Cross-reactivity between antigens from *Amblyomma cajennense* and *A. hebraeum* (Acari: Ixodidae)

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ABSTRACT

Laboratory animals exposed to feeding ticks develop resistance which is reflected by a decline in tick engorgement weight, egg-laying by adults and reduced egg viability. Serum antibodies from these hosts and their reaction with tick antigens have been detected by different methods, including precipitation techniques, immunofluorescent techniques, ELISA and Western blots. However, little is known about the effects of antibodies on ticks that engorge on resistant hosts, or which tissues of the tick body are possibly immunogenic. Some researchers, using immunohistochemistry, have detected host antibodies in the gut, salivary glands and haemolymph of ticks engorged on resistant animals. The same technique has helped considerably in determining antigenic sites or antibody targets in other arthropods. Consequently, immunohistochemistry techniques were used in this study to detect cross-reactivity between sera raised against *Amblyomma cajennense* (Fabricius, 1787) with *Amblyomma hebraeum* (Koch, 1844), and vice versa. The results show the existence of shared antigens between the 2 tick species. In general, our results point more to a 1-way cross-reactivity of *A. hebraeum* with *A. cajennense* than a reciprocal cross-reactivity, suggesting that *A. hebraeum* is more immunogenic than *A. cajennense.*

Key words: *Amblyomma cajennense, Amblyomma hebraeum,* antigens, cross-reactivity.

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INTRODUCTION

Ticks are of medical as well as veterinary importance. As vectors of human diseases, they have been ranked 2nd to mosquitoes. As vectors of animal diseases they are considered to be the most important arthropods. Since they are obligatory blood-sucking ectoparasites with lengthy and physiologically slow life cycles, they constitute very important vectors and reservoirs of rickettsia, spirochaetes, protozoa, bacteria and viruses. The control of ticks has been a major concern because of the very high costs of chemical methods of control usually practiced, the environmental contamination it causes, and the development of tick resistance to parasiticides.

It is well established that various species of ixodid ticks induce a degree of resistance in the host and that resistance is immunologically mediated[14]. The nature of the immunological response following tick infestation varies with the tick–host model. This aspect has been comprehensively reviewed.[3,13]

Laboratory animals repeatedly infested with some tick species have been reported to acquire natural immunity manifested by decreased engorgement weight and decreased number of feeding ticks[7]. Generally, each of the authors reported more profound effects with increasing numbers of successive infestations.

Serum antibodies from both naturally infested and immunised hosts and their reaction with tick antigens have been detected by different methods, including precipitation[8] immunofluorescence[1] and Western blots[4]. However, little is known about the effects of antibodies on ticks that engorge on resistant hosts, or which tissues of the tick body are possibly immunogenic. Some researchers, using immunohistochemistry, have detected host antibodies in the gut, salivary glands and haemolymph of ticks engorged on resistant animals[1,17]. The same technique has helped considerably in determining antigenic sites or antibody targets in arthropods[2,6]. In the light of these studies, we have used these techniques in order to detect the antigenic sites and cross-reactivity between sera raised against *Amblyomma cajennense* and *A. hebraeum.*

MATERIALS AND METHODS

Ticks

Amblyomma hebraeum ticks were obtained from a colony maintained in the humidity chamber (26 °C; 85 % RH) at the University of the Free State, Qwa-Qwa Campus (UNIQWA) South Africa. The *A. cajennense* ticks were from a colony maintained in a biochemical oxygen-demand incubator (29 °C; 85 % RH) at the Department of Animal Pathology, State University of São Paulo (UNESP), Jaboticabal-SP, Brazil.

Rabbits

Rabbits aged 2–4 months were used throughout the experiments. The animals were fed a commercial pellet diet and water *ad libitum.* One group of animals was maintained in the UNIQWA Animal house and the other group in the UNESP Bioterium. The control sera used in the immunoassays were obtained from naive rabbits.

Infestation procedure

The infestations of rabbits were carried out as described previously by Szabó[17] with some modifications. Acrylic feeding chambers with a screw-top lid were glued onto the animal's backs. Twelve ticks of each species (6 females and 6 males) were used per host animal. Serum was collected before and after tick infestation by means of cardiac puncture.

Histological analysis

Ticks were fixed in Bouin-Dubocsq fixative for 24 h and sectioned through a longitudinal sagittal plane after the legs had been amputated for better penetration of the fixative. The ticks were processed, paraffin-embedded, 3–4 μm sections were cut in a microtome, and stained with haematoxilin and eosin, Giemsa and Masson's trichrome stain, in the Department of Animal Pathology, UNESP. This was done in order to study the histology of the tick. Sections of adult female and

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male unfed and partially fed *A. hebraeum* ticks were used for immunohistochemical analysis.

Immunohistochemical analysis

Unstained sections of unfed ticks were used for the indirect immunohistochemical assay previously described by Manyasi[9]. Tick sections in triplicate were first de-parafinised and rehydrated in xylene, alcohol and tap water. The slides were then washed in PBS. This was followed by the inactivation of the endogenous tissue peroxidase with DAKO® Peroxidase Blocking reagent (DAKO Corporation, Carpinteria, CA, USA) for 15 min. In addition, slides were incubated for 1 h with the DAKO® Protein Block Serum-Free reagent to prevent non-specific staining. The sections on the slide were incubated for 1 h with different concentrations of the primary antibody raised in rabbits (1:50 and 1:100). After several washes, the material was incubated for 30 min with biotinilated goat anti-rabbit IgG (1:300) (DAKO®). After several washes, it was incubated with streptavidin peroxidase for 30 min and washed. Finally, the sections were incubated with a substrate (DAKO® Liquid DAB substrate-chromagen system) for a few seconds. The reaction was stopped by washing with distilled water followed by tap water. The sections were then counter-stained with Harris haematoxilin for 30–60 sec and washed with tap water. The sections were dehydrated in alcohol and xylene, and the slides mounted with Glycergel mounting medium (DAKO®).

The final result of this enzymatic reaction was the development of a brown colour in the structures to which the swine anti-rabbit peroxidase conjugated antibody had bound. The activity was scored as follows: +, light brown; ++, dark brown, and both considered positive. When the tissue presented the same colour as the negative control, it was considered negative and indicated by (–) in Tables 1–3. Each serum was tested at least twice.

RESULTS

Data for the indirect test using sections from unfed ticks of each species with various sera from naive and infested hosts are summarised in Tables 1–3.

The data in Table 1 indicate that sera from rabbits naturally infested with *A. hebraeum* reacted positively with most of the internal organs of this tick species, including malpighian tubules and haemolymph. It is noteworthy that serum obtained from rabbits infested with *A. hebraeum* also reacted positively with some internal organs from *A. cajennense* (Table 2). A few internal organs from *A. hebraeum* reacted positively to sera from rabbits infested with *A. cajennense* as shown in Table 3.

Table 1: **Indirect immunohistochemistry of sections of unfed adult *Amblyomma hebraeum* ticks incubated with sera from naive and infested rabbits.**

Structure	Naive rabbit serum*	Infested rabbit serum*
Midgut		
Epithelial cells	–	+
Lumen	++	++
Basement membrane	–	+
Haemolymph cells	–	+
Salivary glands		
Ducts	–	–
Type I acini	–	++
Type II acini	–	++
Type III acini	–	++
Synganglion	–	+
Muscle		
Basement membrane	–	++
Malpighian tubules		
Epithelial cells	–	+
Lumen	–	–
Basement membrane	–	++
Ovary		
Basement membrane	–	–

*Result of enzymatic reaction: + = light brown; ++ = dark brown; – = same colour as negative control.

DISCUSSION

Our results showed the existence of shared antigens between *A. cajennense* & *A. hebraeum.* The sera raised against both tick species reacted with the salivary glands in both ticks. Hard ticks require a long time to engorge, and to make this possible they have developed a sophisticated feeding behaviour, based on a varied salivary secretion repertoire that enables them to obtain an appropriate meal[3]. Within this context, antibody containing sera from tick-infested hosts are expected to recognise salivary gland tissues efficiently. Walker and Fletcher[12], studying the diversity of responses of *Rhipicephalus appendiculatus* salivary glands depending on the degree of host resistance, concluded that salivary glands are capable of responding selectively to diverse conditions at the feeding site.

Table 2: **Indirect immunohistochemistry of sections of unfed adult *Amblyomma cajennense* ticks incubated with sera from naive rabbits and those infested with *Amblyomma hebraeum.***

Structure	Naive rabbit serum*	Infested rabbit serum*
Midgut		
Epithelial cells	–	+
Lumen	–	–
Basement membrane	–	+
Haemolymph cells	–	–
Salivary glands		
Ducts	–	–
Type I acini	–	+
Type II acini	–	+
Type III acini	–	+
Synganglion	–	–
Muscle		
Basement membrane	–	–
Malpighian tubules		
Epithelial cells	–	–
Lumen	–	–
Basement membrane	–	–
Ovary		
Basement membrane	–	–

*Result of enzymatic reaction: + = light brown; ++ = dark brown; – = same colour as negative control.

Table 3: **Indirect immunohistochemistry of sections of unfed adult *Amblyomma hebraeum* ticks incubated with sera from naive rabbits and those infested with *Amblyomma cajennense.***

Structure	Naive rabbit serum*	Infested rabbit serum*
Midgut		
Epithelial cells	–	+
Lumen	+	+
Basement membrane	–	++
Haemolymph cells	–	+
Salivary glands		
Ducts	–	–
Type I acini	–	++
Type II acini	–	+
Type III acini	–	+
Synganglion	–	–
Muscle		
Basement membrane	–	+
Malpighian tubules		
Epithelial cells	–	–
Lumen	–	–
Basement membrane	–	–
Ovary		
Basement membrane	–	–

*Result of enzymatic reaction: + = light brown; ++ = dark brown; – = same colour as negative control.

This is probably a refined evasion technique used by ticks in order to immunomanipulate host response, and relying on their degree of immunity. Moreover, tick salivary gland-derived material can modulate host cytokine, antibody and cell-mediated immune responses[15].

Midguts of *A. cajennense* reacted more strongly against sera raised against *A. hebraeum,* whereas midguts of *A. hebreaum* reacted poorly against sera raised against *A. cajennense.* It appears as though structures of *A. hebraeum* tick gut may act as good immunogens. In this respect, researchers have used purified proteins of the tick midgut to immunise hosts against ticks[5,16]. It should be noted that salivary gland acini type I were always positive. The reason for this is difficult to explain since these acini have an osmoregulatory function and have a lesser or no role during tick feeding. Their intervention in the process of feeding is unknown[11].

Sera from rabbits infested with *A. hebreaum,* against its sections reacted with more intensity on the majority of structures, which was the case with other similar studies on different tick species[2,9].

In general, our results point to a 1-way cross-reactivity of *A. hebraeum* with *A. cajennense* rather than a reciprocal cross-reactivity, suggesting that *A. hebraeum* is more immunogenic than *A. cajennense. Sera raised against A. hebraeum,* when tested with an indirect immunohistochemistry assay, clearly showed that tick-resistant rabbits can certainly recognise probable antigenic sites in tick tissues. Further studies are needed to isolate and characterise the potential antigens identified in this study and to assay their ability to immunise rabbits against tick feeding.

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