



***Amblyomma cajennense* ticks induce immediate hypersensitivity in horses and donkeys**

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Abstract. Since host immune reaction to ticks interferes with tick-borne pathogen transmission, it is important to recognize naturally occurring tick–host immune relationships to better understand the epidemiology of such infectious diseases. *Amblyomma cajennense* is an important tick-borne disease vector in the Neotropical region and horses maintain it in domestic environments. In the present work intradermal testing of *A. cajennense* tick exposed horses and donkeys using crude tick antigens was used to evaluate the type of hypersensitivity induced by infestations. Animals sensitized by *A. cajennense* infestation displayed an immediate hypersensitivity reaction at the antigen inoculation site. Foals sensitized with experimental infestations and field sensitized horses presented the most intense reactions (40% of ear thickness increase). Field sensitized donkeys presented less intense reaction reaching no more than 22% of mean thickness increase. Control horses (non-sensitized) had the least intense reaction, with a peak of no more than 12% of increase. The presence of a prominent immediate hypersensitivity in equids sensitized experimentally or by field infestations indicates that *A. cajennense* ticks induce in this host an immune response that is associated with IgE production and which is known to be inappropriate against intracellular pathogens. Differences observed between horses and donkeys are discussed.

Introduction

Ixodid and argasid ticks are of medical and veterinary importance due to direct damage caused by feeding and transmission of disease causing agents. *Amblyomma cajennense*, the cayenne tick, is the main vector of *Rickettsia rickettsii*, the agent of spotted fever in the Neotropical region (reviewed by Campos Pereira and Labruna 1998). This tick species may also be involved in the transmission of the unknown causative agent of Lyme-simile disease of humans in Brazil (Guimarães et al. 2001).

Amblyomma cajennense, is widely distributed throughout the American continent, from the south of the US, through Central America to northern Argentina (Robinson, 1926). This tick species is commonly found on many domestic and wild

animals, but has also been found on humans (Guimarães et al., 2001). Although the native capybaras and tapirs are believed to be the main natural host for *A. cajennense*, horses are also known to act as primary host for all parasitic stages of this tick, and are capable of maintaining high tick populations in pastures (Aragão, 1936; Labruna et al., 2001).

Tick infestation sites on hosts are characterized by an inflammatory reaction modulated by both the host immune response and tick saliva (Wikel, 1996; Willadsen and Jongejan, 1999). The outcome of such interaction depends on the host and tick species involved and, at one end, ticks might engorge fully and develop into the next stage or, on the other end, they may be severely affected and even die. Following tick infestation, susceptible mice hosts develop an immune reaction characterized by Th₂ cytokine response (Ferreira and Silva, 1999; Mejri et al., 2001). This type of immune response of the host is associated to susceptibility to intracellular pathogens transmitted by ticks (Zeidner et al., 1997). Since a strong and effective host immune reactions to ticks interfere with tick-borne pathogen transmission (Wikel et al., 1997), it is important to recognize naturally occurring tick–host immune relationships to better understand the epidemiology of such infectious diseases.

It has been recently observed that donkeys, but not horses, are strongly resistant to *A. cajennense* ticks (Castagnolli et al. 2003). Considering the importance of *A. cajennense* tick in disease vectoring and its maintenance by horses in the domestic environment, it would be worthwhile to evaluate immune reaction induced by this tick species in horses. Moreover, if alternative control measures such as vaccination against *A. cajennense* is to be developed, information of naturally occurring immunity might be necessary.

In the present work, intradermal testing of *A. cajennense* tick-exposed horses and donkeys using crude tick antigens was used to evaluate animal hypersensitivity induced by infestations. Hypersensitivity diseases are commonly classified according to the type of immune response and the effector mechanism responsible for cell and tissue injury (Abbas et al., 2000); hence cutaneous hypersensitivity reactions elicited by intradermal testing can be used to broadly determine prevailing immune responses in hosts to antigens.

Materials and methods

Ticks

An *A. cajennense* tick colony was set up in the laboratory to obtain unfed ticks. Initially, engorged females were collected from horses of Instituto de Zootecnia de Colina, São Paulo State, Brazil. The colony was maintained at approximately 29 °C, 85% RH (held over saturated KCl), and on a 12:12 photoperiod. For continuous tick supply colony ticks were fed on previously uninfested New Zealand white rabbits. Unfed ticks used in experiments were from 1 to 3 weeks old in the case of immature stages and at most 4 weeks old in the case of adults.

Hosts

Horses (*Equus caballus*) and donkeys (*Equus asinus*) were used as experimental hosts. All animals were either lent by neighboring farmers or donated by a highway maintenance company (Autovias S/A). For the experiments horses and donkeys were taken to Franca University's Veterinary Teaching Hospital or to Centro de Pesquisa em Sanidade Animal-UNESP (CPPAR), Jaboticabal-SP, stalls.

Tick-bite naïve, control, horses were obtained by breeding initially, acaricide treated pregnant mares, and later their offsprings in tick-free stalls as described elsewhere (Castagnolli et al., 2003). Field sensitized horses and donkeys used for experiments had natural *A. nitens* and *A. cajennense* tick infestations as well as endoparasites (Strongyle fecal eggs, one donkey had also *Parascaris equorum* fecal eggs). These animals were not treated against ecto or endoparasites to closely represent field prevailing conditions. All animals were given commercial ration according to their weight and water '*ad libitum*'.

Infestation procedures

For each experimental infestation two feeding chambers (5 cm diameter) were fixed to the shaved back of the hosts with an adhesive glue (Brascoplast[®] Brascola Ltda, Brazil), each. The next day 100 larvae, 30 nymphs and six pairs of adults were added to each chamber.

Experimental groups

Six tick-bite naïve foals (three males and three females), 5–7 month old, were infested three times with an approximate 30 day interval between the beginning of each infestation; 10 field sensitized horses (three males and seven females) and five field sensitized donkeys (three males and two females) were infested once. The control group consisted of four tick-bite naïve foals.

Tick extract

Approximately 100 unfed adults of *A. cajennense* were killed by freezing, homogenized with a ground glass homogenizer in phosphate buffered saline (PBS, pH 7.2) and then sonicated (Branson Sonifier 250). The extract was centrifuged at 4 °C for 1 h at 12,000 × *g*, the supernatant filtered through a 0.22 µm Millex-GV (Millipore) filter and stored at –40 °C until use. The protein concentration of this 'unfed adult extract' (UAE) was 2.1 mg/ml (technique of Lowry et al., 1951).

Intradermal test

Intradermal testing were performed from 15 to 30 days following the last experimental infestation of each host. Animals were sedated with 0.04 mg/kg

Romifidine[®] (Sedivet Boehringer Ingelheim, Brasil). UAE was injected into the dermis of the left ear of horses and donkeys (50 µg in 0.1 ml PBS) and PBS only into dermis of the right ear. Preliminary tests with horses had shown that such a dose of antigen induces an intense local reaction without systemic signs. Right ear reactions were considered non-specific inflammation. Reactions were evaluated by measuring ear thickness at 10 min and then 1, 2, 6, 24, 48, 72 and 96 h post UAE injection. The thickness of both ears was measured three times at each interval with a Mitutoyo[®] caliper (designed originally for the measurement of sheet of paper thickness) and results were expressed as mean percentual changes in the ear thickness in relation to pre-inoculation values. Release of the caliper pressure over the ear at the injection sites was done gently to avoid much squeezing of the edematous tissues, very soft at initial periods. The increase in ear thickness induced by UAE at each time was calculated by subtracting values measured in the right ear from those of the left ear.

Data analysis

Differences in ear thickness within groups at various post-inoculation times were determined using repeated measures analysis of variance. Means were compared by Tukey's Test. Differences between groups were determined by Kruskal–Wallis test and means were compared using Dunn's multiple comparison test. Differences were considered significant when *p* values were less than 0.05.

Results

Tick infestations

Many of the ticks released inside the feeding chambers engorged in all animals, although ticks on field sensitized donkeys and horses fed for, respectively, 3 and 4 days longer. Hence all infested animals were sensitized. Details on tick biological parameters from all experimental infestations are presented elsewhere (Castagnolli et al., 2003).

Intradermal test

All animals sensitized by *A. cajennense* infestations, except for one field sensitized horse (anergic), displayed an immediate hypersensitivity reaction at the antigen inoculation site. Significance of this immediate reaction could be observed by the significant decrease of mean ear thickness from the initial measurements (10 min – 2 h) towards the last ones (72–96 h) when the reaction had almost subsided (Figure 1 and Table 1). Control horses (non-sensitized) had a small reaction with a small peak of no more 12% of increase 1 h post inoculation. In these animals ear

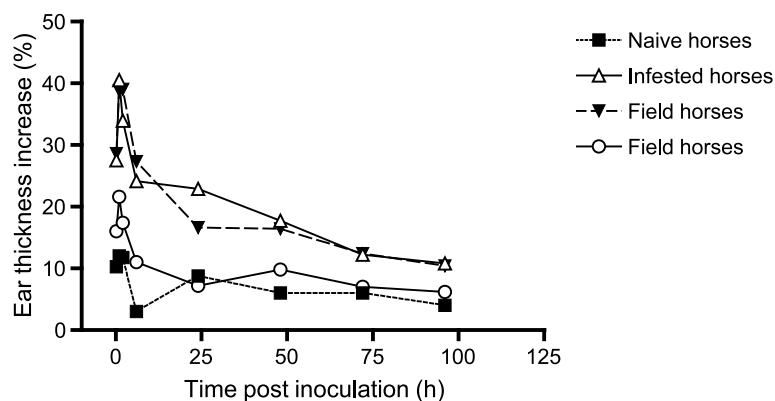


Figure 1. Percent ear thickness increase during an intradermal skin test with 50 µg of *A. cajennense* tick extract in experimentally or field sensitized equids.

thickness did not vary significantly along the measuring period. The immediate reaction of sensitized equids was characterized by a soft swelling irregular area (wheal) with an approximate diameter of 2–3 cm. The peak of this reaction was observed at 1–2 h post inoculation. A second peak of thickness increase was seen only in one field sensitized horse. Foals sensitized with experimental infestations and field sensitized horses presented the most intense reactions (around 40% of ear thickness increase) and which decreased rapidly at first (6 h) and more slowly afterwards. Donkeys displayed a less intense reaction attaining no more than 22% increase in mean ear thickness. This reaction decreased rapidly and from 24 h on was similar to that observed in control animals. A high degree of variation was observed within each group at each time interval and for this reason only the ear increase of foals 10 min after the inoculation of UAE did differ significantly from control group.

Discussion

Our results showed that sensitized equids reacted to tick extracts with an immediate reaction consisting of a soft swelling at the antigen inoculation site. This type of reaction is similar to the immediate hypersensitivity reaction of humans also called type I hypersensitivity. Such reaction is initiated by antigen binding to IgE pre-attached to mast cells or basophils and that leads to inflammatory mediator release. Vascular leakage of plasma, vasodilatation inflammatory infiltrates enriched in eosinophils, basophils and Th₂ cells are characteristic of this type of reaction (Abbas et al. 2000).

In the case of sensitized equids, *A. cajennense* tick attachment sites had in the dermis an inflammatory infiltrate rich in eosinophils and a few basophils (Szabó, M.P.J., Santana, D.A, Castagnolli, K.C., Castro, M.B. and Romano, M.A.

Table 1. Means, standard deviation and statistical analysis of the percent ear thickness increase during an intradermal skin test with 50 µg of *A. cajennense* tick extract in experimentally or field sensitized equids.

Time post inoculation	10 min	1 h	2 h	6 h	24 h	48 h	72 h	96 h
Naive horses ($n=4$)	10.2 ^{aA} ± 10.0	12.00 ^{aA} ± 10.4	11.7 ^{aA} ± 10.4	3.0 ^{aA} ± 2.4	8.7 ^{aA} ± 6.7	6.0 ^{aA} ± 5.7	6.0 ^{aA} ± 8.0	4.0 ^{aA} ± 4.7
Foals ($n=6$)	32.2 ^{acB} ± 15.3	40.5 ^{aA} ± 20.0	33.9 ^{abcA} ± 23.8	24.2 ^{abcA} ± 24.5	22.9 ^{abcA} ± 14.6	17.7 ^{cA} ± 8.7	12.2 ^{bcA} ± 11.8	10.8 ^{bcA} ± 8.4
Field Horses ($n=10$)	28.6 ^{abAB} ± 14.5	38.8 ^{aA} ± 22.5	39.0 ^{aA} ± 25.3	27.3 ^{abA} ± 12.1	16.6 ^{bcA} ± 15.5	16.4 ^{bcA} ± 15.8	12.4 ^{bcA} ± 10.8	10.4 ^{cA} ± 9.1
Donkeys ($n=5$)	16.0 ^{abcAB} ± 7.4	21.6 ^{aA} ± 3.9	17.4 ^{abA} ± 4.1	11.0 ^{bdA} ± 8.8	7.2 ^{cdA} ± 7.2	9.8 ^{bdA} ± 7.1	7.0 ^{cdA} ± 4.3	6.2 ^{dA} ± 7.7

Means in a row followed by the same lower case letter do not differ significantly ($p < 0.05$). Means in a column followed by the same capital letter do not differ significantly ($p < 0.05$).

unpublished data). Horses are known to present hypersensitivity I associated immune reactions (Magro et al., 1988) and diseases (Lorch et al., 2001). Moreover, Th₂ associated immune response in horses has already been described (Aggarwal and Holmes, 1999; Lavoie et al., 2001). Thus observations described above and data from the present work suggest that *A. cajennense* ticks induce in equids a type I hypersensitivity and we can speculate that a Th₂-like response is then a feature of this sensitization.

Even though immune mechanisms induced by tick-bite in all experimental groups from the present work seemed to be similar, some important differences have to be considered. For example, antigen presentation differed considerably among groups. One group of foals was submitted to three controlled infestations in 3-months time. Such infestation regimen differed from field sensitization because it was discontinuous, ticks attached to a restricted host surface and infestation intensity was lower than those usually observed in the field. It must be noted that these animals also had decreased stimuli of other environmental parasites such as worms and soil bacteria. Field sensitized animals, on the other hand, were submitted to naturally occurring parasitic stimuli including continuous tick infestations and one experimental infestation. Considering such differences in the antigen exposure in these two groups and also that reaction of field sensitized horses and that of foals, sensitized only experimentally, were similar, it is possible to suggest that *A. cajennense* tick-bite sensitization is not significantly altered by the many other antigenic inputs existing under field conditions. It must be observed however that field conditions might still allow for some variation in individual hypersensitivity reactions as seen by the presence of an anergic horse and another exhibiting a second peak among field sensitized animals.

Other important difference among experimental groups refers to resistance to ticks. Donkeys have been shown to express a certain degree of resistance to *A. cajennense* ticks when compared to horses (Castagnolli et al. 2003) but also to display, even though not significantly, a less intense ear thickness increase at intradermal testing site. Such a decreased thickness could be attributed, in this host, to a diminished swelling in the harder ear tissues which were then less prone to dilation or, alternatively, to a less prominent hypersensitivity I reaction.

Overall results described in the present work corroborate with previous reports which showed that naturally occurring tick host relationships are characterized by only a faint resistance of the host and development of an immediate hypersensitivity to tick antigens (Szabó et al., 1995; Bechara et al., 2000). These very same relationships are also characterized by the maintenance of important and widespread tick-borne diseases, as is the case of Ehrlichiosis in the dog–*Rhipicephalus sanguineus* relationship and Babesiosis in the case of European cattle and its tick *Boophilus microplus* (Guimarães et al., 2001). Taken together these observations suggest that immediate hypersensitivity might be a pattern in the immune response elicited by susceptible hosts to ticks and which favors transmission of tick-borne diseases. Further investigations are needed to characterize such immune reaction in greater detail for each host species and to determine its importance for pathogen vectoring of *A. cajennense* ticks to horses and donkeys.

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