Tritrichomonas foetus in bulls in the State of Pernambuco, Brazil*

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ABSTRACT. de Oliveira J.M.B., Batista Filho A.F.B., Borges J. de M., Soares L.B.F., Ortega-Mora L.M., Brandespim D.F., Mota R.A. & Pinheiro Júnior J.W. ***Tritrichomonas foetus* in bulls in the State of Pernambuco, Brazil.** [*Tritrichomonas foetus* em touros no estado de Pernambuco, Brasil.] *Revista Brasileira de Medicina Veterinária, 38(4):449-453, 2016*. Programa de Pós-Graduação em Sanidade e Reprodução de Ruminantes. Unidade Acadêmica de Garanhuns, Universidade Federal Rural de Pernambuco, Av. Bom Pastor s/n, Boa Vista, Garanhuns, Pernambuco, 55296-901, Brazil. E-mail: juniormariobaltazar@gmail.com

The aim of the present study was to investigate the occurrence of *Tritrichomonas foetus* in bulls in the state of Pernambuco, Brazil. In total, 105 samples of preputial smegma were collected from bulls in service belonging to different herds (n=63) and slaughterhouses (n=42). Genomic DNA extraction from collected samples was carried out to identify the agent in the samples and they were also submitted to the polymerase chain reaction. A frequency of 6.6% (2.7 - 13.2%; C.I. 95%) was found for *T. foetus*. None of the slaughterhouse samples were positive. With regards to the number of foci, 21.8% (7/32) of the properties contained animals that were positive for *T. foetus*. In conclusion, *T. foetus* infection occurred in bulls in this way, this agent should be included in the diagnosis in animal health control programs. Therefore, as the removal of infected bulls and their replacement by younger animals, should be implemented in order to avoid the dissemination of the agent in herds.

KEY WORDS. Bovines, trichomonosis, PCR, prevention.

RESUMO. Objetivou-se com este trabalho pesquisar a ocorrência de *Tritrichomonas foetus* em touros do estado de Pernambuco, Brasil. No total foram colhidas 105 amostras de esmegma prepucial de animais em serviço, procedentes de propriedades (n=63) e matadouros (n=42). Para a identificação do agente realizou-se a extração do DNA genômico das amostras e as mesmas foram submetidas à reação em cadeia da polimerase. Observou-se uma frequência de 6,6% (2,7% - 13,2%; I.C. 95%) para *T. foetus*. Nenhuma amostra de matadouro foi positiva. Em relação ao número de focos, 21,8% (7/32) das propriedades apresentaram animais positivos para *T. foetus*. Conclui-se que a infecção por *T. foetus* ocorre em touros na região e que o agente deve ser incluído no diagnóstico de programas de controle de saúde animal. Além disso, o descarte dos touros infectados e reposição por animais jovens devem ser implementadas a fim de evitar a disseminação do agente nos rebanhos.

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PALAVRAS-CHAVE. Bovinos, tricomonose, PCR, prevenção.

INTRODUCTION

Bovine Trichomoniasis (BT) is a venereal disease responsible for severe economic losses in the cattle industry caused by a protozoan called *Tritrichomonas fetus* (Alves et al. 2011). Among males, the agent are restricted to preputial and penile mucosa (Cobo et al. 2011), while are restricted to the vagina, cervix, uterus and oviduct in females (Cobo et al. 2004). However, infected males do not exhibit any signs of the infection and become carriers for the remainder of their lives. For these reasons, they are considered as the main responsible for the maintenance of the agent in herds (BonDurant 2005).

The most significant epidemiological form of transmission of the *T. foetus* is through natural breeding, in which one infected animal transmits the agent to a non-infected animal during copulation (BonDurant 2005). However, transmission can also occur through artificial insemination with contaminated semen (Eaglesome & Garcia 1997) as well as from cats (Dos Santos et al. 2015).

The disease exhibit a cosmopolitan distribution, with prevalence values ranging from 0.3% to 32.0% (Rodning et al. 2008, Madoroba et al. 2011, Mendoza-Ibarra et al. 2011, Molina et al. 2013). In Brazil, studies carried out in different regions of the country have reported prevalence values ranging from 1.6% to 2.6% in the South and Southeast regions (Jesus et al. 2004, Silva et al. 2009).

The risk factors associated with the ocurrence of the infection are the utilization of shared pastures, use of old bulls in the reproductive management of animals (Mendoza-Ibarra et al. 2011), bulls loan (Mardones et al. 2008); lack of disease knowledge of farmers about the disease, which promotes their occurrence due to faults in the sanitary management of properties (Rae et al. 2004), resulting in several economic losses for owners (Collantes-Fernández et al. 2014).

Bearing in mind the importance of bulls in the epidemiological chain and the scarcity of data about the disease in Brazil, the aim of the present study was to investigate the occurrence of *T. foetus* infection in bulls in the state of Pernambuco, Brazil.

MATERIALS AND METHODS

This study was approved by the Animal Rights Ethics Committee of the *Universidade Federal Rural de Pernambuco* under license number 022/2013.

In total, 105 preputial smegma samples were collected from bulls in service from properties and slaughterhouses in the state of Pernambuco. The sampled animals were a crossbreed of zebu and taurine with varying degrees of blood. They were bred in intensive, semi-intensive and extensive systems.

The properties were selected based on convenience. In total, 63 bulls were analyzed (*Agreste*=28; *Sertão*=20; *Zona da mata*=15) from 32 herd spread over 18 districts in the state of Pernambuco (Figure 1). In addition, 42 samples of preputial smegma were collected from four slaughterhouses in the *Agreste* region of the state (Figure 2).

An investigative questionnaire containing objective questions was applied on each property to identify possible risk factors. The questions referred to the type of farming, characteristics of the reproductive, productive and sanitary management of the property.

Before collecting material from the breeding bulls on the herd, a request was made to prohibit them from engaging in sexual activity for at least 15 days. Prior to sample collection, the animals were restrained and the hair around the preputial ostium was cut. The area was cleaned with water and the ostium was then dried with paper towels. A previously sterilized plastic scraper was inserted into the foreskin of the animal and smegma of preputial and penile mucosa was scraped out.

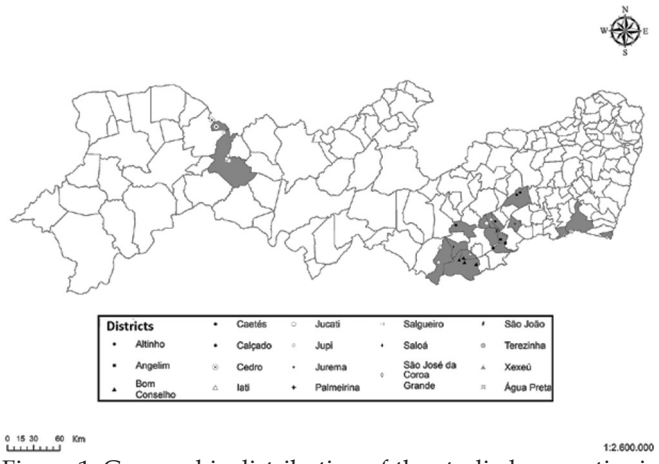

Figure 1. Geographic distribution of the studied properties in the State of Pernambuco.

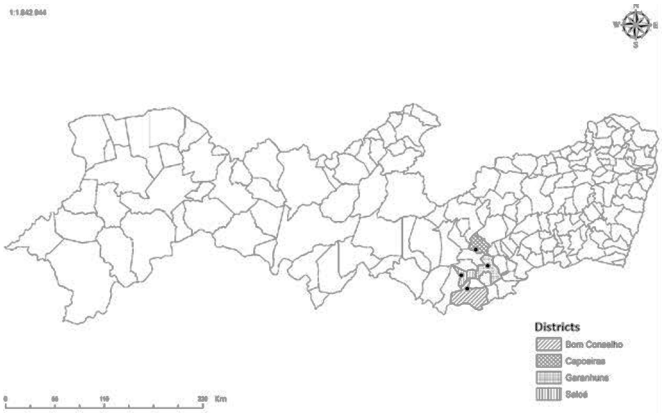

Figure 2. Geographic distribution of the studied slaughterhouses in the State of Pernambuco.

In the slaughterhouses, samples were only collected from animals that had previous sexual experience. The foreskin of males was collected after the slaughter of the animals. The penis and preputial mucosa were then exposed and mucosa was scraped to obtain smegma.

After collection, the samples were placed in buffered saline solution (PBS, pH 7.2) and then sent in cool boxes containing recyclable ice to the laboratory for processing. This trip took no more than three hours.

The samples were submitted to DNA extraction using the "Qiagen DNA Easy Blood and Tissues Kit" (Qiagen®) commercial kit, following the manufacturer's instructions. The extracted DNA was analyzed and quantified in agarose gel 0.8%, with a 1Kb marker of molecular weight. It was then stained with *Blue Green* (LGCbio), visualized in ultraviolet light and photodocumented to confirm its quality.

After DNA extraction, the amplification reactions of the genomic material to *T. foetus* was performed using the oligonucleotides TFR3 (5'CGGGTCTTCCTATATGAGAC- AGAACC3) and TFR4 (5'CCTGCCGTTGGATCAGTTTCGTTAA3'), following the protocol established by Felleisen (1997). For each reaction was used 2,75 µL of ultrapure water, 0,5 µL of each primer to 10 pmol, 6,25 µL Master Mix (Qiagen®) e 2,5 µL of DNA. Positive and negative controls were also used for both of the reactions. The amplified product was detected using electrophoresis in agarose gel 2,0%, stained in *Blue Green* (LGCbio), visualized in ultraviolet light and photodocumented.

The dispersion of absolute and relative frequencies was used in the descriptive statistical analysis. Univariate analysis of the variables of interest was conducted to study the factors associated with *T. foetus* infection. This was done using Fisher's exact test. EPIINFO™ 7 software was used for statistical calculations and Quantum GIS version 1.8.0 was used to design the figures.

RESULTS

It was detected a frequency of 6.6% (7/105) of DNA samples positive for *T. fetus*. With regards to *T. foetus* infection, all of the positive samples came from breeding bulls from herds in the *Agreste* region. All of the slaughterhouse samples were negative. The number of foci was 21.8% (7/32) distributed over 38.8% (7/18) of the districts studied. It was considered as disease focus the property that had at least one positive animal (Figure 3).

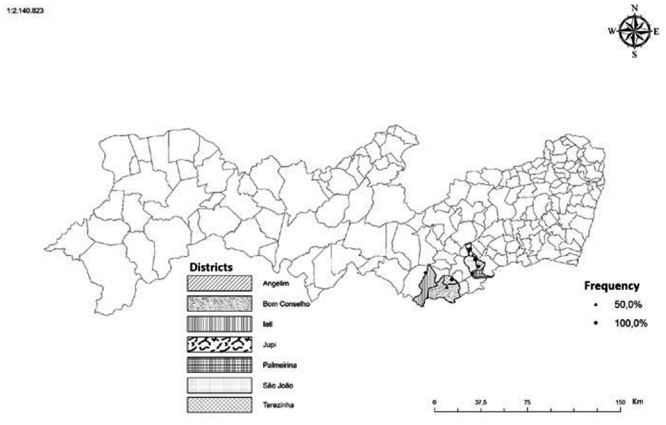

Figure 3. Geographic distribution of the *Tritrichomonas foetus* foci in the State of Pernambuco, Brazil.

No significant associations were found between the hygiene/sanitation management variable and *T. foetus* infection (Table 1).

Table 1. Analysis of the factors associated with infection by Tritrichomonas foetus in bulls in the state of Pernambuco, Brazil.

Variables	N	PCR	Univariate analysis	p-value
		Positive	OR (CI 95%)	
Breeding system:				
Extensive	30	3 (10.00%)	-	0.333
Intensive	10	0 (0.00%)	**	
Semi-intensive	23	4 (17.39%)	1.9 (0.4 – 9.4)	
Size of the herd:				
< 100 animals	35	5 (14.29%)	2.1 (0.4 – 12.1)	0.447
> 100 animals	28	2 (7.14%)		
Animals are acquired from other herds:				
Yes	50	5 (10.00%)	0.6 (0.1 – 3.5)	0.626
No	13	2 (15.38%)		
Reproductive management:				
Natural breeding	37	5 (13.51%)	1.7 (0.3 – 9.6)	
Artificial Insemination	2	0 (0.00%)	**	0.721
Both	24	2 (8.33%)	-	
Lending of bulls:				
Yes	24	1 (4.17%)	0.3 (0.1 – 2.1)	0.236
	39	6 (15.38%)		

Conventions: N – Total samples; OR – (Odds Ratio); CI – Confidence Interval; * Significant Association (p<0.05).

DISCUSSION

Studies of the occurrence of BT are scarce in Brazil. De Jesus et al. (2004) reported a prevalence of 9.9% (173/1736) in samples of preputial smegma using cultures in the State of Rio de Janeiro. In the State of Rio Grande do Sul, a frequency of 2.6% (1/38) was reported in preputial lavage samples from bulls in herds that had a history of high levels of repetition of estrus (Silva et al. 2009). In the State of Pernambuco, Paz Junior et al. (2010) analyzed samples from seven bulls in herds in the district of Sanharó and did not find the agent in any of the samples.

Different *T. foetus* infection rates have been reported in other regions of the world. In South Africa, a prevalence of 4.1% (142/3458) was reported in preputial smegma samples using PCR (Madoroba et al. 2011). Mendoza-Ibarra et al. (2011) reported a prevalence of 32.0% (33/103) in bull samples from herds in the north of Spain using PCR. In Argen-

tina, Molina et al. (2013) reported a prevalence of 1.1% (309/29.178) in smegma samples using direct immunofluorescence.

The differences between the results of the present study and those of other studies may be due to the different types of experimental designs, transportation and diagnostic methods used. Rae & Crews (2006) reported that variations in the sensitivity of diagnostic tests could be associated with the length of time that passes between sample collection and sample processing, as well as the temperature, type of culture and type of transport used. Furthermore, a low concentration of agent in smegma sample can negatively influence the result of the PCR, increasing the number of false negative results.

None of the slaughterhouse samples were positive in the PCR. This may have happened due to the absence of sexual rest. Furthermore, according to Cobo et al. (2007), the implementation of three consecutive trials, with equal intervals and sexual rest between collections, increases agent detection chance because the further development of *T fetus* in preputial mucosa of animals.

In this study it was not possible to measure the losses incurred as a result of this infection, however the presence of infected females in herds negatively affects productivity in the herds. Collantes-Fernández et al. (2014) reported in Spain the economic impact resulting from infection by *T. foetus* in beef herds and these losses are related to increasing birth interval, decreased numbers of cubs and increased time of growth and slaughter of cubs.

Greater positivity was observed in herds that used natural breeding in their reproductive management. This type of activity is considered the main form of transmitting the agent (Rae & Crews 2006). Furthermore, practices such as lending bulls to other herds (Mardones et al. 2008) and using old bulls in reproductive programs may also affect the prevalence of BT (Alves et al. 2011). Although no significant association was found ($p>0.05$), acquiring animals from other herds without knowledge of their sanitary status could favor the introduction and dissemination of the pathogen (Mendoza-Ibarra et al. 2013).

CONCLUSION

The results of the present study confirmed the presence of *T. foetus* infection in bovine herds in the region studied and the diagnosis should be implemented in animal health control programs. Prophylactic and control measures, such as the removal of infected bulls and their replacement with younger animals, should be implemented in order to avoid the dissemination of the agent in herds.

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REFERENCES

Alves T.M., Stynen A.P.R., Miranda K.L. & Lage A.P. Campilobacteriose genital bovina e tricomonose genital bovina: epidemiologia, diagnóstico e controle. *Pesquisa Veterinária Brasileira,* 31:336-344, 2011.

BonDurant R.H. Venereal diseases of cattle: natural history, diagnosis, and the role of vaccines in their control. *Veterinary Clinics of North America: Food Animal Practice*, 21:383-408, 2005.

Cobo E.R., Campero C.M., Gimeno E.J. & Barbeito C.G. Lectin Binding Patterns and Immunohistochemical Antigen Detection in the Genitalia of *Tritrichomonas foetus*-infected Heifers. *Journal of Comparative Pathology*, 131:127-134, 2004.

Cobo E.R., Favetto P.H., Lane V.M., Friend A., VanHooser K., Mitchell J. & BonDurant R.H. Sensitivity and specificity of culture and PCR of smegma samples of bulls experimentally infected with *Tritrichomonas foetus*. *Theriogenology,* 68:853-860, 2007.

Cobo E.R., Corbeil L.B. & BonDurant R.H. Immunity to infections in the lower genital tract of bulls. *Journal of Reproductive Immunology*, 89:55-61, 2011.

Collantes-Fernández E., Mendoza-Ibarra J.A., Pedraza-Díaz S., Rojo-Montejo S., Navarro-Lozano V., Sánchez-Sánchez R., Ruiz-Santa-Quiteria J.A., Ortega-Mora L.M. & Osoro K. Efficacy of a control program for bovine trichomonosis based on testing and culling infected bulls in beef cattle managed under mountain pastoral systems of Northern Spain. *Veterinary Journal*, 1:140-145, 2014.

Dos Santos C.S., De Jesus V.L.T., McIntosh D., Berto B.P. & Lopes C.W.G. Co-infection by *Tritrichomonas foetus* and *Pentatrichomonas hominis* in asymptomatic cats. *Pesquisa Veterinária Brasileira*, 35:981-989, 2015.

Eaglesome M.D. & Garcia M.M. Disease risks to animal health from artificial insemination with bovine semen. *Revue Scientifique Technique*, 16:215-225, 1997.

Felleisen R.S.J. Comparative sequence analysis of 5±8S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. *Parasitology*, 115:111-119, 1997.

Jesus V.L.T., Pereira M.J.S., Alves P.A.M. & Fonseca A.H. Fatores intrínsecos do hospedeiro associados à prevalência de tricomonose genital bovina. *Revista Brasileira de Parasitologia Veterinária*, 13:159-163, 2014.

Madoroba E., Awoke G., Tiny H. & Mkhevu M. Prevalence of *Campylobacter foetus* and *Trichomonas foetus* among cattle from Southern Africa. *African Journal of Biotechnology*, 10:10311-10314, 2011.

Mardones F.O., Perez A.M., Martinez A. & Carpenter T.E. Risk factors associated with *Tritrichomonas foetus* infection in beef herds in the province of Buenos Aires, Argentina. *Veterinary Parasitology*, 153:231-237, 2008.

Mendoza-Ibarra J.A., Ortega-Mora L.M., Pedraza-Díaz S., Rojo-Montejo S., Ruiz-Santa-Quiteria A., García-Peña F.J., Navarro-Lozano V., Cuevas-Martín M.C., Osoro K. & Collantes-Fernández E. Differences in the prevalence of *Tritrichomonas foetus* infection in beef cattle farmed under extensive conditions in northern Spain. *Veterinary Journal*, 196:547-549, 2013.

Mendoza-Ibarra J.A., Pedraza-Díaz S., García-Peña F.J., Rojo-Montejo S., Ruiz-Santa-Quiteria J.A., Miguel-Ibáñez E.S., Navarro-Lozano V., Ortega-Mora L.M., Osoro K. & Collantes-Fernandez E. High prevalence of *Tritrichomonas foetus* infection in Asturiana de la Montaña beef cattle kept in extensive conditions in Northern Spain. *Veterinary Journal*, 193:146-151, 2011.

Molina L., Perea J., Meglia G., Angón E. & García A. Spatial and temporal epidemiology of bovine trichomoniasis and bovine genital campylobacteriosis in La Pampa province (Argentina). *Preventive Veterinary Medicine*, 110:388-394, 2013.

Paz Júnior C.J., Almeida H.J.O., Júnior H.A.F., D´Alencar A.S., Galindo M.K.F., Jesus V.L.T., Alves L.C. & Faustino M.A.G. Frequência de infecção por *Tritrichomonas foetus* (Riedmuller, 1928) em bovinos leiteiros do município de Sanharó-PE. *Medicina Veterinária*, 4:6-11, 2010.

Rae D.O. & Crews J.E. *Tritrichomonas foetus*. *Tritrichomonas foetus*. *Veterinary Clinics: Food Animal Practice*, 22:595-611, 2006.

Rae D.O., Crews J.E., Greiner E.C. & Donovan G.A. Epidemiology of *Tritrichomonas foetus* in beef bull populations in Florida. *Theriogenology*, 61:605-618, 2004.

Rodning S.P., Wolfe D.F., Carson R.L., Wright J.C., Stockdale H.D., Pacoli M.E., Busby H.C. & Rowe S.E. Prevalence of *Tritrichomonas foetus* in several subpopulations of Alabama beef bulls. *Theriogenology*, 69:212-217, 2008.

Silva A.S., Zanette R.A., Oliveira C.B., Gallio M., Pereira P.L., Fernandes M.B., Tonin A.A., Badke M.R.T. & Monteiro S.G. Leptospirose e tritricomonose: isolamento em propriedade com problemas reprodutivos no sul do Brasil. *Arquivos de Ciências Veterinárias e Zoologia,* 12:87-90, 2009.