



Occurrence of brucellosis, leptospirosis and neosporosis in cows with retained placenta in Southwest Paraná, Brazil¹

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ABSTRACT.- Porto Y.F., Pinto Neto A., Bernardi F., Possa M.G., Mota M.F., Martinez A.C., Merlini L.S. & Berber R.C. 2018. **Occurrence of brucellosis, leptospirosis and neosporosis in cows with retained placenta in Southwest Paraná, Brazil.** *Pesquisa Veterinária Brasileira* 38(8):1537-1542. Curso de Medicina Veterinária, Universidade Federal da Fronteira Sul, Campus Realeza, Av. Edmundo Gaievsky 1000, Realeza, PR 85770-000, Brazil. E-mail: adalgiza.uffs@gmail.com

The aim of this study was to measure the occurrence of brucellosis, leptospirosis and neosporosis in cows from 25 family farms milk, with semi-intensive grazing system, located in the Southwest region of Paraná, Brazil. Eighty-four cows with retained placenta, between July 2013 to July 2014, diagnosed by the presence of fetal membranes in the uterus up to 12 hours after partum, decrease of appetite and of milk production, as well increase of rectal temperature were included into the study. The animals were submitted to blood collection for evaluating the seropositivity for brucellosis, leptospirosis and neosporosis. The blood was collected at 15 to 45 days postpartum, avoiding false negative results due to immune deficiency observed in the transition period. After collection, the blood was centrifuged, the serum was packed in three aliquots, identified and subsequently frozen. Serology was performed to diagnose brucellosis (technique of buffered acidified antigen), leptospirosis (through the microscopic agglutination test - MAT) and neosporosis (indirect immunofluorescence test). Animals were considered positive when presenting titer >1:100 (leptospirosis) and 1:200 (neosporosis). No animal with placenta retention presented *Brucella abortus* bacteria; 39% (33/84) were reactive to one or more *Leptospira* serovars, 15% (13/84) were positive to *Neospora caninum*, and 4% (4/84) had both diagnosis, being reactive for leptospirosis and neosporosis. In conclusion, data from this experiment inspires greater attention to leptospirosis and neosporosis in dairy cattle presenting placenta retention in southwest Paraná.

INDEX TERMS: Brucellosis, leptospirosis, neosporosis, cattle, placenta, Paraná, Brazil, dairy cattle, reproductive diseases, reproductive efficiency, bacterioses, parasitoses.

RESUMO.- [Ocorrência de brucelose, leptospirose e neosporose em vacas com retenção de placenta no Sudoeste do Paraná, Brasil]. Objetivou-se com esse estudo

avaliar a ocorrência de brucelose, leptospirose e neosporose em fêmeas bovinas de 25 propriedades leiteiras de agricultura familiar, mantidas em sistema de pastejo semi-intensivo, localizadas na Região Sudoeste do Paraná, Brasil. Para tanto, incluiu-se nesse estudo, 84 vacas diagnosticadas com retenção de placenta (RP) pela presença parcial ou total dos anexos fetais no útero após 12 horas do parto, diminuição do apetite e da produção de leite, como também aumento da temperatura retal. Os animais foram submetidos à coleta de sangue 15 a 45 dias pós-parto, a fim de evitar resultado falso negativo devido à imunodeficiência observada no período de transição. Após coleta, o sangue foi centrifugado, o soro aliquotado em três amostras, identificado e congelado. Para o diagnóstico sorológico de Brucelose utilizou-se o exame

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sorológico do antígeno acidificado tamponado, de leptospirose a técnica de soroaglutinação microscópica, e neosporose pela técnica de imunofluorescência indireta. Os animais foram diagnosticados positivos quando apresentaram título igual ou superior à 1:100 (leptospirose) e 1:200 (neosporose). Em nenhum dos 84 animais com RP foi detectada a presença da bactéria *Brucella abortus*. Desses, 39,28% (33/84) foram reativos para um ou mais sorovares de *Leptospira*, 15,47% (13/84) foram positivos para *Neospora caninum* e 4,76% (4/84) foram reativos para sorovares de *Leptospira* sp. e *N. caninum*, concomitantemente. Em conclusão, os dados deste estudo inspiram maior atenção à leptospirose e neosporose em bovinos leiteiros que apresentam retenção de placenta no sudoeste do Paraná.

TERMOS DE INDEXAÇÃO: Brucelose, leptospirose, neosporose, placenta, Paraná, gado de leite, doenças reprodutivas, eficiência reprodutiva, bovinos, bacterioses, parasitoses.

INTRODUCTION

Placenta retention decreases the reproductive efficiency (RE) of dairy herds, due to mechanical, nutritional, infectious and management issues. Among the infectious factors, reproductive diseases such as brucellosis, leptospirosis and neosporosis are well documented in Brazilian literature (Juffo & Driemeier 2010, Nascimento & Santos 2011, Nobre et al. 2012, Rezende et al. 2013), and that their identification is essential for both the reproductive success and the health of the herd. These diseases are considered zoonoses of importance to public health (Greca Junior 2010, Nascimento & Santos 2011, Nobre et al. 2012).

Brucellosis is an infectious disease caused by *Brucella abortus* affecting farm animals, resulting in abortion in the final third of pregnancy, as well as premature births, sterility and low milk production. It causes losses and decreased reproductive efficiency (Zago 2011). The prevalence in Brazil ranges from 0.06% to 10.2% and becomes significant when considering the cattle herd of 220 million animals (Clementino & Azevedo 2016).

Similarly, Leptospirosis is a bacterial disease caused by *Leptospira* sp. affecting various animal species and humans. Particularly in cattle, it promotes reproductive disorders such as placenta retention, infertility, decreased milk production and abortion, as well as contributing to the reduction of productivity and profitability in the global livestock (Magajevski et al. 2007, Rifatbegović & Maksimovic 2011).

Neosporosis is a disease caused by the *Neospora caninum* protozoan, an obligate intracellular parasite, which affects several intermediate hosts in its cycle, such as sheep, horses, goats and cattle, and has dogs as the definitive host (Parra et al. 2008, Tembue et al. 2011, Carvalho et al. 2014). It also causes abortion in female bovines. In California, neosporosis is responsible for 42% of abortions and the damage is estimated at US\$ 25 million only in the production of milk. In Texas, the losses amount to approximately US\$ 24 million annually (Negrão 2006).

These diseases cause economic impact and decrease in reproductive rates (Laven & Peters 1996, Santos 2010). The aim of this study was to measure the occurrence of brucellosis, leptospirosis and neosporosis in cows affected by placenta retention in southwest region from Paraná, Brazil.

MATERIALS AND METHODS

The study was carried out in 25 family farm milk farms, with small and medium-sized herds, in a semi-intensive grazing system, located in the municipalities of Nova Prata do Iguacu, Nova Esperança do Sudoeste, Realeza and Salto do Lontra, all localized in southwest region of Paraná, Brazil (Fig.1). The southwest Paraná region is characterized by humid subtropical climate and annual average temperature of 19°C (IAPAR 2015).

From July 2013 to July 2014, eighty-four dairy cattle with retained placenta were diagnosed, being 7,14% (6/84) from Nova Esperança do Sudoeste, 25% (21/84) from Realeza, 27,38% (23/84) from Nova Prata do Iguacu and 40,48% (34/84) from Salto do Lontra. Retained placenta were considered in the presence of fetal membranes in the uterus up to 12 hours after partum, associated to decrease of appetite and milk production, as well increase of rectal temperature.

The animals were submitted to blood collection for evaluating the seropositivity for brucellosis, leptospirosis and neosporosis. Blood was collected at the medial coccygeal artery, with prior asepsis, using disposable needles and vacuum siliconized tubes without anticoagulant for each animal (Dirksen et al. 1993). The blood was collected at 15 to 45 days postpartum, avoiding false negative results due to immune deficiency observed in the transition period. After collection, the blood was centrifuged (200rpm), and the serum was packed in three aliquots, identified and subsequently frozen.

Serology for brucellosis was performed by the antigen screening technique on buffered plate - AAT, in a certified veterinarian laboratory. The examination of leptospirosis was conducted at the Department of Preventive Veterinary Medicine and Animal Reproduction of the Paulista State University, Jaboticabal, through the microscopic agglutination test (MAT). Animals were considered positive if presenting titer >1:100 (Hashimoto et al. 2012). Serology for neosporosis was performed at IMUNODOT (Imunodot Indústria Pesquisa Produtos para Diagnósticos Ltda.), Jaboticabal, by the indirect immunofluorescence technique (IIF). Animals presenting titer >1:200 were considered as positive (Teixeira et al. 2010).

Data were subjected to descriptive analysis by PROC FREQ SAS (Statistical Analysis System, Versão 9.2).

RESULTS

No animals with placenta retention presented brucellosis; 39.3% (33/84) were positive for leptospirosis; 15.5% (13/84) were positive for neosporosis; and 4.8% (4/84) were positive for both leptospirosis and neosporosis, simultaneously. All animals were negative for brucellosis.

From the animals evaluated, 39% (33/84) were positive for leptospirosis. The serovars detected were: Icterohaemorrhagiae at 33% (11/33), Hebdomadis at 27% (9/33), Wolffi at 24% (8/33), Gryppothyphosa at 18% (6/33), Pyrogenes at 15% (5/33), Canicolaem at 12% (4/33), Pomona at 12% (4/33), Autumnalis at 9% (3/33), Australis at 6% (2/33), Hardjo at 6% (2/33), Shermani at 6% (2/33), Coppenhagi at 3% (1/33), Tarassovi at 3% (1/33), and Patoc at 3% (1/33). In this study, concurrent infection with more than one type of serovar was found in 39% (13/33) of the animals studied.

Vaccination for leptospirosis was performed in 26% of the analyzed cattle.

Only 4% of animals in this study presented an association between leptospirosis and neosporosis.

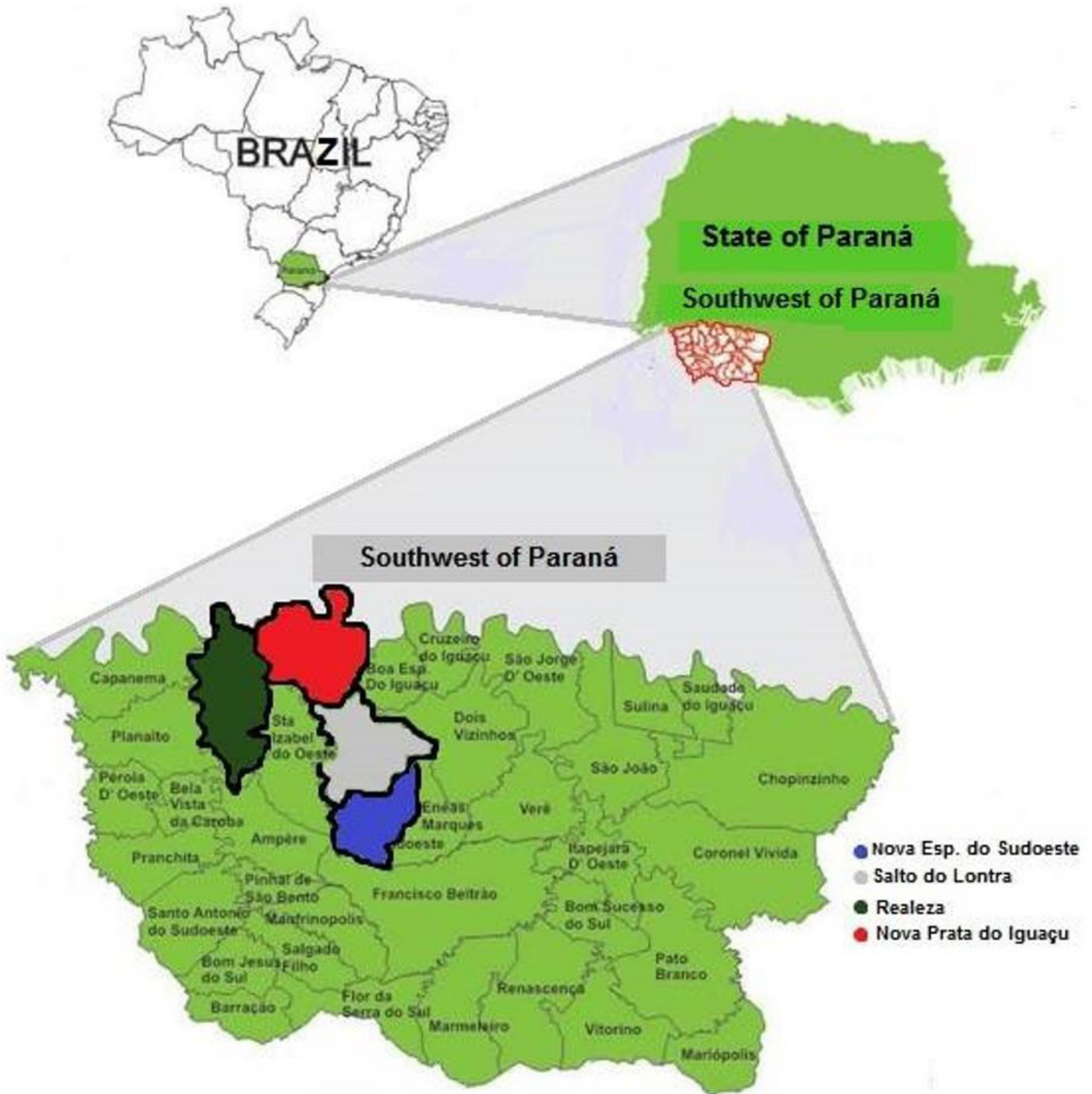


Fig.1. Map of the Southwest region of Paraná, highlighting the municipalities studied. Source: IBGE (2017).

DISCUSSION

The results found that brucellosis were according to the study conducted with dairy cattle in Paraná by Santos et al. (2005). This suggests that the mandatory measures required by the National Program for Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) implemented in 2006, is effective. Still, it must be noted that the animals come from properties that are in compliance with Instruction 62/2011, which established strict control of brucellosis, requiring regular prophylactic measures in dairy cattle (Brasil 2011). In Nigeria,

Alhaji et al. (2016) found prevalence of brucellosis in 10% of animals studied. In that country there are no official control programs for this disease, demonstrating that some public policies may be able to reduce the incidence of diseases that can be controlled, as reported by Bannantine et al. (2013).

Although no animal showed brucellosis, this disease affects approximately 5% of cattle (Brasil 2006) and is also related to retained placenta. Palmquist (2001) reported 7.9% prevalence of brucellosis in dairy cattle from the north and east of Paraná. In other Brazilian studies, prevalence was found as 4% (Dias et al. 2009), 0.14% (Sabedot et al. 2009), 0.32%

(Sartori & Luguesi 2011), 0% (Nascimento et al. 2008) and 3.5% (Viana et al. 2009), while rich countries have already controlled the occurrence of this disease (Nielsen & Yu 2010).

Although it seems small, when considering the national cattle herd, the prevalence of brucellosis can cause extensive damage, since this disease is related to a decrease in production and reproduction of animals (Sartori & Luguesi 2011).

Mufinda et al. (2017) reported that brucellosis control measures include observing biosafety measures in the workplace, especially for professionals exposed to the risk of infection. Even if brucellosis does not occur in the animals studied, it is common to perform veterinary procedures in cows with placenta retention, which if carried out in animals infected with brucellosis and/or other infectious agents could result in contamination and/or dissemination of these agents.

The prevalence of icterohaemorrhagiae and hebdomadis serovars for leptospirosis in this study differs from the one reported in Hashimoto et al. (2010, 2012).

Hardjo is the most prevalent serovar and it correlates with reproductive disorders in the region (Mineiro et al. 2007, Hashimoto et al. 2012, Menegas et al. 2013, Peiter et al. 2015); however, in our study, this correlation did not occur. In a study conducted in the micro-region of Francisco Beltrão, 41% of cattle was diagnosed with leptospirosis, with the Hardjo serovar being present in 81% (104/129) of the samples, and Wolffi serovar present in 29% (38/129) (Peiter et al. 2015).

The Hebdomadis and Wolffi serovars had similar prevalence to the one reported in other Brazilian studies (Peiter et al. 2015). Menegas et al. (2013) studied aborted animals and found 53% seropositivity, presenting titers for one or more serovars. These authors indicated leptospirosis as the cause of abortions, as well as reporting the Hardjo serovar as the most common one in Paraná.

Concurrent infection with more than one type of serovar was found in 39% (13/33) of the animals studied, than shorter than Menegas et al. (2013) reported that 52% of the animals presented reactivity for more than one serovar. Note that this study suggests a public health issue, since the Icterohaemorrhagiae serovar is very common in humans and can result in more severe patients (Guidi 2006, Pelissari et al. 2011).

Vaccination for leptospirosis, performed in 26% of the analyzed cattle, indicates that there is vaccine interference in the results, and this variation depends on the serovars tested, Pereira et al. (2013) reported that vaccination against leptospirosis is a way to increase reproductive efficiency and should be implemented in herds that will carry out artificial insemination programs. However, most serovars do not induce nonspecific reaction. It is also known that after 60 days of vaccination this influence decreases, but it reaches up to 240 days, as in the case of the Icterohaemorrhagiae serovar (Nardi Júnior et al. 2006). While most animals were not vaccinated (73%), which minimizes the interference on the results, the vaccination period was not evaluated, which could explain the higher prevalence of the Icterohaemorrhagiae serovar.

About neosporosis, detected in 15% (13/84) of animals, Langoni et al. (2013) reported 24% (23/94) of bovines with anti-*Neospora caninum* antibodies with titer ranging from 25 to 400, in the state of Paraná. Camillo et al. (2010) reported a prevalence of 24% (431/1778) of animals with neosporosis, higher than that found in the present experiment. However,

the authors consider seropositive animals those with titer greater than or equal to 1:100. Despite the seropositive samples in a large number of the animals of the herd, a study in Canada carried out by Waldner (2005) stated that the use of large-scale diagnostic tests is questionable because of the high cost.

Santos et al. (2005) studied the prevalence of brucellosis, leptospirosis and neosporosis in animals with a history of miscarriage in Paraná, and found that 60% (21/35) were reactive to one of the diseases. From the 21 positive animals, 14.3% were for neosporosis, 42% for leptospirosis and no animals were reactive to brucellosis, resembling the results obtained herein. For the seropositivity diagnosis, Santos et al. (2005) considered titers higher than or equal to 1:200, similar to the one as used in this study. Langoni et al. (2013) reported that the titration used is one of the important factors when estimating the prevalence of *Neospora caninum*, along with other factors such as race and test used.

Macedo et al. (2013) used ELISA diagnosis and found 15% of seropositive beef cattle, similar to the prevalence found in this study, showing that race and dairy ability were not relevant for the prevalence of this agent.

Immunosuppression may be related to the synergism of infectious agents (Santos et al. 2005). Thus, the concomitant infection of different infectious agents may result in increased reproductive losses associated with abortion and retained placenta. On the other hand, only 4% of animals in this study presented an association between leptospirosis and neosporosis.

CONCLUSION

The population of dairy cattle with retained placenta of this study presented seropositivity for leptospirosis and/or neosporosis; no animals were diagnosed as positive for brucellosis.

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