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Humoral response and evolution of *Echinococcus* infection in experimentally infected sheep

Resposta humoral e evolução à infecção por *Echinococcus* em ovinos experimentalmente infectados

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Abstract

There is little information on the humoral response of sheep experimentally infected with *Echinococcus granulosus*. Thus, the objective of this study was to evaluate this response and measure its evolution. Doses of 10, 100, 1000 and 10000 *E. granulosus* eggs were prepared and inoculated via intraruminal puncture. Blood samples were obtained before inoculation and every 48 h after inoculation, until they became seropositive. Thereafter, they were taken monthly for the first year and then every three months until 1700 days of observation had been completed. An ELISA test, with total hydatid fluid antigen, was used for immunodiagnosis. The average optical density of the 12 inoculated sheep was found to be above the mean cutoff value 10 days after inoculation, went on increasing until 180 days after inoculation and remained above the cutoff level until the end of the observation period. This confirms that the antibody response of sheep to *E. granulosus* infection occurs before production of hydatid fluid and that activation, mobilization and establishment of oncospheres in the tissues generates a persistent response from the host's immune system.

Keywords: Echinococcosis, humoral response, sheep.

Resumo

Existe pouca informação sobre a resposta imune humoral de ovinos experimentalmente infectados por *Echinococcus granulosus*. O objetivo deste estudo é avaliar a resposta imune por anticorpos em ovinos infectados. Os ovinos receberam doses de 10, 100, 1.000 e 10.000 ovos de *E. granulosus* por via intrarruminal. Amostras de sangue foram colhidas antes e após infecção, a cada 48 horas, até a detecção de anticorpos anti-*E. granulosus* e após, colheram-se amostras mensal e trimestralmente, no primeiro ano até 1.700 dias de infecção. No imunodiagnóstico, utilizou-se o ensaio imunoenzimático indireto (ELISA-teste) e como antígeno total, líquido hidático. Na detecção de anticorpos anti-*E. granulosus* no soro das 12 ovelhas, a densidade ótica esteve acima do ponto de corte, após 10 dias de infecção, aumentando até 180 dias pós-infecção, e permanecendo acima desses dias até o final do experimento. Isso confirma que a resposta por anticorpos em ovinos infectados por *E. granulosus* antecede a produção de líquido hidático, e que a ativação, mobilidade e permanência das oncosferas nos tecidos possibilita a resposta imune dos hospedeiros.

Palavras-chave: Equinococose, resposta imunológica, ovinos.

Introduction

Cystic echinococcosis (CE) or hydatidosis is a parasite zoonosis produced by a cestode of the Taeniidae family, *Echinococcus granulosus*, which was first described in 1786. It needs two mammalian hosts to complete its life cycle: one definitive host (a carnivore, especially dogs), where it develops the adult or strobilar phase, and an intermediate host, where it develops the larval or metacestode phase (THOMPSON; McMANUS, 2001).

When *E. granulosus* eggs are eliminated by a dog or some other carnivore, and are ingested by a susceptible host (especially sheep), they reach the stomach and there undergo disruption of the embryosphere and activation of the oncosphere. They penetrate the intestinal microvilli and pass into the lymphatic and venous systems, to lodge definitely in a visceral organ, preferably the liver or lungs.

The larva or metacestode that develops is typically unilocular, polymorphic and full of fluid. It has a complex structure consisting of an internal germinal membrane composed of cells with a circular or oval nucleus, and an external cuticular, acellular elastic membrane

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surrounded by a fibrous adventitious membrane produced by the host (ECKERT et al., 2001)

It is one of the main zoonotic diseases, with the highest prevalence among such diseases in Argentina, Uruguay, Chile, Peru and southern Brazil. It produces high regional costs in farm animal production relating to the value of confiscated viscera and loss of wool, milk and meat production; and for the health system, relating to the costs of hospitalization and treatment of infected people (LARRIEU; ZANINI, 2012).

The humoral response to *E. granulosus* infection in sheep has been poorly studied. Antigen 5 antibodies were detected in experimentally infected sheep from 120 days after infection onwards (CONDER et al., 1980). A humoral response was detected 20 days after infection of adult sheep, by means of ELISA (LARRIEU et al., 2009).

There is little information on the continuity of this humoral response. Therefore, the objective of this study was to evaluate the humoral response and its evolution over time in sheep experimentally infected with different doses of *E. granulosus* eggs.

Materials and Methods

Experimental lambs were randomly selected from a flock in General Pico, Province of La Pampa, and were numerically identified using ear-tags. Four groups of three animals each and one group with three animals (control) were established.

Adult tapeworms of the species *E. granulosus* were obtained from 54 dogs using arecoline hydrobromide and were kept in normal saline solution with penicillin at 8-10 °C.

Inoculums of 5 ml of saline solution with doses of 10, 100, 1000 and 10000 *Echinococcus granulosus* eggs, isolated from the mature proglottids in inverse microscopy, were prepared and individually applied using intraruminal puncture in each group of three animals. The control group received 5 ml of saline solution.

Blood (10 ml) was obtained by means of venipuncture of the jugular vein, from animals in the standing position with their heads held to one side, using disposable syringes and 25/8 gauge needles. The first blood sample was taken prior to inoculation, and post-inoculation samples were taken every 48 h until the animals became seropositive. Samples were then taken monthly for the first year and subsequently every three months until 1700 days of follow-up had been completed.

An enzyme immunoassay (ELISA) was carried out for immunodiagnosis, using the antigen from total hydatid fluid (EIE.LHT), in accordance with a previously described technique (GATTI et al., 2007; LARRIEU et al., 2009).

During the study, the animals were maintained on a cattle farm, without dogs. There were no deaths and/or serious diseases among the animals.

Calculations for regression analysis, one-way ANOVA and the Tukey and Fisher tests were carried out using the Minitab 15 software.

Results

All the animals were seronegative before experimental inoculation.

Two animals (16.7%) inoculated with 10 and 1000 eggs, showed a humoral response seven days after infection. At 30 days, four more sheep (33.4%) were detected, at 90 days, eight sheep (66.8%), and at 150 days, 10 sheep (83.3%). The two sheep that did not initially react showed a humoral response at 540 days (inoculated with 100 *E. granulosus* eggs) and at 1200 days (inoculated with 10000 *E. granulosus* eggs) (Figure 1).

The mean optical density of the 12 inoculated sheep exceeded the average cutoff value by the tenth day after inoculation, went on increasing until 180 days after inoculation and then remained above the cutoff level until the end of the observation period (Figure 2). All the sheep maintained the humoral response from

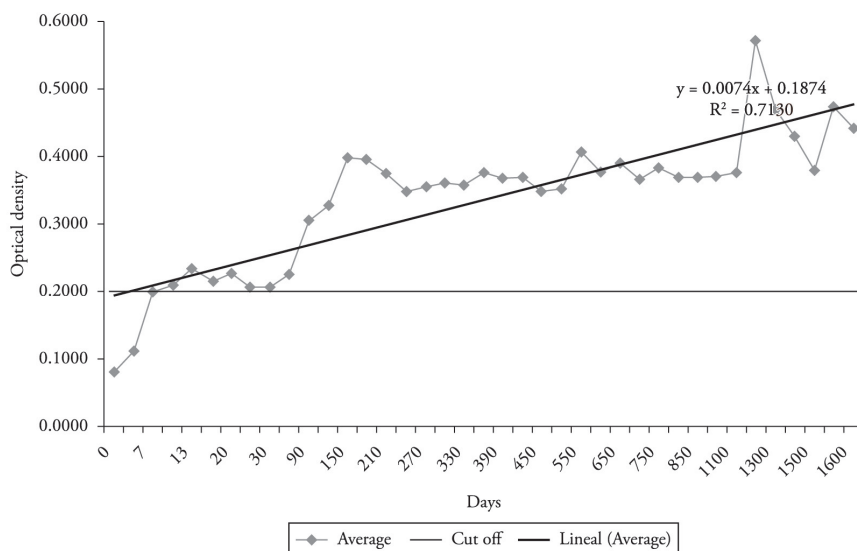


Figure 1. Longitudinal follow-up of the response to infection among sheep experimentally inoculated with different doses of *Echinococcus granulosus* eggs.

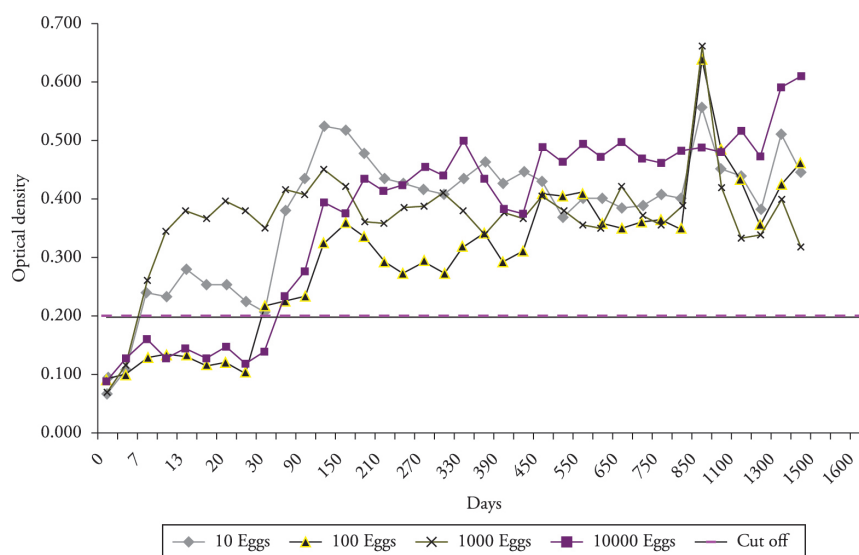


Figure 2. Longitudinal follow-up of the response to infection among sheep experimentally inoculated with *Echinococcus granulosus*.

the time when it was first manifested until 1700 days had been reached. The regression line for the mean optical density showed a tendency to rise both at the beginning of the observations and during the period after 180 days, although the coefficient (R^2) presented a high association (71%). A significantly high measurement was observed at 1400 days.

Significant differences in humoral response were observed with regard to the number of inoculated eggs, considering the whole observation period ($F = 3.01$, $p = 0.032$). From analyzing 95% confidence intervals for the average individual, 95% confidence intervals simultaneously (Tukey) and 95% confidence intervals (Fisher test), differences were only observed between doses of 100 eggs and the remaining doses.

Discussion

The present study followed the humoral response in sheep experimentally infected with *Echinococcus granulosus* eggs over a period of 1700 days, detecting the humoral response from the time of entry of the infecting oncospheres, which occurred seven days after infection in 16.7% of the sheep, reached 100% at 1200 days and was then maintained over time.

Previous observations on 500-day post-inoculation follow-ups showed similar behavior (LARRIEU et al., 2009), thus demonstrating, when associated with histological studies, that the cysts became fertile with the presence of protoscolices 300-360 days after inoculation and that live oncospheres can be identified in viscera 300 days after inoculation and in the presence of formed hydatid cysts.

In the same study, a humoral response was detected 10 days after infection and was maintained over the whole period of observation. Initially, the humoral response was proportional to the quantity of inoculated eggs, but the differences diminished with time (LARRIEU et al., 2009). This observation was confirmed

in the present study, i.e. there was little relationship between the dose and response.

Thus, this confirms that in sheep the antibody response to *E. granulosus* infection occurs before production of hydatid fluid and shows that activation, mobilization and establishment of oncospheres in tissues generates a persistent response from the host's immune system.

Many serological reactions, in sheep or in humans, may be classified as false positives because of the impossibility of finding a hydatid cyst in the host. However, these reactions could be due to *E. granulosus* infection in the oncosphere stage or in the early phase of the metacystode development process.

Various sources of antigen stimulation capable of producing antibodies have been described for hosts: exposure to infecting oncospheres, subsequent development of immature cysts and then development of fertile cysts (LIGHTOWLERS; GOTTSTEIN, 1995; HOLCMAN; HEATH, 1997). Once the metacystodes have become established with their laminar layer, the antigens found in the germinal membrane, hydatid liquid and protoscolices remain sequestered within the hydatid cyst, thus ending the stimulation of the host. In humans, this usually means that there will be a negative serological response. Macromolecules leaving through microfissures of the laminar layer of mature metacystodes form a second source of stimulation for hosts, which will usually mean a positive serological response (LIGHTOWLERS; GOTTSTEIN, 1995).

In the light of the results of this study, it remains necessary to ascertain the time required for serological negativization, once the laminar layer has formed, in order to properly interpret the results from serological tests on sheep and humans.

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