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Original Article

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Abstract

Brucellosis and Leptospirosis play a particularly significant role when present in cattle herds, as they can adversely affect the reproductive performance of the herd, therefore causing direct and indirect losses. Because bulls can mate with many cows, they can spread these diseases throughout the herd. It is therefore important to assess the reproductive health status of the herd. The purpose of this study was to identify the frequency of anti-*Leptospira* spp. and anti-*Brucella abortus* antibodies in bulls in the microregion of Araguaína, as well as identify the contributing factors for the occurrence of such diseases. Samples were taken from 165 breeding bulls from ten rural producers in eight cities of this microregion. They underwent andrological examination and standard serological tests to diagnosis B. *abortus* (AAT) and *Leptospira* spp. (SAM). Among bulls that underwent *B. abortus* (AAT) and *Leptospira* spp. (SAM) tests, 98.1% were considered to be fit for reproduction. Among the 165 breeding bulls that underwent SAM testing, 67.3% (111) were serum-reagent for at least one *Leptospira* spp. serovar, and *Hardjo* and *Wolffi* serovars were more frequently found. None of the animals displayed serological findings for *B. abortus* as a result of AAT. Among the sampled bulls, the frequency of anti-Leptospira antibodies in tested bulls was high, but none of the animals tested positive for *B. abortus*, this result was associated with the use of PNCEBT by the producers surveyed. As for the predisposition elements assessed, only herd size was considered to be significant, with a 2.32 fold increase in the likelihood of occurrence of leptospirosis in farms with more than 1,000 animals.

Keywords: Bulls, infectious diseases, serology.

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Introduction

Brazil holds the largest commercial beef herd on the planet. The players in the production chain strive to increase processes efficiency at the lowest possible cost. Calf production is basically dependent on natural mating. This form of reproductive management may create a need to use in excess of 1.5 million breeding animals per breeding cycle and, which demands continuous annual evaluation of these breeding animals conducted by qualified professionals (Freneau 2011).

By mating with a large number of females, the males may disseminate sexually transmitted diseases within the herd. Moreover, an infected animal can become sub-fertile or infertile, leading to serious losses for the beef and dairy cattle production system, with a reduction in pregnancy and birth rates. Nonetheless, serological surveys and studies focusing on this category of animal are rare (Junqueira & Alfieri 2006).

Brucellosis and Leptospirosis assume particular importance when present in the herds due to the direct and indirect losses they bring (Miranda *et al.*, 2008; Xavier *et al.*, 2009).

Bovine brucellosis is a chronic, infectious disease that affects bovines, mainly expressing itself through the occurrence of miscarriages in the final third of the gestation period and the birth of weak calves. This is zoonosis of major importance globally, causing health risks and economic losses in various countries around the world (Pappas *et al.*, 2005).

The disease is caused by *Brucella abortus.* Other species of *Brucella* such as *B. suis* and *B. melitensis* can also attack bovines when they come into contact with pigs, goats or sheep, which are respectively, natural carriers of these pathogens (Acha & Szyfres 2003; OIE/WHO 2008). It should be stressed that *B. melitensis* is exotic to Brazil (Brasil 2006; Poester, Gonçalves and Lage 2002).

In Brazil, the National Program for Control and Eradication of Animal Brucellosis and Tuberculosis (PNCEBT), promoted by the Ministry of Agriculture, Livestock and Food Supply (MAPA), instituted in 2001, established that the fight against Brucellosis should be centered on vaccinating calves between three and eight months of age, and on the control of animal transport, though herd sanitation is not mandatory (Brasil 2009).

In a serological investigation conducted by PNCEBT/MAPA in 15 states, prevalence varied among different regions of the country, finding rates as low as 0.06% in the state of Santa Catarina (Sikusawa *et al.*, 2009), but as high as 12% in the state of Mato Grosso do Sul (Chate *et al.*, 2009).

A serological survey for research into *Brucella abortus* in dairy cows in the microregion of Araguaína, in the state of Tocantins, revealed a seropositive rate of 4.1% of animals, demonstrating that the infection has spread across this herd category in the region studied (Ramos *et al.*, 2010).

Viana *et al.*, (2010) conducted a serum study of 845 bovines sent to a federally accredited slaughterhouse in Tocantins, and 142 samples (16.8%) were found to be positive for *Brucella abortus* in a buffered acidified plate antigen test (BAPA).

Baptista *et al.*, (2012) analyzed 1,818 serum samples from cows aged 24 months or older, from 102 herds in the microregion of Araguaína, via the buffered acidified plate antigen test (BAPA) and the 2-Mercaptoethanol (2-ME) test, and prevalence rates found for herds and animals were 43.5% (42.3-44.8%) and 6.2% (6.1-6.2%), respectively.

In the case of bulls, pathogenicity of the agent is associated with infection of accessory glands and testicles (Jainudeen & Hafez 2004), mostly manifesting as vesiculitis and, secondarily, by symptoms of orchitis and epididymitis, frequently leading to infected animals becoming sub-fertile or infertile (Radostits *et al.*, 2007).

Leptospirosis is also a bacterial zoonosis affecting mammals all over the world. Their etiological agents are spirochete bacteria of the genus *Leptospira*, of major importance in artificial insemination procedures due to their ability to withstand semen freezing temperatures and to cause infection (Eaglesome & Garcia 1992).

In Brazil, the seroprevalence of leptospirosis in herds ranges from 74% to 100% (Thompson *et al.*, 2006; Lage *et al.*, 2007) and in animals from 45.56% to 62.3% (Langoni *et al.*, 2000; Favero *et al.*, 2001). Serological investigations conducted on bovine populations in the Brazilian territory have

found the serovars *Hardjo*, *Wolffi*, *Pomona*, *Grippotyphosa*, *Icterohaemorrhagiae* and *Canicola* to be significant, with the greatest prevalence of serovar *Hardjo* (Araújo *et al.*, 2005; Castro *et al.*, 2008; Figueiredo *et al.*, 2009; Hashimoto *et al.*, 2012; Lage *et al.*, 2007; Thompson *et al.*, 2006).

In females, the disease affects the reproductive tract causing miscarriages, the birth of debilitated calves, premature births, retained placenta, metritis, infertility and sterility, which may also develop in males (Lemos & Almeida 2005).

Silva *et al.*, (2012) investigated the prevalence and risk factors of bovine leptospirosis in the state of Maranhão and observed that the serovars *Hardjo* and *Wolffi* were the most frequent, and that the variables identified as risk factors for leptospirosis were the presence of horses (p=0.000), capybaras (p=0.034) and bovine herds with 32 or more adult females (p=0.002).

Studies involving the two bacteria in breeding bulls are still rare, and the few that have been conducted have focused on the females. Considering the economic importance of these diseases to livestock in Brazil in general, and Tocantins in particular, the aim of the present study was to identify their frequency amongst breeding males in the microregion of Araguaína, Tocantins.

Materials and Methods

A total of 165 bulls were sampled, from the microregion of Araguaína, which is located in the *Amazonia-Cerrado* ecotone, in the western mesoregion of Tocantins, situated in the northern part of the state, covering a total area of 26,493,499 km^2, with mainly tropical climate, and harboring a beef herd of approximately 1.1 million heads, encompassing 10 (georeferenced) rural properties, spread across eight of the seventeen municipalities, comprising this microregion, as follows: Araguaína, Nova Olinda, Babaçulândia, Wanderlândia, Piraquê, Santa Fé do Araguaia, Muricilândia, Araguanã. The properties were chosen for convenience reasons, as result of the need for a minimum infrastructure in terms of water, electricity and containment pens for the collection of samples from the breeding animals.

During a visit to the properties, once the animals had been appropriately enclosed in the pen, each bull was subjected to the collection, in vacuum tubes without anticoagulant, of 10 ml of blood per puncture of the coccygeal vein. The blood was centrifuged in order to obtain the serum, which was packed in sterile 1.5 ml microtubes and kept in thermal boxes with recyclable ice for transportation to the laboratory where they were stored in a freezer at -20ºC awaiting the serological analysis. Subsequently, the scrotal perimeter was measured and semen was collected using the electroejaculation method, as per the technique recommended by the Brazilian College of Animal Reproduction (CBRA) (1998) so as to verify the reproductive status of the animals.

Andrological examination of the breeding animals – the animal semen was evaluated in the laboratory under an optical microscope immediately after collection in accordance with the CBRA andrological examination manual (1998). The lab analyses were conducted in the Animal Reproduction Laboratory at the School of Veterinary Medicine and Zootechny (EMVZ) at the Federal University of Tocantins (UFT).

Serology for the diagnosis of *Brucella abortus* – Serum samples from all the bulls in the study were subjected to buffered acidified plate antigen test (BAPA), conducted in the JV Brucellosis Diagnostic Laboratory located in the town of Araguaína, in the state of Tocantins, as described in the PNCEBT Technical Manual (Brasil 2006).

Serology for the diagnosis of *Leptospira* spp. – Serum samples collected from the animals were subjected to the Microscopic Agglutination Test (MAT) conducted in the Animal Leptospirosis Animal Diagnostic Laboratory at the Federal University of Goiás. The technique is based on the addition of suspect serum in increasing dilutions to cultures of various *Leptospira* sp. serovars (Brasil 1997). The serum was submitted to a set of 18 serovars: *Andamana, Australis, Autumnalis, Bratislava, Butembo, Canicola, Castellonis, Copenhageni, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogenes, Shermani, Tarassovi* and *Wolffi*. As a cut-off point in the titration stage, dilution greater than or equal to 1:100 up to a limit of 1:800 was employed. In each property sampled, a questionnaire was distributed addressing questions

related to production and breeding procedures and health management of the herd, preferably answered by the owner, after such owners had agreed, via consent form, to inclusion of the property in the collection of animal blood and semen. The project was approved by the Animal Ethics Committee at the Federal University of Tocantins under protocol number 23101.002903-2012-34. The data obtained were analyzed using Epi Info Version 3.5.4 developed by the Centers for Disease Control and Prevention (CDC), Atlanta GA, USA, with a determination of frequency and performance of bivariate analyses.

Results

The reproductive status of the sampled bulls, a determining factor for inclusion in the study, was determined through andrological examinations.

Serum was analyzed from a total of 164 breeding animals of which 111 (67.3%) were serum-reactive for at least one *Leptospira* spp. serovar; however none of them showed a positive serological result for *Brucella abortus* (Table 1).

Table 1: Serological results for the detection of *Leptospira* spp. antibodies via Microscopic Agglutination Test (MAT), and for *Brucella abortus* using the buffered acidified plate antigen test (BAPA), in bulls from the microregion of Araguaína, Tocantins, Brazil, 2012.

Result	*Leptospira* (MAT)			*Brucella* (AAT)		
	Nº	%	IC	Nº	%	IC
Reactive	111	67.3	59.5-74.4	0		
Not Reactive	54	32.7	25.6-40.5	165	100	100-100
Total	**165**	**100.0**		**165**	**100**	

Assessed via MAT method, 12 of the 18 *Leptospira* serovars were found amongst the animals tested, *Hardjo*, *Wolffi* and *Gryppotyphosa* were the most frequent (Table 2), and all of the properties sampled were positive for at least one serovar found in the study. Of the 111 seropositive animals, 73 presented with coagglutination.

Table 2: Serological results for the detection of *Leptospira* antibodies via microscopic agglutination test (MAT), *versus* serovars tested in bulls, by sampled property in the microregion of Araguaína, Tocantins, Brazil, 2012.

Serovar	Property										Total			
	A	B	C	D	E	F	G	H	I	J	+ve	%	-ve	%
Autunalis	1	0	0	0	0	0	2	0	1	0	4	2.42	161	97.58
Butembo	0	0	0	0	0	0	1	0	0	0	1	0.60	164	99.40
Canicola	0	1	0	0	0	0	1	0	1	0	3	1.80	162	98.20
Castellonis	0	0	1	0	1	0	1	0	0	0	3	1.80	162	98.20
Copenhageni	0	0	0	5	2	0	1	0	2	0	10	2.42	155	97.58
Gryppotyphosa	1	2	0	2	0	1	3	0	6	1	16	9.69	147	90.31
Hardjo	5	6	3	22	3	7	10	12	4	1	73	44.20	92	55.80
Hebdomadis	1	1	0	1	0	1	0	0	1	1	6	3.93	159	96.07
Icterohaemorrhagiae	0	0	0	3	3	0	1	0	0	0	7	4.24	158	95.76
Pomona	0	0	2	1	0	0	0	2	5	0	10	6.06	155	93.94
Pyrogenes	0	1	0	0	0	2	1	1	4	0	9	5.45	156	94.55
Wolffi	4	9	0	17	3	7	7	8	12	5	72	43.60	93	56.40

Discussion

In serological investigations carried out on bovine populations in various regions of the country, namely the states of Minas Gerais, São Paulo, Mato Grosso do Sul, Paraná and Paraíba, the serovars *Hardjo* and *Wolffi* rank amongst the most important (Araújo *et al.,* 2005; Castro *et al.,* 2008; Figueiredo *et al.,* 2009; Genovez *et al.,* 2004; Hashimoto *et al.,* 2012; Lage *et al.,* 2007).

Campos Jr. *et al.,* (2006), in a study on the sero-prevalence of anti-Leptospira agglutinins in the breeding bulls of Goiânia, found 74.28% of animals to be serum reactive from a total of 140 sampled, producing titrations with dilutions varying from 1:100 to 1:800. The prevalence of serovars was as follows: *Wolffi* (19.23%); *Hardjo* (15.38%); *Djasiman* and *Grippotyphosa* (5.76%); *Shermani* (4.80%); *Patoc* (1.92%); *Andamana, Castellonis, Copenhageni, Hebdomadis, Sentot* and *Tarassovi* (0.96%).

The data found in the present study are similar if we consider that the serovars *Hardjo*, *Wolffi* and *Grippotyphosa* also rank amongst the most frequent (Table 2). However the percentages and some serovars differed from the present study, the most notable being the serovars *Hardjo* (44.2%) and *Wolffi* (43.6%) which, in addition to showing a high frequency amongst the animals, were both present in 90% of the studied herds.

The other serovars found in the study have other domestic and wild species as reservoirs, thus causing accidental infection in bovines, depending on the opportunity of susceptible animals coming into contact with *Leptospira* (Araújo *et al.,* 2005; Rodrigues *et al.,* 1999). Silva *et al.,* (2012), using samples derived from the PNCEBT/MAPA serum bank, where 4,832 bovine females from the state of Maranhão, aged 24 months or older, were studied, found using the MAT, that 1,904 were reactive to at least one *Leptospira* spp. serotype, with titrations ranging from 100 to 6,400.

The high frequency of anti-*Leptospira spp.* agglutinins found in bulls in Tocantins, substantiated by the high frequencies obtained in various bovine categories in other Brazilian states (Campos Jr. *et al.,* 2006; Figueiredo *et al.,* 2009; Juliano *et al.,* 2000; Silva *et al.,* 2012) points to the endemic character of infections by *Leptospira spp.* in the micro-region of Araguaína.

In the serological analysis conducted to check for anti-*Brucella abortus* antibodies using the BAPA, of the 165 animals examined, none tested positive. The preferred location for bacteria, in the accessory glands and testicles of bovines, may induce the presence of low concentrations or even the absence of serum immunoglobulin concentrations in infected bulls, making the diagnosis of brucellosis difficult in males through conventional serological methods (Aguiar *et al.,* 2001; Radostits *et al.,* 2007).

This fact explains the divergence in results compared with those found in the study conducted by Ramos *et al.,* (2010) with dairy cows from herds in the same microregion, in which, of the 893 samples studied, using the BAPA, 37(4.1%) were found to be serum reactive; a percentage identical to that found in official prevalence reports for the Northern region of Brazil (Brasil 2009).

According to the PNCEBT, however, the animals that tested negative in the BAPA have a good chance of being truly negative for *B. abortus* infection, thus do not require other tests to confirm the results obtained (Brasil 2006).

The fact that no bulls tested positive for Brucellosis in the study could also be related to the fact that, in the studied herds, the measures advocated in the PNCEBT were strictly adhered to, with vaccination of females aged between three and eight months being carried out, and transportation of animals exclusively upon issuance of an animal transportation permit (Brasil 2009).

The data relating to the characteristics of production and management of bulls, and their cohabitation with other species, as answered in the questionnaires, and the results of the anti-leptospiral microscopic agglutination tests (MAT) were submitted to bivariate analysis and a significant association was found with the number of animals in the herd (OR=2.32; [1.08-5.02] and p=0.0436), indicating that properties with over 1,000 animals have a 2.32 fold increase in the likelihood of presenting *Leptospira* infections. In relation to analysis of cohabitation with other species, an association with seropositivity was only found with poultry and swine (OR=0.18; [0.05-0.51] and p=0.0018) which, having an OR value of less than 1, is considered to be a protection factor, unlike that

reported by Lilenbaum and Sousa (2003) in which contact with swine afforded a 3.17 times greater chance of bovines presenting with the infection (OR=3.17; p<0.04).

Conclusion

The frequency of anti-*Leptospira* antibodies in the bulls analyzed was high (67.3%), with the most prevalence of serovars *Hardjo* and *Wolffi*, thus characterizing an endemic distribution of the agent in the properties studied for this category of animal.

No animals tested seropositive for *Brucella abortus* amongst the sampled breeding animals, which was associated with the adoption of the PNCEBT by the properties studied.

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