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Contrasting effects of sera from rabbits and cattle infested with ticks on the in vitro feeding performance of the tick *Rhipicephalus appendiculatus*

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ABSTRACT

Lösel, P.M., Guerin, P.M. and Diehl, P.A., 1993. Contrasting effects of sera from rabbits and cattle infested with ticks on the in vitro feeding performance of the tick *Rhipicephalus appendiculatus*. *Vet. Parasitol.*, 47: 355–360.

The in vitro feeding activity of partially engorged *Rhipicephalus appendiculatus* (Neumann) (Acari: Ixodidae) females fed on sera from uninfested hosts was compared to that of ticks fed serum of hosts which had previously been infested with ticks. Although ticks fed best on sera from bovid hosts which had no prior exposure to this ectoparasite, two infestations of a bovid had no significant effect on the acceptability of its serum. In contrast, ticks fed sera from rabbits which had twice been infested with ticks gained significantly less weight than those fed serum obtained from the same animals before the infestations. Clearly there is a difference between natural host resistance and that of laboratory animals.

INTRODUCTION

Rhipicephalus appendiculatus (Acari: Ixodidae) is a vector of African East Coast fever, a disease of cattle caused by the protozoan *Theileria parva*, which seriously constrains the economic viability of livestock-farming in much of Southern, Eastern and Central Africa. The pathogen develops in the salivary glands of infected ticks, and is introduced into the host via saliva released by the tick during feeding. Attacking the vector by regular dipping or spraying of

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cattle with acaricides disrupts the disease cycle and is the principal control strategy currently employed. The high costs of chemical control agents, concern over the environmental damage they may cause and the appearance of acaricide-resistant tick strains have stimulated research into alternative control methods. One of these is to exploit the natural tick-resistance of the host.

Wikel and Allen (1982) distinguished genetically determined tick-resistance, innate to the host, from naturally acquired resistance induced in susceptible hosts by prior contact with the ectoparasite. Hope of exploiting the latter as a model for anti-tick vaccines has encouraged an intensive search for resistance-triggering tick antigens (cf. Kemp et al., 1986; Nyindo et al., 1989; Dhadialla et al., 1990). Although knowledge of naturally acquired resistance mechanisms remains incomplete, both cellular and humoral immunological mediators are involved (Wikel, 1988). By the passive transfer of such resistance in the serum of a resistant guinea pig to a tick-susceptible one, Trager (1939) demonstrated the importance of the humoral component. Results of a recent study by Rechav et al. (1991) on in vivo effects of induced resistance to *Rhipicephalus evertsi evertsi* in rabbits and guinea pigs corroborates this finding. In this study, a very simple in vitro feeding assay, of a type which might be employed for laboratory 'screening' for tick-resistance, is used to test the effect on *R. appendiculatus* of sera from multiple-infested rabbits and bovids.

MATERIALS AND METHODS

Female *R. appendiculatus* (35–70 mg) which had fed on rabbits for 6 days prior to the experiment were used in all tests. These females, arising from a mixed infestation of 50 males and 50 females, were known from previous experiments to show the most rapid in vitro weight gains. Full details of the feeding method are given in Lösel et al. (1992). Ticks were fed test sera from glass capillaries (Chabaud, 1950) for a period of 18 h (8:10 h light:dark regime at 28 ± 1 °C and 98% RH in an environmental cabinet). The weight of the ticks was determined at the beginning and end of the test period and the weight changes over this time were expressed as a percentage of the tick's initial weight. The amounts of excrement released by these partially engorged ticks during feeding were negligible.

Supply of test sera

Rabbit sera were prepared by centrifugation (3000 rpm for 15 min) of blood samples (8–12 ml) collected from the marginal ear vein of four, 2.5 kg, male, New Zealand White rabbits. Control blood samples were collected 2 weeks before the first infestation with 50 pairs of unfed, 3-month-old male and female ticks which were confined to the rabbits' ears in cloth bags. The 'in-

fested' blood sample was taken 2 weeks after removal of the last tick from a second such infestation.

Samples of both control and infested bovine serum from Africa (Ayrshire breed) and Europe (Simmentaler breed) were generously provided by Dr. S. Essuman of the International Centre for Insect Physiology and Ecology (I.C.I.P.E.), Nairobi, Kenya, and Dr. B. Rutti of this Institute. The procedure with cattle differed from that in rabbits in that 100 pairs of ticks were confined to the hosts' tails by means of taped cloth bags.

The non-parametric Mann–Whitney *U*-test was employed for statistical comparison of weight gains for ticks fed control and infested sera.

RESULTS AND DISCUSSION

Partially engorged female ticks gained significantly less weight on infested rabbit serum than on Swiss and African bovid sera (Table 1). Ticks feeding on serum from twice-infested rabbits gained three times less weight than did those feeding on serum from the same rabbits before the first infestation ($P<0.001$). In contrast, ticks feeding on serum of the tick-naïve Swiss bovid showed weight gains averaging 52% compared with 84% for ticks feeding on serum of the same animal post-infestation. Similarly, for ticks feeding on sera prepared from the African bovid blood samples, the mean weight gain of 71% on serum from the tick-naïve animals was little different from that recorded for ticks fed the post-infestation serum sample (65%). Thus, for both bovid hosts, weight gain differences between ticks fed control and infested sera were not significant.

TABLE 1

Rhipicephalus appendiculatus weight gains expressed as a percentage of the initial weight in 18 h in vitro feeding experiments with serum from naïve and infested rabbit and bovine hosts. Glass capillaries served as the serum reservoir

Host animal	% weight gains (mean ± S.E.)		
	Rabbit	Swiss bovid[1]	Kenyan bovid[2]
Control serum	60.84 (±7.7)	51.36 (±8.97)	71.23 (±8.48)
Infested serum	24.71 (±3.7) $n=31$	84.18 (±9.29) $n=8$	65.39 (±13.1) $n=10$
Significance by Mann–Whitney *U*-test	$P<0.001$	$P>0.05$	$P>0.05$

[1]Simmentaler breed.
[2]Ayrshire breed.

The results of the in vitro tests on rabbit sera correlate with observations, reviewed by Wikel (1988), of tick-feeding inhibition in vivo on various small mammal hosts made resistant by prior infestations, and similar observations made in this laboratory with ticks on infested hosts. Notwithstanding the discrepancy in the number of ticks per kg body weight on the bovids and rabbits in this study, naturally acquired immunity in the rabbit clearly involves an as yet unknown, significant and long-lasting humoral component which disrupts the tick's feeding either by causing it physical injury or by altering its feeding behaviour.

Walker and Fletcher (1986) have shown that expansion of the gut, and consequently the size of the blood meal, are limited by the severe disruption of gut epithelium stem cells in *R. appendiculatus* feeding in vivo on resistant hosts. Host resistance in this case seems to correlate with increased concentrations of eosinophils and basophils at the feeding site. Being pre-fed on susceptible hosts, it may be assumed that the gut of ticks used in the present study had developed normally, and indeed weight gains of over 50% were recorded on five of the six serum types tested. The fact that feeding is so strongly disrupted with serum from multiply infested rabbits points to the presence of agents capable of directly interfering with normal feeding. The absence of white blood cells in the serum precludes their involvement. A role for complement, a component of both serum and extravascular fluids in the skin (Jose, 1987), as a mediator of induced resistance has been proposed (cf. Kemp et al., 1986; Brossard and Papatheodorou, 1990). Many other inflammatory mediators, e.g. cytokines, interleukins and arachidonic acid metabolites (reviewed by Camp and Greaves, 1987), released either systemically or locally as part of the host's immune response to tick infestations may also be involved. These substances may act directly on the tick's feeding process or through changes effected in the host's metabolism resulting in changes in the serum composition, as for example a reduction in albumen and increase in "acute phase plasma proteins" discussed by Billingham (1987). Lösel et al. (1992) discuss evidence for a sensory basis for the 'antifeedant' effect of the infested rabbit serum. Gustatory sensilla on the tick's cheliceral denticles may 'sense' factors in resistant serum which deter feeding.

The markedly different in vitro feeding responses of *R. appendiculatus* to infested sera of bovids and rabbits are surprising. Walker and Fletcher (1986) have shown that the effects of acquired resistance on the host's immune response and its pathological consequences on the tick in subsequent feeding attempts are similar in both species. Subsequent comparison of the salivary gland ultrastructure of partially engorged *R. appendiculatus* by the same authors revealed Type II acini of ticks fed on rabbits to be of larger diameter and richer in secretory granules than those of ticks which had fed on a bovid (Walker and Fletcher, 1990); values for both parameters, however, were greater for rabbit- and bovid-fed ticks which had been in contact with an in-

fested host. Manipulation of the host immune response by effectors secreted in tick saliva has been proposed by several authors (Chinery and Aitey-Smith, 1977; Ribeiro et al., 1985, 1990). From the results of Walker and Fletcher (1990), it appears that salivary gland secretory activity may vary depending on the immune-response of the host. The effects of 'rabbit adaptation' which might have been expected in these ticks, originating from an I.C.I.P.E. culture maintained exclusively on rabbits for some 30 years, make the strength of response induced in this host all the more surprising. The above conflict in natural host and laboratory animal responses underlines the difficulties and limits with which resistance phenomena observed in small mammal studies can be projected onto the case of the actual bovine host.

Unlike the attachment phase where selectivity for a feeding site or food 'quality' would confer an advantage on the unfed adult, the partially fed female used in the above tests is committed to the feeding attempt. Since fecundity will depend upon the size of the blood meal assimilated, a tendency to maximise uptake of serum can be expected. The fact that ticks fed infested rabbit sera feed little as compared to infested bovid sera in this study underlines the strength of the immune response which can be induced in this unusual host by prior exposure to ticks.

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