

Original Article

Detection of Humoral Immune Response of Calves to *Boophilus annulatus* by ELISA

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Abstract

Background: Ticks are blood feeder acarions that feed on variety of animals and introduce a wide variety of molecules to their host immune system and some of them may stimulate host immune system to produce antibodies. This study was carried out to detect humoral immune responses following *Boophilus annulatus* infestation.

Methods: Seven cattle were each experimentally infested with 10000 *B. annulatus* larvae and their humoral immune response to salivary gland; ovary and larval extracts were determined by ELISA. Measurements of serum antibodies level were recorded weekly, in a period of nine weeks post infestation.

Results: An increase of the antibody level was observed in all animals at one week post infestation and reached in a peak at week ninth, then decreased in week 9.

Conclusion: Sera of infected animals showed approximately similar reactions to all of tissue extracts that might be due to the presence of common proteins in tick tissues and could be a candidate for immunization.

Keywords: *Boophilus annulatus*; Salivary gland; Ovary; Larva; ELISA, Humoral response

Introduction

Ticks are the most important arthropod transmitting pathogens to other animal species. The tick *Boophilus annulatus* is a bovine ectoparasite and a vector of some tick-borne disease agents such as *Babesia bigemina*, *B. bovis* and *Anaplasma marginale* in West and Central Africa, Asia and certain parts of Southern Europe (1). Since Johnston and Bancroft (2) have reported the acquisition of natural resistance by cattle to *B. microplus* after repeated infestations, much investigation has been undertaken to elucidate

the role of naturally acquired immunity in tick control. Cattle had been reported to acquire natural resistance against different tick species resulting cellular and humoral immune responses, especially *B. microplus* in Australia, *Amblyomma americanum* in the USA and *Hyalomma anatolicum anatolicum*, *Rhipicephalus appendiculatus*, *Rh. evertsi* and *A. hebraeum* in Africa (2- 5). The presence of immunoglobulins of the host animal in the tick hemolymph and antibody activities against tick tissues have been observed in different species of ticks (6), but there is still a gap of knowledge

about *B. annulatus* which is one of the most prevalent tick in the north of Iran (7).

The aim of this study was to determine the cattle humoral immune response to salivary gland, ovary and larval extracts antigens.

Materials and Methods

Ticks

Engorged females of *B.annulatus* ticks were collected from healthy cattle, cleaned up with 70% ethanol alcohol and then washed with sterile distilled water three times, in order to harvest tick larvae, treated ticks kept under a constant temperature of 28 °C, a relative humidity of 85% and a light- dark photo period [12:12].

Animals

Seven healthy 3-5 months old Holstein calves negative in ELISA against tick antigen were selected and provided from Research Institute of Tehran Veterinary Faculty, housed in tick-proof pens.

Tick infestation

Each of calf was infested with about 10000 *B. annulatus* larvae (8). Ticks culture tube containing larvae fastened to a shaved flank of animal with adhesive tape and the surface area covered with a piece of cloth.

Antigen preparation

Partially fed female ticks were removed from hosts and their ventral surface placed on a drop of melted wax on a glass slide, which placed on hot plate. The tick dorsal surface was separated and removed from the body by cutting the lateral edge of the cuticle with a fine tipped scissors, the exposed organs were immersed in phosphate buffer saline (0.5M NaH₂PO₄, 0.5M Na₂HPO₄) (PBS) pH 7.2 to facilitate observation and prevent dissection of organs. Then salivary gland and ovary were removed while viewing through a dissecting microscope with fine tipped forceps and removed intact organs were rinsed in fresh cold PBS pH 7.2. Those organs and whole body of tick larvae were separately homogenized in PBS pH 7.2 containing

1 mM phenyl methyl sulphonyl fluoride at 4 °C, followed by sonication for 30 min on ice with 40 W. The homogenates were centrifuged at 10000 g for 30 minute at 4 °C, supernatants were collected as antigens and stored at -70 °C (8). The protein concentration of each antigen determined using the method of Warburg (9).

Sera collection

Sera were collected from blood sampled from animals before and after inoculation. The positive sera were obtained by infesting seven 3 months-old calves with 10,000 of *B.annulatus*, blood was collected by jugular vein during 9 consecutive weeks and allowed to clot for 2 h at room temperature and centrifuged at 800g for 15 min, finally extracted serum aliquot and kept at -20 °C.

ELISA

ELISA plates were coated with 5 µg of antigen per well in 20 mM carbonate buffer (pH 9.6) per well by incubation overnight at 4°C (13). They were washed three times and incubated for 1 h at 37 °C with 5% skim milk- PBS and test sera diluted 1/200 were incubated for 1 h at 37°C. Then the plates were washed three times and 100 µl of 1/2000 sheep anti-bovine IgG conjugated with peroxidase were added to the individual wells. After incubation at 37°C for 1 h the plates were washed. The color developed with 2, 2', Azino-bis 3-ethyl Benz- Thiazoline-6-Sulfonic acid (ABTS) as a substrate. The optical density was determined at 405 nm. Sera from seven cattle were tested against *B. annulatus* antigens at one-week intervals during nine weeks period of post infection.

Results

The experimental infestation of seven calves with *B.annulatus* larvae was performed to characterize the humoral immune response to tick's antigens extracts. During the experimental period, 762±153 ticks were collected per animal. Ticks were collected between days 21 and 30 after inoculation.

Fig. 1 shows antibody level changes, which occurred against salivary gland, ovary and larval body antigens. Collected sera showed positive reaction against all antigens from the first week

of tick infestation. An increase of the antibody level was approximately observed during a period of eight weeks post infection then antibody level started to decrease.

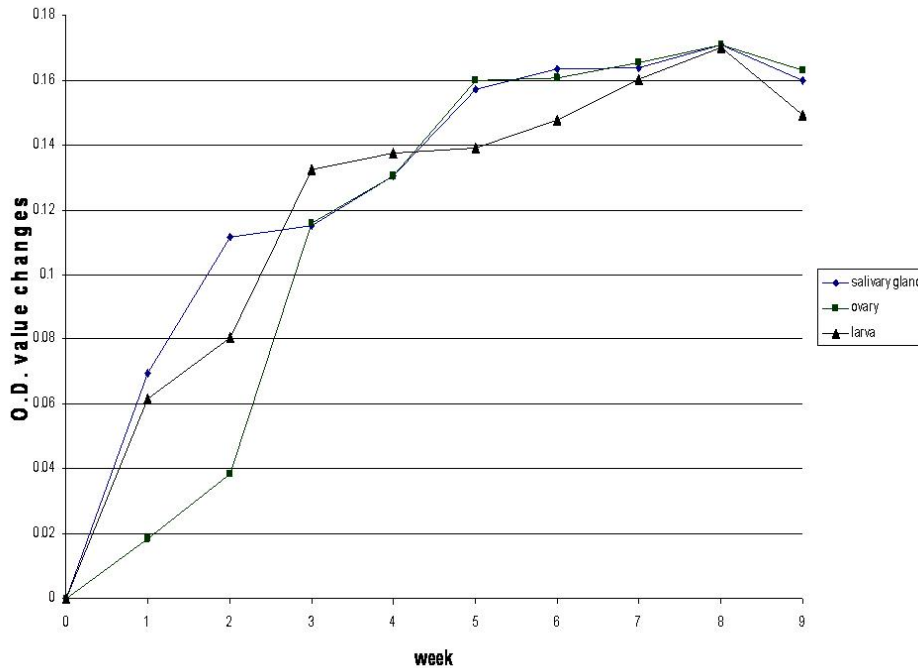


Fig. 1: Comparison of serum antibody response changes in calves infested with *Boophilus annulatus* using different tissues antigens. Results expressed as mean of seven infested bovine sera.

Discussion

ELISA indicated that calves infested with *B. annulatus* develop antibodies to components of tick salivary gland, ovary and larva during the first infestation. Antibodies reactive with tick extracts have been described by several researches (10-12). All of these studies have primarily focused towards an immunological tick-control program based on the development of protection against the respective arthropods. It is extremely important to have defined immunogenic molecules in order to dissect the events involved in acquisition and expression of tick resistance. Some studies clearly show that a number of tissues can be used to induce artificial resistance to tick feeding, an approach that holds significant promise as an alternative me-

thod for tick control (13, 14). The present study provides some basic information about these antigens of *B. annulatus*, which were recognized by sera of infested cattle, it means that the organs of *B.annulatus* could be also candidate as a target of tick antigen in this part of world. Subsequent studies on characterization of antigen point out some probable antigenic sites in specific tick tissues.

Salivary glands, gut and larval body extracts have been tested as immunogens to control tick infestation (15, 16). *B.microplus* infested cattle develop antibodies to components of tick salivary gland, gut, embryo and larva during the first infestation (17). Our data indicated that *B.annulatus* infested cattle developed antibodies to component of tick salivary gland, ovary and larval body extracts within the first infesta-

tion. There are some similarities in the sera reactivity to different antigens. One possible explanation of these similarities is that the animals inoculated with vast varieties of antigens hence tick feeding process take place. It seems that there are some common proteins in different tissues of *B.annulatus*. Our previous study on *B.annulatus* has also showed that some proteins of salivary gland changed in different temperatures but some of them were constant (18). Common proteins in various organ of *B.annulatus* may exist at different stages of tick life cycle. The ability of extracts of whole tick body homogenates and various organs of *Amblyomma maculatum* to induce tick resistance in the host was studied by McGowan (19). However, the vaccination of hosts with the antigen of *B.annulatus* may provide an alternative method to control of this tick.

It can be concluded that for potential vaccine development needs further studies on the isolation of constant proteins and testing for antigenicity and immunogenicity followed by purification and subsequent *in vitro* expression.

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