Short communication

Naturally acquired antibodies against Clostridium perfringens epsilon toxin in goats

Josir Laine A. Veschi a, Octavio A. Bruzzone b, Daniela M. Losada-Eaton c, Iveraldo S. Dutra d, Mariano E. Fernandez-Miyakawa e, *

a Laboratório de Sanidade Animal, Embrapa Semi-Árido, BR 428, Km 152, 56302-970 Petrolina, PE, Brazil
b Laboratorio Ecologia de Insectos Forestales, INTA EEA Bariloche, CC 277, 8400 Bariloche, Argentina
c Laboratorio de Fisiopatogenia, Departamento de Fisiologia, Universidad de Buenos Aires, Paraguay 2155, C1121ABG Buenos Aires, Argentina
d Departamento de Produção e Saúde Animal, Universidade Estadual Paulista, Rua Clóvis Pestana, 793, Aracatuba, SP 16050-680, Brazil
e Instituto de Patobiología, CICV y A, CNIA, Instituto Nacional de Tecnología Agropecuaria, CC 25, 1712 Castelar, Pcia. Buenos Aires, Argentina

Received 14 February 2008; received in revised form 15 April 2008; accepted 22 April 2008

Abstract

Clostridium perfringens type D-producing epsilon toxin is a common cause of death in sheep and goats worldwide. Although anti-epsilon toxin serum antibodies have been detected in healthy non-vaccinated sheep, the information regarding naturally acquired antibodies in ruminants is scanty. The objective of the present report was to characterize the development of naturally acquired antibodies against C. perfringens epsilon toxin in goats. The levels of anti-epsilon toxin antibodies in blood serum of goat kids from two different herds were examined continuously for 14 months. Goats were not vaccinated against any clostridial disease and received heterologous colostrums from cows that were not vaccinated against any clostridial disease. During the survey one of these flocks suffered an unexpectedly severe C. perfringens type D enterotoxemia outbreak. The results showed that natural acquired antibodies against C. perfringens epsilon toxin can appear as early as 6 weeks in young goats and increase with the age without evidence of clinical disease. The enterotoxemia outbreak was coincident with a significant increase in the level of anti-epsilon toxin antibodies.

# 2008 Elsevier B.V. All rights reserved.

Keywords: Clostridium perfringens; Epsilon toxin; Immune response; Caprine

1. Introduction

Clostridium perfringens is considered as part of normal flora in different animal species including sheep and goats (McClane et al., 2006). C. perfringens type D is a common cause of death in sheep and goats worldwide, and younger animals usually are the most affected (Blackwell et al., 1983; Finnie, 2003; Itodo and Ike, 1990). Epsilon toxin produced by C. perfringens type D is the cause of enterotoxemia in sheep and probably in goats (Payne et al., 1997). It has been proposed that if the intestine is altered by sudden changes in diet or other not well defined factors, C. perfringens type D proliferates rapidly and generates large amounts of epsilon toxin, which are required to produce the disease (Songer, 1996).
Immunity against enterotoxemia in sheep is readily produced by vaccination with toxoids (McClane et al., 2006). However, although vaccination has reduced its prevalence, the disease still occurs commonly and vaccination with toxoid is poorly protective in goats (Finnie, 2003; McClane et al., 2006). Anti-epsilon toxin serum antibodies have been detected in non-vaccinated sheep beyond the age they would carry a level of maternal immunity (Thomson and Batty, 1953; Griner, 1961). In goats, Blackwell et al. (1983) observed that in unvaccinated herds without history of clinical disease resembling enterotoxemia, up to 54% of the animals presented serum epsilon antitoxin antibodies and they suggested that subclinical infection in goats may affect vaccination response. Although epsilon toxin is able to stimulate the production of specific antibodies in infected animals (Songer, 1996), the detailed kinetics of changes in anti-epsilon toxin antibody levels in clinically healthy goat kids or after a clinical episode of enterotoxemia, are not known. The aim of the present study was to provide information about the development of natural anti-epsilon toxin antibodies in a cohort of newborn goats raised under commercial farming conditions. During the survey one of these flocks suffered an unexpectedly severe type D enterotoxemia outbreak.

2. Materials and methods

2.1. Animals

Male and female Saanen, Alpine and Saanen × Boer goats from two different herds were used in the trial. All goats were born from females that were never vaccinated against enterotoxemia and were separated from their mothers immediately after birth to avoid horizontal transmission of Caprine arthritis encephalitis virus. They were fed heterologous colostrum and milk, from cows that were not vaccinated against clostridiosis. The experiments were approved by the Committee for Ethics in Animal Experimentation (CEEA) at Universidade Estadual Paulista (UNESP) and were conducted according to the principles of the “Protocolo para uso de animais em experimentação científica ou ensino” of CEEA-UNESP.

Goats in group A came from a herd of 380 animals in the Goat Breeding Sector at UNESP, in Jaboticabal, SP, Brazil. Blood was collected by means of puncture of the jugular vein into Vacutainer® tubes and kept at room temperature until the coagulum was completely formed. Serum samples were kept frozen until they were used in serological tests. The first collection was performed between 5 and 9 (mean of 7) days of life (month 1) in all goats. 13 goats (from a total of 76 newborn) were randomly chosen to be submitted for blood sample collection every month during 14 months. Although there were no reports of enterotoxemia cases in goats of this herd, there were sudden changes in feeding plans and outbreaks of diarrhea in goats of all ages, with no diagnosis of endoparasites or enterotoxemia.

Goats that came from a commercial breeding unit located in São José do Rio Preto, SP, Brazil were defined as group B. This herd of 504 goats was geographically localized at 180 km from the group A herd. Blood and serum samples were obtained as described for goats in group A. However, the first collection was performed between 42 and 47 (mean of 45) days of life (month 2) in all goats. 16 goats (from a total of 82 newborn) were randomly chosen to be submitted for blood sample collection every month for 15 months. Between collections number 9 and 11 an outbreak of enterotoxemia produced by C. perfringens type D occurred and 72 of 504 goats of different ages died. All the sampled goats survived the enterotoxemia attack.

2.2. ELISA

An indirect ELISA was used for the detection of antibodies against epsilon toxin (Uzal et al., 1997). Purified epsilon toxin was obtained as previously described (Sayeed et al., 2005) and a single band was observed in the final product using SDS-PAGE with 12% (w/v) acrylamide gels and stained with Coomassie Blue. Immunoplate (Nunc-Immunoplate Maxisorb) were coated with 10 μg/ml of purified epsilon toxin diluted in carbonate buffer (pH 9.6) and incubated overnight at 4 °C. After washing the plates with phosphate-buffered saline containing 0.05% Tween 20 (PBST), test and control sera diluted in PBST were added to the plates and incubated for 1 h at 37 °C. The plates were washed between steps with PBST, pH 7.4. A hyperimmune serum from vaccinated goats diluted in PBST and calibrated in IU/ml of antitoxin activity (measured by the mouse neutralization test as previously described in the British Pharmacopoeia, 1993) was used as positive control. Test and negative control serum from a neonatal goat deprived of colostrum were diluted 1:50. All serum dilutions were performed in duplicate. Caprine IgG immunoglobulins were detected using peroxidase-conjugated monoclonal antibody (Sigma–Aldrich) diluted 1:500 in PBST. The reaction was developed with ABTS (Sigma–Aldrich) diluted in citrate buffer (pH 9.4) as chromogen substrate and the intensity of staining was read at 414 nm after 20 min of incubation. Titters were calculated with a regression
curve built with the ELISA results of six positive goat control sera with different known epsilon antitoxin titers and the log of the IU of epsilon antitoxin/ml of these samples. The log of the IU/ml values of the test sera were calculated by a regression equation, and then transformed into IU/ml. Other reagents were purchased from Sigma–Aldrich.

2.3. Statistical analysis

For analysis of antibodies kinetics, the data from groups A and B were fitted to a model \( y(x) = a + b \times (1 - \exp(-k \times x)) \), where \( x \) is the goat age in days, \( y \) is the antibodies level (UI/ml), \( a \) is the antibodies level at \( x = 0 \), \( k \) is the daily rate of increment of antibodies, and \( a + b \), is the asymptotic value of the model. The model was fitted using weighted least squares method, with the inverse of variance as a weighting variable using Gnuplot version 4.0 (Williams and Colin, 1998). After fitting, a Monte Carlo test was used to determine which collections from group A had an average antibodies level higher than expected by the model. After 100,000 randomizations, the average was considered significant, if the level of antibodies was higher than the randomized one in at least 95,000 times (corresponding to a \( P < 0.05 \), in one tailed test).

3. Results and discussion

Antibodies against epsilon toxin were detected in goat kids from both flocks about 1.5–2 months after birth (Fig. 1). As observed in Fig. 1, the increasing levels of anti-epsilon toxin antibodies became slower after months 4–5 and kept relatively stable in the group A during the following 9 months. In contrast in group B, although the titers kept relatively stable during 5 months, titer media increased significantly at month 11 (\( P < 0.05 \)) after being analyzed using Monte Carlo test. This is coincident with an unplanned change in farming conditions in flock B that lead to an enterotoxemia outbreak. The incident killed 14% of the goats and suggests that many of the dairy goats in the flock were exposed to elevated levels of type D toxins, including epsilon toxin and proteotoxin.

The present study showed by the example of two flocks, that natural acquired antibodies against \( C. \) perfringens epsilon toxin can appear relatively early in young goats without evidence of clinical disease and increase with the age. These antibodies are an indirect evidence of intestinal epsilon toxin secretion by \( C. \) perfringens under apparently non-pathological conditions. It also suggests that goat kids could be readily colonized by \( C. \) perfringens producing epsilon toxin after birth. Naturally acquired immunity against clostridial toxin is well documented for tetanus toxin (Ehrengut et al., 1983; Matzkin and Regev, 1985; Veronesi et al., 1983). Similar to the present results, it was shown that the chances for achieving immunity against tetanus increase with age, at such level that some non-vaccinated individual reach serum levels of antitoxin higher than the accepted protective titer (Matzkin and Regev, 1985; Veronesi et al., 1983). It has
been hypothesized that natural acquired immunity to tetanus toxin may be produced by colonization of the digestive system (Matzkin and Regev, 1985).

The findings of the present report raise the hypothesis that enterotoxemia increases the sera antitoxin titers in surviving animals. The hypothesis is also partially supported by a previous observation of increased anti-epsilon toxin levels in a sheep experimentally inoculated with \textit{C. perfringens} type D toxins in the intestinal tract (Bullen and Batty, 1957). Although the proportion of goats with titers higher than 0.2 IU/ml was almost constant in group A (percentage variation was between 10 and 30), this fraction increased after month 10 in group B (Fig. 2), temporally coincident with the moment that the herd was affected by enterotoxemia. Blackwell et al. (1983) considered that goats with antibody titers below 0.10 IU/ml would be unprotected, while goats with antibody titers between 0.10 and 1 IU/ml might still be at risk from the disease. When the enterotoxemia outbreak occurred, the titers ranged from 0.11 to 0.35 IU/ml. It is possible that goats in this randomly selected group survived to enterotoxemia because the anti-epsilon toxin titers were beyond the minimal protective threshold.

In conclusion, natural acquired antibodies against \textit{C. perfringens} epsilon toxin can appear early and increase with the age in young goats. Enterotoxemia outbreaks are likely to boost this specific response. Further studies should determine the effects of these antibodies in the immune response induced in goats by vaccination against epsilon toxin.

Acknowledgments

We thank Ana Eaton for her manuscript correction, Dr F. Uzal for providing antiserum and S. Mahfuz and K. Rezende for de goats. M.F.M. is a Fellow of CONICET, Argentina. Project supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Brazil.

References


