

## SEROLOGICAL EVALUATION OF POLYVALENT COMMERCIAL VACCINES AGAINST ENTEROTOXEMIA IN GOATS

### AVALIAÇÃO SOROLÓGICA DE VACINAS COMERCIAIS POLIVALENTES CONTRA A ENTEROTOXEMIA EM CAPRINOS<sup>1</sup>

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#### SUMMARY

We evaluated the serological response of five commercial polyvalent vaccines containing epsilon toxoid of *Clostridium perfringens* type D. For this, 84 young goats were randomly divided into six groups, with 14 animals in each group. The goats in the control group were not vaccinated, while goats of Groups 1 to 5 received two vaccine shots four weeks apart. The first shot was administered when the goats were 45 ( $\pm$  3) days old and the second at 75 ( $\pm$  3). Blood samples for serological tests were collected on days zero, and 30, 60, 90, 120 and 150 after the start of the experiment. The Indirect ELISA technique was used to quantify the epsilon antitoxin antibodies for *Clostridium perfringens* type D. In general, the mean serum antibody titers of goats on day 60 increased as a response to the two vaccine shots received on days zero and 30. The largest number of animals considered protected was also detected on day 60, in response to the two vaccine shots. Only five animals from Group 1 and one goat from Group 3 displayed antibody titers that are considered protective up to 150 days after vaccination. Based on these results, we concluded that the evaluated vaccines showed poor ability of stimulate a protective immune response in the assessed goats.

**KEY-WORDS:** *Clostridium perfringens* type D. ELISA. Epsilon toxoid. Immune response. Vaccination.

#### RESUMO

Foram avaliadas as respostas sorológicas a cinco vacinas comerciais polivalentes que continham o toxóide épsilon do *Clostridium perfringens* tipo D na sua formulação. Para isso, foram utilizados 84 caprinos jovens, divididos aleatoriamente em seis grupos experimentais com 14 animais em cada grupo. Os caprinos do Grupo Controle não receberam nenhuma dose de vacina e os dos Grupos 1 a 5 receberam duas doses de vacina com intervalo de quatro semanas entre elas. A primeira dose de vacina foi aplicada aos 45 ( $\pm$  3) dias de vida dos animais (início do experimento - dia zero) e a segunda aos 75 ( $\pm$  3 - dia 30). As amostras de sangue para a realização dos testes sorológicos foram colhidas antes (dia zero), e nos dias 30, 60, 90, 120 e 150 após o início do experimento. Utilizou-se a técnica de ELISA Indireto para quantificação dos anticorpos antitoxina épsilon do *C. perfringens* tipo D. De maneira geral ocorreu um aumento nos valores médios do título de anticorpos séricos dos caprinos no dia 60 em resposta às duas doses de vacina recebidas nos dias zero e 30, sendo que o maior número de animais considerados protegidos também foi detectado neste dia. Apenas cinco caprinos jovens do Grupo 1 e um do Grupo 3 permaneceram com títulos de anticorpos considerados protetores até o dia 150. Diante dos resultados obtidos, concluiu-se que as vacinas avaliadas apresentaram baixa capacidade de estimular uma resposta imune protetora nos caprinos avaliados.

**PALAVRAS-CHAVE:** *Clostridium perfringens* tipo D. ELISA. Resposta imune. Toxóide épsilon. Vacinação.

<sup>1</sup> Project supported by FAPESP

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## INTRODUCTION

Enterotoxemia or pulpy kidney disease is caused by the effect of intestinal absorption of epsilon toxin which is produced by *Clostridium perfringens* type D (UZAL, 2004). It is one of the most important non-contagious, infectious diseases that affect goats (UZAL et al., 2004). It is recognized worldwide since it inflicts high mortality rates in small ruminant herds.

It affects animals from any age; however, susceptibility to the disease is higher for younger animals (UZAL et al., 2003). The persistence of *C. perfringens* type D in the environment derives from earlier cases of enterotoxemia or constant fecal contamination by animals that have the microorganism installed in their intestinal flora, a fact that renders impossible to eradicate the disease, thus making prophylactic measures very important to its control (EHRETH, 2003). However, the natural exposition of goats to epsilon toxin does not stimulate, in general, the production of antibodies with the magnitude necessary to protect the animals against the challenges posed by this toxin (VESCHI et al., 2008).

The prevention of enterotoxemia requires two measures of great importance: systematic vaccination of all animals in the herd and appropriate nutritional management (UZAL & KELLY, 1998). Vaccines with high immunogenic power combined with effective immunization strategies are capable of protecting goats and sheep against enterotoxemia (UZAL et al., 1998). However, in goats, the usual vaccination produces lower serum antibody titer that lasts shorter periods compared to those of sheep (UZAL & KELLY, 1999).

Lobato et al. (2010) evaluated in goats, the recombinant epsilon toxoid potency of *C. perfringens* type D expressed in *Escherichia coli* and concluded that it produced 14.3 UI/mL when administered in two shots of 0.2 mg on day zero and 21 days later, respectively.

Uzal et al. (1998) stated that effective protection of goats can be obtained when using a vaccine with high immunogenic power in two initial shots four to six weeks apart. However, in Brazil, there are no commercial vaccines produced specifically to protect goats, the multipurpose formulations prepared for cattle and sheep are routinely used for goats. Aiming to check the response of epsilon and beta antitoxins of *C. perfringens* type D, Lobato et al (2000) evaluated the immune response of rabbits and cattle to six commercial vaccines used against clostridial diseases.

Since prevention of enterotoxemia in goats is highly important, the antibody response of five commercial polyvalent vaccines containing the epsilon toxoid of *C. perfringens* type D was evaluated over time for the species using the Indirect ELISA technique.

## MATERIAL AND METHODS

We used 84 young goats ( $45 \pm 3$  days old) whose parents had no history of previous vaccination

against clostridial diseases, of the Alpine and Saanen breeds, reared in total confinement system in a rural property located in the country side of São Paulo state, Brazil. The animals were individually identified and maintained under the usual management conditions of the property.

The goats were randomly divided into six groups of 14 animals each. A total of five commercial polyvalent vaccines that contained in their formulation between 5 and 9 agents, which could be bacterins and/or toxoids, were tested. These vaccines, commercially available in veterinary shops, all had in their formulation the epsilon toxoid of *C. perfringens* type D.

The animals from the control group (CG) were not vaccinated, while the animals from Group 1 to 5 (G1, G2, G3, G4 and G5) were vaccinated with two shots of the vaccines being tested four weeks apart, that is, the first dose on day zero and the second, on day 30. The intravenously dose given was that recommended for sheep by the manufacturer.

Blood samples were harvested by jugular vein puncture using disposable needles and vacuum tubes at six different times, as follows: day 0 (before the first vaccination), 30 (before the second shot), 60, 90, 120 and 150 days after vaccination, totaling 504 blood samples.

For the assessment of humoral immune response, we used the indirect ELISA technique described by Uzal et al. (1997), where the value of 0.25 UI/mL was arbitrarily set by Uzal & Kelly (1998) as the minimum quantity of epsilon antitoxin antibodies necessary to protect the goats against the effects of epsilon toxin of *C. perfringens* type D. All serum samples were analyzed in duplicate on the ELISA micro-plate.

The data were then transformed into log (x+1) and submitted to analysis of variance with repeated measures. Group means were compared by Tukey test and the means at a certain time with respect to time zero were compared by Dunnett test. Fisher exact test was used to assess the association between immune status and group. The results were considered significant when  $P < 0.05$ . Statistical analyses were performed using SAS software (Statistical Analysis System).

## RESULTS AND DISCUSSION

Immunization of goats and the control of predisposing factors are the prophylactic measures indicated to prevent enterotoxemia, since the eradication of this disease is impossible. Uzal et al. (1998) stated that goats need the minimum serum levels of 0.25UI of antitoxin epsilon/mL to be protected from the challenges of enterotoxemia caused by the epsilon toxin of *C. perfringens* type D. Although this figure has been suggested based on observations and assessment of experimental vaccination against the disease, its real meaning is still unknown when the disease occurs naturally. However, it should be used as

reference for laboratories that produce commercial vaccines, followed by observations on their effect on the epidemiological behavior of the disease and when facing the natural challenge.

Table 1 shows the mean values for serological response results of young goats evaluated over time. The mean antibody titer of goats from G1 were significantly different from animals of the control group from day 60, according to Tukey test, showing that the vaccine was able to induce the synthesis of antibodies, reaching values higher than the 0.25UI of antitoxin epsilon/mL, until day 120, which is considered adequate for the protection of the animal. However, at day 150 these values were already below the minimum required, indicating a relatively short protection period.

The mean antibody titer of animals from G2 differed from control group means on days 0 and 60 (30 days after the booster shot) indicating that before the vaccination G2 animals had already titer higher than the control group and that, although on day 60 antitoxin levels were higher than 0.25 UI/mL, the response induced by the vaccination can be considered inconsistent. Likewise, the analysis of the results of G3 shows that even before the first vaccine shot, the animals from this group already had antibody titer higher than the control group and although the vaccination increased the titer, especially on days 60 and 90, the values were not significantly different from the CG and did not reach the minimum required of 0.25 UI/mL.

The antibody titer analysis of animals from G4 that got vaccine 4 also shows inconsistent results because antitoxin epsilon titers of vaccinated animals prior to vaccination on days 30 and 60 were higher than the control group, decreasing again to the level of the control group from day 90 and on. It is also observed that these values never reached the minimum required to be considered protected.

Regarding the goats vaccinated with vaccine 5 (G5), titer means were not significantly different from

the control group at any evaluated time. Although the two shots of this vaccine increased antibody titers on days 60 and 90 compared to time zero in the same group, this increase can be considered insufficient to protect the animals.

Table 2 shows the number of young goats with levels of antibody titers that are considered protective. According to the results obtained in the present study, at the time of the first serological evaluation, that is, approximately 45 days after birth (day zero) none of the goats had titers high enough to be considered protected. Some goats had protective levels of titers 30 days after the first shot, but those numbers were not significantly different according to Fisher exact test.

The analysis results for day 60, that is, 30 days after the booster shot, show that the number of protected animals increased in groups 1, 2 and 4 compared to CG, but not among them.

The results seen in Table 1 show that the production of antibodies induced by the vaccines, in general, increased from day 60, that is, 30 days after the booster shot (except for G4, where the increase was observed as early as day 30). While the results of Table 2 also show that the number of protected animals increased only after day 60, a result that reinforces the need for two vaccine shots in goats. This has been stated by Uzal & Kelly (1999) and Veschi et al. (2006) who observed that the animals that got two shots four weeks apart had higher titers of serum antibodies compared to goats that got a single shot.

The serological evaluation of six commercially available vaccines against clostridial diseases in cattle performed by Lobato et al (2000) showed that only two of the tested vaccines produced detectable levels of antitoxin epsilon antibodies on day 56 after the first shot. Results similar to the ones reported in this study with goats on day 60, where from the five vaccines tested, only three had antibody levels that may be considered protective.

**TABLE 1** - Mean antibody titer values (IU/mL) of young goats vaccinated with different commercial products (Group 1 to Group 5) and Control Group (CG), assessed by ELISA-I at different times.

Day	Group (mean ± standard deviation)					
	GC	G1	G2	G3	G4	G5
0	0,086 ± 0,037 <sup>C</sup>	0,119 ± 0,023 <sup>AB</sup>	0,134 ± 0,029 <sup>A</sup>	0,123 ± 0,012 <sup>A</sup>	0,115 ± 0,031 <sup>AB</sup>	0,094 ± 0,018 <sup>BC</sup>
30	0,116 ± 0,052 <sup>B</sup>	0,168 ± 0,060 <sup>AB</sup>	0,148 ± 0,039 <sup>AB</sup>	0,123 ± 0,021 <sup>AB</sup>	0,175 ± 0,066 <sup>*A</sup>	0,126 ± 0,050 <sup>AB</sup>
60	0,115 ± 0,052 <sup>C</sup>	0,345 ± 0,127 <sup>*A</sup>	0,260 ± 0,142 <sup>*AB</sup>	0,182 ± 0,052 <sup>*BC</sup>	0,241 ± 0,096 <sup>*AB</sup>	0,188 ± 0,094 <sup>*BC</sup>
90	0,147 ± 0,116 <sup>*B</sup>	0,291 ± 0,134 <sup>*A</sup>	0,158 ± 0,067 <sup>B</sup>	0,218 ± 0,127 <sup>*AB</sup>	0,197 ± 0,067 <sup>*AB</sup>	0,153 ± 0,069 <sup>*B</sup>
120	0,120 ± 0,073 <sup>B</sup>	0,265 ± 0,131 <sup>*A</sup>	0,114 ± 0,060 <sup>B</sup>	0,181 ± 0,120 <sup>AB</sup>	0,163 ± 0,045 <sup>*B</sup>	0,126 ± 0,049 <sup>B</sup>
150	0,105 ± 0,066 <sup>B</sup>	0,205 ± 0,093 <sup>*A</sup>	0,096 ± 0,047 <sup>B</sup>	0,125 ± 0,075 <sup>B</sup>	0,107 ± 0,041 <sup>B</sup>	0,101 ± 0,043 <sup>B</sup>

\*significantly different when compared to day 0 "Before vaccination" by the Dunnett test (P<0.05).

<sup>A</sup> means followed by different letters in the row differ by Tukey (P < 0.05).

**TABLE 2** - Number of young goats considered protected (antibody titer above 0.25 IU/ml) of a total of 14 animals/group, after response to different vaccines (G1-G5) and Control (GC).

Day	Number of protected animals					
	GC	G1	G2	G3	G4	G5
0	0	0	0	0	0	0
30	0 <sup>A</sup>	1 <sup>A</sup>	1 <sup>A</sup>	0 <sup>A</sup>	3 <sup>A</sup>	1 <sup>A</sup>
60	0 <sup>D</sup>	11 <sup>A</sup>	8 <sup>AB</sup>	2 <sup>CD</sup>	6 <sup>ABC</sup>	3 <sup>BCD</sup>
90	2 <sup>B</sup>	9 <sup>A</sup>	1 <sup>B</sup>	5 <sup>AB</sup>	2 <sup>B</sup>	2 <sup>B</sup>
120	2 <sup>B</sup>	8 <sup>A</sup>	0 <sup>B</sup>	3 <sup>AB</sup>	0 <sup>B</sup>	0 <sup>B</sup>
150	1 <sup>AB</sup>	5 <sup>A</sup>	0 <sup>B</sup>	1 <sup>AB</sup>	0 <sup>B</sup>	0 <sup>B</sup>

<sup>A</sup> means followed by different letters in the row differ by Fisher exact test (P<0.05).

From day 90, the number of protected animals started to decrease in all vaccinated groups, a fact that was more evident in G2, G3 and G4. The fact that some animals in the control group had protective antibody levels on days 90, 120 and 150 can be explained by the outbreaks of enterotoxemia in the herd that happened during the experiment. The presence of animals with titers that are considered protective is in agreement with Veschi et al (2008) who reported that non-vaccinated goats may present natural epsilon antitoxin, which can be explained by the presence of *C. perfringens* type D in the intestinal tract, producing epsilon toxin sufficient to induce antibody production, but not to protect these animals from the disease or to trigger it (Uzal, 1997).

One hypothesis that can be suggested to explain the reduced antibody response of the tested vaccines would be that for commercial reasons, the immunogens against clostridial are having their formulations changed aiming at polyvalent vaccines targeted for multi-species, which has certainly contributed to reduce their efficacy.

## CONCLUSION

The results of the present study, where five commercially available polyvalent vaccines against enterotoxemia were evaluated, showed that the vaccines were greatly heterogeneous regarding their ability to induce the production of antibodies in goats; while only one was able to induce protective antibody levels during 120 days. Therefore, caution should be exercised by goat producers prior to acquiring a vaccine against enterotoxemia caused by *C. perfringens* type D.

## ACKNOWLEDGEMENTS

The authors are thankful to Dr. Sérgio Mahfuz, owner of Capril Rio Preto, São José do Rio Preto, SP for having kindly provided his flock for the experiment. Thanks are also due to Prof. Dr. Francisco Alejandro Uzal for his valuable contributions in the experimental design of this study.

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