SOROLOGICAL EVALUATION OF COMMERCIAL VACCINES AGAINST ENTEROTOXEMIA IN GOATS[1]

JOSIR LAINE A. VESCHI (2), IVERALDO S. DUTRA (3), MARIA ANGÉLICA B. ALVES (4), MARIANO E. FERNANDEZ-MIYAKAWA (5), SILVIA HELENA V. PERRI (6), FRANCISCO A. UZAL (7)

1 Project financed by FAPESP

2 Researcher - Animal Health - Embrapa Tropical Semi-Arid, BR 428, Km 152, Petrolina–PE, Brazil

Corresponding author - E-mail: josi.veschi@cpatsa.embrapa.br

3 Assisting teacher - Animal Health and Production Dept. - UNESP - Araçatuba-SP, Brazil

4 Scholarship from FAPESP - Course of Veterinary Medicine - UNESP - Araçatuba–SP, Brazil

5 Researcher - California Animal Health and Food Safety – School of Vetrinary Medicine, UCDAVIS– California, USA

6 Assisting Teacher - UNESP - Araçatuba–SP, Brazil

7 Teacher – California Animal Health and Food Safety – School of Veterinary Medicine, UCDAVIS – California, USA.

ABSTRACT

Enterotoxemia in sheep and in goats is caused by the effects of the epsilon toxin of *Clostridium perfringens* type D, being considered the main infectious cause of mortality in those animal species. The main prophylactic measures include adequate nutritional management and vaccination of all animals using vaccines of high immunogenic power. Six commercial vaccines containing in its formulation the epsilon toxoid of C. *perfringens* type *D* were sorogically evaluated. Eighty four female goat kids, whose mothers had no previous vaccination history against clostridioses were used. They were divided into six groups of 14 animals each. The animals of the control group didn't receive any vaccine dose and the animals from the groups 1 to 5 received two vaccine doses. The first vaccine dose was applied at 45 days of life (day zero) and the second dose at 75 days (30 days after the first dose). Blood samples were collected from the goat kids at the days zero, 30, 60, 90, 120 and 150 after the beginning of the experiment, in order to evaluate the immunologic response. The Indirect ELISA technique was used for the quantification of the antibodies against epsilon toxin in the samples of blood serum of the animals. In day zero, no animal presented titre considered protector. The largest number of animals considered protected was found at day 60, in response to the two initial doses of the vaccine (days 0 and 30, first and second doses, respectively). Only five animals which received the vaccine 1 and one animal which received the vaccine 3 stayed with titres of antibodies considered up to 150 days after the first vaccine dose. Based on the results, it was concluded that the evaluated vaccines showed small amount of epsilon toxoid in the commercial formulations, a crucial fact for the low efficiency of the vaccines. For commercial reasons, the vaccines against the clostridioses present versatile formulations, with several toxoid types, used for various animal species, which certainly contributed to reduce their effectiveness in preventing the illnesses caused by the clostridia or their toxins.

KEYWORDS

Clostridioses, *Clostridium perfringens* type D; epsilon toxin; immune response; vaccination.

INTRODUCTION

Enterotoxemia caused by the epsilon toxin of *Clostridium perfringens* type D (Pulpy Kidney Disease) is the infectious disease of greater economic and sanitary importance on sheep and goat farming worldwide (Niilo, 1980; Kriek et al., 1994) and is probably the most important cause of sudden death in sheep and goats at different ages. Several factors have been pointed out as predisposing for the occurrence of this disease, the most important including sudden dietary changes and a reduction in intestinal function speed (Smith & Sherman, 1994). The persistence of C. *perfringens* in the environment is the result of previous cases of enterotoxemia or constant fecal contamination by various animal species that harbor the

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microrganism as part of their normal intestinal flora (Smith & Sherman, 1994). Normally, epsilon toxin can be produced in small amounts in the intestine of animals carrying *C.perfringens* type D; in this circumstance, the toxin does not cause any deleterious effect and stimulates the formation of antibodies (Blackwell et al., 1983). Two measures are mentioned as being of great importance in the prophylaxis against enterotoxemia: vaccination of all animals (Uzal & Kelly, 1999) and adequade nutritional management. Since no enterotoxemia vaccine produced specifically for goats is commercially available, polyvalent vaccines produced for other animals are generally used (Uzal & Kelly, 1998). Vaccines of high immunogenic power, combined with adequate immunization strategies which use two initial vaccine doses, with interval of thre to four weeks between them protect sheep against pulpy kidney disease (Uzal et al., 1998). However, in goats, usual vaccination produces lower and shorter duration serum antibody titres than in sheep and the animals require booster doses every 3 or 4 months throughout their life after first double vaccination (Uzal & Kelly, 1999). The objetive of the present study was to serologically evaluate six commercial vaccines containing epsilon toxoid in goat kids by Indirect ELISA technique.

MATERIALS AND METHODS

Eighty four female goat kids, whose mothers had no history of vaccinations against clostridioses but with a history of occurrence of the disease in the herd, from the breeds Saanen and Alpine, were raised in confinement system in a goat farm countryside the State of São Paulo, Brazil. The animals were randomly divided into six groups of 14 animals each. Five commercial vaccines against clostridioses were used, obtained at stores of veterinary products, containing in its formulation the epsilon toxoid of *Clostridium perfringens* type D. The female goat kids which constituted the control group did not receive any vaccine dose. The animals from group 1 through 5 received two initial doses of one of the commercial vaccines used in the experiment. The first vaccine dose was applied at the day zero (45 days of age) and the second at the day 30 (75 days of age of the goat kids). According to manufaturers' recommendation, the vaccines were applied subcutaneously. The blood samples of the goat kids were collected by puncturing the jugular vein at six different times: at the beginning of the trial, at day zero (45 days of age), before the first vaccination; at day 30 (75 days of age), before the application of the second doses of the vaccine, and at days 60 (105 days of age), 90 (135 days of age), 120 (165 days of age) and 150 (195 days of age), for checking the immune kinetics. Serum was stored at -20°C until the time of the sorological tests. For evaluation of the immunologic answer, the sera were processed by Indirect ELISA technique to detect antibodies against epsilon toxin and titres are reported as international units per milliliter (UI/ml) (Uzal et al., 1997). A value of 0.25 IU/ml was arbitrarily adopted as the minimum protector (Uzal & Kelly, 1998). The obtained data were transformed in log (x+1) and submitted to analysis of variance with repeated measures. To check association between the immunologic status and the groups, the Fisher test was used. Differences were considered to be significant when $P<0.05$. The statistical analyses were run using SAS program.

RESULTS AND DISCUSSION

Based on the results obtained in the present study, in the serological evaluation of the samples colleted at day zero (45 days of age), beginning of the experiment, no animal presented titers considered protectors arbitrarily fixed at 0.25 IU/ml (Uzal et al, 1998). At day 30, i.e., 30 days after the first vaccine dose and before the application of the second vaccine dose, only one animal in the groups 1, 2 and 5, and three animals in the group 4 presented protective antibody titres (Table 1). However, there was no statistical significative difference by Fisher test ($P<0.05$).

Table 1. Number of goat kids considered protected (titre of antibodies ≥ 0.25UI/ml) from a total of 14 animals/group (Groups 1-5) and Control Group (C).

Day	Number of protected animals					
	Control*	Group 1*	Group 2*	Group 3*	Group 4*	Group 5*
0	0	0	0	0	0	0
30	0 ^{A}	1 ^{A}	1 ^{A}	0 ^{A}	3 ^{A}	1 ^{A}
60	0 ^{D}	11 ^{A}	8 ^{AB}	2 ^{CD}	6 ^{ABC}	3 ^{BCD}
90	2 ^{B}	9 ^{A}	1 ^{B}	5 ^{AB}	2 ^{B}	2 ^{B}
120	2 ^{B}	8 ^{A}	0 ^{B}	3 ^{AB}	0 ^{B}	0 ^{B}
150	1 ^{AB}	5 ^{A}	0 ^{B}	1 ^{AB}	0 ^{B}	0 ^{B}

*means followed by different letters, in the same line, differ from each other by the Fisher test ($P < 0.05$).

At 60 days from the beginning of the experiment, that is, 30 days after the second vaccine dose, the number of animals considered as protected increased in all the groups, except in the control, reinforcing the importance of the use of two initial vaccine doses against enterotoxemia in goats (Uzal & Kelly,1999; Veschi et al., 2006). At day 90 (135 days of age), the number of protected animals decreased in groups 1, 2, 4 and 5, results which agree with Jansen (1967) and Uzal & Kelly (1998) and increased in the the groups control and 3, which can be explained by the occurrence of the outbreak of enterotoxemia in the flock of the experiment. In the samples of days 120 and 150, no animal presented titre considered as protector in the groups 2, 4 and 5. Five goat kids of group 1 presented titre of antibodies considered protected when evaluated at 150 days, demonstrating that the vaccine 1 induced protection to the animals for a longer period when compared with the other evaluated vaccines. The fact of some animals from the control group having shown titres of antibodies considered as protectors at days 90, 120 and 150 can be explained by the history of occurrence of outbreak of enterotoxemia in the flock during the experiment, since, according to Blackwell et al. (1983), up to 54% of non-vaccinated animals can present natural antibodies against the epsilon antitoxin. The levels of antibodies began to decrease from the day 90, that is to say, 60 days after the second vaccine dose, making clear that the immunity produced by the vaccines in the animals of groups 2, 3, 4 and 5 was not efficiently long. At days 120 and 150, there was no more animal considered protected in groups 2, 4 and 5, allowing to state that the tested vaccines were not efficient in maintaining the immunity of the animals for a long time. Even in group 1, where the animals showed a better response to the vaccine, there was a decrease in the number of animals considered protected, and at 150 days, only 45% of the goat kids presented protector titres. Green et al. (1987) obtained similar results when evaluated multivalent vaccines in sheep and goats. For the vaccine tested in the animals of group 1, it is possible to adopt the management technique recommended by Uzal & Kelly (1999), who propose that the goats should receive two initial doses of the vaccine, with four to six weeks of interval, besides reinforcement doses at each three or four months during the lifetime of the goat. In this condition, the vaccination of the animals loses the practical sense due to the number of vaccine applications and the practical consequences, as, for example, the reactions in the site of vaccine application and the high cost of handling. It is worth to stand out that this vaccine is not commercially indicated for goats.

CONCLUSIONS

Based on the results of this study with the vaccines evaluated, it can be concluded that the little amount of epsilon toxoid in the commercial products is the crucial fact which reduces the

efficiency and effectiveness of the products. It is known that for commercial reasons, the immunogens against the clostridioses produced formulations of multivalent vaccines, containing several toxoid types for various animal species, which certainly contributed to reduce their effectiveness related to certain illnesses caused by the clostridia or their toxins.

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