

Cross Reactivity between Instars of the *Rhipicephalus sanguineus* (Latreille, 1806) Tick

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INTRODUCTION

The brown dog tick *Rhipicephalus sanguineus* is probably the most prevalent of ixodid species and is confined almost entirely to domestic dogs.¹ It has been linked to several tick-borne diseases such as spotted and boutonneuse fever² and ehrlichioses³ in man, and babesiosis and ehrlichioses in dogs.⁴ Dogs, but not guinea pigs, are unable to develop resistance against *R. sanguineus* even after repeated feeding.⁵⁻⁷ Intradermal skin testing with whole unfed adult *R. sanguineus* extract of previously infested hosts⁸ induced an immediate and a delayed hypersensitivity reaction in guinea pigs. Dogs, on the other hand, developed a strong immediate reaction, but a second peak in skin reaction was not observed, suggesting that cellular immunity (Th1-type) is an important mechanism of resistance to *R. sanguineus* ticks.

The objective of the work reported here was to detect cross resistance among larvæ, nymphs, and adults of *R. sanguineus* ticks fed on guinea pigs, as well as determine the type of hypersensitivity reaction elicited by adult tick antigens on larva- or nymph-infested guinea pigs.

MATERIAL AND METHODS

All instars of *R. sanguineus* ticks were supplied from a laboratory colony, kept at 29°C, relative humidity 80%, and 12:12 L:D photoperiod. Four-month-old tick bite naïve female guinea pigs, were used as hosts.

Ticks were released inside a plastic feeding chamber glued on the shaved back of each guinea pig. Primary and challenge infestations were separated by a 30-day interval. Infestation of each guinea pig consisted of 250 larvæ, 100 nymphs, or five pairs of adults, unless otherwise stated.

Two experiments were performed. In the first, guinea pigs were infested with either larvæ, nymphs, or adults and challenged with adults. In the second experiment, guinea pigs were infested with larvæ or nymphs and submitted to a cutaneous hypersensitivity test (CHT) with unfed adult tick extract (UATE) in the ear. Control, tick bite-naïve guinea pig groups were included in the challenge infestations and

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CHT. Cross reactivity was evaluated by comparing feeding and reproductive performance of ticks in challenge infestations and CHT to UATE.

The unfed adult tick extract (UATE) was prepared as described elsewhere⁸ and injected into the dermis of the left ear (15 µg in 0.03 ml of PBS) of guinea pigs 20 days after an infestation with 400 larvæ or 200 nymphs. Local reactions were evaluated according to Szabó *et al.*⁸

An *F*-test was used for ANOVA. Means were compared by using the Tukey test ($p < 0.05$).

RESULTS

Primary Infestation with Larvæ, Nymphs, or Adults and Challenge Infestation with Adult Ticks

Decreased female and egg mass weights were observed during challenge infestations following primary infestation with larvæ, nymphs, or adult ticks (see TABLE 1). Female and egg mass weight drops were significant following primary infestation with adults and nymphs, respectively.

Cutaneous Hypersensitivity Reaction Induced by Unfed Adult Tick Extract on Larvæ and Nymph Infested Guinea Pigs

The results are shown in FIGURE 1. Larvæ infested guinea pigs developed a delayed reaction but nymph infested guinea pigs displayed a small immediate reaction (38% increase in ear thickness two hours postinoculation) and a strong delayed reaction (61% increase in ear thickness by 18 hours). Ear thickness increase of larva sensitized animals was significantly different from that of naïve animals 48 hours after extract inoculation. In the case of nymph sensitized guinea pigs, the ear thickness increase was significant 2, 18, 24, and 48 hours postextract inoculation. Control, tick bite naïve guinea pigs developed only mild reactions throughout the experiment.

TABLE 1. Biological parameters of *Rhipicephalus sanguineus* ticks fed on guinea pigs following primary infestation with larvæ, nymphs, or adults. Sample numbers for each parameter are bracketed

	Larvæ	Nymphs	Adults	Control
Female weight	37.5 ab	25.0 ab	27.7 b	63.1 a
± SD (mg)	21.5 (14)	27.8 (6)	30.8 (14)	35.1 (22)
Egg mass weight	10.7 ab	0.8 b	14.3 ab	26.5 a
± SD (mg)	13.5 (14)	0.7 (6)	15.0 (9)	19.4 (22)

NOTE: Thirty female ticks were released on each experimental group of six guinea pigs. Means in a row followed by the same letter do not differ significantly ($p < 0.05$).

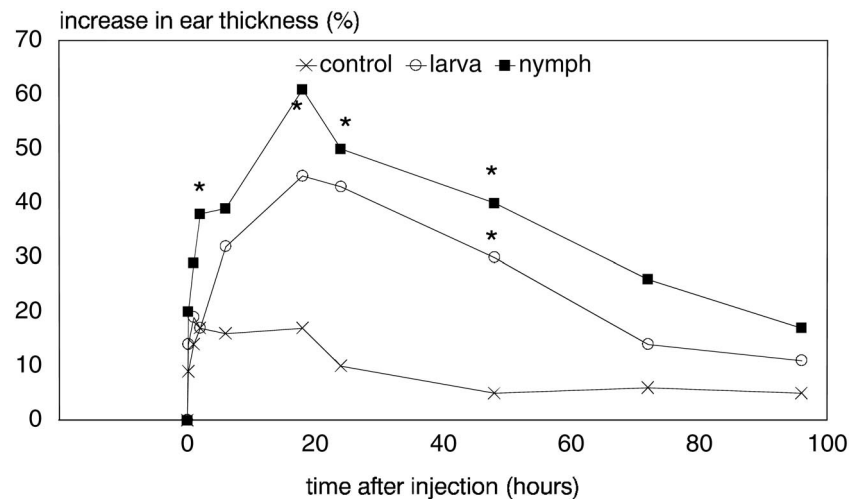


FIGURE 1. Cutaneous hypersensitivity induced by unfed adult *Rhipicephalus sanguineus* tick extract in the ear of guinea pigs sensitized by infestation with larvæ or nymphs. Results are expressed as mean percentage change in ear thickness following extract inoculation in relation to preinoculation values. Means refer to seven (control group) or ten (larva or nymph infested groups) guinea pigs. *Significantly different from control means ($p < 0.05$).

DISCUSSION

Previous work had demonstrated that dogs are unable to develop resistance to adult *R. sanguineus* ticks, even after repeated feeding^{1,5-7} whereas guinea pigs develop strong resistance after one infestation of adult ticks.⁷ Resistance induction in dogs against *R. sanguineus* tick by immunization is being investigated,^{10,11} and knowledge of cross reactivity among *R. sanguineus* instars is necessary to aid in the development of more efficient immunization protocols. In the present work, guinea pigs were chosen for the evaluation of cross resistance among *R. sanguineus* tick instars since this host is more likely to develop resistance against immature stages of this tick species.

It was shown here that a primary infestation with nymphs or adults induces resistance in guinea pigs to subsequent infestation with adult *R. sanguineus* ticks. Nymphs induced the strongest immunity, as observed by the greatest decrease in female and egg mass weight. Adults also induced a strong resistance to a subsequent challenge infestation, as shown by significantly reduced female weights. On the other hand, although female and egg mass weights were greatly decreased, primary infestation with larvæ did not lead to statistically significant differences in the observed parameters for female ticks during challenge infestation.

Immunity of hosts to parasites depends on the recognition of proper antigen target. However, a proper immune mechanism against this target antigen is also needed for the development of resistance. After the description of two types of murine helper T cell clones, T helper 1 (Th1) and T helper 2 (Th2), by Mosmann and coworkers,¹²

it became clear that an immune reaction to the same antigen may lead to a different outcome in terms of immunity (resistance) depending on the T cell clone (Th1 or Th2) involved in the reaction.^{13,14}

The intradermal skin testing of whole unfed adult *R. sanguineus* extract in previously infested hosts⁸ with adult ticks induced a small immediate and an intense delayed hypersensitivity reaction in guinea pigs. Dogs, on the other hand, developed a strong immediate reaction, but a second peak in skin reaction was not observed,⁸ suggesting that cellular immunity (Th1-type) is an important mechanism of resistance to *R. sanguineus* ticks. Failure to mount a delayed hypersensitivity could be attributed to the host species, but delayed cutaneous reactions in dogs to flea¹⁵ and *Leishmania antagens*¹⁶ have been reported. Furthermore, a link between a Th2 immune response and lack of protective immunity to this tick species was demonstrated recently.¹⁷

It was concluded that guinea pigs recognize appropriate adult *R. sanguineus* antigen targets and also mount an efficient immune reaction against them. In the present work guinea pigs, sensitized with infestations with larvæ or nymphs and submitted to cutaneous hypersensitivity test (CHT) to unfed adult *R. sanguineus* extract, developed a delayed hypersensitivity reaction, but only a slight immediate reaction was seen in nymph infested guinea pigs. These results indicate that immature and adults stages of *R. sanguineus* tick share common antigenic determinants that induce similar cutaneous hypersensitivity in guinea pigs.

The results from cross resistance and CHT experiments showed that it is worthwhile to search for an antigen common to the three stages of *R. sanguineus* tick and to include this in a vaccine for the immunization of dogs. This antigen, however, needs to be one that induces a delayed type hypersensitivity in dogs. This type of immune reaction may also be enhanced by an appropriate adjuvant.

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