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VETERINARY MICROBIOLOGY - RESEARCH PAPER

Seroprevalence of bovine vaccinia in cows and its correlation with the productive profile of affected farms in Distrito Federal, Brazil

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Abstract

Bovine vaccinia (BV) is an infectious disease caused by Vaccinia virus (VACV) characterized by vesicular and exanthematic lesions, mainly in cattle. Although BV has been described in some Brazilian regions in the last decades, official information regarding the current prevalence in bovine herds of Midwestern Brazil is lacking. Thus, the current study aimed to estimate the seroprevalence and risk factors associated with BV in cattle in the Distrito Federal (DF), Brazil. Sera of 312 cows of 64 herds were tested by virus-neutralizing test for VACV antibodies. Herd and animal seroprevalence were estimated to be 33.3% (CI 95%: 18.2–48.3%) and 10.6% (CI 95%: 1.0–20.2%), respectively. Seropositive cows were detected in dairy, beef, and mixed-purpose farms. The results of an epidemiological questionnaire showed that no risk factor analyzed was positively associated with seropositivity to VACV. There was no significant association between type of milking (manual/mechanic) and seropositivity to VACV; however, most seropositive cows were present in farms with high daily milk production and high number of lactating and adult cows. Our results indicate that VACV circulates in many regions of DF with considerable prevalence in dairy cows. Control measures to restrict VACV circulation and consequences of the infection may be advisable.

Keywords *Orthopoxvirus* · Poxviruses · VACV · Diseases of cattle · Epidemiology

Introduction

Bovine vaccinia (BV) is an infectious disease caused by *Vaccinia virus* (VACV), a zoonotic orthopoxvirus that causes vesicular and exanthematic lesions, mainly in cattle [1–3]. VACV may also infect other species, such as horses, pigs, rabbits, mice, opossums, sheep, and monkeys [4–7]. There is some evidence that wild rodents, dogs, and cats are also susceptible to VACV, but apparently do not develop clinical signs [6, 8–11].

Outbreaks and single cases of BV in cattle and milkers have been described in some Brazilian regions, such as

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Southeastern [1, 12–14] and Midwestern [3, 15]. Additionally, coinfections involving VACV and other poxviruses have been reported in cattle from Brazilian herds [3, 15–18]. These data are somewhat scaring and detrimental to the national dairy industry and public health services [19, 20].

In cattle, BV courses with vesicles, papules, pustules, erythema, edema, erosions, ulcers, and crusts usually on the teats, udder, mouth, tongue, muzzle and hoof [3, 15, 21]. VACV infection generally occurs in lactating cows and, especially, in farms that practice manual milking [1, 12, 13, 22]. Virologic and molecular tests have been preferentially used to diagnose the disease. However, serological assays are useful to evaluate and quantitate the presence and circulation of VACV in bovine herds [23, 24], generating seroepidemiological data of interest to design animal health programs [25].

Although some studies have detailed important aspects of BV in Brazil in recent years, such as epidemiological interactions, clinical and pathological findings, and diagnosis, no official data regarding to its seroprevalence in local bovine herds is available. Thereby, the current study aimed to estimate the seroprevalence and risk factors associated with BV in cattle in the Distrito Federal (DF), Brazil.

Distrito Federal, located in the Brazil Midwest region, has some particularities, such as small farms and herds. In 2015, the DF herd was equivalent to 0.1% of the bovine population in the Midwest region [26]. The herds/farms are typically small and mostly devoted to milk production or mixed exploration [27]. Furthermore, it is an important route of animal transport among the Brazil Midwest, Southeast, and Northeast [3].

Given the relevance of studying this poxvirus in a place with important particularities and location, the objective of this work is to estimate seroprevalence and risk factors associated with BV in herds and animals in DF in 2015.

Material and methods

Location and sampling

The serum samples from cows used in this study were kindly provided by the Secretaria de Estado da Agricultura, Abastecimento e Desenvolvimento Rural (SEAGRI), from DF, Brazil. The samples were collected during the seroepidemiological survey for bovine brucellosis and tuberculosis in 2015.

The target population of this study included farms with adult cows (over 24-month-old) in the DF. The age range was established due to the fact that the cows were in reproductive period and, consequently, in dairy production.

The number of sampled farms was calculated using the software Epitools® (SERGEANT, 2018), in which it was considered that in 2015 there were 2,727 herds in the DF with adult cows. The prevalence of herds affected by the VACV was estimated at 20%, with a sampling error of 10% and a confidence level of 95%. The calculation generated a minimum number of 62 farms selected in the DF. Thereby, samples of 64 out of 344 farms represented in the serum bank of SEAGRI were used. Samples of the five operational units (OU) of the animal health service from DF (Brazlândia, Gama, Planaltina, Rio Preto, and Sobradinho) were studied. As a result, the random sampling led to the analysis of 20 farms in Brazlândia, 17 in Sobradinho, 15 in Rio Preto, 8 in Planaltina, and 4 in Gama.

The number of selected cows in each farm was determined assuming an intra-herd prevalence of 40% in herds with more than 5 adult animals and 50% in smaller farms, and values of 92.7% sensitivity and 90.8% specificity for virus neutralization (VN), using as reference the statistical bases observed previously [28]. Using the Epitools®, simulations were performed with different sample sizes in order to define a minimum number of animals in the herd to be tested that would allow to rank the farm as positive or negative for VACV infection. The farm was considered positive when at least one cow was tested positive. The sample size chosen would yield values of sensitivity and specificity of the herd of at least 88% and 70%, respectively. Then, 1 to 7 cows were sampled per farm, totaling 312 sera, which represented approximately 20% (312/1565) of serum samples bank. These samples were stored in isothermal boxes and immediately sent to the laboratory, where they were stored at − 20 °C until performing the VN test. The samples were considered viable for analysis.

This study was approved according to the Ethical Principles of Animal Care and Research and under Ethics Committee on Animal Use (protocol number 122/17) of the Universidade Federal de Goiás, Brazil. The samples were thawed and incubated in a water bath at 56 °C for 30 min, to inactivate the complement system, and subsequent performance of the VN.

Cell line and virus

Vero cells (*African Green Monkey* kidney) were used for viral amplification and quantification and for the VN test. Cells were cultured in RPMI medium supplemented with penicillin (10,000 IU/mL), streptomycin (10 mg/mL), amphotericin B (250 µg/mL), ciprofloxacin (1 mL/L) and 10% bovine fetal serum.

The *Vaccinia virus* Pelotas 2 (P2V) was used as the standard viral strain, which had been isolated from horses in southern Brazil [4, 17]. Cell cultures and virus growth were performed at 37 °C with CO_2 at 5%.

Virus-neutralization (VN) assays

After inactivating the complement system, serum samples were submitted to a standard VN assay in 96-well plates. For this, samples were diluted (1:10) in RPMI medium, tested against a fixed dose of virus (100–200 $TCID_{50}$/well), and incubated at 37 °C for 2 h. A suspension of Vero cells was added and the plates were incubated for 120 h at 37 °C with CO_2 at 5%. In all tests, fetal bovine serum was used as a negative control and a serum from a rabbit experimentally infected with the VACV as a positive control [5]. The microplates were read in an inverted optical microscope. The test was considered valid when the control cells, the viral titration, the absence of serum cytotoxicity, and the cytopathic effect of the virus on the control gave expected results. The samples were considered positive for antibodies when no cytopathic effect was observed.

Positive serum samples in the qualitative VN test were assigned to the quantitative VN to determine the antibody titer. For this, serial twofold dilutions were tested against a fixed dose of the virus, as described in the qualitative test. VN titers were considered the reciprocal of the highest dilution of serum that prevented the production of the cytopathic effect indicator in Vero cells.

Statistics

Data analysis was performed with STATA® software, version 12 (STATACORP, 2011). The results of the VN and the epidemiological survey were used for statistical analysis. The farm was considered positive when at least one seropositive cow was detected.

The estimate herd and animal seroprevalence of BV was based on the ratio between farms or animals classified as positive in the sample and the total of samples in DF, considering the dimension of each OU. Since each primary and secondary sample unit represents respectively a set of farms and animals within the sample, weights were calculated as follows (Formulas 1 and 2).

In Formula 1, the weight 1 (P1), exercised by each sampled farm in relation to its OU, for the purpose of calculating herd prevalence in DF, was given according to the expression:

$$P1 = \frac{\text{Total number of farms in the OU}}{\text{Number of sample farms in the OU}}$$

For estimating the animal prevalence of BV, the calculation considered conglomerate sampling made in two stages. Therefore, in Formula 2, a weighting was performed, considering the weight (P2) exercised by each sampled cow in relation to its herd and, posteriorly, to its respective OU.

$$P2 = \frac{\text{Females } \geq 24 \text{ months present on the farms}}{\text{Females } \geq 24 \text{ months sampled on the farms}} \times \frac{\text{Females } \geq 24 \text{ months in the OU}}{\text{Females } \geq 24 \text{ months present in the farms in the OU}}$$

The frequency of animals affected in each BV positive herd was estimated by the ratio of the number of positive samples to the total number of collected samples.

In addition to the serological analysis, we analyzed the results of an epidemiological questionnaire applied by the veterinarians of the SEAGRI in each farm (supplementary material). As a result, it was possible to obtain information on the type of exploration, raising, and management practices employed, especially in relation to dairy management. In this study, variables such as number and breeds of cattle, milk production and disposal of subproducts, presence of other animals (domestic and wild), veterinary assistance, purchase and sale of animals, items or employees' shares, and physical characteristics of the property, among others factors were analyzed.

All the information generated by the field and laboratory study was inserted in a specific database. Thus, possible risk factors were studied after exploratory data analysis. Quantitative variables were converted into categorical variables for use in bivariate analysis.

Using the chi-square test (χ^2), a bivariate analysis of the variables in the questionnaire was performed, whose association with the presence or absence of VACV in the herd presented biological or epidemiological plausibility.

Results

Out of the 64 farms sampled, 23 were positive, i.e., presented at least one animal seropositive to VACV. Thus, the herd prevalence for VACV in DF was 33.3% (CI 95%: 18.2–48.3%). Table 1 shows the total and sampled drawn number of farms and cows used in this study by OU.

The animal prevalence was estimated at 10.6% (CI 95%: 1.0–20.2%). The confidence interval (CI) is quite wide, reflecting the type of cluster sampling and also due to the reduced number of farms and cows studied in some regions, such as in Gama.

Among the 23 positive farms, the percentage of seroreagent cows within the farms ranged from 14.2 to 66.6%, with an average intra-herd prevalence of 27.2%. In these 23 farms, 30 cows were seropositive for VACV. The number of positive sera in each farm varied from one to three animals. Virus titers between 2 and 256 were found, with a median of 8.

In relation to the characteristics of the analyzed farms, most of them were small-scale, since 50% of the farms (32/64) had a maximum of 18 cattle in the herd and seven adult cows. In addition, 40.6% (26/64) of the farms had only two cows.

Most evaluated farms were dairy (29/64) and mixed/dual-purpose (25/64) herds, followed by 10 beef herds (Table 2).

Table 1 Number of farms and cows analyzed in the seroprevalence study for VACV in the different operational units (OU) from Distrito Federal, Brazil, in 2015

Operational units	Total number of farms	Number of sampled farms	Number (and percentage) of positive farms analyzed	Total number of cows	Number of sampled cows	Number (and percentage) of positive cows analyzed
Brazlândia	544	20	7 (35%)	8021	81	7 (8.6%)
Gama	700	4	1 (25%)	8651	26	3 (11.5%)
Planaltina	495	8	3 (37.5%)	8734	39	3 (7.6%)
Rio Preto	524	15	4 (26.6%)	12,583	74	7 (9.4%)
Sobradinho	464	17	8 (47%)	5684	92	10 (10.8%)
DF	2727	64	23 (35.9%)	43,673	312	30 (9.6%)

DF Distrito Federal

Table 2 Productive characteristics of 64 cattle farms sampled in Distrito Federal, Brazil, in 2015

Variables	Amount	Frequency (%)
Production purpose		
Dairy	29	45.3
Mixed (dual-purpose)	25	39.1
Beef	10	15.6
Herd management		
Extensive	38	59.4
Semi-intensive	25	39.0
Intensive	1	1.6
Milking type		
Manual	45	70.3
Absent	15	23.4
Mechanic	4	6.3
Number of cows over 24 months		
3 or more cows	38	59.4
1 or 2 cows	26	40.6

In general, 49 farms performed milking (manual [45/49] or mechanized [4/49]). Forty-four farms (44/64) had lactating cows present in the herd in the last 12 months, in which half (22/44) had only three lactating cows and a daily dairy production up to 14.5 L. Table 2 summarizes the distribution of farms according to the type of farm, type of breeding, and type of milking and number of cows over 24 months old.

Considering the consumption of raw milk or related dairy products, 29.69% (19/64) of the farmers claimed to consume these products. Most farmers did not deliver milk to industries (54/64), but they produced cheese, butter, or other dairy products on farm (38/64), for their own consumption (34/38) and/or for informal sale (8/38). Most of the mixed and dairy farms did not cool down the milk (45/54). Of those who cooled milk (9/64), eight used coolers or their own expansion tank, and only one utilized a collective tank.

In addition, 78.13% of the farms did not have veterinary assistance (50/64). In the other cases, nine and five farms were assisted by private and cooperative veterinarians, respectively. Most of the farms did not rent pasture (53/64), or shared items (56/64) nor drinking fountains with animals from another farm (60/64) did not concentrate cattle in any region (43/64) nor had wetlands (52/64). Only eight farms shared items (8/64), mainly equipment, and three also shared employees. Only 16 farms declared to have bought cattle and 21 properties to have sold animals in the last 12 months prior to the survey, and 46 properties did not slaughter animals (46/64). Most farms (57/64) did not use artificial insemination and had no abortions in the last 12 months (56/64).

In relation to other animals present in the properties, the domestic animals cited were dogs (55/64), poultry (52/64), horses (44/64), pigs (33/64), cats (33/64), and small ruminants (11/64). The described wild animals included primates (35/64), marsupials (32/64), deer (14/64), capybaras (14/64), and felids (5/64) (Table 3).

Table 3 shows the bivariate analysis of possible risk factors most discussed for BV in all 64 farms analyzed in the current study.

The presence of others domestic or wild animals in the evaluated farms was analyzed as potential risk factors, such as horses ($p=0.504$), pigs ($p=0.552$), poultry ($p=0.835$), dogs ($p=0.861$), cats ($p=0,552$), cervids ($p=0.984$), capybaras ($p=0.201$), marsupials ($p=0.434$), wild felids ($p=0.844$), and primates ($p=0.409$), but no one of them were considered significant. Only sheep and goats were statistically relevant ($p=0.035$).

In addition, other analyzed factors were not significant, such as introduction of breeders animals ($p=0.855$), slaughter of animals at the end of reproductive life ($p=0.395$), use of artificial insemination ($p=0.203$), abortion of cows in the last 12 months ($p=0.111$), wetlands on the farms ($p=0.646$), sold cattle in the last 12 months ($p=0.801$), milk delivery and cooling in dairy and mixed farms ($p=0.313$ and 0.186, respectively), and consumption of raw milk or dairy products made with raw milk ($p=0.297$). On the other

Table 3 Analysis of potential risk factors for bovine vaccinia in farms from Federal District in 2015

Variables	Bovine Vaccinia diagnosis			*P*
	Negative*	Positive	Total	
Exploration type				0.384
Mixed	5 (12.2%)	5 (21.7%)	10	
Milk	21 (51.2%)	8 (34.8%)	29	
Beef	15 (36.6%)	10 (43.5%)	25	
Creation type				0.728
Extensive	25 (61.0%)	13 (56.5%)	38	
Semi-containment/confinement	16 (39.0%)	10 (43.5%)	26	
Farm classification				0.638
Rural	38 (92.7%)	22 (95.7%)	60	
Urban periphery	3 (7.3%)	1 (4.3%)	4	
Total number of cows over 24 months of age				0.068
≤ 7 females	24 (58.5%)	8 (34.8%)	32	
> 7 females	17 (41.5%)	15 (65.2%)	32	
Milking type				0.112
Absent	8 (19.5%)	7 (30,4%)	15	
Mechanics	1 (2.4%)	3 (13%)	4	
Manual	32 (78%)	13 (56%)	45	
Herd size				0.093
< 20 cattle	25 (61%)	9 (39%)	34	
≥ 20 cattle	16 (39%)	14 (61%)	30	
Daily milking number				0.536
No milking	9 (22%)	8 (34.8%)	17	
Once a day	30 (73.2%)	14 (60.9%)	44	
2 or 3 times a day	2 (4.8%)	1 (4.3%)	3	
Bovine breeds				0.349
Zebuine	4 (9.7%)	5 (21.7%)	9	
European	4 (9.7%)	3 (13.0%)	7	
Mixed	33 (80.5%)	15 (65.2%)	48	
Rent pasture				0.158
No	36 (87.8%)	17 (73.9%)	53	
Yes	5 (12.2%)	6 (26.1%)	11	
Shares an item with other farms				0.922
No	36 (87.8%)	20 (87%)	56	
Yes	5 (12.2%)	3 (13%)	8	
Veterinary assistance				0.201
No	30 (73.1%)	20 (87%)	50	
Yes	11 (26.8%)	3 (13%)	14	
Bought cattle in the last 12 months				0.880
No	31 (75.6%)	17 (73.9%)	48	
Yes	10 (24.4%)	6 (26.1%)	16	
Have areas where cattle remain grouped			0.762	
No	27 (65.8%)	16 (69.6%)	43	
Yes	14 (34.2%)	7 (30.4%)	21	

*Absolute number (% of total)

hand, farms that did not produce cheese, butter, or other dairy product showed significant risk ($p = 0.032$).

Analyzing the milk production in mixed and dairy farms, the positive farms showed higher daily production (mean = 42.2 L, median = 30 L), higher number of lactating cows (mean = 8.2, median = 9), higher number of adult cows (mean = 16.6, median = 10), and higher total number of herd (mean = 40, median = 24) ($p > 0.05$). Negative farms, on the

other hand, showed an average daily milk production of 35.5 L and an average of 12 L, a number of lactating cows of an average of 6.3 and a median of 3, an average number of adult females of 12.5 and a median of 1 and total herd mean of 28.2 and median 14.

Discussion

We studied the seroprevalence and epidemiology of BV in DF in Brazil, in order to know the possible risk factors related with this poxvirus in cattle in this region. A recent study showed that BV is the most common poxvirus of cattle in this Brazilian region [3]. Although this disease has been studied in some countries and in some Brazilian regions (mainly Southeast), few epidemiological investigations have been conducted, especially in the Midwestern region of Brazil, including a serological evaluation of the herds. For the first time, a study with this scope was carried out in this region.

In the current study, a seroprevalence for VACV of 33% in farms and 10.6% in cattle was observed. Regardless of the farm, an intra-herd prevalence of 14.2% up to 66.6% was determined. A previous similar serological study was performed in another Brazilian state (Minas Gerais). The authors found a higher prevalence of 75.7% in dairy cattle, detecting antibodies against orthopoxvirus and intra-herd involvement of 20 to 100% [10]. Possibly, the high prevalence found in this last investigation may be related to the target population of the study. In addition, this region appears to be endemic for VACV. Similarly, small and relatively small properties, with few cows in their herds, were analyzed in our and in this previous study [10]. Some limitations of the current study also need consideration, such as the restricted number of analyzed samples and the expressive number of small farms with few cows.

According to some studies, BV is a disease commonly found in dairy cattle, mainly during lactation [14, 15, 29]. The current investigation did not demonstrate significant difference of seropositivity to VACV between dairy and beef farms in DF. Similar data were obtained in another study describing the clinical and pathological aspects of 27 cases of BV in DF between 2015 and 2018 [3]. In an investigation performed in Minas Gerais, Brazil, of the 78 cows that presented antibodies against the VACV, only 8 had a history of lesions compatible with BV [10].

In the current study, there was no clinical history and/or officially notified cases of BV in the herds of this region in 2015. However, Alonso et al. [3] observed clinical cases of VACV with highest number of cases in the UO in Planaltina, followed by Brazlândia, between 2017 and 2018. Possibly, BV can be underdiagnosed in beef cattle due the usual handling practices favor that the oral or cutaneous lesions, sometimes mild, are not identified by the farmers and rural workers. Oral lesions are generally reported in outbreaks of BV, especially affecting suckling calves [1, 3, 15, 30, 31], and this percentage can be even higher, since the mouth is not evaluated clinically in details, in most cases [1].

VACV infection occurs frequently in farms that use manual milking, because apparently milking is the main reason for the transmission of the virus among animals [1, 12, 13, 22]. A study demonstrated that 92% and 25–30% of the farms that performed manual or mechanical milking were affected, respectively [1]. In other investigation, only lactating cows and calves that suckled directly on these cows became infected by VACV, while bulls, cows that were not being milked, and calves that were fed in buckets did not show lesions [22]. In the current study, there was no significant relation between type of milking and positivity to BV, although the serological diagnosis of VACV was predominant in farms that had the following characteristics: high milk production, high number of animals, and high number of lactating cows. It is possible that a new investigation analyzing a higher number of samples in this region can verify this relation.

Some studies indicated that VACV may be introduced in the farms by contaminated milkers who work in different properties [1, 32]. Nevertheless, in the current study, the sharing of employers among farms was not a significant risk factor. Animal movement and migration of workers in dairy farms were considered major causes of VACV spread in Brazilian Amazon biome [33], whereas milk truck was indicated as a probable route of transmission and dissemination of this poxvirus in outbreaks diagnosed in Midwestern Brazil [15]. Other authors showed that an inadequate destination of garbage in public collection site was associated to VACV-positive seroprevalence [6]. In the current study, there were no significant associations between seropositivity and animal trade or delivery of milk. However, we observed higher positivity in large farms housing a high number of animals. Another recent study performed in DF indicated that the circulation and introduction of infected cattle of neighboring states may be an important epidemiological factor to the disease [3]. In addition, DF is an important route of animal transport among Brazilian states of the Southeast, Midwest, and Northeast.

The current study has showed that consumption of raw milk is common in 30% of farms in DF. This situation represents an important risk to the local public health. Scientific evidence indicates a possible animal and human infection by ingestion of VACV-contaminated milk [34]. Infectious particles and/or DNA of the VACV have already been detected in milk of cows naturally [35] and experimentally [36] infected, besides milk experimentally infected and subjected to heat treatment [37]. Furthermore, there is a risk for humans associated with the consumption or manipulation of

contaminated cheese [38, 39]. In the current study, there was no significant relationship between the farm positivity and the manipulation of cheese and their derivatives by farmers. However, significant association between seropositivity and the non-production of dairy derivatives was observed ($p < 0.05$), but this association does not seem to have biological significance.

Wild rodents have been considered possible reservoirs and transmitters of the disease and risk factors to the infection [6, 11, 35, 40, 41]. Moreover, other domestic and wild animals, such as dogs, cats, horses, opossums, coati, marsupials, pigs, rabbits, sheep, and monkeys also appear to be linked to the VACV transmission cycle [4, 6, 9, 10, 42, 43]. In the present study, there was correlation only between seropositivity and the presence of sheep and goats ($p < 0.05$), but this significance does not seem to have biological relevance, since these animals do not seem to act as reservoirs for BV. In addition, there is no data related to bovine poxviruses in small ruminants of the region.

The current study showed that veterinary assistance is not common in cattle farms in DF. No beef and mixed positive farms claimed to have, at the moment of the study, this assistance. When veterinary assistance occurs on the farms, management and prophylaxis are generally more appropriate, and according to Megid et al. [14], the percentage of infection by VACV is directly correlated with the introduction of control measures in the property. The technification of the farms, including mechanized milking, is another factor considered positive for minimizing infections by VACV [10]. In addition, most regional dairy farms have some particular features of production, such as own consumption of the produced milk and the fact that dairy production is not the main activity of many properties.

The serological results of the current study indicate the circulation of VACV in the cattle herds from DF, although no clinical case of BV had been officially notified in the region in 2015 [3]. Thus, according to the considerable seropositivity for VACV and current viral circulation in the region, more epidemiological studies are needed to provide additional data that elucidate the origin, zoonotic potential, dissemination dynamics of BV, and possible domestic and wild reservoirs, as has been studied in other regions [35].

Abbreviations VACV: Vaccinia virus; BV: Bovine vaccinia; DF: Distrito Federal; VN: Virus-neutralization; SEAGRI: Secretaria de Estado da Agricultura, Abastecimento e Desenvolvimento Rural; OU: Operational units; CI: Confidence interval

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Lorena Ferreira Silva, Stephan Alberto Machado de Oliveira, Ana Lourdes Arrais de Alencar Mota, Vitor Salvador Picão Gonçalves, Carolina de Oliveira Freitas, Juliana Felipetto Cargnelutti, Eduardo Furtado Flores, and Fabiano José Ferreira de Sant'Ana. The first draft of the manuscript was written by Lorena Ferreira Silva and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Ethics approval Sera analyzed in this study were processed according to the regulations of the Animal Ethics and Use Committee of Universidade Federal de Goiás (Brazil).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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