Pesq. Vet. Bras. 30(3):249-254, março 2010

Anaplasma marginale infection in cattle from south-western Amazonia[1]

Luciana Gatto Brito[2*], Márcia Cristina de Sena Oliveira[3], Rodrigo Barros Rocha[2], Francelino Goulart da Silva Netto[2], Adriana Denise Marim[4], Gislaine Cristina Rodrigues de Souza[4], Fabiano Benitez Vendrame[5] and Maria Manuela da Fonseca Moura[4]

ABSTRACT.- Brito L.G., Oliveira M.C.S., Rocha R.B., Silva Netto F.G., Marim A.D., Souza G.C.R. & Moura M.M.F. 2010. ***Anaplasma marginale* infection in cattle from south-western Amazônia.** *Pesquisa Veterinária Brasileira 30(3):249-254.* Embrapa Rondônia, BR 364 Km 5,5, Porto Velho, RO 78900-970, Brazil. E-mail: luciana@cpafro.embrapa.br

The present study provides the first epidemiological data regarding infection by *Anaplasma marginale* in cattle reared in south-western Brazilian Amazonia. One simple procedure was adapted for the extraction of DNA from blood clots collected in seven microregions of Rondônia State and two mesoregions of Acre State. PCR method was used to asses the frequency of *A. marginale* infections in 4 to12-month-old cattle. The cattle infection was investigated by polymerase chain reaction (PCR) using the specific primer "msp5" for *A. marginale*. The DNA amplifications revealed that the mean frequency of *A. marginale* infection was 98.6% (1,627/1,650) in samples from Rondonia, and 92.87% (208/225) in samples from Acre. The high frequency of *A. marginale* infections in 4 to 12-month-old cattle indicate a situation of enzootic stability in the studied areas and are comparable to those detected by immunodiagnosis in different endemic regions in Brazil. The DNA extraction of clotted blood method described here can be used for epidemiological studies on anaplasmosis and other bovine hemoparasites.

INDEX TERMS: *Anaplasma marginale*, epidemiology, cattle, south-western Amazonia, Brazil.

RESUMO.- [**Infecção por *Anaplasma marginale* em bovinos na Amazônia Sul Ocidental, Brasil.**] O presente estudo fornece os primeiros dados epidemiológicos relativos a infecção por *Anaplasma marginale* em bovinos criados na Amazônia Sul Ocidental brasileira. Foi adaptado um procedimento simples para a extração de DNA a partir de coágulos sanguíneos coletados em sete microrregiões do estado de Rondônia e duas mesoregiões do estado do Acre. A técnica da reação em cadeia da polimerase (PCR) foi aplicada para avaliar a freqüência da infecção por *A. marginale* em bovinos com idade entre 4 e 12 meses. Após a extração do DNA de cada amostra, a infecção nos bovinos foi investigada pela amplificação do gene "msp5" *de A. marginale*. As técnicas de amplificação do DNA revelaram que a freqüência de infecção por *A. marginale* foi de 98,6% (1.627/1.650) nas amostras provenientes de Rondônia e de 92,87% (208/225) nas amostras do Acre. A alta freqüência da infecção por *A. marginale* nos animais com idade entre 4 e 12 meses indica uma situação de estabilidade enzoótica nas regiões estudadas, as quais são comparáveis às detectadas por técnicas de imunodiagnóstico em outras regiões endêmicas no Brasil. A extração do DNA através do método aqui descrito pode ser utilizado em estudos epidemiológicos sobre a anaplasmose bovina e outros hemoparasitas.

TERMOS DE INDEXAÇÃO: *Anaplasma marginale*, epidemiologia, bovinos, Amazônia Sul Ocidental, Brasil.

[1] Received on April 30, 2009
Accepted for publication on December 8, 2009.

[2] Embrapa Rondônia, BR 364 Km 5,5, Porto Velho, RO 78900-970, Brazil. *Corresponding author: luciana@cpafro.embrapa.br

[3] Embrapa Pecuária Sudeste, Rodovia Washington Luiz Km 234, Fazenda Canchim, Cx. Postal 339, São Carlos, SP 13560-970, Brazil.

[4] Universidade Federal de Rondônia, BR 364, Km 9,5, Porto Velho, RO 78900-000.

[5] Agência de Defesa Sanitária Agrosilvopastoril do Estado de Rondônia, Avenida Presidente Dutra, 2701, Porto Velho, RO, 76801-974.

INTRODUCTION

The Brazilian south-western Amazonia, which includes the states of Rondonia and Acre, is located at 7° 06' 32" North, 13° 41' 36" South, 66° 37' 10" East and 73° 59' 27" West (IBGE 2007). The climate is characterized by annual averages of 28ºC temperature, 3,500-6,000mm rainfall, and 90% relative humidity (SIPAM 2009). In Acre and Rondonia, livestock activity has great economic importance, especially for beef and milk production.

The parasitism by cattle ticks is favored through environmental conditions; in many cases sick animals associated with tick parasitism have been observed and reported by farmers. Since now, epidemiological studies have not yet been conducted in the south-western Amazon region with the specific purpose to characterize the epidemiological data of cattle hemoparasitoses in the region.

Anaplasma marginale is a rickettsial hemoparasite transmitted to cattle biologically by *Rhipicephalus microplus* and mechanically by flies and fomites (Aguirre et al. 1994, Stiles 1936). Anaplasmosis is an economically important disease affecting dairy and beef cattle in most tropical, subtropical and many temperate countries (Morel 1989, Dalgliesh et al. 1990). In Brazil, the anaplasmosis prevalence varies between 12.4 and 100% (Artiles et al. 1995, Vidotto & Marana 2001).

The cattle that are recovering from anaplasmosis infection become healthy carriers by maintaining low levels of infection and contributing to the maintenance of the anaplasmosis' endemy. In these cases, the microscopic detection diagnostic is less reliable than the diagnosis based on techniques of greater sensitivity and specificity. Among these techniques, those based on PCR stands out and is already being used for detection of animal carriers in several studies conducted on hemoparasitism of cattle (Almeria et al. 2001, Oliveira et al. 2005, Oliveira-Sequeira et al. 2005, Carelli et al. 2007).

The serological tests that detect antibodies have limited use because they indicate exposure to the agent, but no information on the stage of infection. (Wagner et al. 1992).

The application of PCR-based tests to the study of the epidemiology of anaplamosis is still incipient, but characteristics of high sensitivity and specificity have been verified by several authors (Fahrimal et al. 1992, Figueroa et al. 1992, Smeenk et al. 2000, Almeria et al. 2001) for the detection of infection both in the vertebrate hosts and ticks.

The development of practical techniques to prepare DNA samples are widely studied for applied in epidemiological studies such as for forensic medicine (Jones et al. 1999, Gonzales et al. 2006, Lin et al. 2006). In this study, PCR reactions were prepared with DNA samples extracted directly from blood clots. These clots were recovered from blood samples obtained for implementation of serological tests for brucellosis realized by the Animal Haelth Services of Rondonia and Acre states, and can be considered a random representative sample of the cattle reared in this region.

This study presents the first epidemiological data about *A. marginale* infection in cattle from Brazilian Amazonia and describes the technique for DNA extraction from blood clots used in seroepidemiological studies developed by Animal Health Services of Rondonia and Acre.

MATERIALS AND METHODS

Blood sample collection

The study was developed with blood clots donated by Animal Health Services of Rondonia and Acre states. All evaluated cattle with 4-12 months of age were from eight microregions of Rondonia: Alvorada do Oeste, Ariquemes, Colorado do Oeste, Cacoal, Guajara-Mirim, Ji-Parana, Porto Velho and Vilhena, and two mesoregions of Acre: Rio Branco Valley and Jurua Valley.

The microregions of Rondonia and the mesoregions of Acre were defined according to the Brazilian Institute of Geography and Statistics (IBGE 2006). The blood collections were performed from August 2004 to November 2004, using a vacuum system without the use of anticoagulant.

Determination of sample size

The sample size was determined by the formula of the Pan American Center for Zoonoses (1979), for the study of chronic infectious diseases, in which:

$$n = \frac{p(100-p)Z^2}{\left(\frac{dp}{100}\right)^2}$$

where: n = number of samples, p = expected prevalence, Z = degree of trust and d = margin of error.

Based on an estimated prevalence of cattle 90.0% positive for *Anaplasma marginale*, determined in a pilot study with 100 blood clots samples with a degree of confidence of 95.0% and a margin of error of 10.0%, was found for a sampling of 45 samples by region. As there was availability of a larger number of blood clot samples per microregion and the analysis were executed separately, 166 samples were analyzed from Ariquemes, 118 from Alvorada do Oeste, 319 from Cacoal, 331 from Guarajá-Mirim, 328 from Ji-Paraná, 309 from Porto Velho, and 79 samples from Vilhena, in Rondonia. In Acre state the blood clots samples colleted from mesoregions analyzed were: 79 from Jurua Valley and 146 samples from Rio Branco Valley.

Blood sample processing

The DNA extraction from clotted blood was made according to the methodology described by Garg et al. (1996) adapted by Brito et al. (2006). The first step for DNA extraction was the mechanical break of the 3g of the blood clots in small pieces through use of the scalpel blades, as proposed by Tas (1990). For the soluble clot DNA extraction the methodology proposed by Garg et al. (1996) was adapted by Brito et al. (2006) with the use of nylon textile with pores of 200µm. The pores size of the textile was measured with micrometric objective in microscope Leica DM1000. The nylon textile were cut in circles of 10cm diameter and was attached to the top of a 50 mL conical-bottom polypropylene centrifugal tube and shaped into a form of a funnel, being held in place with a rubber band**.** The blood clot was poured into the nylon textile funnel and Parafilm® (American Can Co., Neenah, WI) was wrapped around the top of the tube. The clot was then forced through the nylon textile by centrifugation at

7,000rpm for 15 minutes. Then, blood DNA was extracted from 300µL of each sample using the GFX™ Genomic Blood DNA Purification kit (Amersham Biosciences©) as recommended by the manufacturer. All samples were analyzed by spectrophotometric measurement at *260* and 280nm for assessment of purity of *DNA*.

The DNA samples were submitted to PCR amplification of the *msp*5 gene of *A. marginale*, by specific primers sequences (*msp*5 F: 5' CGC AGA TCT AGC AAA ATC GGC GAG AGG TTT ACC ACT TC 3' and *msp*5 R: 5' GCG CTG CAG TGG CGC AAA ATG CCC GAC ATA CC 3'). PCR amplifications were done in a Mastercycler Gradient Termocycler (Eppendorf AG, Hamburg, GE). PCR was performed in a 25mL solution containing 10mM TRIS-HCl; 50mM KCl; 1.5mM $MgCl_2$; 1.5 U Taq DNA polymerase; 0,2mM of each nucleotide; 10pm of each primer and 5mL of DNA samples. The amplified fragments were then separated through electrophoresis in 1.5% agarose gel. The gel was prepared with Tris Borate EDTA buffer (1X), and visualized by ethidium bromide staining. *A. marginale* positive samples had bands visible at 458 bp.

For confirmation that the fragments visualized by agarose gel electrophoresis were really from *A. marginale*, random samples were sequenced in automated DNA sequencer ABI 3100 (Applied Byosystems ©) as additional control and were confirmed to correspond to the genbank accessions AY 355282.1

RESULTS AND DISCUSSION

The 1,650 bovine blood clot samples from Rondonia state, correspond to 1.19% of the state bovine herd with 4-12 months of age. The molecular diagnosis showed that 98.6% (1627 animals of 1650) were positive for *Anaplasma marginale* with the amplification of the msp5 gene. The prevalence according to the regions is showed in Table 1. The *A. marginale* mean infection rate of 98.6% indicates the studied regions as being enzootically stable, according to Mahoney (1975) and Coleman et al. (2001). In Acre state was observed that 92.44% of cattle (208 from 225 animals) were positive by the amplification of the msp5 gene of *A. marginale* (Table 2).

Table 1. Prevalence of *Anaplasma marginale* in microregions of Rondonia state

Microregions of Rondônia state	Positive samples	Negative samples	Prevalence of *Anaplasma marginale*
Alvorada do Oeste	117	1	99.15%
Ariquemes	163	3	98.19%
Cacoal	315	4	98.74%
Guajará-Mirim	325	6	98.18%
Ji-Paraná	323	5	98.47%
Porto Velho	305	4	98.7%
Vilhena	79	0	100%
Total	1,627	23	98.6%

Table 2. Prevalence of *Anaplasma marginale* in mesoregions of Acre state

Mesoregions of Acre state	Positive samples	Negative samples	Prevalence of *Anaplasma marginale*
Rio Branco Valley	133	13	91.10%
Juruá Valley	75	4	94.90%
Total	208	17	92.44%

The results obtained in Rondonia and Acre states not differ from other Brazilian regions, where *A. marginale* infection is also high and independent of others factors like age and racial traits. However, similar epidemiological patterns are expected in other regions between the parallels 32° South and 32° North, where the main vector of *A. marginale,* the *Rhipicephalus microplus* tick, is amply diffused (Mahoney & Ross 1972, Kuttler 1988, Leite et al. 1989).

Since the *R. microplus* tick is considered the main transmitter of anaplasmosis in Brazil, and its epidemiological traits are similar to those of babesiosis; the concept of enzootic stability/instability proposed by Mahoney & Ross (1972) can also be applied in the study of bovine anaplasmosis. With this concept in mind, the microregions and the mesoregions of Rondonia and Acre can be considered as enzootic stable areas for *A. marginale,* due to the high prevalence observed in the cattle reared in these regions.

In Rondonia and Acre states, the *A. marginale* infection rate was similar among the sampled regions (Table 1). The presence of *R. microplus* can be observed in these regions, which favors the infection of cattle raised in these states. The *A. marginale* presence in these regions favors the cattle reared in these regions considering that the animals were asymptomatic for anaplasmosis at the moment of collection of the blood samples, indicating that the immunological system of these animals are already sensitized by the presence and circulation of the parasite in the environment. Ribeiro (1991) and Kessler et al. (1992) demonstrated the importance of the presence of *R. microplus* for the maintenance of immunity against *A. marginale* in the cattle.

Few Brazilian regions have a situation of *A. marginale* instability, such as the state of Sergipe, where, due to the local climatic differences, the average prevalence is 16.3% (Dalagnol et al. 1995). In the municipality of Bagé, localized in the extreme south of the state of Rio Grande do Sul, an anaplasmosis prevalence of 64% was observed through the card test (TC) (Artiles et al. 1995). In these regions of instability, the ecological and climatic factors determined the seasonal variation of *Rhipicephalus microplus* and of *hematophagous flies,* transmitters of *A. marginale*, with possible presence of other forms of transmission of the disease.

In Santa Cruz de la Sierra, Bolivia, the seroprevalecence observed in humid subtropical, dry tropical and flooded ecological regions through ELISA, was of 20.5%, also characterizing the area as of enzootic instability (Carrique et al. 2000); the dry subtropical region of this locality is the most unstable, with risks of disease; in South Africa the infection prevalence rate varied from 50 to 75%, according Masika et al. (1997). In two different areas evaluated in Switzerland, Kinhm (2002) detected 8.2% of positive samples for *A. marginale*, utilizing the ELISA technique.

In Norte de Veracruz, Mexico, studies indicate that cattle infected with *A. marginale* presented a prevalence of 69% (Cossio et al. 1997), associated with a mortality of 26% of the herd in the year of 1995 and with the dislocation of susceptible cattle to areas with high prevalence of the infirmity with subsequent infection.

Alonso et al. (1992) considered that for determination of the profile of enzootic stability/instability of a region, inherent factors to the animals should be evaluated, such as decrease in immunity due to reduction in the number of ticks, introduction of animals from free areas, and other factors of interaction with the environment, such as breed, climatic variations, stress, management, and kind of pastures (Kessler et al. 1987, Dalgliesh et al. 1990).

In Brazil, in spite of the difficulty in eradicating the cattle tick, it is important to highlight that its presence in the herd allows for the maintenance of the immunity against anaplasmosis, and it is important for the herd to live with the parasite under sufficient levels so as to maintain the immunity, minimizing economic losses. This situation occurs in some regions of the Brazilian Southeast and Midwest, where the animals acquire the infection during the first weeks of life when in contact with the vector, keeping them infected throughout the years (Furlong & Evans 1991, Furlong, 1993).

Vidotto et al. (1995, 1997, 1998) and Andrade et al. (2001) verified that anaplasmosis is endemic to the northern and northwestern region of the state of Paraná, with instability situations or enzootic stability, depending on the situation. Isolated cases of the disease or outbreaks are generally associated to seasonal increase of the population of *R. microplus*, flaws in its control, or the introduction of susceptible cattle from tick-free areas, independently of the breed involved. A research on an endemic region in Paraná using the indirect immunofluorescence technique (IFI), verified a prevalence of 68% for anaplasmosis. Later, in the same area, 87.6% of positive cattle were found with the competitive ELISA technique. Under this epidemiologic condition, the risk of anaplasmosis outbreak is low.

In a serologic research conduct by Payne & Osorio (1990) in Paraguay, the prevalence found was of 92%. The parasite is also endemic to Argentina. In areas where the *R. microplus* tick has been eradicated, sporadic outbreaks of the disease have happened (Guglielmone 1994), since the contact with the vector is essential for the development of acquired immunity.

Studies made in other Brazilian regions also demonstrated the state of enzootic stability for the bovine anaplasmosis. In Minas Gerais, the prevalence obtained by TC for the regions of Alto Paranaiba, Metalurgica, Sul de Minas and Triangulo Mineiro, was of 86.5%, 93.1%, 91.6, and 86.1%, respectively, while that for the region of Zona da Mata evaluated by IFI, the proportion was of 81.1% (Ribeiro & Reis 1981, Ribeiro et al. 1984, Dalagnol et al. 1995).

Research on anti-*A. marginale* antibodies of the IgG class, by means of indirect ELISA in samples of bovine serum of the Medio Paraiba (Rio de Janeiro) mesoregion, revealed the prevalence of 98.2% (Souza et al. 2001). Later, at this same area, Vidotto et al. (1997) observed through the competitive ELISA technique, 87.5% of positive samples. The seroprevalence of *A. marginale* evaluated in cattle from nine municipalities of Norte Fluminense, Rio de Janeiro, through the indirect ELISA, revealed that 91.1% were positive, demonstrating that the infection was high and homogeneous throughout the municipalities, characterizing the region as an area of enzootic stability (Souza et al. 2000). The prevalence rates observed between the different microregions of the states of Rondonia and Acre demonstrate that the infection by *A. marginale* is homogeneous, which is corroborated by the different means of mechanical transmission, besides the biological transmission (Ribeiro 1991, Kessler et al. 1992).

In three microregions of the state of Bahia, the observed prevalence through indirect ELISA was 96.9%, not differing significantly from the result provided by IFI (97.2%) and of 86% in Santa Catarina, according to Dalagnol et al. (1995), and in the serologic samples from cattle from rural nesting of the municipality of Corumba, Mato Grosso do Sul showed the presence of antibodies anti-*A. marginale* in 96% of the cases. Madruga et al. (1986) found prevalence of 91.2%, 100%, 100%, and 96.9%, respectively for the municipalities of Tamarineiro II, Paiolzinho, Mato Grande and Taquaral, what characterizes these regions as endemically stable, with results very close to those obtained in studied with meso and micro regions of Rondonia and Acre states.

REFERENCES

Aguirre D.H., Gaido A.E., Viñabal S., Torione de Echaide S. & Guglielmone A.A. 1994. Transmission of *Anaplasma marginale* with adult *Boophilus microplus* ticks fed as nymphs on calves with different levels of rickettsaemia. Parasite 1:405-407.

Almeria S., Castella J., Ferrer D., Ortuno A., Estrade-Pena A. & Gutierrez J.F. 2001. Bovine piroplasma in Minorca (Baleric Islands Spain): A comparison of PCR-based and light microscopy detection. Vet. Parasitol. 99:249-259.

Alonso M., Arellano-Sota C., Cereser V.H., Cordoves C.O., Guglielmone A.A., Kessler R., Mangold A.J., Nari A., Patarroyo J.H., Solari M.A., Vega C.A., Vizcaíno O. & Camus E. 1992. Epidemiology of bovine anaplasmosis and babesiosis in Latin America and the Caribbean. Rev. Sci. Technol. Off. Int. Epiz. 11:713-33.

Andrade G.M., Vidotto O., Vidotto M.C., Yoshihara E., Kano F.S. & Amaral C.H.S. 2001. Seroprevalence of *Anaplasma marginale* in dairy cattle and studies on the dynamics of natural infection of Holstein calves in southern Brazil. Semina, Ciênc. Agrárias 22:155-159.

Artiles J., Alves-Branco F.P.J., Martins J.R., Correa L.B. & Sapper M.F.M. 1995. Prevalência de *Babesia bovis* e *Babesia bigemina* em bovinos no estado da Bahia. Bras. J. Vet. Parasitol. 4:187-189.

Brito L.G., Oliveira M.C.S, Moura M.M.F., Silva Netto F.G., Marim A.D., Souza G.C.R. & Silva J.L. 2006. Extração de DNA a partir de coágulos sanguíneos bovinos. Boletim de Pesquisa e Desenvolvimento 43, Embrapa Rondônia. 13p.

Carelli G., Decaro N., Lorusso A., Elia G., Lorusso E., Mari V., Ceci L. & Buonavoglia C. 2007. Detection and quantification of *Anaplasma marginale* DNA in blood samples of cattle by real-time PCR. Vet. Microbiol. 1241(2):107-114.

Carrique J.J., Widdowson M.A., Cuéllar A.M., Ribera H. & Walker A.R. 2000. Risk of babesiosis and anaplasmosis in different ecological zones of Santa Cruz Department, Bolivia. Vet. Parasitol. 93:29-38.

Centro Pan-Americano de Zoonoses 1979. Procedimientos para Estudios de Prevalencia por Muestreo. Nota Técnica 18, Rev.1, Ramos Mejia, Buenos Aires. 35p.

Coleman P.G., Perry B.D. & Woolhouse M.E.J. 2001. Endemic stability a veterinary idea applied to human public health. Lancet 357:1284-1286.

Cossio B.R., Rodriguez S.D., Garcia-Ortiz M.A., Garcia-Tapia D. & Aboytes-Torres R. 1997. Bovine anaplasmosis prevalence in northern Veracruz state, Mexico. Prev. Vet. Med. 32:165-170.

Dalagnol C.A., Martins E. & Madruga C.R. 1995. Prevalência de anticorpos contra *Babesia bovis*, *Babesia bigemina*, *Anaplasma marginale* em bovinos de corte na região de clima Cfb. Revta Bras. Parasitol. Vet. 4(Supl.1):220.

Dalgliesh R.J., Jorgensen W.K. & de Vos A.J. 1990. Australian frozen vaccines for the control of babesiosis and anaplasmosis in cattle: A review. Trop. Anim. Hlth Prod. 22:44-52.

Fahrimal Y., Goff W.L. & Jasmer D.P. 1992. Detection of *Babesia bovis* carrier cattle by using plymerase chain reaction amplification of parasite DNA. J. Clin. Microbiol. 30(6):1374-9.

Figueroa J.V., Chieves L.P., Johnson G.S. & Buening G.M. 1992. Detection of *Babesia bigemina*-infected carriers by polymerase chain reaction amplification. J. Clin. Microbiol. 30(10):2576-82.

Furlong J. & Evans D. 1991. Epidemiologia do carrapato *Boophilus microplus* no Brasil: necessidade de uma abordagem compreensível para seu estudo realístico. Anais 7º Seminário Brasileiro de Parasitologia Veterinária e 2º Simpósio sobre a Mosca-dos-Chifres *Haematobia irritans,* São Paulo, p.48-50.

Furlong J. 1993. Controle do carrapato dos bovinos na região sudeste do Brasil. Cad. Téc. Vet. UFMG 8:49-61.

Garg U., Hanson N., Tsai M. & Eckfeldt J. 1996. Simple and rapid method for extraction of DNA from fresh and cryopreserved clotted human blood. Clin. Chem. 42:647-648.

Gonzales J.L., Loza A. & Chacon E. 2006. Sensitivity of different Trypanosoma vivax specific primers for the diagnosis of livestock trypanosomosis using different DNA extraction methods. Vet. Parasitol.136:119-126.

Guglielmone A. 2004. Epidemiologia y prevencion de los hemoparasitos (*Babesia* y *Anaplasma*) em la Argentina, p.460-479. In: Nari A. & Fiel C. (Eds), Enfermidades Parasitarias de Importancia Econômica em Bovinos. Hemisferio Sur, Montevideo, Uruguay.

IBGE s/d. Microrregiões geográficas dos estados de Rondônia e Acre. Disponível em http://www.sidra.ibge.gov.br/bda/tabela/protabl.asp?z=t&o=3&i=P. Acessado em 24 de novembro de 2006.

IBGE 2007. Pontos extremos, segundo as Grandes regiões e Unidades da Federação - 2007. Anuário estatístico do Brasil 67. Disponível em http://www.ibge.gov.br/home/geociencias/geografia/default_div_int.shtm?c=1. Acessado em 9 de março de 2009.

Jones R.N., Low D.E. & Pfaller M.A. 1999. Epidemiology trends in nosocomial and community-acquired infections due to antibiotic-resistant Gram-positive bacteria: The role of streptogramins and other newer compounds. Diagn. Microbiol. Infect. Dis. 33:101-112.

Kessler R.H., Madruga C.R., Jesus E.F. & Semprebom D.V. 1987. Isolamento de cepas puras de *Babesia bovis*, *Babesia bigemina* e *Anaplasma marginale* em área enzoótica. Pesq. Agropec. Bras. 22:747-752.

Kessler R.H., Schenk M.A.M., Madruga C.R., Sacco A.M.S. & Miguita M. 1992.Tristeza parasitária dos bovinos (TPB), p.1-30. In: Charles T.P. & Furlong J. (Eds), Doenças Parasitárias dos Bovinos de Leite. Embrapa-CNPGL, Coronel Pacheco, MG.

Kinhm U. 2002. Anaplasmosis bovina en Suiza. Informaciones Sanitárias 15:177.

Kuttler K.L. 1988. World-wide impact of babesiosis, p.1-15. In: Ristic M. (Ed.), Babesiosis of Domestic Animals and Man. CRC-Press 1, Florida.

Leite A.M.O., Arnoni J., Silva S.S., Farias N., Cruz H. & Nishikawa H. 1989. Serological study of bovine babesiosis in a marginal area of Brasil, p.624-35. Proc. 8th National Veterinary Hemoparasite Disease Conference, St Louis, Missouri.

Lin Y.C., Yao S.M., Yan J.J., Chen Y.Y., Hsiao M.J., Chou C.Y., Su H.P., Wu H.S. & Li S.Y. 2006. Molecular epidemiology of *Bordetella pertussis* in Taiwan, 1993-2004: Suggests one possible explanation for the outbreak of pertussis in 1997. Microbes Infect 8:2082-2087.

Madruga C.R., Kessler R.H. & Sacco A.M.S. 1986. Produção de antígenos e análise preliminar do teste de imunofluorescência indireta para diagnóstico de anticorpos anti-*Anaplasma marginale*. Embrapa-CNPGC, Campo Grande, MS. 4p.

Mahoney D.F. & Ross D.R. 1972. Epizootiological factors in the control of bovine babesiosis. Aust. Vet. J. 48:292-298.

Mahoney D.F. 1975. The diagnosis of babesiosis in Australia, p.49-62. In: Wells E.A. (Ed.), Workshop on Hemoparasites (Anaplasmosis and Babesiosis). CIAT.

Masika P.J., Sonandi A. & Van Averbeke W. 1997. Perceived causes, diagnosis and treatment of babesiosis and anaplasmosis in cattle by livestock farmers in communal areas of the Central-Eastern Cape Province, South Africa. J. South Afr. Vet. Assoc. 68:40-44.

Morel P.C. 1989. Manual of Tropical Veterinary Parasitology. CAB International, Wallingford, UK. 473p.

Oliveira M.C., Oliveira-Sequeira T.C., Araujo Jr J.P., Amarante A.F. & Oliveira H.N. 2005. *Babesia* spp. infection in *Boophilus microplus* engorged females and eggs in São Paulo State, Brazil. Vet. Parasitol. 130:61-67.

Oliveira-Sequeira T.C.G., Oliveira M.C.S., Araujo Jr J.P., Amarante A.F.T. 2005. PCR-based detection of *Babesia bovis* and *Babesia bigemina* in their natural host *Boophilus microplus* and cattle. Int. J. Parasitol. 35:105-111.

Payne R.C. & Osorio O. 1990. Tick-borne diseases of cattle in Paraguay. I. Seroepidemiological studies on anaplasmosis and babesiosis. Trop. Anim. Hlth Prod. 2:53-60.

Ribeiro M.F.B. & Reis R. 1981. Prevalência da anaplasmose em quatro regiões do estado de Minas Gerais. Arq. Esc. Vet. UFMG 33:57-62.

Ribeiro M.F.B., Patarroyo J.H., Santos J.L. & Farias J.E. 1984. Epidemiologia da anaplasmose bovina no estado de Minas Gerais. I. Prevalência de anticorpos aglutinantes e fluorescentes na Zona da Mata. Arq. Bras. Med. Vet. Zootec. 36:425-432.

Ribeiro M.F.B. 1991. Morfologia, evolução e reprodução do *Anaplasma marginale* (Theiler, 1910) em células epiteliais intestinais de teleóginas de *Boophilus microplus* (Canestrini, 1887). Estudo ao microscópio óptico e eletrônico. Tese de Doutorado, UFMG, Belo Horizonte. 134p.

Smeenk I., Kelly P.J., Wray K., Musuka G., Trees A.J. & Jongejan F. 2000. *Babesia bovis* and *Babesia bigemina* DNA detected in cattle and ticks from Zimbabwe by polymerase chain reaction. J. S. Afr. Vet. Assoc. 71(1):21-4.

SIPAM s/d. Banco de Dados. Disponível em http://www.sipam.gov.br/content/view/41/50/. Acessado em 9 de março de 2009.

Souza J.C.P., Soares C.O., Massard C.L., Scofield A. & Fonseca A.H. 2000. Soroprevalência de *Anaplasma marginale* em bovinos na mesorregião Norte Fluminense. Pesq. Vet. Bras. 20:97-101.

Souza J.C.P., Soares C.O., Madruga C.R. & Massard C.L. 2001. Prevalência de anticorpos anti-*Anaplasma marginale* (Rickettsiales: Anaplasmataceae) em bovinos na mesorregião do Médio Paraíba. Ciência Rural 31:309-314.

Stiles G. 1936. Mechanical transmission of anaplasmosis by unclean instruments. North Am. Vet. 17:39-41.

Tas S. 1990. Purification of DNA from clotted blood. Clin. Chem. 36:1851.

Vidotto O., Yamamura M.M., Andrade G.M., Barbosa C.S., Freire R.L. & Vidotto M.C. 1995. Ocorrência de *Babesia bigemina*, *B. bovis* e *Anaplasma marginale* em rebanhos de bovinos leiteiros da região de Londrina, PR. Revta Bras. Parasitol. Vet. 4(Supl.1):184.

Vidotto O., Andrade G.M. & Amaral C.H.S. 1997. Freqüência de anticorpos contra *Babesia bigemina*, *B. bovis* e *Anaplasma marginale* em rebanhos de bovinos leiteiros da região de Londrina, PR. Arq. Bras. Med.Vet. 49:659-65.

Vidotto M.C., Vidotto O. & Andrade G.M. 1998. Seroprevalence of *Anaplasma marginale* on cattle in Parana State, Brasil, by major surface protein 5 competitive inhibition enzyme-linked immunosorbent assay. Ann. N.Y. Acad. Sci. 849:424-426.

Vidotto O. & Marana E.R.M. 2001. Diagnóstico em anaplasmose bovina. Ciência Rural 31:361-368.

Wagner G.G., McGuire C. 1992. *Babesia bigemina*: Quantification of infection in nymphal and adult *Boophilus microplus* using DNA probe. Ex. Parasitol. 74:117-26.