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Prevalence of Bovine Genital Campylobacteriosis in beef cattle in Brazil

Dionei Joaquim Haas[1], Karina Leite Miranda-Guimarães[1], Elaine Maria Seles Dorneles[2] and Andrey Pereira Lage[1]*

[1]*Department of Preventive Veterinary Medicine, School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil*

[2]*Department of Veterinary Medicine, Federal University of Lavras, Campus Universitário, Lavras, MG, Brazil*

***Corresponding Author:** Andrey Pereira Lage, Department of Preventive Veterinary Medicine, School of Veterinary Medicine, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.

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Abstract

The aim of the present study was to estimate the national and regional prevalence of Bovine Genital Campylobacteriosis in beef herds and bulls in Brazil. Samples were collected from bulls in the most important beef cattle raising States in Brazil: Bahia, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraná, Rio Grande do Sul, Rondônia, São Paulo and Tocantins. Direct Fluorescence Antibody Test was performed in preputial washings from 1191 bulls from 120 beef herds in the 12 sampled States. A questionnaire was applied in each farm to collect information on herds and animals. The prevalence of infected beef herds and bulls were, respectively, 50.8% (95%CI: 41.6% - 60.1%) and 19.7% (95%CI: 13.3% - 25.1%). None of the studied herd variables showed association with *C. fetus* infection. In conclusion, BGC is widely disseminated in Brazilian beef herds and bulls and may be one of the causes of the low efficiency of Brazilian beef cattle industry.

Keywords: *Campylobacter fetus; Bull; Direct Immunofluorescence; Natural Breeding*

Abbreviations

BGC: Bovine Genital Campylobacteriosis; *C. fetus*: *Campylobacter fetus*; DIF: Direct Immunofluorescence; OIE: World Organization for Animal Health; PCR: Polymerase Chain Reaction; PBS: Phosphate Buffered Saline; UFMG: Universidade Federal de Minas Gerais; USA: United States of America

Introduction

Campylobacter fetus, a microaerophilic spiral-shaped Gram-negative bacterium, is the etiologic agent of bovine genital campylobacteriosis (BGC), a disease that causes abortions but, mainly, early embryonic death and repetition of estrous in cattle [1,2]. When *C. fetus* reaches the uterus, the bacterium adheres to the epithelium and induces chemokine production and leukocyte infiltration, which culminates in endometrial dysfunction and infertility [1,3]. BGC significantly impacts the reproductive efficiency and earnings of cattle production, causing great economic losses to beef cattle industry [2,5-7]. It is sexually transmitted and infected bulls, which are asymptomatic, are the main spreaders of the disease, disseminating *C. fetus* by venereal route to cows during mating [2,4]. BGC is also transmittable through artificial insemination via contaminated semen, what brings important restrictions to international trade and makes it a statutory disease with mandatory reporting by the World Organization for Animal Health - OIE [8].

BGC is widespread in the world [7-9] and is usually of high economic importance in the largest beef producing countries as Argentina [10,11], Australia [12], Brazil [2], Uruguay [14] and USA [15], that usually raises beef cattle extensively and under natural breeding. Epidemiological data from these countries provided valuable information on the conditions and magnitude of BGC, which are essential to better health decisions in BGC control programs, highlighting risk factors as natural breeding, use of old bulls and cows, presence of clean-up bulls in the herd, frequency of purchase of new animals, trespassing of animals from neighbor herds, lack of a defined breeding season, property area, cattle density, and absence of a BGC vaccination program [2,5,10-15].

In Brazil, BGC has been diagnosed since the 1950s, when *C. fetus* was first identified in the country [16]. Analyses of the beef cattle producing systems used in Brazil, with herds with large cattle population, raised extensively, with reproductive management based on natural breeding, usually without a restricted breeding season, indicate that bovine venereal diseases could be widespread in the country [2,15,17,18].

However, most data on BGC in Brazil come from clinical case reports, convenience sampling or small sampling studies [19-22], that usually does not capture the broad picture of BGC prevalence in the country. Methodological differences among studies, including different experimental designs, animal categories involved (cows or bulls), diagnostic tests used (culture, PCR, direct fluorescence antibody test (DFAT) or mucus agglutination test), participation of farms with historical reproductive disorders and different productive systems (beef or dairy), also pose important problems and make comparisons among studies and Brazilians regions unfeasible [2,17]. Findings of other studies, although they could reflect the location where they were carried out [5,23-26], could not be extrapolated to represent BGC in the country.

Therefore, although BGC is recognized in the country for almost 70 years, the real magnitude of BGC in Brazilian beef cattle is unknown. Thus, the objectives of this study were (i) to estimate the prevalence of BGC in herds and bulls and (ii) to evaluate the risk factors for *C. fetus* infection among beef cattle in the Brazil.

Materials and Methods

Study design

This cross-sectional epidemiological study was designed to estimate the prevalence of BGC at animal and herd levels. The twelve most important Brazilian beef production states, Bahia, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraná, Rio Grande do Sul, Rondônia, São Paulo and Tocantins, were included in the study (Figure 1A). Those states corresponded to 89.1% of the Brazilian's cattle production [27] and the sampled area, 5,575,933 Km^2, corresponds to 65.5% of the total national territory. All five Brazilian regions had herds sampled.

Two-level cluster sampling was used. First, the number of herds sufficient to obtain an estimate of the prevalence of herds with the desired precision was determined. To classify these herds as BCG-positive, a random selection of animals was carried out in each of them. The sample was calculated using an estimated prevalence of 52% [5], 95% confidence and 15% error [28]. The average number of animals sampled per cluster was estimated at 10. The sample size was calculated using correction for cluster sampling [28,29]. Based on those parameters a sample of 1200 animals in 120 properties was calculated. Ten herds were sampled per each of the 12 most important Brazilian beef production states, from February to October 2000. Herds that are exclusively beef producers and had 10 or more bulls were randomly selected from the database of Programa de Sanidade para Bovinos (Cattle Health Program) - Probov (Pfizer Saúde Animal - Brazil).

Preputial washings

Preputial washings, one per bull, were collected as previously described [30] in phosphate buffered saline (PBS, pH 7.4). All preputial washings were stored at 4°C until the time of the examination. Samplings were performed after a period of sexual rest of at least 15 days [2].

Direct fluorescence antibody test

Direct Fluorescence Antibody Test (DFAT) was performed according to Figueiredo., *et al.* [31], using 20 μL of a 1/128 dilution of anti-*C. fetus* subsp. *venerealis* prepared in rabbit with the *C. fetus* subsp. *venerealis* strain NCTC 10354^T and conjugated with fluorescein (Sigma-Aldrich, USA). The DFAT used had sensitivity and specificity of 92.6% and 88.9%, respectively [31].

Statistical procedures

Herd prevalence's and confidence intervals were calculated for simple sampling, using the exact binomial method [32] (Epi Info 7.1.5 software) (33). Weighted animal prevalence's and confidence intervals were calculated [28,34] weighting the data obtained by the total number of animals per herd and by state [28]. The true prevalence was estimated [35,36] adjusting the apparent prevalence obtained to the previously determined specificity (88.9%) and sensitivity (92.6%) of the DFAT [31]. Prevalence's were calculated for each of the five Brazilian geographic regions, North, Northeast, Midwest, Southeast and South (Table 1 and 2, Figure 1B) and for total area sampled, that represents the national prevalence.

The determination of the cut-off point to be used to classify the herd as positive was performed using simulations of herd sensitivity and specificity [35]. It was established that the cut-off point to classify the herd as positive was the presence of two or more bulls positive to DFAT, which guaranteed the best balance between herd sensitivity and herd specificity, 70% and 99%, respectively.

Evaluation of risk factors was performed by univariate analysis using the chi-square test with alpha at 0.05% [32]. Numerical variables were grouped into quarters for the analyses. Herd variables analyzed were: origin of the replacement bulls, number of bulls, total number of cows, total number of animals, area of property, breed group, presence and type of zootechnical bookkeeping, vaccinations, use of young bulls, presence and duration (with natural breeding or artificial insemination) of restricted breeding season, use of artificial insemination, use of clean-up bulls and age of bulls.

Results

Beef bull and herd prevalences

The prevalences of herds and beef bulls with BGC for the five Brazilian geographic regions and in the total area sampled are summarized in table 1 and 2 and figure 1B. Overall, herd prevalences range from 5.0% to 70% among regions with a national prevalence of 50.8% (95% CI: 41.6% - 60.1%). True prevalence of infected beef bull was 24.1%, ranging from 0.4% to 31.9% among regions. The lowest rates were observed in Northeast region, whereas the highest ones were observed in the Midwest, Southeast and South regions. Design effects and intracluster correlation coefficients calculated for each region and for the country were presented in table 2.

Region	Positive Herds	Tested Herds	Prevalence Herd	95% CI (%)	
				Min.	Max.
North	12	30	40,0	22,7	59,4
Northeast	1	20	5,0	0,3	26,9
Midwest	20	30	66,7	47,2	82,7
Southeast	14	20	70,0	45,7	88,1
South	14	20	70,0	45,7	88,8
Total	61	120	50,8	41,6	60,1

Table 1: *Prevalence of beef cattle herds with bovine genital campylobacteriosis in the North, Northeast, Midwest, Southeast and South regions of Brazil.*

Region	D[1]	rho[2]	Positive Bulls	Tested Bulls	True Prevalence	Apparent Prevalence	95% CI (%)	
							Min.	Max.
North	12,26	0,35	42	297	13,6	11,2	4,3	18,1
Northeast	4,70	0,08	3	200	0,4	0,4	0,0	1,2
Midwest	14,25	0,42	81	297	31,9	26,1	15,8	36,5
Southeast	24,77	0,49	51	198	28,5	23,3	10,3	36,4
South	13,92	0,26	47	199	24,7	20,2	10,9	29,5
Total			224	1191	24,1	19,7	13,3	25,1

Table 2: *Prevalence of beef bulls with bovine genital campylobacteriosis in the North, Northeast, Midwest, Southeast and South regions of Brazil.*

1 - D - Design effect.

2 - rho - Intracluster correlation coefficient.

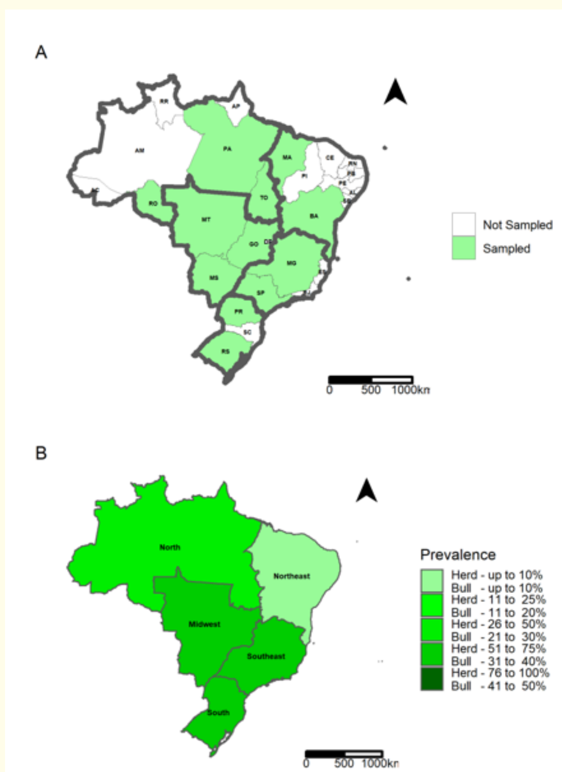

Figure 1: *Sampling area and prevalence of bovine genital campylobacteriosis at beef herd and bull levels for the five Brazilian geographic regions. A - States sampled for the BGC survey; AC: Acre; AL: Alagoas; AM: Amazonas; AP: Amapá; BA: Bahia; CE: Ceará; DF: Distrito Federal; ES: Espírito Santo; GO: Goiás; MA: Maranhão; MG: Minas Gerais; MS: Mato Grosso Do Sul; MT: Mato Grosso; PA: Pará; PB: Paraíba; PE: Pernambuco; PI: Piauí; PR: Paraná; RJ: Rio De Janeiro; RN: Rio Grande Do Norte; RO: Rondônia; RR: Roraima; RS: Rio Grande Do Sul; SC: Santa Catarina; SE: Sergipe; SP: São Paulo; TO: Tocantins. B - BGC prevalence at herd and bull level for each region.*

Risk factor analyses

The profile of the studied herds concerning the animal population, property area, breeding season and age of the bulls is shown in table 3 and 4. The median number of bulls, cows and cattle per studied herd was 70, 2465 and 2520, respectively. Bull median age was 70 months in the sampling. There was no significant difference among bulls' age groups on the rate of *C. fetus* infection (Table 4). The distribution of the analyzed herds characteristics was shown in table 5. Categorical variables were shown as the data were collected and numerical data were grouped into quarters.

Variables	Mean	Median	Minimum	Maximum	Q1[1]	Q3[2]
Bulls[3]	113,1	70	10	2000	40	120
Females[4]	3426	2465	740	18000	1800	3987
Animals[5]	3539	2520	765	18400	1905	4095
Area (ha)	8787	4000	700	110000	2361	8150
Breeding season - NM[6]	100,7	90	60	210	90	120
Breeding season - AI[7]	86,2	90	30	210	60	112,5
Age of bulls (months)[8]	71,6	70	28	192	53	84

Table 3: *Profile of the sampled herds regarding the numerical variables studied.*

1 - Q1 - First quartile.

2 - Q3 - Third quartile.

3 - Total number of bulls.

4 - Total number of cows.

5 - Total number of animals.

6 - Duration of breeding season in the natural mating system (days).

7 - Duration of breeding season in the artificial insemination system (days).

8 - Age of bulls sampled (months).

Age of bulls	NI[1]	I[2]	Total
Up to 53 months	241	62	303
54 to 70 months	245	60	305
71 to 84 months	261	44	305
Upper to 84 months	219	58	277

Table 4: *Age distribution of bulls in relation to Campylobacter fetus infection.*

1 - NI - Herd without Campylobacter fetus infection.

2 - I - Herd with Campylobacter fetus infection.

Characteristics	NP[1]	NI[2]	I[3]	Total
Area of property	120			
Up to 2361 ha		17	15	32
2362 to 4000 ha		17	16	33
4001 to 8150 ha		12	14	26
Upper to 8150 ha		17	12	29
Total of animals	120			
Up to 1905		17	13	30
1906 to 2520		10	20	30
2521 to 4095		19	11	30
Upper to 4095		17	13	30
Total of bulls	120			
Up to 40		16	16	32
41 to 70		17	16	33
71 to 120		15	11	26
Upper to 120		15	14	29
Total of cows	120			
Up to 1800		17	14	31
1601 to 2465		10	19	29

2466 to 3987		20	10	30
Upper to 3987		16	14	30
Breed group	120			
European		3	5	8
Cross-breed		2	9	11
Zebu		58	43	101
Zootechnical control	120			
Perform		59	54	113
Not perform		4	3	7
Type of bookkeeping	119			
Book or card		18	19	37
Computer		20	24	44
Both		20	11	31
Replacement of bulls	104			
Own herd		39	37	76
Other herds		3	2	5
Auction		1	0	1
All		11	11	22

Table 5: *Characterization of the studied herds in relation to the area of property, herd size, breed group, zootechnical control and type of bookkeeping, and origin of the bulls in relation to the presence of Campylobacter fetus infection.*

1 - NP - Number of herds that answered the question.

2 - NI - Herds without Campylobacter fetus infection.

3 - I - Herd with Campylobacter fetus infection.

The reproductive management data of the studied herds were shown in table 6. All sampled herds used natural breeding, although 74.2% of them also used artificial insemination.

Characteristics	NP[1]	NI[2]	I[3]	Total
Artificial insemination - AI	90			
Use		45	44	89
Not use		1	0	1
Breeding season - BS	117			
Use		57	50	107
Not use		2	5	7
In part of the cattle		1	2	3
Duration BS with natural mating	104			
Up to 90		35	33	68
91 to 100		0	0	0
101 to 120		14	8	22

Upper 120		6	8	14
Duration AI with natural mating	80			
Up to 60 days		16	18	34
61 to 90 days		14	12	26
91 to 113 days		0	0	0
Upper 113 days		11	9	20
Clean-up bulls	90			
Use		42	44	86
Not use		4	0	4
Use of young bulls	110			
Only in heifers		16	19	35
Only in cows		4	6	10
In heifers and cows		35	30	65

Table 6: *Reproductive management characteristics of the properties studied in relation to the presence of infection by Campylobacter fetus.*
1 - NP - Number of herds that answered the question.
2 - NI - Herds without Campylobacter fetus infection.
3 - I - Herds with Campylobacter fetus infection.

None of the studied herds had previously been diagnosed with BGC, therefore no herd vaccinated animals against BGC, but most of them vaccinated heifers against brucellosis. Data on vaccinations performed by the sampled herds were shown on table 7.

Vaccines	NP[1]	NI[2]	I[3]	Total
Brucellosis	119			
Vaccinated		55	55	110
Not vaccinated		7	2	9
IBR and BDV	119			
Vaccinated		18	21	39
Not vaccinated		44	36	80
Leptospirosis	119			
Vaccinated		19	27	46
Not vaccinated		43	30	73

Table 7: *Vaccinations against reproductive diseases performed on the properties studied in relation to the presence of infection by Campylobacter fetus.*
1 - NP - Number of herds that answered the question.
2 - NI - Herd without Campylobacter fetus infection.
3 - I - Herd with Campylobacter fetus infection.

Among the studied variables - origin of the replacement bulls, number of bulls, total number of cows, total number of animals, area of property, breed group, presence and type of zootechnical bookkeeping, vaccinations, age of bulls, presence and duration (with natural breeding or artificial insemination) of restricted breeding season, use of artificial insemination, and use of clean-up bulls- none showed a significant association with the presence of BGC.

Discussion

BCG is for long recognized as an important cattle disease in Brazil [2,17], causing high economic losses to the Brazilian beef cattle industry [2,17]. However, its real magnitude and levels of infection among cattle and herds were mostly unknown in the country. This is the first study that shows the prevalence of BGC in the national beef herd and across the five Brazilian geographic regions.

Data on average herd prevalence in the Midwest, Southeast and South regions do not differ statistically from each other and showed very high infection rates, around 70% of herds positive for *C. fetus*, and reveal a national average prevalence of 50.8% (95% CI: 41.6% - 60.1%) (Table 1). Hence, it shows that one of two beef cattle herds in Brazil is infected with *C. fetus*.

This disturbing health scenario observed at the herd level also occurs at the animal level. Analyzing the average animal prevalence in four of the Brazilian geographic regions, North, Northeast, Midwest, Southeast and South, statistically similar high infection rates were observed, from 11.2 to 26.1% infected bulls, with a national prevalence of 19.7% (95% CI: 13.3%-25.1%), and a true prevalence estimate of 24.1% (Table 2). Therefore, about one fifth to one quarter of the beef bulls were infected by *C. fetus*.

The Northeast region exhibited the lowest prevalence of infected beef bulls and herds (Table 1 and 2, figure 1B), which was significantly different from the other regions. However, it cannot be said that BGC is absent in the region, especially when observing that the prevalence of infected herds in the region is 5.0% and the average animal prevalence is 0.4%, with 1.2% upper limit for the confidence interval. In fact, those prevalences are of concern, as important losses can occur at those rates of infection. For example, in the provinces of La Pampa and Buenos Aires, both in Argentina, where similar livestock production systems are in use, prevalence rates of 0.5 to 1.5% of bulls and 2.3 to 4.2% of herds were observed [11,37]. BGC at those prevalences have been implicated in significant gestational losses, being the second most important bacterial cause of abortion, just behind *Brucella abortus* infection [38]. Moreover, as *C. fetus*-infected bulls could transmit the infection to 50% to 100% of the cows they copulate [2,5,15,19,20] and each bull is able to mate with dozens of cows in the breeding season, usually 25 to 40 cows, an important number of females is expected to manifest reproductive problem on the herds. Thus, although the observed lower prevalence of *C. fetus*-infected bulls in the Northeast region, it could still constitute an important problem for beef production in the region.

The observed intracluster correlation coefficients, but the one for the Northeast region, were greater than 0.20, the value suggested for moderated contagious infectious diseases [29,39] and also greater than that found for *C. fetus*-infected bulls in the Pantanal of Mato Grosso do Sul state [5]. Those findings reflect differences in the distribution of infected animals among herds in the studied regions. While in Pantanal of Mato Grosso do Sul state most of the herds showed high rates of *C. fetus*-infected bulls [5] and data from the Northeast region in the present study showed that most herds did not exhibit BGC, in all other Brazilian regions there was a greater diversity in the rates of infected bulls and herds in each region (Table 2), resulting in higher intracluster correlation coefficients. Due to those high intracluster correlation coefficients, high study design effects were also observed (Table 2). Besides being quite important for the right estimation of sampling size for future studies, the values of study design effects, which represents the increase in the variance due to cluster sampling, are needed to correct data analyses for this kind of sampling, because the standard error is larger in those studies [39].

The absence of significance for any of the variables in the evaluation of risk factors for BGC could be due to the experimental design. Although cross-sectional studies, as the present one, could contribute to understand factors that promote infection, they are not the best ones to study cause and effect association, which are best studied by prospective studies [36].

The sample design may have introduced bias in the risk factor analysis, not allowing an association between the variables studied and the risk of infection by *C. fetus* to be revealed, even among those variables that have already been observed to be associated with a higher risk of occurrence of BGC, as is the case of natural breeding, use of clean-up bull, age of bulls and property size [5,10,13,20,25,40]. The selection of properties that had bulls and, consequently, natural breeding, may have had its effects masked in relation to the presence of BGC due to variables that could cause confusion, such as the presence of clean-up bulls and the age of the bulls employed. This was the main limitation of the present study.

The widespread distribution of BGC in Brazilian beef herds reflects characteristics of the Brazilian livestock productive system and farmers' cultural behaviors. Most Brazilian farms raise cattle extensively on pasture under natural breeding, do not diagnose BGC in the herd and do not vaccinate against the disease. This is the profile of all studied farms in this study (Table 4-7). Moreover, most of studied herds also used clean-up bull and older bulls, over 4 ½ years old, two factors that were observed to increase the risk for BGC in other studies (Table 3, 4 and 6) [13,20]. Old bulls were prone to infection by *C. fetus*, for having deeper preputial crypts, which provides an ideal microaerophilic environment for the multiplication of bacteria and for establishing chronic infection [4,5,41]. Hence, as observed in the present study, the beef production system used in Brazil preserves factors favoring the maintenance and proliferation of bovine venereal diseases [15].

Beef herds infected with BGC suffer from low reproductive efficiency with conception rates as low as 35%, infertility and estrus repetitions, which consequently increase calving intervals, resulting in a smaller number of calves produced and weaned and, consequently, decreasing the total meat production by unit of time [5,6,20,42,43]. Together, the findings of high prevalence and widespread distribution of BGC among Brazilian beef herds, highlight BGC as one of the probably major causes of the low reproductive rates found in beef cattle in Brazil [5,18, 44,45].

Taking into consideration that none of the studied beef herds had previously been diagnosed with BGC, the high prevalence of BGC at bull and herd levels and that bulls are asymptomatic and usually carry the infection for life [2,4,41], Brazilian beef farmers should take strict measures to control and prevent BGC. As treatment of *C. fetus*-infected bulls has an unfavorable cost-benefit effect, a program based on the diagnose of BGC and culling of infected bulls could be established [2,42]. However, such a program can also bring economic and financial consequences due to the need of acquisition of replacement uninfected bulls and loss of important genetic lineages. So, in order to lower the prevalence, incidence, clinical disease and to mitigate the losses, it is recommended the implementation of a vaccination program, for both cows and bulls [2,46], the replacement of infected bulls by uninfected ones, and the use of artificial insemination, where it is feasible, strategies that have been shown to be effective where beef cattle are managed extensively [2,10,42,43,46,47].

Conclusion

In this study, the estimated prevalences of BGC at bull and herd levels in the five geographic Brazilian regions, as well as in the country, were observed to be very high, showing that BGC is widely disseminated among Brazilian beef cattle. Such findings support that BGC may be one of the causes of the low reproductive efficiency of the Brazilian beef herd. The extensive breeding system observed in our sample, as in Brazil, with natural breeding and low adoption of measures against BGC, such as artificial insemination, vaccination and diagnosis of the disease, should have contributed to the scenario found in the present study.

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Conflicts of Interest

Preputial sampling collections were performed by veterinarians from Programa de Sanidade para Bovinos (Cattle Health Program) - Probov (Pfizer Saúde Animal - Brazil).

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