Enzootic instability for bovine anaplasmosis on family farms located in southwestern Paraná, Brazil

Instabilidade enzoótica para anaplasmose bovina em propriedades de agricultura familiar do sudoeste paranaense, Brasil

Tatiane Sott[1]; Carina Franciscato[1]; Adolfo Firmino da Silva Neto[1]; Iucif Abrão Nascif Júnior[1]; Fagner Luiz da Costa Freitas[1]*

[1]Laboratório de Saúde Única, Universidade Federal da Fronteira Sul – UFFS, Realeza, PR, Brasil

Abstract

The objective of this study was to assess the occurence of animals seropositive for *Anaplasma marginale* in the municipality of Realeza, Paraná State, Brazil. Blood samples were collected from 344 cows on 18 small farms in the municipality of Realeza-PR. The animals'serum samples were forwarded to the Federal University of Fronteira do Sul, in order to investigate the occurrence of anti-*A. marginale* IgG antibodies by an enzyme-linked immunosorbent assay commercial kit. IgG antibodies to *A. marginale* were detected in cattle from 77.7% of the farms. To the best author's knowledge, this is the first report of occurrence of *A. marginale* in cattle in southwestern Paraná. The serological assay showed that 24.4% of the animals were seropositive, thus characterizing the location investigated as an area of enzootic instability for the disease. The family farms located in the municipality of Realeza-PR showed enzootic instability for bovine anaplasmosis. It is necessary to conduct disease monitoring programs in association with preventive measures in order to ensure the sanitary quality of the herds and to reduce economic losses for the farmers. In addition, it is essential to implement educational extension actions that allow farmers to acquire knowledge, attitudes and perceptions regarding the risk factors that contribute towards herd *A. marginale*-infection.

Keywords: *Anaplasma marginale*, epidemiology, seroprevalence.

Resumo

O objetivo deste estudo foi avaliar a ocorrência de animais soropositivos para *Anaplasma marginale*, no município de Realeza, Estado do Paraná, Brasil. Foram colhidas amostras de sangue de 344 fêmeas bovinas provenientes de 18 propriedades rurais do município de Realeza - PR. Amostras de soro dos animais foram encaminhadas à Universidade Federal da Fronteira Sul, para realização do Ensaio Imunoenzimático Indireto para pesquisa de anticorpos IgG anti- *Anaplasma marginale* por meio de *kit* comercial. Anticorpos IG anti-*A. marginale* foram detectados em 77,7% das propriedades. Trata-se do primeiro registro da ocorrência de *A. marginale* no Sudoeste paranaense. A sorologia evidenciou 24,4% de animais soropositivos, caracterizando o local pesquisado como área de instabilidade enzoótica para a doença. As propriedades de agricultura familiar, localizadas no múnicipio de Realeza-PR, apresentaram instabilidade enzoótica para anaplasmose bovina. É necessário que programas de monitoramento da enfermidade sejam realizados em conjunto com medidas de prevenção, visando garantir qualidade sanitária do rebanho e reduzir perdas econômicas dos produtores rurais. Além disso, é fundamental a realização de ações de extensão que viabilizem a aquisição de conhecimento, atitude e percepção dos criadores diante dos fatores de risco contribuintes para a infecção por *A. marginale* do rebanho.

Palavras-chave: *Anaplasma marginale*, epidemiologia, soroprevalência.

Dairy cattle-rearing is a prominent agricultural activity in the state of Paraná and is responsible for generating a significant portion of the monthly income of agricultural households. However, the investments and technological advances used in this activity over the years have not been sufficient to prevent the presence of pathogens capable of causing economic and production losses relating to dairy herds. Among these, bacteria belonging to the genus *Anaplasma* genus can be highlighted, since this agent is responsible for causing anaplasmosis in cattle (OIE, 2016).

Anaplasma marginale is transmitted by the *Rhipicephalus (Boophilus) microplus* tick, fomites and through bites from *Stomoxys calcitrans, Haematobia irritans* and congenitally (GONÇALVES, 2000). The clinical condition of the infected animals is characterized mainly by hyperthermia, anorexia, tachycardia, tachypnea, pale or icteric

mucosae, lethargy, prostration, reduced rumination movements, decreased milk production and abortion, resulting from hemolysis and anoxia (VIDOTTO et al., 2006). Consequently, productivity and income obtained from the dairy business are compromised due to the presence of this disease in the herd. Expenditure on drugs and specialized professionals is required, and the disease can cause reduction in milk production, temporary infertility in males and females, abortions and death (SOUZA et al., 2001).

Occurrence of bovine anaplasmosis is directly associated to climatic and management characteristics that enable or hinder the survival of the vectors (COSTA et al., 2013). Higher incidence is seen particularly in regions with tropical or subtropical climates (OIE, 2016), in which also contribute to infection with *Babesia* species. In Brazil, the climatic characteristics vary greatly (GONÇALVES, 2000), such that the epidemiological situation of the babesiosis is classified into three groups: unstable areas, where the prevalence is between 20 and 75%; stable areas, where the prevalence is greater than or equal to 75%; and free areas where the survival of biological vectors is not possible (MAHONEY & ROSS, 1972).

Agricultural and livestock production from small producers in Brazil is referred to as family agriculture. This type of activity is an important factor in promoting social equality, economic development and actions that promote more rational and sustainable use of natural resources. In the southwest region of the state of Paraná, where this study was developed, family agriculture has been strongly associated with milk production. In addition, the geographic location, bordering the neighboring country of Argentina, confers strategic importance on this region from the zoosanitary point of view.

However, the systematic information available on the health of the dairy herds of this region is scarce, which hampers health monitoring, and consequently preventive and epidemiological surveillance actions, especially in relation to diseases that are not subject to government control programs. For this reason and because of the specific characteristics of anaplasmosis, this disease is of great epidemiological importance within this scenario. Affected animals do not show clinical signs after the initial phase of the infection, and it is not possible to detect the pathogen through conventional diagnostic methods. Thus, animals carrying the pathogen remain in the environment, which increases the risk of transmission (VIDOTTO & MARANA, 2001).

The objective of this study was to assess the occurrence of antibodies to *A. marginale* in cattle in the municipality of Realeza, southwestern Paraná.

This study was approved by the Ethics Committee on the Use of Animals (CEUA) (protocol 23205.003294 / 2014-98) and the farmers and researchers signed an free and informed consent statement. Following this, blood samples were collected from 344 cows of milk-yielding breeds (310 Holstein-Friesian, 13 Jersey, 19 Holstein-Friesian/Jersey crossbreds, one Holstein-Friesian/Gir crossbred and one Holstein-Friesian/Brown Swiss crossbred) over the age of one year, on 18 small farms that were chosen by convenience in the municipality of Realeza-PR (25° 46' 01" S 53° 31' 37" W). During the sample collection, which was done between May and August 2015, the farmers were asked about some features of their farms and about the individuals who were responsible for dairy activities and management.

Blood samples were taken from the coccygeal vein using sterile, disposable needles of dimensions 40 × 12 millimeters, which were attached to glass tubes under vacuum and without anticoagulant. The tubes were identified with the ear tag number, breed and age of the animal and the owner's name. The tubes were placed in Styrofoam boxes with ice and were sent to the Clinical Analysis Laboratory of the Superintendency Unit of the University Veterinary Hospital (SUHVU) of the Federal University of Fronteira do Sul (UFFS). In the laboratory, the tubes were centrifuged at 3,000 rpm for 10 minutes. After centrifugation, the serum was stored in Eppendorf microtubes, which were placed in an the ultra-freezer at a temperature of –45 °C. Subsequently, the animals' serum samples were forwarded to the UFFS One Health Laboratory in order to detect IgG antibodies to *A. marginale* by a commercial Enzyme-linked immunosorbent assay (ELISA) (Imunodot Diagnostics, Jaboticabal, BR). The preparations and procedures used followed the directions of the instruction manual for the product. At the end of the procedure, the plates were read using an ELISA microplate reader (Multiskan GO UV/VIS spectrophotometer; Thermo Scientific) with a 405 nm filter. The results were obtained by calculating the cutoff index, optical density (OD) and sample coloration, as indicated in the instruction manual. Samples that presented an intense yellow color and OD greater than or equal to the cutoff index were considered to be positive for *A. marginale*, while samples that showed no intense yellow color and OD lower than the cutoff index were considered negative.

The number of animals diagnosed as positive through the ELISA serological test was used as a parameter for classifying the area as one of enzootic instability or stability. This classification followed the conditions proposed by Mahoney & Ross (1972) to bovine babesiosis, in which areas of stability are those where the number of positive animals exceeds 75% of the total sample, while areas of instability are those where the positive animals represent less than 75% of the sample.

In order to evaluate the association between risk factors and exposure to the pathogen, a univariate analysis using the chi-square test and Odds Ratio (OR) with 95% confidence interval was performed. The calculations were performed using the Statcalc from Epi Info™ software, version 7.2.0.1.

Results and Discussion

Herein, antibodies to *A. marginale* were detected in 77.7% of the farms, representing the first report of occurrence of *A. marginale* in southwestern Paraná. The serological evaluation showed that 24.4% of the animals were reactive, thus characterizing the location investigated as an area of enzootic instability for the disease, with seropositivity ranging from 5% to 60% on the positive farms (Table 1).

Table 2 presents the main risk factors possible associated to the occurrence of antibodies to *A. marginale* in cattle on sampled family farms. Most of the owners were over 30 years of age and had a low education level. Dairy farming was the main source of income of the farms, although odds ratio didn't show sufficient strength to establish an association. The use of antiparasitic drugs without veterinary prescription in situations of high ticks and

Table 1. Seroprevalence of *Anaplasma marginale* on family farms in the municipality of Realeza, state of Paraná, Brazil, 2016.

Rural properties	Number of animals examined	Frequency of IgG antibodies to *A. marginale* (%)	Reaction *cut-off*	Optical density (D.O)			
				Positive animals		Negative animals	
				Mean	Variation (min - max)	Mean	Variation (min - max)
1	18	50.0%		1.68	1.198-2.384	0.914	0.706-1.133
2	20	15.0%	1.18375	1.38	1.209-1.520	0.878	0.610-1.163
3	24	25.0%		1.38	1.219-1.622	0.920	0.701-1.162
4	12	50.0%		1.38	1.195-1.522	0.996	0.818-1.140
5	20	40.0%		1.83	1.554-2.710	1.150	0.740-1.483
6	15	13.3%		1.54	1.545-1.546	1.193	0.896-1.526
7	20	5.00%	1.54875	1.81	1.814-1.814	1.061	0.715-1.465
8	20	40.0%		1.86	1.595-2.316	1.167	1.014-1.533
9	20	60.0%		2.05	1.633-3.504	1.009	0.615-1.294
10	32	43.7%		1.66	1.383-2.467	1.033	0.427-1.293
11	19	21.0%	1.36750	1.45	1.366-1.554	1.019	0.689-1.304
12	20	30.0%		1.53	1.371-1.797	1.043	0.810-1.227
13	18	5.50%		1.48	1.483-1.483	0.915	0.491-1.337
14	23	17.4%		2.24	1.757-2.570	0.935	0.424-1.445
15	15	0.00%		0.0	0.0	0.981	0.785-1.546
16	18	0.00%	1.65750	0.0	0.0	0.820	0.491-1.507
17	10	0.00%		0.0	0.0	0.894	0.701-1.112
18	20	0.00%		0.0	0.0	1.003	0.594-1.592

Table 2. Risk factors associated to the occurrence of IgG antibodies to *A. marginale* on family farms in the municipality of Realeza, state of Paraná, Brazil, 2016.

Risk factors	(%)	OR	χ^2
Age group responsible for dairy farming			
0 to 20	5.5	*	-
20 to 30	5.5	*	-
30 to 40	27.7	0.45	0.51
40 to 50	27.7	1.77	0.20
Over 50	33.3	0.66	0.13
Educational level of responsible for dairy farming			
Basic education	83.4	*	-
Incomplete high school	5.5	*	-
High school	11.1	*	-
Main source of income from property			
Dairy farming	88.9	*	-
Agriculture	11.1	*	-
Parasites identified in the herd			
Ticks	100	§	-
Flies	55.5	§	-
Parasite control			
Strategic control	11.1	*	-
Curative control	83.4	*	-
Aleatory control	5.5	*	-
Interval between treatments with acaricides			
15-30 days	11.1	*	-
30-60 days	5.5	*	-
> 60 days	83.4	*	-
Service used			
Veterinarian	77.7	§	-
Farmer	100	§	-

* The Odds Ratios result is zero; § The exposure factor is present in more than one situation.

flies infestation (curative control), possibly, contributed towards the occurrence of IgG antibodies to *A. marginale* on the family farms evaluated in this study, despite the fact that this was not statistically supported.

The risk factors observed in this study may contribute to outbreaks and high mortality rate in the herd in case of prolonged use of acaricides, exploited breed and infestation level (MAHONEY & ROSS, 1972). In the latter case, in order to control *S. calcitrans, H. irritans* and *R. (B) microplus* infestations is essential to maintain populations at acceptable levels that do not cause economic losses to dairy farms, enabling a balance in host-parasite relationship.

Until the present date, few regions of Brazil have been identified as areas of instability for *A. marginale*. Although European breeds are susceptible to infestations and parasitic infections (AZEVEDO et al., 2008), the occurrence of antibodies to *A. marginale* found in the present study was lower than that observed in other regions of Brazil. Studies performed in the State of Rio de Janeiro, southeastern Brazilian territory, showed an occurrence of antibodies to *A. marginale* ranging between 91.16% (SOUZA et al., 2000) and 98.21% (SOUZA et al., 2001). In the state of Bahia, northeastern Brazilian territory, a seropositivity of 94.8% was reported in cattle (BARROS et al., 2005). In other municipalities in the State of Paraná, antibodies anti-*A.marginale* were observed in 92.94% of cattle from Londrina (ANDRADE et al., 2001), 76.1% in Umuarama (YOSHIHARA et al., 2003) and 58.74% in Ponta Grossa, Guarapuava and Laranjeiras do Sul cities (MARANA et al., 2009).

The fact that the municipality of Realeza, Paraná, is geographically located close to the border with Argentina emphasizes the need to monitor *A. marginale* infection in cattle, especially because of the possibility of illegal trade of animals through river crossings, which consequently may make disease control in Brazil difficult.

Conclusion

The family farms located in the municipality of Realeza, Paraná, showed enzootic instability for bovine anaplasmosis. Given the data presented, it is necessary to conduct disease monitoring programs in conjunction with preventive measures in order to ensure the sanitary quality of the herds and to reduce economic losses for the farmers. In addition, it is essential to implement educational extension actions that allow farmers to acquire knowledge, attitudes and perceptions regarding the risk factors that contribute towards herd *A.marginale* infection.

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