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Humoral immune response in cattle experimentally infested with larvae of Dermatobia Hominis
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HUMORAL IMMUNE RESPONSE IN CATTLE EXPERIMENTALLY INFESTED WITH LARVAE OF DERMATOBIA HOMINIS

SUMMARY

Six bovines were infested with 60 first instar larvae of Dermatobia hominis. The animals were bleed weekly, and their antibodies levels to D. hominis L₁, L₂, and L₃ instars measured during the time, following the infestation course. The antisera were submitted to a titration against optimal dilutions of antigen coated wells of microplates, previously sensitized with L₁, L₂, and L₃ preparations, respectively. The ELISA assay was used to test single dilutions of antisera, which results were comparatively analyzed with a control of non-infested animals. Antibodies against L₁ were detected between the first and 21st day post-infestation (DPI) and, from the 42nd DPI on, Anti-L₁ antibodies, could be detected on the 21st DPI and from the 35th DPI until approximately the 49th DPI, when it was observed a decreasing of antibodies titration equivalent to the control group. No antibodies were detected against the L₃ instar-antigens. Antibodies levels against L₁ showed absorbance higher than 1.500 O.D. at 492nm in the ELISA assay, when compared to the 0.096 O.D. observed to the negative animals. High anti-L₂ antibodies were also detected on the 21st DPI, where two animals showed O.D. of 0.450 and 0.900 at 492nm, with a cut-off estimated in 0.110 O.D. It was also demonstrated a rising of anti-L₂ antibodies in the same four animals, which presented antibodies response against L₁ instar. The obtained results, with an estimated prevalence of 50%, were comparatively evaluated, taking the double diffusion immunoprecipitation test as a standard, and showed a concordance of 98%. The association between infestation and presence of specific antibodies was also discussed.

Key words: Dermatobia hominis, myiasis, humoral immune response, ELISA.

INTRODUCTION

Dermatobia hominis (LINNAEUS Jr., 1781), known in Brazil as “berne” fly, is distributed
in Latin America’s tropical and subtropical regions and is one of the most important ectoparasites infesting domestic animals. Its importance in cattle breeding is related to the economic losses caused by its larval forms (ANDERSEN, 1960; LELLO et al., 1982; SANCHO, 1988). Generally the life cycle of *D. hominis* in cattle last 35 days, but it varies according to environmental conditions and to the host, and might extend to over 100 days (LELLO et al., 1982).

The larval development of this fly includes first (L₁), second (L₂), and third (L₃) instars, growing from 1 to 25mm in length and 0.3 to 10mm in width, producing nodules that are easily detected in the host. The literature is limited regarding the host’s immune response to this parasite. MOTA et al. (1980) has demonstrated that the larval antigens of *D. hominis* are immunogenic in rabbits, whereas anti- *D. hominis* antibodies were detected up to 13 weeks post infestation (WPI). CORONADO- FONSECA (1989) has observed an increase in *D. hominis* antibody titres in cattle up to day 12 post infestation (DPI), suggesting a secondary immune response. LELLO et al. (1980) observed inflammatory reactions caused by *D. hominis* larvae in rabbits that had been immunized. They verified that the inflammatory reactions surrounding the parasite occurred earlier and with greater intensity in immunized rabbits.

This study represent the first ELISA investigation of the kinetics of the bovine humoral immune response to *D. hominis*. The aim of this paper was to verify the evolution of the immune response on non-immunized cattle infested with larvae of this parasite.

**MATERIAL AND METHODS**

Raising of *D. hominis* L₁ larvae was carried out on the Institute of Veterinary at Universidade Federal Rural do Rio de Janeiro, Brazil. Frequent collections of L₃ from naturally infested cattle were made from hide of cattle slaughtered as well as by extracting the warble from living cattle. L₃ weighing, over 400mg, were placed in glass flasks, containing moistened sawdust, and kept in an incubator (Fanem, Brazil) at 25 ± 2°C with 70 ± 10% relative humidity. After a period of 26 to 31 days, emerged adults were placed inside wood cages and allowed to mate. Adults of *Musca domestica* (L.), the housefly, were also placed inside the cage and served as vectors for the ovisposition of *D. hominis* adult females. In each cage a ratio of 10 to 30 *D. hominis* adults to 50 to 100 carrier insects was used. The housefly vectors serving as carriers of *D. hominis* eggs were captured, conditioned in assay tubes and placed in an incubator under the conditions previously mentioned. After the incubation period, which varied from 4 to 6 days, the L₁ already at the phase of hatching were kept in an incubator at 20 ± 1°C, until the experimental animal infestation.

Twelve crossbred (*Bos taurus* × *Bos indicus)* male calves free from infestation by *D. hominis* were used which aged 12 to 18 months and weighted 120 to 140kg. The calves were kept in a stable for an adjustment period of 25 days fed on commercial concentrated feed (Purina brand), chopped “elephant” grass (*Pennisetum purpureum* Schum.), and fresh water *ad libitum*. They were treated with levamisol chloride (Ripercol, Cyanamid) at 1 ml/20 kg l.w. and sprayed with deltamethrin (Butox, Hoechst). After 25 days post drugs treatment, infestation was carried out. At this period, the calves were randomized in two groups of six calves, a control group (non-infested) and an infested group. Infested animals were held and their hairs removed on different spots of the dorsal region. The infestation methodology used was the same as carried out by SANAVRIA et al. (1987), wherein the L₁ were encouraged to hatch and were individually removed with a thin point paintbrush and placed on the dorsal area of the calves. Calves were infested with 60 L₁ of *D. hominis* per calf.

The animals were bleed in the mornings of days -7, 0, 7, 14, 21, 28, 35, 42, 49, and 63 after infestation. Blood collection was made by jugular venipuncture, and after clotting, sera were obtained by centrifugation at 3000 rpm for 10 minutes. Sera samples were frozen at -20°C until use.

The ELISA (Enzyme-Linked Immunosorbent Assay) was carried out according classic methodology previously described (SÁNCHEZ-VISCAÍNO & CAMBRA-ALVAREZ, 1987; CROWTHER, 1995, 1998). Antigens were obtained by processing *D. hominis* larvae of three different ages: L₁ newly-emerged from eggs, L₂ (14 days) and L₃ (28 days) obtained from cattle artificially infested. L₁ antigens were prepared by sonication of L₁ at 50 Hz in saline solution (0.85%) for 30 minutes (Fisher, USA). L₂ and L₃ antigens were prepared by cutting larvae on Petri plates, followed by homogenization in saline solution (0.85%) with a homogenizer (Virtis, USA) at 20,000 rev/min for 20 minutes. After 12 hours at 5°C, homogenate was filtered and protein concentrations was determined by method described elsewhere (HUDSON, & HAY, 1989). Bovine albumin (Merck) was used as the protein standard. Purified antigenic preparations were frozen at -20°C until required, without any additive.
Polystyrene microtitre plates, with 96 flat-bottom wells, were sensitized by the addition of 100µl (2.0µg/ml) of each of the three antigens diluted in carbonate buffer, pH 9.6, into each well and incubated at 5 ± 1°C overnight. The sensitized plates were washed twice with PBS 0.01M, pH 7.2 with tween 20 (polyoxyethylene sorbitan monolaurate, Vetec, Brazil) 0.05% (PBS-T) and dried for posterior use. Sensitized plates could be used for approximately 30 days when maintained at 4°C. Test sera were diluted 1:500 in PBS-T containing 1% powdered milk, instead of casein, for blocking irrelevant spaces, according methodology previously described (CROWTHER, 1998). Each serum was added to 100µl into each well in triplicate. Plates were incubated at 37 ± 1°C for 45 minutes in a humid incubator, then washed three times with PBS-T. The anti-bovine conjugate (FIOCRUZ-RJ) diluted at 1:500 was added to 100µl into each well. The reaction was incubated at 37 ± 1°C for 45 minutes in a humid incubator. The plates were then washed for three times with PBS-T and, 100 µl of substrate (phosphate-citrate buffer, pH 3.5, orthophenylenediamine and hydrogen peroxide, Merck) added to each well. The material was kept in the dark for 15 minutes. Color development (absorbance) was read at 492nm in a ELISA-reader (Labsystems, USA). The negative samples, obtained from the control group, constituted of not infested animals, served as a tool for constructing a cut-off, which was based on the average added of two standard deviations for each assessed antigen. A value higher than the cut-off was considered as a really positive. The obtained results were comparatively studied taking precipitation by double immune diffusion as a standard method (CROWTHER, 1995). The correlation of co-opacity and co-negativity was evaluated. The independent variants were also estimated (TOMAN, 1981).

Assays with animals were planned considering a two-way design (infestation status - either present or absent - and periods of evaluations) and six replications per treatment. For assessment of the results regarding the levels of antibodies anti-L₁, anti-L₂ and anti-L₃ of _D. hominis_ the following statistic evaluations were done: ANOVA, Tukey's test and polynomial regression analysis (SNEDECOR & COCHRAN, 1976).

RESULTS AND DISCUSSION

The main aim of this paper was to establish an immunoassay to measure bovine antibodies, which could react against antigenic preparations obtained from different instars of _D. hominis_. For this reason, it was established two groups of animals: the first one, constituted of six bovines which were experimentally infested with 60 _L₁_; and the second, with six control animals, which were not infested at this time. The animals were bleed weekly, and their antibodies levels against soluble antigens of _D. hominis_ _L₁_, _L₂_ and _L₃_ instars measured during the time, following the infestation course of the experimentally inoculated animals. The antisera were submitted to a titration against optimal dilutions of antigen coated wells of microplates, previously sensitized with _L₁_, _L₂_ and _L₃_ preparations, respectively. The reacting antibodies detection was carried out by addition of a constant amount of an antispecie conjugate. Such assays were evaluated fully, from the diagnostic point of view where numbers of animals and experimental antisera, from each experimental group were available. Therefore, the ELISA assay was used to test single dilutions of antisera, and the tests could be adequately controlled using standard positive and negative antisera (CROWTHER, 1995, 1998). The averages of levels of antibodies anti-L₁, anti-L₂ and anti-L₃ of _D. hominis_ observed in the infested animals are found in figure 1. The averages of _L₁_ and _L₂_ preparations, expressed in absorbance values at 492nm, as observed in the infested animals, were significantly higher (P<0.01) than those observed in the control animals. Although it was observed a great variation on the antibodies levels from the experimentally infested group, the anti-L₁ and anti-L₂ qualities could be detected in different periods post-infestation. Antibodies against _L₁_ were detected during the period between the first and 21<sup>st</sup> day post-infestation (DPI) and, from the 42<sup>nd</sup> DPI on. Anti-L₂ antibodies, by their turn, could be detected on the 21<sup>st</sup> DPI and from the 35<sup>th</sup> DPI until approximately the 49<sup>th</sup> DPI, when it was detected a decreasing of antibodies titration which were equivalent to the control group. No antibodies were detected against the _L₃_ instar-antigens on the evaluated period. Some infested animals presented a tendency of rising antibodies levels against _L₁_ and _L₂_ antigenic preparations. Such phenomena was noticed, mainly to the _L₁_ instar, where four animals showed very high antibodies levels, whose absorbance was higher than 1,500 O.D. at 492nm in the ELISA assay, when compared to the cut-off, estimated in 0.096 O.D. Except for two animals, which did not present circulating antibodies, all the others infested animals were always positive against the _L₃_ instar. Nevertheless, between the 21<sup>st</sup> and 28<sup>th</sup> DPI period and, on the 42<sup>nd</sup> DPI, they presented results very close related to the cut-off as well as the control animals. High anti-L₂ antibodies were also detected on the
21st DPI, where two animals showed O.D. of 0.450 and 0.900 at 492nm, respectively, with a cut-off estimated on 0.110 O.D. It was also demonstrated a rising of anti-L 2 antibodies in the same four animals, which presented antibodies response against L 1 instar, on the 42nd and 49th DPI, when titration were higher than 0.300 O.D. The obtained results, with an estimated prevalence of 50%, were comparatively evaluated, taking the double diffusion immunoassay precipitation test as a standard, and showed a concordance of 98%. On the other hand, the predictive values of sensitivity, specificity and accuracy, suggested that ELISA assay against different instars of *D. hominis* (L 1 and L 2 ) should be of relevance to *D. hominis* gested that ELISA assay against different instars of *L. cuprina* and *M. ovinus* should occurs prior to *D. hominis* prior to experimental infestation (CORONADO-FONSECA, 1989). SANDEMAN et al. (1995), as well as BARON & NELSON (1985), seeking for antibodies titres to *Lucilla cuprina* and *Melophagus ovinus* respectively, in experimentally infested sheep observed gradual augments of serum antibodies, which decreased on few weeks after exposition. These authors concluded that adaptive immune response against *L. cuprina* and *M. ovinus* should occurs only when larval infestation was active. Similar results were obtained by PERAÇOLI et al. (1980), LELLO & BOULARD (1990) and LELLO & PERAÇOLI (1993) working with rabbits experi-
mentally infested with *D. hominis*. The present study has a significant importance concerned to the capability of making specific antibodies following a *D. hominis* infestation, independent of class or isotype, which lasted for two months. Such immune response, although needing more detailed work on the antigenic components of each larval instar, suggests the L1 antigen as a representative marker to infestation on bovines, once there was a straight correlation between infestation and presence of specific antibodies. These levels of antibodies suggest that the antigens produced by L1 were more immunogenic than those produced by the other larval instars. The observed average of antibodies titration against L1 was 0.373 (O.D.) such average was significantly higher (*P*<0.05) than those observed for antibodies anti-L2 (O.D. = 0.140) and anti-L3 (O.D. = 0.013). Finally, it can be concluded that ELISA against *D. hominis* larval antigens is an important tool for studies of occurrence of infestations in different regions and periods of time.

REFERENCES


