

# Investigation of campylobacteriosis and genital trichomoniasis in bulls from rural properties in the Pantanal of Mato Grosso state, Brazil

## Investigação de Campilobacteriose e Tricomonose genital em touros de propriedades rurais do Pantanal Mato-Grossense, Brasil

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### Highlights

Campylobacteriosis and bovine trichomoniasis cause significant economic losses.

Information is lacking on the occurrence of these diseases in Pantanal.

Favorable conditions promote the occurrence of these diseases.

Most of the assessed properties were flooded and adopted natural breeding.

*Campylobacter* spp. and *T. foetus* were not found in bulls.

### Abstract

Bovine genital campylobacteriosis (BGC) and bovine trichomoniasis (BT) are diseases of cattle that are primarily transmitted through sexual contact. Although bulls may be asymptomatic, these infectious diseases contribute to reproductive failure, embryonic death, and abortion in cows. Infection in cattle causes significant economic losses. BGC is caused by two bacterial subspecies, *Campylobacter fetus* subsp. *venerealis* and *Campylobacter fetus* subsp. *fetus*, whereas the protozoan *Trichomonas foetus*

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causes BT. Mato Grosso state has the largest bovine herd in Brazil, particularly in the Pantanal region. This area encompasses vast expanses of land characterized by annual floods and a predominant reliance on natural breeding for animal reproduction. These conditions create a favorable environment for the occurrence of BGC and BT within the herd. Given the lack of up-to-date data regarding the prevalence of these diseases, this study aimed to examine the presence of *Campylobacter* spp. and *Tritrichomonas foetus* in samples from 100 bulls in the municipalities of Poconé, Santo Antônio de Leverger, and Nossa Senhora do Livramento located in the Pantanal region of Mato Grosso state. Preputial smegma samples were retrieved using preputial swabs and stored in a saline solution at -80°C for subsequent analysis. Polymerase chain reaction was used to identify the presence of *Campylobacter fetus* subsp. *venerealis*, *Campylobacter fetus* subsp. *fetus*, and *Tritrichomonas foetus*. Despite a questionnaire revealing epidemiological conditions conducive to the persistence and spread of these pathogens, they were not detected in the bulls evaluated on rural properties in the Pantanal of Mato Grosso region.

**Key words:** *Campylobacter fetus*. Reproductive diseases. Polymerase chain reaction. *Tritrichomonas foetus*.

## Resumo

Campilobacteriose genital bovina (CGB) e Tricomonose bovina (TB) são doenças infectocontagiosas de transmissão venérea, assintomáticas nos touros, sendo consideradas como importantes enfermidades causadoras de falha reprodutiva, morte embrionária ou abortamento, ocasionando perdas econômicas significativas em rebanhos bovinos infectados. CGB é causada pela bactéria *Campylobacter fetus* subsp. *venerealis* e *Campylobacter fetus* subsp. *fetus*, e TB pelo protozoário *Tritrichomonas foetus*. O estado de Mato Grosso é detentor do maior rebanho bovino do Brasil, envolve a região do Pantanal Mato-Grossense que possui grandes extensões de terra, com ciclo anual de enchentes e a reprodução dos animais realizada predominantemente por monta natural, condições estas, favoráveis a presença de CGB e TB no rebanho. Considerando a carência de informações recentes sobre a ocorrência dessas enfermidades no estado de Mato Grosso, o objetivo deste estudo foi investigar a presença de *Campylobacter* spp. e *Tritrichomonas foetus* em 100 touros provenientes dos municípios de Poconé, Santo Antônio de Leverger e Nossa Senhora do Livramento, localizados na região pantaneira do estado de Mato Grosso. Amostras de esmegma prepucial foram coletadas por meio de escarificação via swab prepucial e armazenadas em solução salina a -80°C. Para a detecção de *Campylobacter fetus* subsp. *venerealis*, *Campylobacter fetus* subsp. *fetus*, e *Tritrichomonas foetus*, foi realizada a reação em cadeia pela polimerase (PCR). Apesar do questionário aplicado nas propriedades revelar condições epidemiológicas que favorecem a manutenção e disseminação desses patógenos, este estudo não identificou a presença dos referidos agentes em touros avaliados nas propriedades rurais do pantanal Mato-Grossense.

**Palavras-chave:** *Campylobacter fetus*. Doenças reprodutivas. Reação em cadeia pela polimerase. *Tritrichomonas foetus*.

## Introduction

The state of Mato Grosso has the largest cattle herd in Brazil, with approximately 32.8 million herds. The annual meat production from this state reaches a remarkable 1.41 million tons, with approximately 34.8% of this production being exported (Instituto Mato-Grossense de Economia Agropecuária (IMEA, 2022).

Situated in the southern region of Mato Grosso state, the Pantanal stands out as a significant biome because of its remarkable diversity of fauna and flora. This unique ecosystem is characterized by a periodically flooded plain, featuring vast expanses of natural fields that are highly conducive for cattle raising activities (Silva & Abdon, 1998).

The reproductive indices of livestock play a vital role in determining the profitability of cattle farming. Reproductive diseases, in particular, pose a significant threat to profitability as they stealthily spread and compromise the reproductive performance of affected animals, leading to detrimental effects on overall productivity (Pellegrin et al., 2002).

Venereal infections in cattle are prevalent worldwide, with a higher incidence observed in regions and properties where natural breeding remains the primary reproduction method (Sahin et al., 2017). Notably, bovine genital campylobacteriosis (BGC) and bovine trichomoniasis (BT) significantly limit animal productivity in endemic countries (Silveira et al., 2018). These diseases are sexually transmitted, with BGC being caused by the bacteria *Campylobacter fetus* subsp. *venerealis* and

*Campylobacter fetus* subsp. *fetus*, and TB by the protozoan *Tritrichomonas foetus*, which predominantly affects the genital tract of cattle (Alves et al., 2011).

Infected males serve as permanent asymptomatic carriers and play a significant role in the dissemination of these diseases. In females, the impact is evident through various clinical manifestations, including embryonic death, repeated estrous cycles, abortions, the presence of empty cows by the end of the breeding season, prolonged calving intervals, and ultimately a decrease in overall calf production (Pellegrin et al., 2002).

The diagnosis of these diseases relies on a combination of herd reproductive history and the analysis of samples collected through preputial or cervicovaginal swabs for laboratory testing. These tests include culture, direct examination, direct immunofluorescence, agglutination of cervical mucus, matrix-assisted laser desorption/ionization-time of flight mass spectrometry, and polymerase chain reaction (PCR) (Sahin et al., 2017). In recent years, PCR has been increasingly used as a diagnostic tool for the direct detection of microorganisms in samples (Van der Graaf-Van Bloois et al., 2013; Carli et al., 2020). PCR offers high sensitivity and specificity (Groff et al., 2010) and does not require extensive precautions during sample transportation, as the DNA remains intact even if the pathogen is non-viable (Botelho et al., 2018).

In recent decades, only a few studies have focused on diagnosing the occurrence or prevalence of BGC and BT, compared with the large number of epidemiological surveys conducted in the country until the late 1980s (Alves et al., 2011). The lack of official data,

along with discrepancies in the prevalence of these diseases across different geographic regions, can be attributed to various factors. These factors include the breeding locations of the assessed animals; herd management practices; absence of prophylactic vaccines; and variations in sample collection, conservation, and transportation techniques (Carli et al., 2020; Pena-Fernández et al., 2021). According to Carli et al. (2020), the occurrence of the bacteria *C. fetus* subsp. *venerealis* in Brazilian cattle herds range from 1.8% to 51.7%. The variability observed in the detection frequencies might be attributed to the challenges associated with conventional culture methods and immunofluorescence techniques for accurate identification.

In Brazil, surveys have provided insights into the prevalence of BGC in infected animals, particularly in the Midwest region. Alves et al. (2011) reported a detection rate of 50.8% carrier animals using the direct immunofluorescence technique. In addition, Carli (2022) identified a positive animal out of three tested animals (33%) through PCR analysis in the Mato Grosso cattle herd. These findings highlight the presence of BGC in the region and the importance of implementing diagnostic techniques such as immunofluorescence and PCR for accurate detection.

Given the underreported nature of both diseases in Mato Grosso state, coupled with the favorable natural conditions and reproductive management practices

prevalent in the Pantanal region, which contribute to the presence and spread of BCG and BT, the primary objective of this study was to investigate the occurrence of *Campylobacter fetus* subsp. *venerealis*, *Campylobacter fetus* subsp. *fetus*, and *Tritrichomonas foetus* in bulls from rural properties in the Pantanal region of Mato Grosso, Brazil, using PCR.

## Materials and Methods

This research was approved by the Animal Welfare Committee of the University of Cuiabá (CEUA-UNIC, protocol no 007/2019).

Thirteen properties located in the Pantanal of the state of Mato Grosso were carefully chosen. These properties are located in the municipalities of Poconé (16° 15' 26" south, 56° 4' 13" west), Santo Antônio de Leverger (15° 51' 17" south, 56° 37' 29" west), and Nossa Senhora do Livramento (15°46' 30" south, 56°20' 44" west), which are the largest sub-regions of the Pantanal, according to Silva and Abdon (1998). The number of selected properties was based on data provided by the Institute of Agricultural Defense of the state of Mato Grosso, where a 3.4% average selection of existing properties in each municipality was established. As for the sample size, 100 animals aged over 25 months were randomly allocated based on the availability of animals per farm per municipality, as indicated in Table 1.

**Table 1**

**Number of properties and bulls over 25 months old evaluated in the municipalities of Poconé, Santo Antônio de Leverger, and Nossa Senhora do Livramento of the Mato Grosso state between November 2019 and March 2020**

Municipality	Total number of properties per municipality (n)	Total number of males > 25 months of age per municipality (n)	Total number of animals collected (n)	Evaluated properties (n)
Poconé	173	55,754	52	6
Sto. Antônio de Leverger	135	36,735	33	5
N. Senhora do Livramento	71	15,790	15	2
<b>Total</b>	<b>379</b>	<b>108,279</b>	<b>100</b>	<b>13</b>

Source: Instituto de Defesa Agropecuária do Estado de Mato Grosso - INDEA-MT (2019) (Unpublished data registered in SINDESA).

Biological samples from 100 zebu bulls were collected between November 2019 and March 2020. Only non-castrated males aged over 25 months and with access to cow lots intended for breeding were included in this study.

An epidemiological questionnaire was applied to obtain comprehensive epidemiological data on the sampled properties and animals. The questionnaire primarily focused on gathering information concerning property identification; ownership details; property infrastructure, including paddocks; the presence or absence of wetlands; cattle population size; and the type of farming practices employed. In addition, the questionnaire addressed aspects related to nutritional and sanitary management; reproductive systems adopted on the properties; acquisition, sale, or lending of animals for reproduction; leasing practices; use of reproductive biotechnologies; birth and weaning rates; the occurrence of abortions

and fetus disposition; and knowledge level of the property staff concerning the studied diseases.

To ensure minimal interference with the PCR reactions, certain measures were taken prior to sample collection. First, the preputial hair was carefully trimmed. Next, the foreskin was cleaned with paper to ensure the absence of blood and urine. For sample collection, a sterile swab was introduced into the preputial ostium and gently used to scrape the inner penile mucosa, extending up to the region of the cul-de-sac of the prepuce mucosa, as described by Genovez et al. (1986). Each swab was then placed in a 2 mL microtube containing sterile saline solution and stored in an isothermal box with recycled ice to maintain the appropriate temperature during transportation. Upon reaching the laboratory, the samples were promptly transferred to a -80°C freezer for storage until molecular analysis.

DNA extraction and PCR for detection of *T. fetus*, *C. fetus* subsp *fetus*, and *C. fetus* subsp *venerealis* were performed as follows:

For DNA extraction, the commercial kit DNeasy® Blood & Tissue Kit (QIAGEN, Netherlands) was used, following the manufacturer's specifications, with 1 mL of each sample. After DNA extraction, samples were quantified by fluorimetry using Qubit 2.0 Invitrogen® and stored at -20°C.

The detection of *T. foetus* was performed using the simplex PCR technique, according to the methodology described by Felleisen et al. (1998) (Table 2). The reactions

had a final volume of 25 µL, containing 2x Taq DNA Polymerase Master Mix RED 1.5 mM MgCl<sub>2</sub> (Ampliquon), 1 µL of each primer (10 pmol/µL), 0.5 µL of H<sub>2</sub>O, and 10 µL of DNA template (170 ng). All reactions were performed in a thermocycler (Axygen® MaxyGene II Thermal Cycler), and the cycling conditions were 94°C for 5 min, 35 cycles of 94°C for 30 s, 67°C for 30 s, 72°C for 1 min, and 72°C for 5 min. A strain of *T. foetus*, provided by the Instituto Biológico of São Paulo, was used as positive control, and free H<sub>2</sub>O as negative control.

**Table 2**

**Primers used to perform PCR for *T. foetus*, *C. fetus* subsp. *fetus*, and *C. fetus* subsp. *venerealis* and amplification products**

Microorganism/ gene	Sequence 5' to 3'	Amplification products	Reference
<i>Tritrichomonas fetus</i> ITS1	TFR3- 5'CGGGTCTTCCTATATGAGACAGAACC-3' TFR4- 5'CCTGGCGTTGGATCAGTTTCGTTAA-3'	347 pb	Felleisen et al. (1998)
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	MG3F- 5'GGTAGCCGCAGCTGCTAAGAT-3' MG4R- 5'TAGCTACAATAACGACAAC-3'	960 pb	Hum et al. (1997)
<i>Campylobacter fetus</i> subsp. <i>venerealis</i>	VenSF- 5'CTTAGCAGTTTGCGATATTGCCATT-3' VenSR- 5'GCTTTTGAGATAACAATAAGAGCTT-3'	142pb and 960pb	

**Source:** Hum et al. (1997) and Botelho et al. (2018).

To detect *C. fetus* subsp *fetus* and *C. fetus* subsp *venerealis*, the multiplex-PCR technique was performed according to the methodology described by Hum et al. (1997) (Table 2). The reactions had a final volume of 25 µL, containing 5 µL 10x buffer (Invitrogen), 0.75 µL 50 mM MgCl<sub>2</sub> (Invitrogen), 4 µL of dNTPs (200 µM of each nucleotide), 0.25 µL of Taq DNA polymerase (5 U) (Invitrogen), 1.25 µL of each primer (30 pmol/µL) and 5 µL

template DNA totaling 60 ng in the reaction. All reactions were performed in a MJ Research PTC200 thermocycler (Marshall Scientific®), and the cycling conditions were 94°C for 5 min, 30 cycles of 94°C for 60 s, 57°C for 60 s, 72°C for 60 s, and 72°C for 10 min. *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis*, provided by Instituto Biológico of São Paulo, were used as positive control, and free H<sub>2</sub>O as negative control.

The results were analyzed using electrophoresis on a 1.5% agarose gel. The gel was then documented using the Electrophoresis Documentation and Analysis System 120 (Kodak®) and further analyzed using the ID Image Analysis software (Kodak® Digital Science).

## Results and Discussion

Based on the data collected via the epidemiological questionnaire, we observed that all 13 properties under evaluation exploited beef cattle, with a notable predominance of Nelore cattle. Among the sampled bulls, 85% were within the age range of two and a half to five years, while the remaining 15% were aged between five and seven years.

Of the total evaluated properties, 77% (10 out of 13) practiced natural breeding, where the bulls had access to the females throughout the year. Three properties (23%) used artificial insemination, followed by bull introduction. In addition, four properties (30.7%) were involved in selling breeding bulls, and we observed that only these properties conducted regular andrological examinations. There were no records of lending or transferring bulls to other properties. However, it was observed that 82% of the evaluated bulls were acquired from external herds.

None of the evaluated properties, accounting for 100%, immunized bulls against reproductive diseases, such as infectious bovine rhinotracheitis, bovine viral diarrhea, leptospirosis, and campylobacteriosis. Surprisingly, the individuals responsible for the 13 properties were not aware of bovine

campylobacteriosis and trichomoniasis, nor the detrimental effects that these diseases can have on their herds.

Analysis of the epidemiological questionnaire revealed that 46% (6 out of 13) of the properties reported cases of abortion within their herds. Unfortunately, in these cases, the fetuses or fetal appendages were left in the pastures.

Furthermore, wetlands were present in 77% (10 out of 13) of the evaluated properties. These areas posed challenges or restricted access during rainy seasons.

The analysis of preputial smegma samples collected from the 100 bulls revealed negative results for both *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus*, as well as *T. foetus*. This indicates the absence of BGC and BT in animals from the 13 evaluated properties in the Pantanal of Mato Grosso.

During the study, the bulls did not engage in sexual intercourse. It noteworthy that before sample collection, the Pantanal experienced wildfires during the dry season, which destroyed pastures and internal structures of the properties. Consequently, it was not possible to divide the herds into different lots.

According to Pellegrin et al. (2002), regular sexual activity has been associated with a decrease in pathogen concentration. In this study, the collection of preputial secretions from bulls was conducted during their reproductive season. This timing may have contributed to the absence of identified agents in the sampled bulls. Therefore, to ensure accurate results, bulls should undergo a rest period before and during the collection interval.

It is important to highlight that the sample collection method used for animals involved in natural breeding was not the determining factor in obtaining the negative results reported in this study. This is because the diagnostic method used in this study was PCR-based, which is known for its high sensitivity and specificity.

It is noteworthy that previous studies that investigated the occurrence or prevalence of BGC and BT have used various diagnostic techniques with varying degrees of sensitivity and specificity. These techniques include isolation, culture, and direct immunofluorescence (Sahin et al., 2017).

Culture of BGC bacteria and the BT protozoan is challenging because of their fastidious characteristics and viability, respectively, requiring specific transport and culture media for each agent, as well as careful attention to transport time (Van der Graaf-Van Bloois et al., 2013). Moreover, the frequency of identification may be related to the difficulty of detection due to the lower concentration of pathogens when conventional culture and immunofluorescence methods are used to diagnose these diseases (Schmidt et al., 2010).

Currently, molecular assays are the most used methods in laboratory routines for the diagnosis of *Campylobacter* sp. and *Tritrichomonas* sp. These methods are highly specific and sensitive, independent of pathogen viability (Groff et al., 2010; Carli et al., 2020). They do not require extensive precautions during sample transportation since the DNA remains intact even if the agent is nonviable, overcoming the limitations of traditional diagnostic methods (Botelho et al., 2018).

Another factor to consider when using conventional culture and immunofluorescence diagnostic methods is the age of the bulls. Older bulls are more prone to natural infection by *T. foetus* and *C. fetus* because of the deepening of preputial crypts as they age, which creates a microaerophilic environment in the preputial mucosa that is conducive to the biology of these agents (Bondurant, 2005; Botelho et al., 2018). In this study, the use of conventional diagnostic methods could potentially hamper the accurate identification of these agents, particularly because a significant majority (85%) of the assessed bulls were in the age range of two and a half to five years old.

Pellegrin et al. (2002) found no significant difference in the frequency of infection between older and younger bulls on four farms in the state of Mato Grosso do Sul. This can be attributed to the fact that in this region, younger bulls generally start mating at four years of age, a period in which their preputial crypts are probably well-developed thereby facilitating the transmission of agents.

The negative findings for both diseases in this study align with the results reported by Rodrigues et al. (2017) who investigated the presence of these pathogens in Nelore and Senepol breeds during a breeding season in Mato Grosso do Sul. They used preputial smegma swab samples for culture and direct examination; this was conducted in triplicate with 15-day intervals.

In a study by Rocha et al. (2009), negative results were also obtained for *T. foetus* when examining young bulls aged 30 months to 5 years using immunofluorescence. This investigation



took place in properties located in Rio de Janeiro and was characterized by a history of reproductive issues, such as high rates of estrus repetition, abortions, and prolonged calving intervals. Similarly, Leal et al. (2012) did not observe any prevalence of BT in the uterus, vagina, or prepuce of animals slaughtered at a slaughterhouse in the Federal District. The diagnostic methods used in their study included culture and direct examination.

The negative result for *C. fetus* in bulls in this study differs from those reported by some authors. Pellegrin et al. (2002), using the direct immunofluorescence technique, found an occurrence of 56% (74 out of 132 animals) for *C. fetus* in Nelore bulls with an average age of 7 years, following 45 days of sexual rest. Haas et al. (2020), also using the immunofluorescence technique, recorded an occurrence of 27% (81 out of 297 animals) for *C. fetus* in bulls from properties in the midwestern region (Goiás, Mato Grosso, and Mato Grosso do Sul). In both studies, the collection method involved swabs with mechanical removal of mucus by scraping the preputial mucosa, as described by Genovez et al. (1986). It is noteworthy that the same collection process was employed in this study.

Botelho et al. (2018) obtained positive results of 13.5% (27 out of 200) for *Campylobacter fetus* subsp. *venerealis*, 17.5% (35 out of 200) for *Campylobacter fetus* subsp. *fetus*, and 8% (16 out of 200 bovines) for *Tritrichomonas foetus* using PCR on samples collected by scraping preputial smegma from animals older than 2 years slaughtered in abattoirs. They attribute the success of the results to the collection method and the PCR technique employed.

In this study, we observed that in the evaluated Pantanal properties, there was a predominance of natural breeding (77%), and the bulls remained in contact with the cows throughout the year. This reproductive management can probably be explained by the difficulty of separating the herd into lots in marshy areas. The presence of flooded areas in 77% of the evaluated properties further reinforces this explanation.

Moreover, the evaluated properties introduced animals from other properties, as 82% of the assessed bulls were acquired from different herds. This practice may contribute to the introduction and persistence of BGC and BT infections in the herd, as documented by Genovez (1986), Lage (2001), Pellegrin et al. (2002), and Carli et al. (2020). However, despite the presence of these predisposing conditions for infection, no positive cases of these diseases were observed in the bulls.

The occurrence of abortions in 46% (6 out of 13) of the properties, where fetuses or fetal annexes were left in the pasture, is possibly associated with the presence of other reproductive diseases that cause abortion.

The characteristics and clinical signs of BGC and BT diseases are not specific and further laboratory tests are needed to differentiate them from other reproductive diseases (Carli, 2022). According to Cobo et al. (2011), vaccination against BGC and BT can effectively prevent or eliminate the infection in most bulls by stimulating the production of genital and systemic IgG. This is particularly relevant considering the challenges of implementing other control measures in Brazilian breeding conditions. However, it is important to note that the

use of vaccines should be accompanied by the implementation of appropriate sanitary control measures, as highlighted by Alves et al. (2011). Vaccination alone should not be employed indiscriminately, and a comprehensive approach involving both vaccines and sanitary measures is recommended for effective control.

## Conclusion

The questionnaire applied in the properties identified the predominance of natural breeding throughout the year, presence of wetland areas, lack of knowledge regarding sanitary control measures and prophylaxis for reproductive diseases, and acquisition of bulls from other herds without proper sanitary control. These epidemiological conditions facilitate the maintenance and spread of the studied pathogens. However, *C. fetus* subsp. *venerealis*, *C. fetus* subsp. *fetus*, and *T. foetus* were not found in the bulls evaluated in the Pantanal region of Mato Grosso.

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