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SHORT NOTE

PROBIOTIC INCREASE THE ANTIRABIES HUMORAL IMMUNE RESPONSE IN BOVINE

PROBIÓTICO AUMENTA A RESPOSTA IMMUNE HUMORAL ANTI-RÁBICA EM BOVINOS

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ADDITIONAL KEYWORDS

Cattle. Probiotic. Rabies vaccine. Humoral response.

PALABRAS CHAVE ADICIONAIS

Bovino. Probiótico. Vacina anti-rabica. Resposta humoral.

SUMMARY

The effect of a probiotic on the immune response of cattle immunized with a dose of rabies vaccine was evaluated in this study. The Nellore bovines (N= 75) were divided randomly into 5 groups: supplemented with 0, 2, 3, 4 and 8 g the probiotic (Proenzime®)/animal/day, groups Gc, G_2, G_3, G_4 and G_8 respectively, for 60 days. All the animals were immunized with a single dose of rabies vaccine on the first day of the experiment (day 0). The obtained results showed that there was significant increase ($p<0.05$) in the titers of antirabies antibodies and frequency of animals immunized in the groups G_4 and G_8 on 30 and 60. In conclusion, the probiotic (Proenzime®) increase the humoral immune response of bovine immunized with rabies vaccine.

RESUMO

Foi avaliado o efeito do probiótico sobre a resposta imune de bovinos imunizados com uma dose de vacina anti-rábica. Utilizou-se bovinos Nelore (N= 75) divididos randomicamente em 5 grupos: suplementados com 0, 2, 3, 4 e 8 g probiótico (Proenzime®)/bovino/dia, grupos Gc, G_2, G_3, G_4 e G_8, respectivamente, por 60 dias. Todos os animais foram imunizados com uma de vacina anti-rábica no dia zero do experimento. Os resultados obtidos mostraram que houve aumento significativo ($p<0,05$) nos títulos de anticorpos anti-rábicos e na freqüência de animais imunizados nos grupos G_4 e G_8 no dia 30 e 60. Conclui-se que o probiótico (Proenzime®) aumentou a resposta immune humoral em bovinos imunizados com vacina anti-rabica.

INTRODUCTION

Rabies is an infection caused by a virus of the genus *Lyssavirus*, order Rhabdoviridae. It's a major animal disease in the world because causes fatal encephalitis in mammals, including humans, has wide geographical distribution and constitutes a serious health problem (Consales *et al.*, 2006). The vaccination is the half most effective and economical instrument for the control of disease (Oliveira *et al.*, 2000).

The use of probiotics is a strategy to increase the immunological response of animals vaccinated or infected by virus or bacteria (Hamilton-Miller, 2004). Havenaar and Huis In't Veld defined probiotics in 1992 as viable microorganisms, including lactic bacteria and yeast, like lyophilized cells or fermented products that have beneficial effects on host health after being ingested. These substances are used as growth pro-

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moters (Arenas *et al.*, 2007).

In the present study was evaluated the effect of different concentrations of probiotic added to the mineral diet, in the immunological response of bovine immunized with rabies vaccine.

MATERIALS AND METHODS

Nellore cattle (*Bos taurus indicus*) aged 18 months, from a farm in Presidente Bernardes, SP, Brazil was utilized in this study. These animals were divided randomly into 5 groups (N= 15), animals from the control group (Gc) fed pasture and received mineral salt (Premix R®) *ad libitum* and the other groups were fed in a similar system, but each one received 2, 3, 4 or 8 g probiotic (Proenzime®) added to 70 g of mineral salt, groups G_2, G_3, G_4 and G_8 respectively. This probiotic/salt ratio was chosen because in observations taken for 20 days before the experiments the daily average salt intake was estimated at 70 g per bovine. All the animals were immunized with 1 dose of rabies vaccine on the first day of the experiment (day 0).

The probiotic (Proenzime®) is produced by EMBRAUPEC, Paranavaí, PR, Brazil. It is composed of amylase, cellulase, protease, lipase, pectinase, *Lactobacilus acidophilus*, *Streptococus faecium*, *Bifidobacterium thermophilum*, *Bifidobacterium longum* and zinc.

A commercial rabies vaccine with satisfactory antigenic values contains a suspension of fixed rabies PV (Pasteur virus) obtained in BHK-21 cells, inactivated by beta-propiolactone, adsorbed in aluminum hydroxide and preserved with thimerosol at 1:10000.

On days 0, 30 and 60, blood (10 ml) was collected from the jugular vein in vacuum tubes with no anticoagulant. After the blood samples were clotted and centrifuged 2500 rpm, 10 min, serum samples were stored at -20 °C for further determination of rabies-neutralizing antibodies in BHK-21 cells.

The neutralizing antibodies were determined by serum neutralization in BHK-21 clone 13 cells. This test is based on the Rapid fluorescent focus inhibition test (RFFIT) (Smith *et al.*, 1996) and the fluorescent inhibition microtest (FIMT RFFIT) (Zanlan *et al.*, 1979).

Data were compared by repeated measures analysis of variance; further multiple

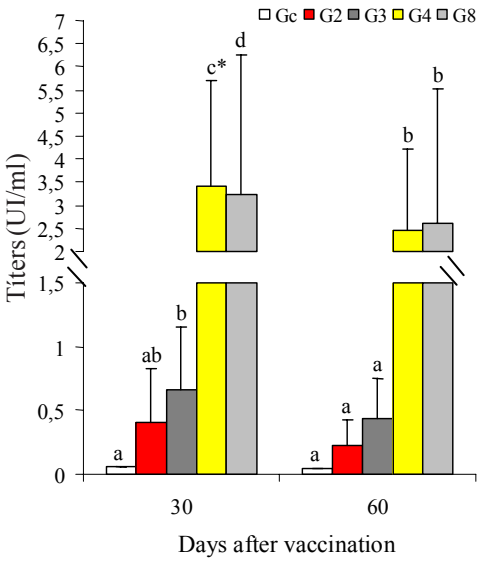

Figure 1. *Mean titers (± standard deviation) of rabies antibodies in first-time-vaccinated cattle receiving feed supplementation with 0 (Gc), 2 (G_2), 3 (G_3), 4 (G_4) or 8 (G_8) g of probiotic (Proenzime®)/animal/day. In a day means followed by the same letter indicate no significant difference among groups ($p<0.05$). *indicates significant difference between days 30 and 60 within a same group ($p<0.05$).* (Títulos médios (± desvio padrão) de anticorpos anti-rabicos de bovinos primo-vacinados com supplementação alimentar de 0 (Gc), 2 (G_2), 3 (G_3), 4 (G_4) ou 8 (G_8) g de probiótico (Proenzime®)/animal/dia. Médias seguidas por a mesma letra indicam, dentro de um mesmo dia, diferença não significava entre os grupos ($p<0,05$). *indica diferença significativa entre dias 30 e 60 dentro de um mesmo grupo ($p<0,05$)).

comparisons were carried out by LSD test. In all the analyses, outliers were excluded. To compare the frequency of animals immunized against rabies among the experimental groups as a function of observation day, the Goodman test was used. In all the analyses, probability of error was set at 5% (Zar, 1999).

RESULTS AND DISCUSSION

The World Health Organization (WHO) recommends rabies-neutralizing antibody titers of at least 0.50 UI/ml for effective prevention in humans against rabies virus contamination. Some studies argue that this neutralizing antibody titer is the minimal level required to protect cattle (Albas *et al.*, 2005).

On day 0, the results from titration of rabies neutralizing antibodies, sampled from the serum of cattle under the different experimental treatments, were not reactive, indicating that none of the animals had either prior contact with the rabies virus or vaccination against rabies. Thus, the variations found among rabies antibody titers were induced by rabies vaccination as well as by the probiotic treatment.

Significant interaction was found between the probiotic level and vaccination time ($F_{(4,63)}$= 2.58; p= 0.046). After rabies vaccination, all the cattle had rabies-antibody titers, irrespective of whether or not they had received probiotic added to the mineral mixture. However, animals from groups G_4 and G_8 had a significant increase ($p<0.05$) in the production of rabies antibodies 30 and 60 days after vaccination **(figure 1)**, and also the frequency of immunized animals (antibody titers= 0.50 UI/ml) **(figure 2)** on both observation days. In this way, these supplementations caused a persistent immunostimulant effect. But their action mechanism is not completely known (Silva *et al.*, 2006). These results agree with previous studies in swine (Ávila *et al.*, 1998) and poultry (Kabir *et al.*, 2004) which indicate that the immune response is improved when probiotic administration is associated with vaccination.

In group G_3, there was a significant increase ($p<0.05$) in rabies antibody titers on day 30, but on day 60 the titers decreased significantly **(figure 1)**. Thus, the immu-

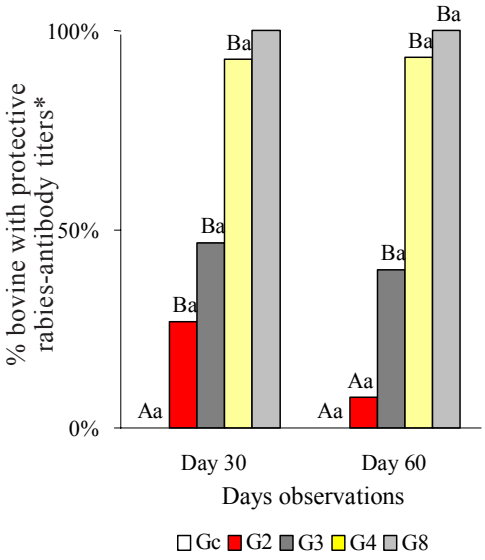

Figure 2. *Frequency of rabies-immunized cattle. The cattle received daily feed supplementation with 0 (Gc), 2 (G_2), 3 (G_3), 4 (G_4) or 8 (G_8) g probiotic (Proenzime®)/ animal/day. Means followed by same upper-case letter indicate that no significant difference was found between the groups ($p<0.05$) within the same day. Means followed by the same lower-case letter indicate that no significant difference was found between the days ($p<0.05$) within the same group.* (Freqüência de bovinos imunizados contra a raiva. Os bovinos receberam supplementação alimentar diária com 0 (Gc), 2 (G_2), 3 (G_3), 4 (G_4) ou 8 (G_8) g de probiótico (Proenzime®)/animal/dia. Médias seguidas por uma mesma letra maiúscula indicam que, dentro de um mesmo dia, diferença não significativa entre os grupos ($p<0,05$). Médias seguidas por uma mesma letra minúscula indicam que, dentro de um mesmo grupo, diferença não significativa entre os dias ($p<0,05$).

nostimulant action caused was not persistent.

In group G_2, there was not a significant increase ($p<0.05$) in the titers of rabies antibodies **(figure 1)**. Moreover, in this group, the frequency of immunized animals decreased significantly ($p<0.05$) 60 days after vaccination, similarly to group Gc **(figure 2)**. The supplementation was not an immunostimulant.

In group control cattle had the protective rabies antibody titers in any of the two observation days, i.e., all the animals were exposed to the risk of rabies contamination after 30 and 60 days of vaccination **(figure 2)**. In fact, Albas *et al.* (2005), and Reis *et al.* (2008) report that cattle which are first-vaccinated against rabies require a booster vaccination to achieve protective titers of rabies anti-bodies.

The results found in the present study lead to the conclusion that the probiotic (Proenzime®) increases both the humoral immune response and the frequency of cattle immunized by rabies vaccine.

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