Reproductive diseases in a small dairy farm and its importance in regional development

Doenças reprodutivas em uma pequena propriedade leiteira e sua importância no desenvolvimento regional

Enfermedades reproductivas en una pequeña granja lechera y su importancia en el desarrollo regional

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Abstract

Losses due to low reproductive efficiency can economically affect production in a small dairy farm. The aim of this study was to detect the presence of anti-*Leptospira*, anti-*Toxoplasma gondii*, and anti-*Neospora caninum* antibodies in dairy cows with history of reproductive problems at a small dairy farm in Umuarama, Paraná, Brazil. The evaluated herd presented with history of abortion and return to estrus. Of the 37 samples analyzed, 10 (27%) were positive in microscopic serum agglutination, with titers ranging from 100 to 400, 21 (56.75%) were positive for *Toxoplasma gondii* in the indirect immunofluorescence test (IIFT), with titers ranging from 16 to 256, and four (10.8%) were positive for *Neospora caninum* in IIFT,

with titers ranging from 25 to 50. No variables were associated with any infection. Leptospirosis, toxoplasmosis, and neosporosis are widespread in herds and may have a negative impact on reproductive parameters of dairy cattle. Periodic serological evaluations are important, as they may guide the choice of preventive disease control measures in the herd and also alert and educate small producers and the local population on the possible presence of zoonoses spread by these infectious agents, in addition, it can be concluded that infectious diseases can have negative implications on the reproductive parameters of dairy cattle causing return to heat and abortion.

Keywords: Dairy production; Diagnosis; Health; Leptospirosis; Neosporosis; Toxoplasmosis.

Resumo

Perdas devido à baixa eficiência reprodutiva podem afetar economicamente a produção em uma pequena fazenda leiteira. O objetivo deste estudo foi detectar a presença de anticorpos anti-*Leptospira*, anti-*Toxoplasma gondii* e anti-*Neospora caninum* em vacas leiteiras com histórico de problemas reprodutivos em uma pequena fazenda leiteira em Umuarama, Paraná, Brasil. O rebanho avaliado apresentou histórico de aborto e retorno ao estro. Das 37 amostras analisadas, 10 (27%) foram positivas em soroaglutinação microscópica, com títulos variando de 100 a 400, 21 (56,75%) foram positivas para *Toxoplasma gondii* no teste de imunofluorescência indireta (IIFT), com títulos variando de 16 para 256, e quatro (10,8%) foram positivos para *Neospora caninum* em IIFT, com títulos variando de 25 a 50. Nenhuma variável foi associada a qualquer infecção. Leptospirose, toxoplasmose e neosporose são comuns em rebanhos e podem ter um impacto negativo nos parâmetros reprodutivos do gado leiteiro. Avaliações sorológicas periódicas são importantes, pois podem orientar a escolha de medidas preventivas de controle da doença no rebanho e também alertar e educar pequenos produtores e a população local sobre a possível presença de zoonoses disseminadas por esses agentes infecciosos, além disso, pode se concluir que as doenças infecciosas podem ter implicações negativas sobre os parâmetros reprodutivos de bovinos leiteiros causando retorno ao cio e abortamento.

Palavras-chave: Diagnóstico; Leptospirose; Neosporose; Produção de laticínios; Saúde; Toxoplasmose.

Resumen

Las pérdidas debidas a la baja eficiencia reproductiva pueden afectar económicamente la producción en una pequeña granja lechera. El objetivo de este estudio fue detectar la

presencia de anticuerpos anti-*Leptospira*, anti-*Toxoplasma gondii* y anti-*Neospora caninum* en vacas lecheras con antecedentes de problemas reproductivos en una pequeña granja lechera en Umuarama, Paraná, Brasil. El rebaño evaluado tenía antecedentes de aborto y regreso al estro. De las 37 muestras analizadas, 10 (27%) resultaron positivas en la aglutinación microscópica de suero, con títulos que variaron de 100 a 400, 21 (56,75%) fueron positivas para *Toxoplasma gondii* en la prueba de inmunofluorescencia indirecta (IIFT), con títulos que variaron entre 16 para 256, y cuatro (10,8%) fueron positivos para *Neospora caninum* en IIFT, con títulos que oscilaron entre 25 y 50. Ninguna variable se asoció con ninguna infección. La leptospirosis, la toxoplasmosis y la neosporosis son comunes en los rebaños y pueden tener un impacto negativo en los parámetros reproductivos del ganado lechero. Las evaluaciones serológicas periódicas son importantes, ya que pueden orientar la elección de medidas preventivas para el control de la enfermedad en el rebaño y también alertar y concienciar a los pequeños productores y a la población local sobre la posible presencia de zoonosis propagadas por estos agentes infecciosos, además, se puede concluir que las enfermedades infecciosas pueden tener implicaciones negativas sobre los parámetros reproductivos del ganado lechero provocando el retorno al celo y el aborto.

Palabras clave: Diagnóstico; Leptospirosis; Neosporosis; Producción láctea; Salud; Toxoplasmosis.

1. Introduction

Brazil has gradually increased milk production in the last decade, growing 43% since 2005 to be the 5th largest milk producer in the world, at 35.17 billion L per year. In Brazil, Paraná is the 3rd largest milk producer, at 4.5 billion L, with a herd of approximately 1.7 million cows milked daily (Brasil, 2016).

Dairy farming is an economically challenging activity in which errors can lead to economic loss. Reproductive efficiency is a major factor influencing productivity. Management and adequate health conditions are essential, and reproductive parasitic diseases play a vital role in production efficiency (Castro et al., 2008).

Leptospirosis is an infectious disease that affects animals and humans and is caused by pathogenic species of the genus *Leptospira*. The disease causes reproductive disorders, such as infertility, abortions, weak calf syndrome, mastitis/blood in milk, and decreased milk production (Faine et al., 1999; Hashimoto et al., 2012; Santana et al., 2013; Alfieri & Alfieri, 2017). The prevalence of bovine leptospirosis ranges from 13% to 70% in Brazil (Figueiredo

et al., 2009; Castro et al., 2008; Oliveira et al., 2009; Hashimoto et al., 2010; Hashimoto et al., 2012; Santana et al., 2013).

Toxoplasmosis is a cosmopolitan non-contagious zoonosis caused by the protozoan *Toxoplasma gondii*, an obligate intracellular parasite of the Apicomplexa group (Albuquerque et al., 2011; Joaquim et al., 2016). The prevalence of toxoplasmosis ranges from 16% to 49% in Brazil, depending upon the state (Albuquerque et al., 2011; Ferreira Meirelles et al., 2014; Guerra et al., 2014).

Bovine neosporosis is caused by *Neospora caninum*, an obligate intracellular coccidian protozoan. The prevalence of bovine neosporosis ranges from 7% to 35% in Brazil, depending upon the state (Martins et al., 2012; Langoni et al., 2013; Santana et al., 2013; Rocha et al., 2015; Lorenzett et al., 2016).

Among these diseases, some stand out, with higher prevalences demonstrated in the literature and with greater responsibilities for reproductive problems, such as neosporosis, being pointed out as a major cause of abortions, in addition to leptospirosis and toxoplasmosis (Santana et al. 2013; Ferreira Meirelles et al 2014; Alfieri & Alfieri, 2017).

In a small dairy farm, economic losses caused by low reproductive efficiency can raise production costs. Therefore, the objective of this study was to detect anti-*Leptospira*, anti-*Toxoplasma gondii*, and anti-*Neospora caninum* antibodies in dairy cows at a small dairy farm to promote regional development in Umuarama, Paraná, Brazil.

2. Methodology

Blood samples were collected from Holstein cows at a small dairy farm in Umuarama, Paraná, Brazil, from October to December 2016. The animals were not vaccinated, in their reproductive age, and had a history of reproductive problems.

Approximately 10 mL of blood was collected through coccygeal venipuncture and immediately sent to the Laboratory of Preventive Veterinary Medicine and Public Health of the Paranaense University, where the samples were centrifuged to obtain the sera and fractionated into three aliquots of equal volume, stored in sterile flasks at -20°C for subsequent serological tests.

Anti-*Leptospira*, anti-*Toxoplasma gondii*, and anti-*Neospora caninum* antibodies were tested at the Leptospirosis Laboratory of the Department of Preventive Veterinary Medicine of the State University of Londrina.

Microscopic serum agglutination (SAM) with live antigens was used to detect anti-*Leptospira* antigens (Faine et al., 1999). Twenty-two reference serovars were used: Australis, Bratislava, Autumnalis, Fortbragg, Butembo, Castellonis, Bataviae, Canicola, Whitcombi, Cynopteri, Grippotyphosa, Hebdomadis, Copenhageni, Icterohemorrhagic, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Sentot, Tarassovi, and Shermani. Antigens were maintained at 28°C for 5–10 days in Difco™ Leptospira Enrichment (DIFCO®-USA) medium modified with rabbit serum (Alves, 1996). A dilution of 1:100 was used as the cut-off point (Myers, 1985).

Sera with ≥50% of agglutinated leptospires were considered positive. Two-fold serial dilutions were prepared with positive serum to determine the maximum positive dilution. The analysis considered the most probable serovar as the one with the highest titer (Vasconcellos et al., 1997), and the sera that presented coagglutination at the highest dilution were considered only *Leptospira* positive (Almeida et al., 1994).

The indirect immunofluorescence test (IIFT) was used to detect anti-*Toxoplasma gondii* antibodies (Camargo, 1974) using a commercial anti-bovine IgG conjugate (FITC, Sigma®, USA). The sera were tested from four-fold to 1:4096 sequential dilutions, considering the samples presenting with fluorescent tachyzoites at a titer ≥ 64 as positive.

Further, IIFT was used to detect anti-*Neospora caninum* antibodies, using slides containing *Neospora caninum* forms, a commercial anti-bovine IgG conjugate (FITC, Sigma®, USA), and a positive control of naturally infected animals. Titers ≥ 25 were considered positive.

Variables associated with the three infections were investigated in an interview with the animal owner using an epidemiological questionnaire collecting information including the productive purpose of breeding, vaccination, animal breed, animal age, animal exchange with other farms, presence of reproductive problems, presence of dogs and/or cats in the farm, and source of water used for the animals.

Results obtained from the investigation of variables associated with the three infections were statistically analyzed using the Yates's chi-squared (χ^2) or Fisher's exact test on the Statistical Package for the Social Sciences software version 21.0 at a significance level of 5%.

3. Results and Discussion

Parasitic diseases should be detected in bovine herds for their control and eradication to reduce the economic losses resulting from decreased milk production, birth defects, and abortions and to reduce the medication costs to treat the diseases. According to Santana et al. (2013), dairy cattle undergoes intensive management to prevent the transmission of parasitic diseases caused by close contact among animals.

Of the 37 animals, 5 (13.5%) had a history of abortion and return to estrus, 26 (70.2%) had return to estrus alone, and 6 (16.0%) had no reproductive problems. The mean animal age was approximately six years, and the mean milk production was 12 L/day. The farm had no veterinary assistance, and the reproductive management included estrus observation and artificial insemination. At the farm, the herd was in direct daily contact with cats, horses, and sheep, but not with dogs.

Of the 37 samples analyzed, 10 (27%) were positive for *Leptospira* in SAM with titers ranging from 100 to 400. However, the most probable serovar was detected in only seven (18.9%) samples, of which four (57.1%) were Pomona, and one (14.3%) was each of Canicola, Icterohemorrhagic, and Butembo. In Paraná, two studies reported higher prevalence compared to this study. Hashimoto et al. (2012) analyzed animals from 274 farms and reported that 181 (66%) had at least one *Leptospira*-positive animal, demonstrating the importance of investigating this etiological agent in the state. Another study analyzed samples from 14,163 females aged ≥ 24 months from 1,926 herds unvaccinated against leptospirosis, reporting a prevalence of 54.28% in farms and 37.70% in positive animals. The variables associated with leptospirosis were presence of horses [odds ratio (OR) = 1.59 (1.28–1.97)], presence of 20 females or more aged ≥ 24 months [OR = 2.25 (1.46–3.49)], and presence of calving pen [OR = 1.32 (1.04–1.68)] (Hashimoto et al., 2015)

The different prevalence values may be associated with intrinsic and extrinsic factors influencing disease occurrence, such as different climatic and environmental conditions among regions and different infection hosts (Ferreira et al., 2017). Correia et al. (2017) conducted a study in Rio de Janeiro to compare the seropositivity of a herd in rainy and dry seasons, reporting a prevalence of 43.6% and 31.8%, respectively. They concluded that incidental infections caused by unadapted serovars significantly increased in the rainy season, which was when the samples of this study were collected, explaining the prevalence found.

For *Toxoplasma gondii*, 21 (56.75%) samples were IIFT positive, 14 (56.75%) had a titer of 16, 5 (23.80%) had a titer of 64, and 2 (9.52%) had a titer of 256. These values are

higher those found by Ferreira Meirelles et al. (2014) and Petry et al. (2017), which were 32.20% and 55%, respectively, both in Paraná. It was not possible to associate toxoplasmosis with the different variables analyzed. However, in a study on the risk factors for toxoplasmosis by Albuquerque et al. (2011), cats in contact with the cattle, cats in contact with the water used by the cattle, and number of cats in the farm (> 3) were associated with *Toxoplasma gondii* infection. This result indicates that the cats present in the farm analyzed in this study may be parasite disseminators, since they were in direct contact with the cattle and also with their water and food (feed, hay, and pasture).

For *Neospora caninum*, four (10.8%) samples were IIFT positive: two with a titer of 25 and two with a titer of 50. Higher prevalence values (20.4%, 46.9%, 35.1%) were reported by Martins et al. (2012), Ferreira Meirelles et al. (2014), and Rocha et al. (2015) in Paraná. The lower values found in this study can be explained by the absence of dogs in the farm, since different authors state that the presence of dogs is a determinant of infection (Hobson et al., 2005; Martins et al., 2012; Langoni et al., 2013).

For reproductive problems, Langoni et al. (2013) detected 24% seroprevalence of *Neospora caninum* and reported that the cows presented with return to estrus and abortion, similar to the present study. Reichel et al. (2013) reported that bovine neosporosis may influence the reproductive parameters and have a negative economic impact. In Brazil, the estimated loss of milk production associated with this disease was of 51.3 million dollars per year (estimates varying from 35.8 to 111.3 million dollars).

Of the five animals that underwent abortion, none were *Leptospira* positive in SAM; three (60%) were *Toxoplasma gondii* positive in IIFT, with a titer of 16; and only one (20%) was *Neospora caninum* positive in IIFT, with a titer of 50. Of the animals presenting with return to estrus, 54.83%, 29.0%, and 9.67% had toxoplasmosis, leptospirosis, and neosporosis, respectively.

It was not possible to associate the analyzed variables to the three infections (Tables 1, 2, and 3).

Table 1 - Variables investigated for leptospirosis in dairy cows with history of reproductive problems at a small dairy farm in Umuarama, Paraná, Brazil, 2016.

Variable		Total positivity (%)	P	OR (CI 95%)
Aborto	Sim	0/5 (0%)	0,295*	1,227 (1,025 – 1,469)
	Não	10/32 (31,25%)		
Idade dos animais	Até 5 anos	2/13 (15,38%)	0,440*	0,364 (0,065 -2,050)
	> 5 anos	8/24 (33,33%)		
Números de partos	Até 3	5/26 (19,23%)	0,125*	0,286 (0,061 – 1,328)
	> 3	5/11 (45,45%)		
Retorno de cio	Sim	9/31 (29,03%)	1,000*	2,045 (0,209 – 20,054)
	Não	1/6 (16,66%)		

p=probability; * Fisher's exact test; **Yates's chi-squared (χ^2); OR=Odds Ratio; CI= Confidence Interval
Source: The authors.

Table 2 - Variables investigated for toxoplasmosis in dairy cows with history of reproductive problems at a small dairy farm in Umuarama, Paraná, Brazil, 2016.

Variable		Total positivity (%)	p	OR (CI 95%)
Aborto	Sim	0/5 (0%)	0,560*	1,200 (0,523 – 1,408)
	Não	7/32 (21,87%)		
Idade dos animais	Até 5 anos	4/13 (30,77%)	0,213*	3,111 (0,575 - 16,833)
	> 5 anos	3/24 (12,5%)		
Números de partos	Até 3	6/26 (23,07%)	0,649*	3,000 (0,317 - 28,434)
	> 3	1/11 (9,09%)		
Retorno de cio	Sim	5/31 (16,13%)	0,315*	0,385 (0,055 - 2,700)
	Não	2/6 (33,3%)		

p=probability; * Fisher's exact test; **Yates's chi-squared (χ^2); OR=Odds Ratio; CI= Confidence Interval
Source: The authors.

Table 3 - Variables investigated for neosporosis in dairy cows with history of reproductive problems at a small dairy farm in Umuarama, Paraná, Brazil, 2016.

Variable		Total positivity (%)	P	OR (CI 95%)
Aborto	Sim	1/5 (20%)	0,456*	2,417
	Não	3/32 (9,37%)		(0,200 – 29,234)
Idade dos animais	Até 5 anos	2/13 (15,38%)	0,602*	2,000
	> 5 anos	2/24 (8,33%)		(0,248 – 16,159)
Números de partos	Até 3	3/26 (11,53%)	1,000*	1,304
	> 3	1/11 (9,09%)		(0,120 – 14,119)
Retorno de cio	Sim	3/31 (9,67%)	0,524*	0,536
	Não	1/6 (16,66%)		(0,046 – 6,240)

p=probability; * Fisher's exact test; **Yates's chi-squared (χ^2); OR=Odds Ratio; CI= Confidence Interval
Source: The authors.

This study showed widespread leptospirosis, toxoplasmosis, and neosporosis in the herd that could have a negative impact on reproductive parameters of dairy cattle, causing return to estrus and abortions. As the animals were brucellosis negative, other reproductive diseases should be studied, such as infectious bovine rhinotracheitis and bovine viral diarrhea.

We concluded that reproductive losses are endemic to the herd, possibly resulting in chronic diseases caused by etiologic agents acting alone or combined with other infections.

Periodic serological evaluations are essential to guide the preventive and control measures to be implemented in farms. Epidemiological data obtained from this study may help establish control strategies based on the analysis of risk factors for infectious diseases, other than alerting and educating small producers and the local population on the possible presence of zoonoses spread by these infectious agents.

The use of simple measures can prevent the exposure of new animals to these infections, such as eradicating dogs and cats from the farm, since they are definitive hosts of *Neospora caninum* and *Toxoplasma gondii*, storing animal feed and hay in a place protected from rodents and cats, more intensive reproductive management, veterinary assistance, and annual vaccination against the major reproductive diseases. In addition to these practices, it is important to educate rural producers on health measures to prevent direct and indirect contact of noninfected animals to animals with unknown health history.

4. Conclusion

With these results, it can be concluded that Leptospirosis, toxoplasmosis and neosporosis are common in herds, and control measures should be adapted to improve reproductive rates. It should be noted that Leptospirosis and toxoplasmosis are potential health-related risk factors.

Declaration of Interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of paper.

Ethics Committee

This project was approved by the Research Ethics Committee Involving Animal Experimentation of the Paranaense University under protocol 32014/2016.

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