 infestations of 8 adult R. appendiculatus each per rabbit

Strain previously infested with	Challenging Instar	Challenging Strain	Number engorged ± S.E.	Mean wt ± S.E.(mg)
LS	Larvae	LS	37 ± 0[a]*	0.33 ± 0.03[a]
		FS1	42 ± 11[a]	0.43 ± 0.03[b]
FS1		LS	32 ± 10[a]	0.33 ± 0.03[a]
		FS1	31 ± 5[a]	0.36 ± 0.02[ab]
LS	Nymphs	LS	41 ± 3[a]	4.0 ± 0.2[a]
		FS1	40 ± 0[a]	5.1 ± 0.2[b]
FS1		LS	43 ± 2[a]	3.7 ± 0.1[a]
		FS1	41 ± 2[a]	4.2 ± 0.1[a]
LS	Females	LS	3.3 ± 1.2[a]	98 ± 24[a]
		FS1	4.0 ± 0.6[a]	239 ± 32[b]
FS1		LS	4.0 ± 0.7[a]	116 ± 25[a]
		FS1	3.3 ± 0.9[a]	117 ± 34[a]

*Values for each instar in each column not having a common letter are significantly different ($P<0.05$).

TABLE 30. Mean number and engorged weight of challenge ticks engorging when applied after 3 infestations of 8 adult R. appendiculatus each per rabbit

Strain previously infested with	Challenging Instar	Challenging Strain	Number engorged ± S.E.	Mean wt ± S.E.(mg)
LS	Larvae	LS	27 ± 7^{a}*	0.38 ± 0.01^{a}
		FS2	65 ± 10^{bc}	0.51 ± 0.02^{c}
FS2		LS	83 ± 5^{c}	0.47 ± 0.01^{b}
		FS2	58 ± 10^{b}	0.46 ± 0.01^{b}
LS	Nymphs	LS	40 ± 2^{a}	4.0 ± 0.2^{a}
		FS2	48 ± 1^{b}	8.9 ± 0.2^{c}
FS2		LS	48 ± 0^{b}	6.7 ± 0.2^{b}
		FS2	43 ± 1^{a}	6.7 ± 0.4^{b}
LS	Females	LS	3.6 ± 0.5^{a}	74 ± 18^{a}
		FS2	4.4 ± 0.4^{a}	230 ± 21^{c}
FS2		LS	4.0 ± 0.6^{a}	193 ± 19^{bc}
		FS2	3.8 ± 0.2^{a}	144 ± 19^{b}

*Values for each instar in each column not having a common letter are significantly different ($P<0.05$).

better on rabbits previously exposed to the LS ticks (heterologous hosts) than on rabbits exposed to the same FS ticks (homologous hosts). The LS ticks, too, fed slightly better on heterologous rabbit hosts than on homologous rabbit hosts (Tables 26-30). When the two field strains were considered separately from the LS with respect to feeding performance, the difference between homologous and heterologous rabbits was less marked. There was no significant difference in feeding performance of larvae between homologous and heterologous rabbits (Table 25 and 28). However, nymphs of field strains, particularly those of FS2, fed better on heterologous rabbits than on homologous rabbits.

Similar results were obtained when cattle, previously exposed to two infestations of 100 adults each in succession, were challenged together with susceptible rabbits and previously exposed rabbits (Tables 31-33). Larvae and nymphs yielded similar results on previously exposed rabbits and cattle, but females yielded equal weights on homologous and heterologous cattle. Egg production was directly related to engorged weights (Tables 34 and 35).

4.4.2. Cross-protection between strains

If it is to be assumed that cross-protection by host resistance occurs when engorged weights of challenge ticks are similar, or nearly so, on homologous and heterologous hosts, the data in Tables 24 and 26 showed that resistance induced by LS larvae did not protect against larvae and nymphs of FS. However,

TABLE 31. Mean number and mean weight of R. appendiculatus larvae engorging during challenge infestation on rabbits and cattle

Host	Strain previously infested with	Challenging strain	Number engorged	Mean wt ± S.E.(mg)
Rabbits	LS	LS	91	0.36 ± 0.02^{a**}
		FS2	148	0.48 ± 0.01^{cd}
	FS2	LS	75	0.44 ± 0.03^{bc}
		FS2	158	0.49 ± 0.04^{cd}
	None	LS	153	0.47 ± 0.02^{c}
		FS2	146	0.54 ± 0.02^{d}
Cattle	LS	LS	-*	-
		FS2	46*	0.46 ± 0.01^{c}
	FS2	LS	13*	0.38 ± 0.00^{ab}
		FS2	14*	0.39 ± 0.01^{ab}

*Many ticks were lost

**Values in this column not having a common letter are significantly different ($P<0.05$).

TABLE 32. Mean number and mean weight of R. appendiculatus nymphs engorging during challenge infestation on rabbits and cattle

Hosts	Strain previously infested with	Challenging strain	Number engorged	Mean wt ± S.E. (mg)
Rabbits	LS	LS	37	$4.0 \pm 0.4^{a**}$
		FS2	34	8.2 ± 0.1^{gf}
	FS2	LS	16	7.0 ± 0.1^{de}
		FS2	42	6.1 ± 0.2^{cd}
	None	LS	48	8.7 ± 0.1^{gh}
		FS2	45	9.3 ± 0.1^{h}
Cattle	LS	LS	18*	3.9 ± 0.2^{a}
		FS2	25*	7.6 ± 0.8^{ef}
	FS2	LS	22*	4.7 ± 0.5^{ab}
		FS2	19*	5.4 ± 0.4^{bc}

*Some ticks were lost during feeding

**Means in this column not having a common letter are significantly different ($P<0.05$).

TABLE 33. Mean number and mean weight of R. appendiculatus females engorging during challenge infestation on rabbits and cattle

Host	Strain previously infested with	Challenging strain	Number engorged	Mean wt ± S.E. (mg)
Rabbits	LS	LS	16	75 ± 14[a]**
		FS2	18	277 ± 29[bc]
	FS2	LS	17	309 ± 22[c]
		FS2	14	197 ± 36[b]
	None	LS	36	371 ± 17[d]
		FS2	36	368 ± 26[d]
Cattle	LS	LS	25*	207 ± 24[b]
		FS2	29*	324 ± 20[cd]
	FS2	LS	12*	190 ± 33[b]
		FS2	28*	326 ± 28[cd]

*Some ticks were lost during feeding

**Means in this column not having a common letter are significantly different ($P<0.05$).

TABLE 34. Mean weight of egg batches from R. appendiculatus females used in a challenge infestation applied after 3 infestations of 8 adults each per rabbit (See Table 29)

Strain previously infested with	Challenging strain	Number of ticks	Mean wt of eggs ± S.E. (mg)
LS	LS	10	47 ± 15[a]*
	FS2	16	121 ± 14[b]
FS2	LS	19	104 ± 12[b]
	FS2	15	67 ± 10[a]

*Means not having a common letter are significantly different ($P<0.05$).

TABLE 35. Mean weight of egg batches from R. appendiculatus females used in a challenge infestation on previously exposed rabbits and cattle (see Table 33)

Hosts	Strain previously infested with	Challenging strain	Number of females	Mean wt ± S.E. (mg)
Rabbits	LS	LS	16	31 ± 9[a*]
		FS2	15	146 ± 22[bc]
	FS2	LS	17	162 ± 16[bc]
		FS2	13	114 ± 24[b]
Cattle	LS	LS	19	132 ± 16[b]
		FS2	27	205 ± 13[d]
	FS2	LS	10	100 ± 27[b]
		FS2	25	183 ± 15[cd]

*Means in this column not having a common letter are significantly different ($P<0.01$).

resistance induced by FS larvae protected against LS larvae and nymphs. Resistance induced by larvae of each of FS protected against larvae of both FS (Table 25).

Resistance induced by nymphs of any of the three strains protected against larvae and nymphs of any strain, though that induced by LS nymphs protected against FS2 larve and nymphs rather poorly (Tables 27 and 28). Resistance induced by nymphs of each of FS protected against larvae and nymphs of both strains, though again resistance induced by FS1 protected against FS2 nymphs poorly (Table 36).

Resistance induced by LS adults protected against larvae and nymphs of FS1 and not against those of FS2 (Tables 29 and 30). It did not protect against adults of either FS. Resistance induced by adults of either FS strain protected against larvae, nymphs and adults of LS. Cross-protection by host resistance induced in cattle was similar to that described above for rabbits (Tables 31-33).

4.5. Duration of feeding

The duration of feeding of larvae, nymphs and females of the different strains was recorded on susceptible rabbits, during successive infestations on the same rabbits and during challenge infestations on previously exposed rabbits. There were differences in the duration of feeding on susceptible rabbits by ticks of different strains (Table 37). FS2 larvae and nymphs fed faster

TABLE 36. Mean scutal lengths of adult R. appendiculatus moulting from challenge nymphs applied after 2 infestations of 50 nymphs each per rabbit

Strain previously infested with	Strain challenged	Number of ticks	Mean scutal length ± S.E. (mm)
		Males	
FS1	FS1	32	2.53 ± 0.05^{ab}*
	FS2	46	2.80 ± 0.05^{c}
FS2	FS1	37	2.65 ± 0.05^{b}
	FS2	46	2.42 ± 0.04^{a}
		Females	
FS1	FS1	43	1.22 ± 0.02^{a}
	FS2	48	1.33 ± 0.01^{b}
FS2	FS1	56	1.26 ± 0.01^{a}
	FS2	51	1.25 ± 0.02^{a}

*Means for each sex not having a common letter are significantly different ($P<0.05$).

TABLE 37. Duration of feeding of R. appendiculatus ticks on susceptible rabbits. One hundred larvae and one hundred nymphs of each strain were applied on one ear of each of four rabbits. Eighty adults were applied on each rabbit

Instar	Strain	Number engorged	Mean ± S.E. (days)
Larvae	LS	297	$4.66 \pm 0.04^{b*}$
	FS1	317	4.75 ± 0.05^{b}
	FS2	364	4.29 ± 0.03^{a}
Nymphs	LS	382	5.17 ± 0.03^{b}
	FS1	388	5.41 ± 0.03^{c}
	FS2	335	4.95 ± 0.03^{a}
Females	LS	35	6.64 ± 0.02^{a}
	FS1	37	7.41 ± 0.02^{b}
	FS2	53	8.42 ± 0.02^{c}

*Values for each instar not having a common letter are significantly different ($P<0.05$).

than either LS or FS1 larvae and nymphs. FS1 larvae and nymphs were the slowest feeders. In contrast, the females of FS2 were the slowest feeders on susceptible rabbits, followed by FS1 females. Other data on feeding duration on susceptible rabbits can be seen in the first infestation of Tables 38-40. The order of duration of feeding described above for the different strains was fairly consistent, though the actual values differed in different experiments. For instance, when 8 adults were used for each rabbit, it took the females nearly twice as long as 80 adults per rabbit to engorge (Tables 37 and 40), suggesting that heavier infestations took shorter to complete than lighter ones.

During the successive infestations of 500 larvae on the same rabbits, the duration of feeding for the LS increased with increasing infestations (Table 38). That of the FS, however, only went up during the third infestation, and then came down again. The successive infestations of 50 nymphs on the same rabbits did not produce any definite pattern for the duration of feeding (Table 39). Successive infestations of 8 adults on the same rabbits, on the other hand, showed that the duration of feeding of LS females was similar on susceptible and on previously exposed rabbits. However, that of the FS females was significantly longer on susceptible rabbits than on previously exposed rabbits and also longer than that of the LS females.

The duration of feeding of ticks during challenge infestations is recorded in Tables 41-43. It is obvious from the

TABLE 38. Duration of feeding (mean days ± S.E.) of larvae during infestations of 500 R. appendiculatus larvae per rabbit in succession

Strain	Infestation number			
	1	2	3	4
(a) LS	4.07 ± 0.02^{a*}	4.36 ± 0.04^{b}	4.84 ± 0.06^{d}	4.84 ± 0.06^{d}
FS1	4.53 ± 0.04^{c}	4.53 ± 0.04^{c}	4.74 ± 0.05^{d}	4.02 ± 0.04^{a}
(b) LS	4.07 ± 0.02^{b}	4.26 ± 0.04^{cd}	4.17 ± 0.05^{c}	4.34 ± 0.05^{d}
FS2	3.90 ± 0.02^{a}	3.92 ± 0.03^{a}	4.07 ± 0.04^{b}	3.94 ± 0.05^{a}

*Values in each of (a) and (b) not having a common letter are significantly different ($P<0.05$).

TABLE 39. Duration of feeding (mean days ± S.E) of nymphs during infestations of 50 R. appendiculatus nymphs per rabbit in succession

Strain		Infestation number			
		1	2	3	4
(a)	LS	$7.9 \pm 0.2^{d*}$	6.9 ± 0.1^{c}	7.5 ± 0.2^{dc}	6.0 ± 0.1^{a}
	FS1	7.0 ± 0.2^{c}	6.6 ± 0.1^{b}	7.7 ± 0.1^{d}	6.0 ± 0.1^{a}
(b)	LS	6.9 ± 0.1^{d}	7.6 ± 0.1^{e}	6.4 ± 0.1^{bc}	6.1 ± 0.1^{a}
	FS2	6.8 ± 0.1^{d}	6.5 ± 0.1^{c}	6.0 ± 0.1^{a}	6.0 ± 0.1^{a}

*Values in each of (a) and (b) not having a common letter are significantly different ($P<0.01$).

TABLE 40. Duration of feeding (mean days ± S.E.) of female ticks during infestations of 8 adults (2 males and 2 females per ear) per rabbit in succession

Strain		Infestation number		
		1	2	3
(a)	LS	10.4 ± 0.6^{a}*	11.0 ± 0.6^{a}	10.2 ± 0.4^{a}
	FS1	13.8 ± 0.7^{b}	10.2 ± 0.5^{a}	10.4 ± 0.4^{a}
(b)	LS	11.8 ± 0.5^{a}	10.6 ± 0.5^{a}	9.9 ± 0.5^{a}
	FS2	17.7 ± 1.0^{b}	10.5 ± 0.3^{a}	10.6 ± 0.5^{a}

*Values in each of (a) and (b) not having a common letter are significantly different ($P < 0.05$).

TABLE 41. Duration of feeding of challenge ticks applied after 4 infestations of 500 R. appendiculatus larvae each per rabbit

Strain previously infested with		Challenging Instar	Strain	Mean ± S.E. (days)
(a)	LS	Larvae	LS	5.2 ± 0.1[a*]
			FS1	5.4 ± 0.1[b]
	FS1		LS	5.0 ± 0.2[a]
			FS1	4.9 ± 0.1[a]
	LS	Nymphs	LS	6.2 ± 0.1[a]
			FS1	6.9 ± 0.1[b]
	FS1		LS	6.0 ± 0.1[a]
			FS1	6.1 ± 0.2[a]
(b)	LS	Larvae	LS	4.7 ± 0.1[d]
			FS2	4.2 ± 0.0[b]
	FS2		LS	4.3 ± 0.0[c]
			FS2	4.0 ± 0.0[a]
	LS	Nymphs	LS	5.7 ± 0.1[b]
			FS2	4.7 ± 0.0[a]
	FS2		LS	4.7 ± 0.1[a]
			FS2	4.7 ± 0.1[a]

*Values for each instar in each of (a) and (b) not having a common letter are significantly different ($P < 0.05$).

TABLE 42. Duration of feeding of challenge ticks applied after 4 successive infestations of 50 R. appendiculatus nymphs each per rabbit

	Strain previously infested with	Challenging Instar	Challenging Strain	Mean ± S.E. (days)
(a)	LS	Larvae	LS	$4.3 \pm 0.1^{a*}$
			FS1	4.8 ± 0.1^{b}
	FS1		LS	4.8 ± 0.1^{b}
			FS1	5.1 ± 0.1^{c}
	LS	Nymphs	LS	5.2 ± 0.1^{a}
			FS1	6.0 ± 0.1^{bc}
	FS1		LS	5.7 ± 0.1^{b}
			FS1	6.2 ± 0.1^{c}
(b)	LS	Larvae	LS	4.8 ± 0.1^{b}
			FS2	4.7 ± 0.0^{a}
	FS2		LS	4.5 ± 0.1^{a}
			FS2	4.6 ± 0.1^{a}
	LS	Nymphs	LS	6.0 ± 0.1^{c}
			FS2	5.2 ± 0.1^{a}
	FS2		LS	5.9 ± 0.1^{bc}
			FS2	5.7 ± 0.1^{b}

*Values for each instar in each of (a) and (b) not having a common letter are significantly different ($P<0.05$).

TABLE 43. Duration of feeding of challenge ticks applied after 3 successive infestations of 8 adult R. appendiculatus each per rabbit

	Strain previously infested with	Challenging Instar	Challenging Strain	Mean ± S.E. (days)
(a)	LS	Larvae	LS	4.0 ± 0.0[a*]
			FS1	5.0 ± 0.1[c]
	FS1		LS	4.3 ± 0.1[b]
			FS1	4.3 ± 0.1[b]
	LS	Nymphs	LS	5.1 ± 0.1[a]
			FS1	6.1 ± 0.1[b]
	FS1		LS	5.3 ± 0.1[a]
			FS1	5.9 ± 0.1[b]
(b)	LS	Larvae	LS	4.1 ± 0.0[a]
			FS2	4.4 ± 0.0[b]
	FS2		LS	4.5 ± 0.0[c]
			FS2	4.7 ± 0.1[d]
	LS	Nymphs	LS	4.7 ± 0.1[a]
			FS2	4.9 ± 0.0[b]
	FS2		LS	5.5 ± 0.1[c]
			FS2	5.5 ± 0.1[c]

*Values for each instar in each of (a) and (b) not having a common letter are significantly different ($P<0.05$).

data that the duration of feeding varied from experiment to experiment. It will also be observed that the data for larvae and nymphs were consistent, at least in one respect. That is, when larvae fed faster on homologous rabbits than on heterologous rabbits, the nymphs did the same, and vice versa. Since larvae and nymphs shared the same rabbit ear and so the factor affecting the duration of feeding may be coming from the host. In the majority of cases the duration of feeding of either instar differed between homologous and heterologous hosts. The data indicated that when a large difference occurred between the LS and a given FS when feeding on homologous hosts, the duration of feeding on heterologous hosts tended to approach that of the homologous strain. This too indicated that the host had some control on the duration of feeding.

4.6. Variation in tick size

The variances of scutal lengths of adults moulting, from nymphs fed on the same rabbits in succession (Tables 44-47) showed that variation in tick size was less during the first infestation (i.e. on susceptible rabbits) than during subsequent infestations. The coefficient of variation (CV) showed that variation of tick size in males and females was similar. CV was calculated as follows:

$$CV = \frac{\text{Standard deviation}}{\text{mean}} \times 100$$ (Sokal and Rohlf, 1969).

CV was only used in cases where means differed considerably. The data also showed that there was no difference between the variation of the size of LS adults and FS adults moulting from nymphs fed on previously exposed homologous rabbits.

Further observations were made from scutal length measurements of nymphs and adults moulting from engorged larvae and nymphs used in challenge infestations. Unfed nymphs and males showed consistently higher size variation on heterologous rabbits than on homologous rabbits previously exposed to larvae and nymphs (Tables 48-52). However, variation of unfed males from rabbits previously exposed to adult ticks were not consistent (Tables 53 and 54). Furthermore, variation of female size did not show any differences between homologous and heterologous rabbits.

TABLE 44. Variation in scutal length (mm) of adult R. appendiculatus moulting from successive infestations of 50 nymphs each per rabbit: LS males versus FS1 males (CV = coefficient of variation; SL = scutal length)

Infestation number	Number of ticks	Mean SL ± S.E.	Variance	CV
		LS Males		
1	111	2.91 ± 0.02	0.05	8
2	79	2.17 ± 0.03	0.08	13
3	81	2.11 ± 0.02	0.05	10
4	65	2.09 ± 0.03	0.07	13
		FS1 Males		
1	127	3.03 ± 0.02	0.05	8
2	100	2.48 ± 0.03	0.09	12
3	86	2.31 ± 0.03	0.07	12
4	84	2.26 ± 0.03	0.07	12

TABLE 45. Variation in scutal length (mm) of adult R. appendiculatus moulting from successive infestations of 50 nymphs each per rabbit: LS males versus FS2 males (CV = coefficient of variation; SL = scutal length)

Infestation number	Number of ticks	Mean SL ± S.E.	Variance	CV
		LS Males		
1	101	3.08 ± 0.03	0.08	9
2	108	2.44 ± 0.02	0.06	10
3	95	2.43 ± 0.02	0.06	10
4	101	2.42 ± 0.02	0.05	10
		FS2 Males		
1	96	3.35 ± 0.02	0.03	5
2	106	2.79 ± 0.04	0.14	13
3	102	2.67 ± 0.03	0.08	10
4	80	2.54 ± 0.03	0.08	11

TABLE 46. Variation in scutal length (mm) of adult R. appendiculatus moulting from successive infestations of 50 nymphs each per rabbit: LS females versus FS1 females (CV = coefficient of variation; SL = scutal length)

Infestation number	Number of ticks	Mean SL ± S.E.	Variance	CV
		LS Females		
1	106	1.30 ± 0.01	0.004	5
2	86	1.07 ± 0.01	0.016	12
3	75	1.05 ± 0.02	0.017	12
4	73	1.06 ± 0.02	0.017	12
		FS1 Females		
1	118	1.33 ± 0.01	0.005	5
2	69	1.21 ± 0.01	0.009	8
3	78	1.16 ± 0.01	0.012	10
4	82	1.12 ± 0.02	0.019	12

TABLE 47. Variation in scutal length (mm) of adult R. appendiculatus moulting from successive infestations of 50 nymphs each per rabbit: LS females versus FS2 females (CV = coefficient of variation; SL = scutal length)

Infestation number	Number of ticks	Mean SL ± S.E.	Variance	CV
		LS Females		
1	129	1.37 ± 0.01	0.005	5
2	100	1.25 ± 0.01	0.009	7
3	95	1.19 ± 0.01	0.014	10
4	101	1.16 ± 0.01	0.012	9
		FS2 Females		
1	117	1.42 ± 0.01	0.004	4
2	122	1.32 ± 0.01	0.015	9
3	93	1.27 ± 0.01	0.012	9
4	81	1.20 ± 0.01	0.011	9

TABLE 48. Variation in scutal length (mm) of adult R. appendiculatus moulting from challenge nymphs applied after 4 infestations of 50 nymphs each per rabbit: LS versus FS1 (SL = scutal length)

Strain previoulsy infested with	Strain challenged with	Number of ticks	Mean SL ± S.E.	Variance
		Males		
LS	LS	53	2.12 ± 0.03	0.04
	FS1	52	2.32 ± 0.05	0.14
FS1	LS1	84	2.22 ± 0.03	0.10
	FS1	101	2.27 ± 0.03	0.06
		Females		
LS	LS	65	1.07 ± 0.01	0.012
	FS1	51	1.11 ± 0.02	0.015
FS1	LS	95	1.09 ± 0.01	0.014
	FS1	81	1.11 ± 0.01	0.013

TABLE 49. Variation in scutal length (mm) of adult R. appendiculatus moulting from challenge nymphs applied after 4 infestations of 50 nymphs each per rabbit: LS versus FS2 (SL = scutal length)

Strain previously infested with	Strain challenged with	Number of ticks	Mean SL ± S.E.	Variance
		Males		
LS	LS	88	2.32 ± 0.02	0.05
	FS2	90	2.76 ± 0.03	0.10
FS2	LS	72	2.47 ± 0.03	0.07
	FS2	70	2.50 ± 0.03	0.06
		Females		
LS	LS	76	1.17 ± 0.01	0.015
	FS2	84	1.27 ± 0.01	0.011
FS2	LS	79	1.23 ± 0.01	0.011
	FS2	96	1.23 ± 0.01	0.008

TABLE 50. Variation in scutal length (mm) of R. appendiculatus nymphs moulting from challenge larvae applied after 4 infestations of 50 nymphs each per rabbit: LS versus FS1 (SL = scutal length)

Strain previously infested with	Strain challenged with	Number of ticks	Mean SL ± S.E.	Variance
LS	LS	84	0.391 ± 0.003	0.0007
	FS1	100	0.421 ± 0.004	0.0014
FS1	LS	135	0.397 ± 0.003	0.0011
	FS1	126	0.420 ± 0.003	0.0009

TABLE 51. Variation in scutal length (mm) of adult R. appendiculatus moulting from challenge nymphs applied after 4 infestations of 500 larvae each per rabbit: LS versus FS1 (SL = scutal length)

Strain previously infested with	Strain challenged with	Number of ticks	Mean SL ± S.E.	Variance
		Males		
LS	LS	40	2.34 ± 0.03	0.04
	FS1	54	2.67 ± 0.05	0.16
FS1	LS	38	2.47 ± 0.04	0.05
	FS1	39	2.43 ± 0.05	0.08
		Females		
LS	LS	69	1.16 ± 0.01	0.011
	FS1	66	1.22 ± 0.02	0.018
FS1	LS	29	1.22 ± 0.02	0.007
	FS1	29	1.17 ± 0.03	0.020

TABLE 52. Variation in scutal length (mm) of adult R. appendiculatus moulting from challenge nymphs applied after 4 infestations of 500 larvae each per rabbit: LS versus FS2 (SL = scutal length)

Strain previously infested with	Strain challenged with	Number of ticks	Mean SL ± S.E.	Variance
		Males		
LS	LS	61	2.28 ± 0.03	0.06
	FS2	104	3.28 ± 0.03	0.11
FS2	LS	67	2.34 ± 0.03	0.06
	FS2	93	2.48 ± 0.03	0.07
		Females		
LS	LS	79	1.18 ± 0.01	0.017
	FS2	129	1.40 ± 0.01	0.005
FS2	LS	58	1.22 ± 0.02	0.016
	FS2	111	1.24 ± 0.01	0.012

TABLE 53. Variation in scutal length (mm) of adult R. appendiculatus moulting from challenge nymphs applied after 3 infestations of 8 adults each per rabbit: LS versus FS1 (SL = scutal length)

Strain previously infested with	Strain challenged with	Number of ticks	Mean SL ± S.E.	Variance
		Males		
LS	LS	81	2.30 ± 0.03	0.07
	FS1	50	2.59 ± 0.04	0.07
FS1	LS	68	2.23 ± 0.03	0.07
	FS1	82	2.33 ± 0.03	0.06
		Females		
LS	LS	61	1.16 ± 0.02	0.018
	FSL	46	1.24 ± 0.02	0.015
FS1	LS	74	1.12 ± 0.02	0.019
	FS1	72	1.17 ± 0.01	0.015

TABLE 54. Variation in scutal length (mm) of adult R. appendiculatus moulting from challenge nymphs applied after 3 infestations of 8 adults each per rabbit: LS versus FS2 (SL = scutal length)

Strain previously infested with	Strain challenged with	Number of ticks	Mean SL ± S.E.	Variance
		Males		
LS	LS	85	2.33 ± 0.03	0.05
	FS2	100	3.27 ± 0.03	0.11
FS2	LS	87	2.87 ± 0.03	0.09
	FS2	64	2.80 ± 0.04	0.13
		Females		
LS	LS	107	1.13 ± 0.01	0.013
	FS2	136	1.41 ± 0.01	0.007
FS2	LS	115	1.32 ± 0.01	0.009
	FS2	94	1.28 ± 0.01	0.013

CHAPTER 5

DISCUSSION

The results presented here show that R. appendiculatus adults collected from the field are smaller than would be expected if they had moulted from nymphs fed on susceptible hosts. For this reason, it is being assumed that, taken as a whole, the field host population contains individuals with a fair amount of host resistance against R. appendiculatus. Furthermore, the laboratory strain and field strains had been exposed to different host species. It is against this background, therefore, that the results have been compared.

Branagan (1974) compared the feeding performance of the same LS of R. appendiculatus and that of a field strain collected at Naro Moru which, for practical purposes, is the same area from where FS2 was collected. He found no difference between them, though presumably he compared them on susceptible hosts only. The results obtained in the present investigation have similarly shown that, if any real difference does exist between the feeding and breeding performance of the LS and FS of R. appendiculatus on susceptible hosts, it would only be in favour of LS. Although LS yielded less egg weight per engorged female weight than that of FS, it yielded a higher proportion of ticks on susceptible rabbits, which together with its much smaller egg, made it have a higher fecundity than FS.

The eggs of LS were found to be smaller than those of the FS. There are at least two possibilities why this is so. 1. The original sample from which LS was raised contained smaller eggs, and has remained so ever since. This is highly unlikely since the two field strains from different areas had similar egg sizes. 2. LS was originally isolated from ticks with egg size similar to those of the FS used here, but was exposed to laboratory conditions favouring smaller egg size. It is known that organisms have the capacity to adapt to a new environment (Waddington, 1966). A good example of this is given by Anderson (1966, 1973) who reported that selection favoured larger body size of Drosophila spp. at lower temperature and smaller body size at higher temperature. In his reports, what started as phenotypic differences caused by temperature eventually became genotypic differences through 'genetic assimilation' over a few years. In the case of R. appendiculatus, female ticks that had engorged on rabbits produced smaller eggs and larvae than those engorged on cattle. This occurred for both LS and FS. Since cattle are a natural host, this indicates that the rabbit host may be exerting selection pressure for smaller size, the present smaller size of LS eggs and larvae probably being the favoured optimum (Mather, 1966) on susceptible rabbits. Similar selection pressure caused by an unnatural host on Schistosoma mansoni was reported by LoVerde et al. (1985). There is the possibility, therefore, that feeding a field strain of R. appendiculatus continually on rabbits eventually reduces the size of its instars.

The data also indicated that female R. appendiculatus fed on susceptible rabbits produced larger larvae than those fed on previously exposed rabbits. Cattle data showed the reverse effect. It is well known that fluids imbibed by feeding ticks from susceptible hosts differ from those imbibed from previously exposed hosts (Trager, 1939; Riek, 1962; Balashov, 1972; Allen, 1973; Randolph, 1979). Moreover, different host species may also differ with respect to the constituents of fluids imbibed from them. Harrison et al. (1984), for instance, showed that sera from mice and cattle hosts of Taenia saginata differed in their antigenic properties. The fact that there were differences between the size of R. appendiculatus larvae from females fed on cattle and on rabbits, and between the size of larvae from females fed on susceptible and on previously exposed hosts, suggests that the factor affecting larval size might be the quality of food provided by the host. The weight of eggs produced per given weight of engorged female was also found to be significantly greater for females fed on cattle than for those fed on rabbits, which also suggests that quality of food may be the factor involved.

All unfed instars of the FS were found to be significantly larger than those of LS. When they engorged on susceptible rabbits, larvae and nymphs of the FS remained larger than those of LS, but the females did not. The reason why the females of FS did not engorge to a bigger mean weight than that of LS is likely to be found in the duration of feeding on susceptible rabbits. Supporting this contention is the fact that serous exudate

characteristic of host resistance was observed at feeding sites of late-feeding females, and also the fact that there was an inverse relationship between mean daily engorged weights and duration of feeding. Boese (1974) reported that infestation of rabbits with Haemaphysalis leporispalustris induced host resistance between days 6 and 10. Assuming this is the case with all hard ticks, host resistance may have caused lower engorged weights than expected in FS, since they took significantly longer to feed than LS on susceptible rabbits.

The unusually low female mean engorged weight produced by LS during the first infestation of cattle with adult ticks was suspected to have been due to the effects of injection of larval homogenate for a skin test done on the animals earlier. Tick homogenates have been shown to immunize laboratory animals and cattle against ticks (Allen and Humpries, 1979; Mongi, 1980; Johnston et al. 1986)

According to Wakelin (1976) there is selection pressure on parasites to effect adaptive changes by which to elicit weaker responses or evade harmful ones. The results obtained here showed that FS yielded heavier engorged weights and higher proportions of ticks engorging on previously exposed hosts than LS. This implies that FS elicited weaker responses against themselves than did LS. Assuming that LS was originally similar to FS, it is conceivable that the ability to elicit weaker responses was lost in the absence of selection pressure from host resistance. Stewart et al. (1982)

carried out similar comparisons with B. microplus and found that a field-derived composite strain had higher fecundity than the laboratory strain, though this happened on both previously exposed and susceptible cattle hosts. This study has shown that R. appendiculatus is unlike Amblyomma americanum (Hunt and Drummond, 1983) in that both CEI and hatchability of eggs of LS females fed on susceptible hosts were similar to those of FS females.

It has been shown elsewhere (Chiera et al. 1985a; Chiera and Punyua, unpublished) that the survival of unfed R. appendiculatus is correlated with the size of ticks. Large adults of Drosophila pseudoobscura have also been shown to have a higher fecundity and longer survival than smaller ones (Anderson, 1973). All other things being equal then, FS ticks would be expected to have better survival while awaiting host than LS ticks, since the size of the former remained significantly larger after feeding on resistant hosts.

Hosts previously exposed to FS had better cross-resistance than those previously exposed to LS. Cross-resistance between the field strains used here was also found to be very high, which suggested that a recently isolated field strain might be the best candidate for use in the search for immunogens for use in tick control. It also suggested that cross-immunity may not present any problem in tick control programmes employing immunogens. Cross-resistance differed in different instars, but nymphs seemed to offer the best cross-protection.

It is not known why the size of the female in R. appendiculatus is affected less by host resistance than that of the male, but it may well be an adaptation. As pointed out above, reduction of the size of the female also affects its survival and egg production. In contrast, despite the fact that reducton of the size of the male affects its survival, it may not affect reproduction. Furthermore, Chiera et al. (1985a) showed that tiny females reproduce poorly, whilst tiny males reproduce normally. The results have indicated that the factors affecting the proportion and the weight of engorged R. appendiculatus ticks feeding successfully on resistant hosts may be different. Brown (1985) has suggested that antigens secreted early on during the feeding process might be responsible for the reduction of the proportion feeding, while other antigens secreted later might cause the reduction of engorged weight.

All the three strains had their lowest size variation when fed on susceptible rabbits, suggesting that susceptible hosts provide optimum conditions for all strains. Although only nymphs and males showed consistently lower size variation on homologous hosts than on heterologous hosts, this is an indication that homologous hosts provide more uniform conditions for the ticks than heterologous hosts.

The duration of feeding particularly of females, was found to be short when heavy infestations were used and long when light infestations were used. Branagan (1969) made similar observations

with the nymphs of the same LS of R. appendiculatus, and suggested that this was due to clustering of ticks before feeding, and the fact that smaller engorged nymphs were produced (smaller ticks take a shorter time to feed). Balashov (1972) reported longer feeding periods on resistant hosts by Rhipicephalus and Hyalomma species, and suggested that the presence of oedema and other lesions may prolong feeding. Results presented here similarly showed that the duration of larval feeding tended to increase with increasing host resistance. In contrast, FS females took consistently longer to feed on susceptible rabbits than on previously exposed ones. These results are therefore an indication that the presence of host resistance against R. appendiculatus influences the duration of feeding.

In conclusion it will be observed that the field strains of R. appendiculatus are better adapted to feeding on resistant hosts than the Muguga Laboratory strain, for as Wakelin (1984) pointed out, adaptation need only be relative since only enough progeny are required to ensure reproduction . Caution is therefore needed when interpreting results on host resistance against ticks obtained with ticks bred and maintained in the laboratory for a long time.

CHAPTER 6

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CHAPTER 7

APPENDICES

Appendix 1

Analysis of variance for y (FS2 females : those fed on cattle versus those fed on rabbits. See Figure 3)

Analysis of covariance

Item	N	x^2	xy	y^2	Correction for regression
Between series	1	44417	8815	1749	
Within series	94	1547415	1061603	742299	728311
Total	95	1591832	1070418	744048	719796

Analysis of variance for y after correction

Item	SS	N	MS	t	P
Between series	10265	1	10265	8.26	<0.01
Within series	13988	93	150		
Total	24253	94			

Appendix 2

Analysis of variance for y (LS females: those fed on cattle versus those fed on rabbits. See Figure 3)

Analysis of covariance

Item	N	x^2	xy	y^2	Correction for regression
Between series	1	320947	143075	6378	
Within series	71	474217	330879	254926	230867
Total	72	795164	473954	318707	282498

Analysis of variance for y after correction

Item	SS	N	MS	t	P
Between series	12150	1	12150	5.95	<0.001
Within series	24059	70	344		
Total	36209	71			

Appendix 3

Analysis of variance for y (LS females versus FS females fed on cattle. See Figure 4)

Analysis of covariance

Item	N	x^2	xy	y^2	Correction for regression
Between series	1	432950	323881	242289	
Within series	108	1467920	1020554	726667	709528
Total	109	1900870	1344435	968956	950884

Analysis of variance for y after correction

Item	SS	N	MS	t	P
Between series	934	1	934	2.41	<0.01
Within series	17139	107	160		
Total	18072	108			

Appendix 4

Analysis of variance for y (LS females versus FS females fed on rabbits. See Figure 4)

Analysis of covariance

Item	N	x^2	xy	y^2	Correction for regression
Between series	1	23000	23926	24890	
Within series	56	553124	369086	256846	246282
Total	57	576124	393013	281735	268100

Analysis of variance for y after correction

Item	SS	N	MS	t	P
Between series	3072	1	3072	4.00	<0.001
Within series	10563	55	192		
Total	13635	56			

Apppendix 5

Anova for Table 11

(a)

Source of variation	df	SS	MS	F
Subclasses (error)	26	0.0365	0.0014	
Infestations	3	0.0804	0.0268	14.1***
Strains	1	0.0481	0.0481	25.3***
Interaction	3	-0.0066	-0.0022	1.6 NS
Total	33	0.1584		

$F_{0.001}(1,26) = 13.7$ $F_{0.001}(3,26)=7.4$

(b)

Source of variation	df	SS	MS	F
Subclasses (error)	32	0.0176	0.0006	
Infestations	3	0.1169	0.0390	70.8***
Strains	1	0.0449	0.0449	81.6***
Interacton	3	0.0010	0.0003	0.6 NS
Total	39	0.1804		

$F_{0.001}(1,32) = 13.1$ $F_{0.001}(3,32) = 6.9$

***$P < 0.001$

Appendix 6

Anova for Table 12

(a)

Source of variation	df	SS	MS	F
Subclasses (error)	27	14.44	0.53	
Infestations	3	194.54	64.85	121.3***
Strains	1	15.09	15.09	28.2***
Interaction	3	-1.69	-0.56	-1.1 NS
Total	34	222.37		

$F_{0.001}(1,27) = 13.6$ $F_{0.001}(3,27) = 7.3$

(b)

Source of variation	df	SS	MS	F
Subclasses (error)	31	9.27	0.30	
Infestation	3	95.44	31.81	106.4***
Strains	1	19.48	19.48	65.2***
Interactions	3	-0.10	-0.03	-0.1 NS
Total	38			

$F_{0.001}(1,31) = 13.2$ $F_{0.001}(3,31) = 7.0$

*** $P<0.001$

Appendix 7

Anova for Table 13
Based on individual tick scutal lengths

Source of variation	df	SS	MS	F
Males				
Subclasses(error)	801	59.49	0.07	
Infestations	3	68.00	22.67	305.2***
Strains	1	13.76	13.76	185.3***
Interaction	3	0.62	0.21	2.8*
Total	808	141.87		
Females				
Subclasses(error)	849	8.253	0.010	
Infestations	3	5.780	1.927	198.2***
Strains	1	0.826	0.826	85.0***
Interactions	3	0.007	0.002	0.2 NS
Total	856			

$F_{0.05}(3,801) = 2.6$ $F_{0.001}(1,801) = 10.9$ $F_{0.001}(3,801) = 5.5$

$F_{0.001}(1,849) = 10.9$ $F_{0.001}(3,849) = 5.5$

*P<0.05; ***P<0.001

Appendix 8

Anova for Table 13

Male percentages versus female percentages. Comparison based on mean scutal lengths for hosts during 2nd, 3rd and 4th infestations as percentage of first infestation

Source of variation	df	SS	MS	F
LS				
Subclasses(error)	24	108.33	4.51	
Infestations	2	50.11	25.06	5.6*
Sexes	1	332.00	332.00	73.6***
Interaction	2	52.85	26.42	5.9**
Total	29	543.29		
FS2				
Subclasses(error)	22	226.00	10.27	
Infestations	2	190.69	95.35	9.3**
Sexes	1	432.93	432.93	42.1***
Interaction	2	11.56	5.78	0.6 NS
Total	27	861.18		

$F_{0.05}(2,24) = 3.4$ $F_{0.01}(2,24) = 5.6$ $F_{0.01}(2,22) = 5.7$

$F_{0.001}(1,24) = 9.3$ $F_{0.001}(1,22) = 9.6$

*$P<0.05$; **$P<0.01$; ***$P<0.001$

Appendix 9

Anova for Table 14

Source of variation	df	SS	MS	F
Males				
Subclasses(error)	725	47.90	0.07	
Infestations	3	85.23	28.41	430.0***
Strains	1	6.53	6.53	98.8***
Interaction	3	1.38	0.46	7.0***
Total	732	141.04		
Females				
Subclases(error)	679	7.859	0.012	
Infestations	3	6.458	2.153	186.0***
Strains	1	1.276	1.276	110.2***
Interaction	3	0.209	0.070	6.0***
Total	686	15.802		

$F_{0.001}(1,725) = 10.9$ $F_{0.001}(3,725) = 5.5$

$F_{0.001}(1,679) = 10.9$ $F_{0.001}(3,679) = 5.5$

***$P < 0.001$

Appendix 10

Anova for Table 15

(a)

Source of variation	df	SS	MS	F
Subclasses(error)	95	1327036	13969	
Infestations	2	1641406	820703	58.8***
Strains	1	87626	87626	6.3*
Interaction	2	61668	30834	2.2 NS
Total	100	3117736		

$F_{0.05}(1,95) = 3.9$ $F_{0.001}(2,95) = 11.6$

(b)

Source of variation	df	SS	MS	F
Subclasses(error)	99	1183154	11951	
Infestations	2	920149	460074	38.5***
Strains	1	136999	136999	11.5**
Interaction	2	144443	72222	6.0**
Total	104	2384744		

$F_{0.01}(2,99) = 4.8$ $F_{0.01}(1,99) = 6.9$ $F_{0.001}(2,99) = 7.4$

*$P<0.05$; **$P<0.01$; ***$P<0.001$

Appendix 11

Anova for Table 17

(a)

Source of variation	df	SS	MS	F
Subclasses(error)	24	1019	43	
Infestations	3	4820	1607	37.9***
Strains	1	626	626	14.8***
Interaction	3	322	107	2.5 NS
Total	31			

$F_{0.05}(3,24) = 3.0$ $F_{0.001}(1,24) = 14.0$ $F_{0.001}(3,24) = 7.6$

(b)

Source of variation	df	SS	MS	F
Subclasses(error)	29	1151	37	
Infestations	3	7269	2423	61.1***
Strains	1	747	747	18.8***
Interaction	3	348	116	2.93*
Total	36	9515		

$F_{0.05}(3,29) = 2.9$ $F_{0.001}(1,29) = 13.4$ $F_{0.001}(3,29) = 7.1$

*$P<0.05$; ***$P<0.001$

Appendix 12

Anova for Table 18

(a)

Source of variation	df	SS	MS	F
Subclasses(error)	27	1983	73	
Infestations	3	926	309	4.2*
Strains	1	132	132	1.8 NS
Interaction	3	370	123	1.7 NS
Total	34	3410		

$F_{0.05}(3,27) = 3.0$

(b)

Source of variation	df	SS	MS	F
Subclasses(error)	30	1462	49	
Infestations	3	682	227	4.7**
Strains	1	41	41	0.8 NS
Interaction	3	262	87	1.8 NS
Total	37	2446		

$F_{0.001}(3,30) = 4.5$

*$P < 0.05$; **$P < 0.01$

Appendix 13

Anova for Table 19

(a)

Source of variation	df	SS	MS	F
Subclasses(error)	24	16.8	0.7	
Infestations	2	4.2	2.1	3.0 NS
Strains	1	0.0	0.0	0.0 NS
Interaction	2	6.2	3.1	4.4*
Total	29	27.2		

$F_{0.05}(2,24) = 3.4$

(b)

Source of variation	df	SS	MS	F
Subclasses(error)	24	19.2	0.8	
Infestations	2	1.8	0.9	1.1 NS
Strains	1	0.3	0.3	0.4 NS
Interaction	2	4.2	2.1	2.6 NS
Total	29	25.5		

*P 0.05