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Vaccination of bulls against Bovine Genital Campylobacteriosis: a therapeutic approach

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Abstract

The therapeutic effect of vaccination with a bacterin produced with Campylobacter fetus subsp.venerealis NCTC 10354 was evaluated in Nelore bulls 5 to 7 years of age. Twenty-seven bulls, that tested positive for C. fetus using the direct fluorescent antibody technique (DFAT), were used in the present experiment. Bulls received two doses of the vaccine separated by an interval of 23 days. After the first vaccination, 15 animals remained positive for C. fetus by DFAT, a significant drop of 44.5% ($P<0.05$) in the frequency of infected bulls. After the second vaccination, 12 animals were positive by DFAT, a significant decrease of 55.6% ($P< 0.05$) in the number of infected-bulls. There was no significant difference in the number of infected bulls between the first and the second vaccinations. These results showed that vaccination of bulls with a bacterin against C. fetus subsp. venerealis may be an important additional strategy to control bovine genital campylobacteriosis.

Keywords: Bovine genital campylobacteriosis, *Campylobacter fetus*, bull, vaccine

Introduction

Bovine genital campylobacteriosis (BGC) is an infectious venereal disease of cattle caused by *Campylobacter fetus* subsp. *venerealis*. The most prominent clinical signs are frequent return to estrus, with increased and irregular duration of the estrous cycle, increased calving interval, increased age at first calving, and temporary infertility in bulls (Dekeyser, 1984; Lage and Leite, 2000). It causes substantial economic losses in countries and regions with large cattle populations that are subjected to natural breeding due to reduction in milk production, reduction in the number of calves produced, irregular lots of calves for market, and increased cull rate (Stoessel, 1982).

The frequency of *C. fetus* infection is reported to range from 8% to 72% in Brazilian herds (Lage and Leite, 2000). Recently, prevalence of BGC in beef bulls was estimated at 18.2% and infection was found to be widespread in beef cattle producing regions (Lage *et al*, 2001). However, in some regions such as Pantanal Matogrossense (Brazilian Pantanal), the prevalence of infection of bulls with *C. fetus* could be as high as 52.3% (Pellegrin *et al.*, 2002). This high prevalence of BGC in Brazil is attributed to the large number of cattle subjected to natural breeding and difficulties in diagnosing the disease in the field (Pellegrin, 1999; Lage and Leite, 2000).

Bulls are considered to play a central role in disseminating and maintaining BGC in herds with a natural breeding program because they are symptomless, and–permanently infected and each bull have the chance to breed a large number of cows can spread the disease to the cows in the herd (Stoessel, 1982; Dekeyser, 1984; Lage and Leite, 2000). Consequently, several measures to control the disease are directed towards bulls, i.e., replacement of *C. fetus* infected animals – free younger ones, introduction of artificial insemination, and treatment.

Vaccination is one of the most effective strategies for the control of BGC especially in areas or herds where artificial insemination is not able to be employed (Dekeyser, 1984; Lage and Leite, 2000) and has already been successfully used in prevention and therapy of BGC (Clark *et al*., 1974; Leite *et al*., 1980; Eaglesome *et al*., 1986). Bacterins with oil adjuvants have been shown to be efficient in reducing the number of animals that return to heat and reducing the frequency of abortion when used in infected cows (Frank *et al*., 1967; Clark *et al.*, 1974; Leite *et al*., 1980; Ramos *et al*., 1986). In infected herds, cows are usually vaccinated annually, 30 to 45 days before the breeding season (Leite *et al*., 1980; Dekeyser, 1984; Ramos *et al*., 1986; Lage and Leite, 2000; Wagenaar *et al*., 2000).

Although vaccination of cows is an already established measure in the control of BGC, its use in bulls is controversial. Some researchers find it very effective in curing and preventing infection by *C. fetus* (Clark *et al*., 1968; Bouters *et al*., 1973; Clark *et al*., 1974; Clark and Dufty, 1982; Hum *et al*., 1993) while others suggest it is not successful (Allan, 1972; Vasquez

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et al., 1983). The purpose of the present study was to evaluate the therapeutic use of a vaccine against BGC in infected bulls.

Materials and Methods

Animals

Twenty-seven, Nelore bulls from a beef herd using a natural breeding program in Corumbá, Mato Grosso do Sul, Brazil, were used. The bulls, 5 to 7 years of age, were in good physical condition and did not present any clinical signs of illnesses. Animals were reared on natural pastures and received water and minerals ad libitum. Bovine genital campylobacteriosis was previously diagnosed in the herd (Pellegrin et al., 1998).

Sample collection

Preputial washings of all bulls were collected according to Leite et al. (1995). Seven samplings were taken during the period of September 5 (Day 0) to December 13, 2000 (Day 97) on days 0, 10, 20, 70, 80, 90 and 97 of the experiment. Bulls began sexual resting approximately six months before the beginning of the experiment and remained in this condition throughout the study.

Direct fluorescent antibody technique (DFAT)

Direct fluorescent antibody technique (DFAT) was carried out according to Figueiredo et al. (2002). Samples were divided in three series: before vaccination (days 0, 10 and 20), after the first vaccination (days 70 and 80), and after the second vaccination (days 90 and 97). A bull was considered C. fetus – positive if fluorescent bacteria, with typical morphology of the Campylobacter sp, were present in at least one preputial washing from the series.

Vaccine

C. fetus subsp. *venerealis* NCTC 10354 strain was cultivated in Brain Heart Infusion agar (BHI agar – Difco, USA) with 10% horse blood at 37ºC in an atmosphere of 85% N2, 10% CO2, and 5% O2 for 48 h. After incubation, the culture was harvested by a swab and inoculated in flasks containing 500 mL of Brewer thioglicolate broth (Difco, USA) and incubated for 72 h at 37ºC. Bacterial growth was then inoculated in flasks containing 1 L of Brewer thioglicolate broth and cultivated during four days at 37ºC. To the pure cultures it was added 0.3% formaldehyde (Labsynth, Brazil) for inactivation during 24 h. Following inactivation, the suspension was centrifuged at 13000 X G for 30 min at 4ºC and the sediment was weighed and suspended in saline to a concentration of 6mg/mL. Vaccine was prepared by adding 15% of oil adjuvant (Emulsigen®, MVP Laboratories Incorporation, USA) to the bacterial suspension. The mixture was agitated at 100 rpm for 12 hours at 37ºC and then bottled in 100 mL – flasks.

Cultures and vaccines were respectively checked for purity and sterility by Gram stain and inoculation on BHI blood agar, thioglicolate broth, and Sabouroud agar (United States of America - USA, 2002a). Three guinea pigs were inoculated subcutaneously with a 2-mL dose of vaccine for safety. They were observed for seven days for adverse reactions (USA, 2002b).

Animals were vaccinated subcutaneously with 3 mL of the experimental vaccine on days 60 and 83 of the experiment and were observed for two hours for symptoms of immediate hypersensitivity (Povey and Carman, 1997).

Statistics

The frequency of infected animals before vaccination and after the first and second vaccinations was analyzed by the McNemar test with an alpha error level of 0.05 (Siegel, 1975).

Results

In samplings done before the first vaccination (days 0, 10, and 20), all twenty-seven bulls were found by DFAT to be infected by *C. fetus*. The number of bulls with positive results from DFAT in the series of tests before and after vaccination against bovine genital campylobacteriosis is shown in Table 1.

After the first vaccination, 15 of the 27 DFAT – positive animals still remained infected at the end of the two samplings (days 70 and 80), showing a significant decrease ($P<0.05$) in the number of *C. fetus* – infected animals (Table 1 and Table 2). After the second vaccination, 8 of the 15 DFAT – positive animals following the first vaccination, remained infected at the end of the two samplings (days 90 and 97). Four animals considered negative after the first vaccination tested positive after the second vaccination (Table 1). The total number of DFAT – positive animals after second vaccination, 12 bulls, was significantly lower (55.6%, $P<0.05$) compared to the number of *C. fetus* – infected animals before vaccination (Table 2). However, no significant difference was observed in the number of infected animals between the first and the second vaccination (Table 2).

Table 1. Number of bulls with positive results in the direct fluorescence antibody technique (DFAT) in the series of tests before vaccination (3 tests) and after vaccination (2 tests after each vaccination) against bovine genital campylobacteriosis.

Number of bulls	Before vaccination (3 tests)	After first vaccination (2 tests)	After second vaccination (2 tests)
1	3	2	2
3	3	2	1
3	3	1	0
1	3	0	1
1	3	0	0
2	2	2	0
1	2	1	2
1	2	1	1
3	2	1	0
2	2	0	2
1	2	0	1
4	2	0	0
2	1	1	1
2	1	0	0

Table 2. Effect of therapeutic vaccination of bulls against *C. fetus* diagnosed by direct fluorescent antibody technique (DFAT).

	Infected (%)	Free from infection (%)	Total
Before first vaccination	27 (100%)	0 (0%)	27
After first vaccination	15 (55.6%)	12 (44.5%)	27
After second vaccination	12 (44.5%)	15 (55.6%)	27

Discussion

Countries and regions with large herds under extensive management and with natural breeding programs are still at high risk for reproductive failure due to venereal diseases like bovine genital campylobacteriosis. Strategies, which reduce the number of *C. fetus* - infected animals, could be very useful in decreasing the economic losses due to this infection. One of those strategies is a therapeutic approach using vaccination of bulls, which was confirmed to be helpful in the control of bovine genital campylobacteriosis by the present study.

One of the key points in assessing the efficacy of a vaccine is the technique used to distinguish infected from non-infected individuals. Direct fluorescent antibody technique has been widely used in the diagnosis of the BGC (Philppot, 1968; Ruckerbauer *et al*., 1974; Leite, 1977; Cipolla *et al*., 1984; El-Jakee *et al*., 1991; Figueiredo *et al*., 2002) and is prescribed by World Animal Health Organization (OIE) for testing bulls for international trade (Wagenaar *et al*., 2000). It was chosen as the diagnostic test in this study based on its specificity (88.9%), high sensitivity (92.6%), low detection limit (being able to detect up to 100 bacteria / mL of prepucial washing) and practicality (Figueiredo *et al*, 2002). Moreover, the use of culture method as the diagnostic tool in this study was not feasible due to the time spent between the collection of the preputial washings and its arrival at the laboratory, usually 48 – 72 h.

The time interval used between samplings, 10 days, was chosen to optimize sensitivity and to avoid interference with the management of the farm. Soto and Dick (1983) obtained better results in the diagnosis of BGC using DFAT and consecutive preputial samplings with intervals of 8 and 15 days. This interval allows the population of *C. fetus* to reestablish itself after suffering a drop during preputial washing thus preventing false-negative results due to low numbers of bacteria (Winter *et al.*, 1967; Philpott, 1968).

To increase the performance of diagnosis, DFAT was done in consecutive preputial samplings, during sexual resting of bulls, and a bull was only considered free from infection when presenting negative results in all consecutive preputial washings from a series. This strategy has been successfully used and recommended by many authors to increase detection of *C. fetus* – infected animals (Dufty and McEntee, 1969; Stoessel, 1982; Soto and Dick, 1983; Lage and Leite, 2000). Thus, the frequency of false-negative results should have been 0.005% and 0.0004% after two and three consecutive tests, based on the reported sensitivity of DFAT (92.6%) (Tarabla, 2000; Figueiredo *et al*., 2002).

All but four bulls had two or three DFAT-

positive test results before vaccination, showing their infection by *C. fetus*. Of those four bulls that had only one positive-test by DFAT, two of them had two positive results after the first and second vaccination, confirming their infection by and the lack of effect of the vaccine against *C. fetus* in these animals. In spite of that, the two other bulls that had only one positive result before vaccination were likely false-positive animals because of the reduction in specificity due to the diagnostic strategy employed. Even if these animals were excluded from statistical analysis, the differences in the number of animals infected before and after vaccination and between the first and second vaccinations remained the same.

The diagnostic strategy, repeating tests in sexual resting bulls, was used to reduce false-negative results that would have overestimated the power of the vaccine in controlling BGC infection in bulls. However, this could also cause the emergence of false-positive animals, reducing the specificity of the diagnosis (Tarabla, 2000). Indeed, this could have been the case in two bulls that had only one positive-DFAT result after the second vaccination but had negative results following the first vaccination. These animals could have been really infected animals, or, on the contrary, false-positive results from DFAT. In the latter case, the performance of vaccination against BGC in bulls may have been better than our present results reported.

Although there was a significant difference between the number of infected animals before vaccination and after the second dose of vaccine, the lack of significance between first and second vaccination in reducing the number of infected bulls could have been the result of the 23-day interval between the two doses of the vaccine. It could possibly have interfered with the immunization of animals that remained infected. Ramos *et al*. (1986), who vaccinated a group of heifers twice with an interval of 14 days, observed that a larger interval between vaccine doses was needed to prevent such interference.

Bouters *et al*. (1973) reported the cure of 70% and 100% of 41 *C. fetus* – infected bulls after the first and second vaccinations, respectively. However, the freedom of 70% of infected animals from *C. fetus* was observed only at day 42 after vaccination, when the second dose of the vaccine was then administered. Although these authors did not report the antigen concentration of the vaccine used in their experiment, their results could explain some of the differences seen in the present study. The interval between vaccinations used in the present study, 23 days, could have been too short for any detectable difference to be expressed.

The short interval between the second vaccination and DFAT testing (14 days) in the present study, due to the beginning of the breeding season, could also have decreased the observed effect of the second vaccination due to an insufficient time to observe the full effect of the vaccination. It was reported that animals take 14 to 56 days to acquire immunity after vaccination and yield negative results to DFAT (Bouters *et al*., 1973; Vasquez *et al*., 1983). Furthermore, Berg *et al*. (1979) have demonstrated that the complete elimination of infection from some animals could take up to 136 days. Thus, infected animals from the present study probably did not have enough time to elicit an immune response capable of eliminating *C. fetus* from the prepuce. In addition, Bouters *et al*. (1973) also observed that DFAT could detect *C. fetus* in prepuce for longer periods after vaccination compared to culture methods, suggesting that, although microorganisms were inactivated by antibodies, they could stay in the prepuce for a long period of time before elimination. Hence, the number of infected animals found positive by DFAT after the second vaccination could have been even fewer, suggesting a better effectiveness of the vaccination of bulls against *C. fetus*.

Another factor that can influence the effectiveness of vaccines is the use of inadequate amount of antigen per dose of the vaccine. Antigen concentration of vaccines against *C. fetus* is usually expressed as milligram of bacteria per dose. The concentration of 18 mg of bacteria per dose of the vaccine used in this study is in the range of concentrations employed by the majority of successful vaccines (10 mg to 40 mg per dose; Schurig *et al*. 1975; Clark and Dufty, 1978; 1982; Bryner *et al*., 1988). Thus, the antigenic concentration of the vaccine used in the present study could not account for the different results observed in the present study compared to previous studies.

Performance of vaccines against bovine genital campylobacteriosis could also be affected by the adjuvants and strain used. The majority of vaccines against *C. fetus* are prepared with oil or alluminium hydroxide adjuvants (Clark *et al*., 1968; Bouter *et al*., 1973; Leite *et al*., 1980; Clark and Dufty, 1982; Ramos *et al*., 1986; Cobo *et al*., 2003). However, the most effective vaccines are prepared with oil adjuvants (Clark *et al*., 1968; Bouter *et al*., 1973; Leite *et al*., 1980; Clark and Dufty, 1982; Ramos *et al*., 1986). The adjuvant used in the present study was an oil adjuvant that produced low side effects and is used in some commercial *C. fetus v*accines yielding good responses in the field.

Commercial vaccines against *C. fetus* are blamed to induce a poor performance, because they are not elaborated with regional isolates of *C. fetus* (Bryner *et al*., 1979; 1988; Cobo *et al*., 2003). The strain used to prepare the experimental vaccine, NCTC 10354, is the type strain for *C. fetus* subsp. *Venerealis.* The antigenic differences among reference and local strains of *C. fetus*, associated the antigenic variation of strains during infection, could have interfered with the protection induced by the vaccine (Schuring *et al*., 1975; Hum *et al.*, 1983).

The results found in the present study indicate that vaccination was effective in eliminating the infection in most of the BGC-infected bulls but cannot be recommended as the sole measure of control in infected herds. Therefore, vaccination of bulls must be performed simultaneously with the vaccination of females (Clark *et al*., 1976; Leite *et al*., 1980; Ramos *et al.*, 1986) to achieve better control of BGC.

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