

Ehrlichiosis in Brazil

Erliquiose no Brasil

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Abstract

Ehrlichiosis is a disease caused by rickettsial organisms belonging to the genus *Ehrlichia*. In Brazil, molecular and serological studies have evaluated the occurrence of *Ehrlichia* species in dogs, cats, wild animals and humans. *Ehrlichia canis* is the main species found in dogs in Brazil, although *E. ewingii* infection has been recently suspected in five dogs. *Ehrlichia chaffeensis* DNA has been detected and characterized in mule deer, whereas *E. muris* and *E. ruminantium* have not yet been identified in Brazil. Canine monocytic ehrlichiosis caused by *E. canis* appears to be highly endemic in several regions of Brazil, however prevalence data are not available for several regions. *Ehrlichia canis* DNA also has been detected and molecularly characterized in three domestic cats, and antibodies against *E. canis* were detected in free-ranging Neotropical felids. There is serological evidence suggesting the occurrence of human ehrlichiosis in Brazil but its etiologic agent has not yet been established. Improved molecular diagnostic resources for laboratory testing will allow better identification and characterization of ehrlichial organisms associated with human ehrlichiosis in Brazil.

Keywords: *Ehrlichia* sp., domestic animals, wild animals, humans.

Resumo

Erliquiose é uma doença causada por rickettsias pertencentes ao gênero *Ehrlichia*. No Brasil, estudos sorológicos e moleculares têm avaliado a ocorrência de espécies de *Ehrlichia* em cães, gatos, animais selvagens e seres humanos. *Ehrlichia canis* é a principal espécie em cães no Brasil, embora a infecção por *E. ewingii* tenha, recentemente, despertado suspeita em cinco cães. O DNA de *E. chaffeensis* foi detectado e caracterizado em cervo-do-pantanal, enquanto que *E. muris* e *E. ruminantium* ainda não foram identificadas no Brasil. A erliquiose monocítica canina causada pela *E. canis* parece ser altamente endêmica em muitas regiões do Brasil, embora dados de prevalência não estejam disponíveis em muitas delas. O DNA de *E. canis* também foi detectado e caracterizado em três gatos domésticos, enquanto anticorpos contra *E. canis* foram detectados em felídeos neotrópicos de vida livre. Evidências sorológicas sugerem a ocorrência de erliquiose humana no Brasil, entretanto, o agente etiológico ainda não foi identificado. A melhoria do diagnóstico molecular promoverá a identificação e caracterização de espécies associadas à erliquiose humana no Brasil.

Palavras-chave: *Ehrlichia* sp., animais domésticos, animais silvestres, humanos.

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Introduction

Ehrlichia are Gram-negative, pleomorphic, obligate intracellular bacteria that infect a wide range of mammals. The genus initially included 10 species classified based on the host cell infected: monocytes (*E. canis*, *E. risticii*, *E. sennetsu*), granulocytes (*E. ewingii*, *E. equi*, *E. phagocytophila*, human granulocytic ehrlichiosis [HGE] agent), and thrombocytes (*E. platys*). Based on sequences of 16S ribosomal RNA (rRNA) and other genes (e.g., *groESL* operon and surface protein genes), the genus *Ehrlichia* was rearranged and currently consists of five species: *E. canis*, *E. chaffeensis*, *E. ewingii*, *E. muris* and *E. ruminantium* (formerly *Cowdria ruminantium*). The *Ehrlichia* genus belongs to the family Anaplasmataceae of the order Rickettsiales (DUMLER et al., 2001). The remaining organisms were reclassified into the *Anaplasma* genus: *A. platys* (formerly *E. platys*) and *A. phagocytophilum* (a combination of organisms previously known as *E. equi*, *E. phagocytophila* and HGE agent), and into the *Neorickettsia* genus: *N. helminthoeca*, *N. risticii* (formerly *E. risticii*) and *N. sennetsu* (formerly *E. sennetsu*). The organisms not currently belonging to the genus *Ehrlichia* will not be further discussed in this review.

The organism was first described in dogs by Donatien and Letosquard (1935) in Algeria. After a major epizootic during the Vietnam War ehrlichiosis was characterized as a dog disease due to the infection and death of many military working German Shepherd dogs (HUXSOLL et al., 1970). In Brazil, *E. canis* was first reported in dogs from the city of Belo Horizonte, State of Minas Gerais, Southeastern Brazil (COSTA et al., 1973).

Ehrlichia organisms are mainly transmitted through the bite of an infected tick. This explains the higher prevalence of ehrlichiosis in tropical and subtropical regions due to the geographical distribution of vectors (ANDEREG; PASSOS, 1999). Thus, the presence of known competent tick vectors as well as reservoir hosts largely determine where ehrlichiosis is found. For instance, *E. canis* infecting dogs is mainly transmitted by *Rhipicephalus sanguineus* (DANTAS-TORRES, 2008), *E. ruminantium* (cattle) by ticks of genus *Amblyomma*, *E. chaffeensis* (deer) and *E. ewingii* (human and dogs) by *A. americanum* and *Dermacentor variabilis* (DUMLER et al., 2001; YABSLEY, 2010), and *E. muris* (rodents) by *Haemaphysalis flava* and *Ixodes persulcatus* (INOKUMA et al., 2007). Other tick species, such as *A. cajennense*, have been suspected to act as vectors of *E. canis* in rural areas (COSTA JR et al., 2007).

Several methods with varying degrees of sensitivity and specificity can be used to detect *Ehrlichia* organisms. In the past, *Ehrlichia* species were identified using light microscopy by finding elementary bodies, initial bodies or morulae in the host cell cytoplasm of Romanowsky-stained blood smears (Figure 1) (HILDEBRANDT et al., 1973). Unfortunately, this technique lacks sensitivity and specificity. Indirect immunofluorescence assay (IFA) was the traditional test to diagnose human and canine monocytic ehrlichiosis (Figure 2) (RISTIC et al., 1972; WANER et al., 2001; AGUIAR et al., 2007a; DUMLER et al., 2007; SAITO et al., 2008). Although this technique is still widely used, a significant number of false positives may occur due to cross-reactivity with other organisms from the genera *Ehrlichia*, *Anaplasma* and *Neorickettsia* (RISTIC et al., 1981; HARRUS et al.,

2002; OLANO; WALKER, 2002; PADDOCK; CHILDS, 2003). Several other serological tests are now commercially available to diagnose ehrlichiosis (e.g., Enzyme Linked Immunosorbent Assay (ELISA), immunoblot, competitive Enzyme Linked Immunosorbent Assay (cELISA)) (OHASHI et al., 1998; WANER et al., 2000; ALLEMAN et al., 2001; LÓPEZ et al., 2007; ZHANG et al., 2008). Diagnostic accuracy has been greatly enhanced by the introduction of culture and molecular techniques. In Brazil, *E. canis* was first cultivated in 2002 using DH82 cells (TORRES et al., 2002) and a Brazilian strain was molecularly characterized in 2008 using *dsb*, 16S rRNA and *p28* genes (AGUIAR et al., 2008). These organisms can all be grown in cell culture with exception of *E. ewingii*. However, this is a time-consuming technique not available in many laboratories. Cultures also require specialized laboratory facilities and highly trained personnel. Molecular detection of the *Ehrlichia* genus by polymerase chain reaction (PCR), nested-PCR and real-time PCR has been used to identify individuals infected either experimentally or naturally in both acute and chronic phase (MACIEIRA et al., 2005; DINIZ et al., 2007; LABRUNA et al., 2007; DAGNONE et al., 2009; FARIA et al., 2010; NAKAGHI et al., 2010). PCR is a more sensitive and specific test compared to other methods (IQBAL et al., 1994; PEIXOTO et al., 2005; LABRUNA et al., 2007), although false-positive results can still occur (APFALTER et al., 2005).

The geographic distribution of some *Ehrlichia* species has not yet been fully established, although *E. canis* and *E. chaffeensis* have been described in most regions of the world (INOKUMA et al., 1999; COCCO et al., 2003; FABURAY et al., 2005; MASTRANDREA et al., 2006; PEREZ et al., 2006; TAMAMOTO et al., 2007; MORO et al., 2009, NDIP et al., 2009). Ehrlichiosis is widely detected across Brazil (Figure 3). Three species have been described to date in Brazil: *E. canis* (AGUIAR et al., 2007a; LABRUNA et al., 2007; UENO et al., 2009; DINIZ et al., 2007; OLIVEIRA et al., 2009a), *E. ewingii* (OLIVEIRA et al., 2009c) and *E. chaffeensis* (MACHADO et al., 2006). This is a review of recent studies on the occurrence of *Ehrlichia* species in domestic and wild animals, ticks and humans in Brazil.

Companion Animals

1. Clinical findings of *Ehrlichia canis* infection in dogs

Canine monocytic ehrlichiosis (CME) is a disease caused by *E. canis* and classically consists of three stages: acute, subclinical and chronic (NEER, 1998). The acute stage lasts two to four weeks and has non-specific clinical signs that may include apathy, anorexia, vomiting, fever, ocular and nasal discharge, weight loss, ocular lesions, lymphadenopathy, hepatosplenomegaly, and dyspnea (MOREIRA et al., 2003, 2005; CASTRO et al., 2004; AGUIAR et al., 2007b; ORÍÁ et al., 2008; BORIN et al., 2009). Hematological findings in this phase may include normocytic normochromic anemia (MOREIRA et al., 2003, 2005; CASTRO et al., 2004; BORIN et al., 2009), leukopenia with a shift to the left (MOREIRA et al., 2003; BORIN et al., 2009) and thrombocytopenia (MOREIRA et al., 2003, 2005;

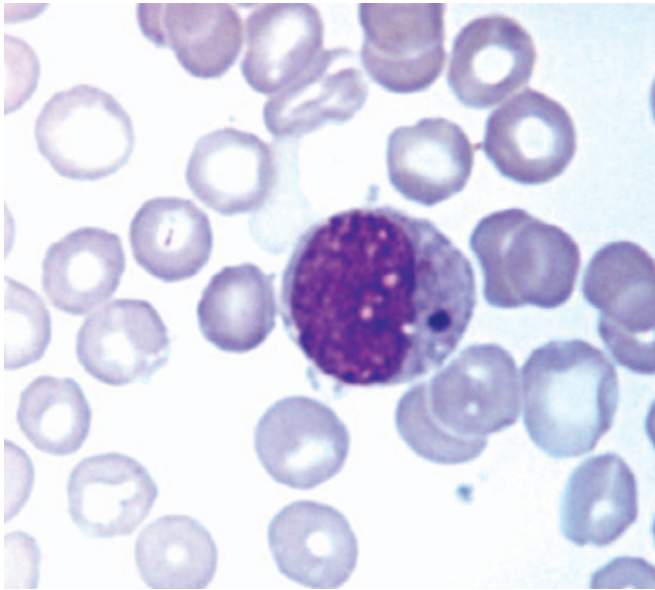


Figure 1. Light micrograph of *Ehrlichia canis*, Jaboticabal strain, inside a macrophage from an experimentally infected dog. Wright's-Giemsa (100 \times). Photograph kindly provided by Profa. Dra. Machado, R. Z.

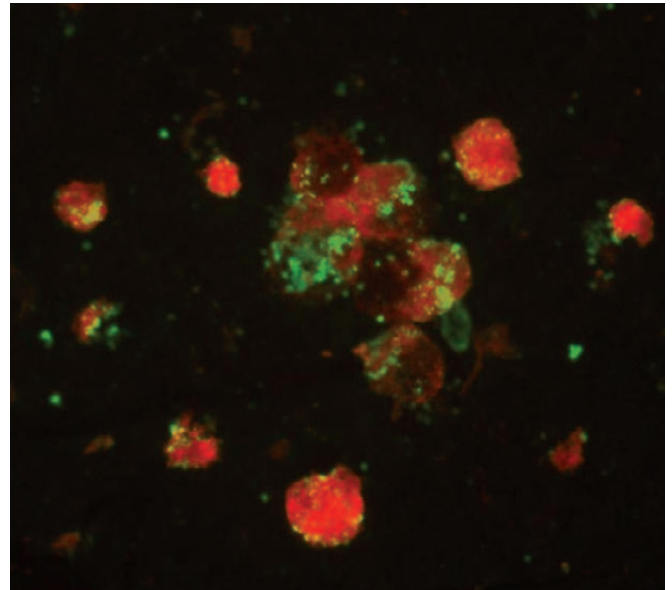


Figure 2. Indirect immunofluorescence assay (IFA) of *Ehrlichia canis* in serum from an infected dog (400 \times). Photograph kindly provided by Prof. Dr. Labruna, M. B.

CASTRO et al., 2004; BORIN et al., 2009; XAVIER et al., 2009). Cardiomyocyte injury has been identified in Brazilian dogs naturally infected with *E. canis*. In this population, dogs with acute ehrlichiosis were at a higher risk of developing myocardial cell injury than other sick dogs (DINIZ et al., 2008).

The subclinical phase usually starts 6 to 9 weeks post-infection. Laboratory findings during the subclinical stage include non-regenerative anemia (MOREIRA et al., 2003, 2005; ORIÁ et al., 2008; BORIN et al., 2009), leukopenia and thrombocytopenia (DAGNONE et al., 2003; BULLA et al., 2004; MOREIRA et al., 2005; ORIÁ et al., 2008; SANTOS et al., 2009; XAVIER et al., 2009). Altered platelet function is likely since dogs may present superficial bleeding such as epistaxis and petechia, even when platelet counts and coagulation profiles are within reference ranges (FRANK; BREITSCHWERDT, 1999; MOREIRA et al., 2005). In chronic cases, infected dogs fail to mount an effective immune response. Bone marrow involvement leads to pancytopenia (WALKER et al., 1970; MOREIRA et al., 2005) and death may occur due to hemorrhage secondary to thrombocytopathy (DAGNONE et al., 2001) or infections in neutropenic patients (HUXSOLL et al., 1970). It appears that CME occurs at any time throughout the year in many parts of Brazil. Tropical weather favors the proliferation of its main biological vector *R. sanguineus* (DANTAS-TORRES, 2008).

2. Prevalence of *Ehrlichia canis* infection in dogs

Ehrlichia canis was first reported in Brazil in 1973 (COSTA et al., 1973). Although the disease is currently described nationwide, prevalence data is only available for some regions (Table 1). The studies differ with respect to population, geographic area, presence of vector, and diagnostic test used. Therefore, comparison of

epidemiological data among studies is difficult or not feasible. IFA and other serological methods may yield false-positive results because these techniques do not differentiate between infection and previous exposure to the organism, whereas false-negatives by PCR in peripheral blood also may occur in subclinically or chronically infected dogs. In the chronic phase, the pathological agent is present inside macrophages in the spleen (HARRUS et al., 1998) and not in peripheral blood (HARRUS et al., 2004), explaining the negative PCR results. The detection range by PCR varies among laboratories (MAANEN et al., 2004).

In Jaboticabal city, São Paulo State, Southeastern Brazil, 30 dogs with clinical signs suggestive of ehrlichiosis were tested by different diagnostic methods; 53% were positive by nested-PCR from the 16S rRNA gene and 73% by serology (63% by IFA and 70% by dot-ELISA). In this study, only 27% of the dogs were tested positive by all three methods (NAKAGHI et al., 2008). The prevalence of *E. canis* infection in dogs from different areas and from selected hospital populations from Southeastern Brazil ranged from 15% (MACIEIRA et al., 2005) to 44.7% (COSTA JR et al., 2007). Table 1 summarizes the occurrence of *E. canis* in dogs from different geographic locations according to the type of population studied, total number of dogs evaluated in each study, and method of diagnosis. When dogs suspected to be infected based on suggestive clinical signs, such as pale mucous membranes, inappetence, apathy, vomiting, fever, lymphadenopathy, splenomegaly, epistaxis and others or laboratory data (anemia, thrombocytopenia) were tested, the prevalence increased from 40% (UENO et al., 2009) to 92.3% (OLIVEIRA et al., 2000). The prevalence of *E. canis* infection in dogs from rural and urban areas of the State of Rio Grande do Sul without abnormal clinical or laboratory findings was 4.8% (SAITO et al., 2008).

Using dot-ELISA an *E. canis* prevalence of 36% was found in a hospital population in the Ilhéus-Itabuna microregion, Bahia State,

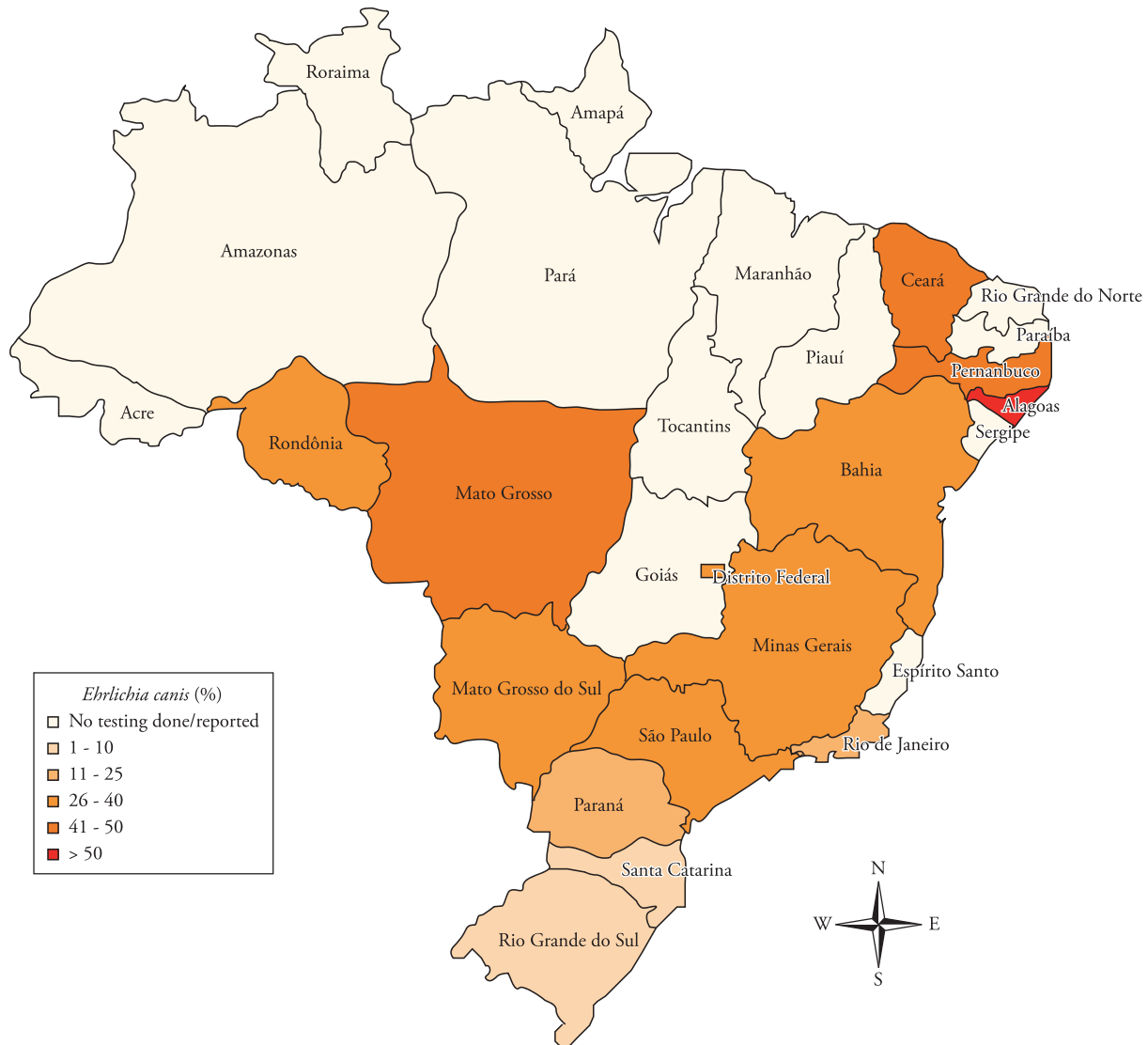


Figure 3. Geographical illustration of serologic and molecular occurrence of *Ehrlichia* spp. infections in dogs from Brazil based on data from Table 1. Geographic Information System, ARCGIS 9, Version 9.2, ERSI.

Northeastern Brazil (CARLOS et al., 2007). Using nested-PCR, only 7.8% of dogs from a hospital population had *E. canis* DNA in their blood in this same microregion (CARVALHO et al., 2008). It is important to notice that since these studies were conducted in a hospital population, prevalence data may not reflect the actual situation of canine ehrlichiosis in that region. A national seroprevalence of *E. canis* and other agents was performed in 2,553 dogs presented to 138 veterinary practices in 12 Brazilian States using dot-ELISA as the diagnostic method. Dogs were presented to the clinic for routine vaccinations, examinations, or other procedures (LABARTHE et al., 2003). The geographical distribution of *Ehrlichia* spp. infections in dogs from Brazil by serologic and molecular methods is illustrated in Figure 1.

In a survey of dogs from rural and urban areas by IFA, the prevalence found in Mato Grosso State (42.5%), Central-West Brazil (SILVA et al., 2010), was higher than in Rondônia State (36%), Northern Brazil (AGUIAR et al., 2007b), Bahia State (35.6%),

Northeastern Brazil (SOUZA et al., 2010), and Rio Grande do Sul State (4.8%), Southern Brazil (SAITO et al., 2008). The reasons for different prevalences in Southern, Central-West and Northern-Northeastern Brazil are unknown, since *R. sanguineus* is abundant throughout these urban and rural areas of Brazil (LABRUNA; PEREIRA, 2001; DANTAS-TORRES et al., 2006). However, *R. sanguineus* ticks can adopt different strategies to seek their hosts and these strategies may vary widely from region to region (DANTAS-TORRES, 2008). As previously described, climatic (KEEFE et al., 1982), or habitat conditions where the animals live (SAINZ et al., 1996) may account for the differences found.

3. Risk factors of *Ehrlichia canis* infection in dogs

In a study at the Veterinary Teaching Hospital (VTH), Universidade Estadual de Londrina, Paraná State, Southern Brazil, a random representative sample of the canine population at that

Table 1. Occurrence of ehrlichial infection in dogs from Brazil.

Geographical area	Population	N. dogs	Diagnostic method	Occurrence	Reference
Southeastern Brazil					
Jaboticabal, SP	Suspect	52	dot-ELISA ¹	48/52 (92.3%)	Oliveira et al. (2000)
	Positive by dot-ELISA	48	Blood smear examination	1/48 (2%)	
Jaboticabal, SP	Suspect	51	IFA	34/51 (66.6%)	Oriá et al. (2008)
			dot-ELISA ¹	44/51 (86.2%)	
Jaboticabal, SP	Suspect	25	Nested PCR for 16S rRNA gene	22/25 (88%)	Dagnone et al. (2009)
Jaboticabal, SP	Suspect	30	IFA	19/30 (63.3%)	Nakaghi et al. (2008)
			dot-ELISA ¹	21/30 (70%)	
			Nested PCR for 16S rRNA gene	16/30 (53.3%)	
Jaboticabal, SP	Suspect	40	Nested PCR for 16S rRNA gene	29/40 (72.5%)	Faria et al. (2010)
		35	Spleen aspiration	17/35 (48.6%)	
		35	Blood smear examination	2/35 (5.7%)	
Botucatu, SP	Suspect	198	PCR for 16S rRNA gene	154/198 (77.7%)	Diniz et al. (2007)
			IFA	145/198 (73.2%)	Diniz et al. (2007)
Botucatu, SP	Suspect	70	PCR for dsb gene	28/70 (40%)	Ueno et al. (2009)
			Blood smear examination	10/70 (7%)	
Botucatu, SP	Hospital	217	Nested PCR for 16S rRNA gene	67/217 (30.9%)	Bulla et al. (2004)
	non-thrombocytopenic	71		1/71 (1.4%)	
	thrombocytopenic	146		66/146 (45%)	
São Paulo State	Random	671	dot-ELISA ²	104/671 (15.5%)	Labarthe et al. (2003)
Rio de Janeiro State	Random	422	dot-ELISA ²	125/422 (29.6%)	Labarthe et al. (2003)
Minas Gerais State	Random	446	dot-ELISA ²	93/446 (20.9%)	Labarthe et al. (2003)
Rio de Janeiro, RJ	Random	226	PCR for 16S rRNA gene	34/226 (15%)	Macieira et al. (2005)
	non-thrombocytopenic	114		4/114 (2.6%)	
	thrombocytopenic	112		30/112 (26.8%)	
Ribeirão Preto, SP	Hospital	221	Nested PCR for 16S rRNA gene	86/221 (38.9%)	Santos et al. (2009)
	non-thrombocytopenic	114		29/114 (25%)	
	thrombocytopenic	107		57/107 (53%)	
Minas Gerais State	Rural area	226	IFA	101/226 (44.7%)	Costa Jr et al. (2007)
Minas Gerais State	Urban area	101	Blood smear examination	16/101 (16%)	Soares et al. (2006)
Minas Gerais State	Hospital	4407	Blood smear examination	251/4407 (5.7%)	Borin et al. (2009)
Southern Brazil					
Rio Grande do Sul State	Random	389	IFA	19/389 (4.8%)	Saito et al. (2008)
Rio Grande do Sul State	Random	356	dot-ELISA ²	6/356 (1.7%)	Labarthe et al. (2003)
Paraná State	Random	43	dot-ELISA ²	2/43 (4.7%)	Labarthe et al. (2003)
Londrina, PR	Random	381	dot-ELISA ²	87/381 (22.8%)	Trapp et al. (2006)
Londrina, PR	Anemic and/or thrombocytopenic	129	PCR for 16S rRNA gene	28/129 (22%)	Dagnone et al. (2003)
Santa Catarina State	Random	142	dot-ELISA ²	1/142 (0.7%)	Labarthe et al. (2003)
Northern Brazil					
Montenegro, RO	Random	314	IFA	97/314 (31%)	Aguiar et al. (2007b)
	Urban area	153		58/153 (38%)	
	Rural area	161		40/161 (25%)	
Northeastern Brazil					
Salvador, BA	Random	472	IFA	168/472 (35.6%)	Souza et al. (2010)
Ilhéus-Itabuna, BA	Hospital	200	dot-ELISA	72/200 (36%)	Carlos et al. (2007)
Ilhéus-Itabuna, BA	Hospital	153	Nested PCR for 16S rRNA gene	12/153 (7.8%)	Carvalho et al. (2008)
Bahia State	Random	117	dot-ELISA ²	42/117 (35.9%)	Labarthe et al. (2003)

¹Immunocomb, Biogal; ²SNAP[®] 3DX, Idexx.

Table 1. Continued...

Geographical area	Population	N. dogs	Diagnostic method	Occurrence	Reference
Ceará State	Random	11	dot-ELISA ²	5/11 (45.5%)	Labarthe et al. (2003)
Pernambuco State	Random	105	dot-ELISA ²	52/105 (49.5%)	Labarthe et al. (2003)
Alagoas State	Random	11	dot-ELISA ²	6/11 (54.5%)	Labarthe et al. (2003)
Central-West Brazil					
Cuiabá, MT	Random	254	IFA	108/254 (42.5%)	Silva et al. (2010)
Campo Grande, MS	Suspect	26	Nested PCR for 16S rRNA gene	10/26 (38.4%)	Dagnone et al. (2009)
Mato Grosso do Sul State	Random	126	dot-ELISA ²	45/126 (35.7%)	Labarthe et al. (2003)
Federal District	Random	101	dot-ELISA ²	24/101 (23.8%)	Labarthe et al. (2003)

¹Immunocomb, Biogal; ²SNAP[®] 3DX, Idexx.

institution was tested. Eighty-seven out of 381 (22.8%) dogs were seropositive for ehrlichiosis using a commercially available immunoenzymatic dot-ELISA (SNAP[®] 3DX, IDEXX Laboratories Inc., Portland, ME, USA) assay. The groups at higher risk of being seropositive to ehrlichiosis than the general population included: dogs older than 1 year, previously exposed to ticks and presence of neurological signs. Dogs seropositive for *B. vogeli* were more likely to be seropositive to *E. canis* and 54/381 (14.2%) of these dogs had antibodies against both agents (TRAPP et al., 2006). In another study using thrombocytopenia and anemia as screeners for *E. canis* infection, 22% of 129 dogs tested positive for *E. canis* DNA by PCR at the same VTH (DAGNONE et al., 2003).

Most veterinary clinicians in Brazil use the presence of clinical and/or laboratorial findings to make a presumptive diagnosis of *E. canis* infection in dogs. At the Botucatu VTH, Southeastern Brazil, 217 canine blood samples randomly obtained from routine tests made at the Clinical Pathology Laboratory were divided into two groups based on platelet count: 71 non-thrombocytopenic and 146 thrombocytopenic. This population has an unexpectedly high prevalence of thrombocytopenia but unfortunately the randomization method was not described in the study. Thrombocytopenic dogs were divided into those with mild thrombocytopenia (62/146 dogs, platelets count between 100,000-200,000/ μ L) and severe thrombocytopenia (84/146 dogs, less than 100,000/ μ L). Sixty-seven (31%) of all dog blood samples in the study were positive using a nested-PCR protocol, whereas 45% of thrombocytopenic dogs were positive. Twenty-one percent of the dogs with mild and 63% of dogs with severe thrombocytopenia had ehrlichial DNA amplified by nested-PCR from the 16S rRNA gene. In this study the authors suggested that in endemic areas dogs with severe thrombocytopenia are more likely to be infected by *E. canis* infection (BULLA et al., 2004). In Rio de Janeiro, Southeastern Brazil, 226 dogs presented at private clinics were sampled and divided into thrombocytopenic (112/226) and non-thrombocytopenic (114/226). *Ehrlichia canis* infection was documented in 30 (27%) dogs with thrombocytopenia, but only in 4 (3%) non-thrombocytopenic animals using a PCR assay specific for the 16S rRNA gene. Anemia was found in 60% of thrombocytopenic dogs that were PCR-positive (MACIEIRA et al., 2005). In Ribeirão Preto, Southeastern Brazil, 86/221 dogs (39%) from private veterinary university hospitals were positive for *E. canis* by nested-PCR for the 16S rRNA gene, 57/107 (53%) were thrombocytopenic and 29/114 (25%) were non-thrombocytopenic (SANTOS et al., 2009).

In the city of Jaboticabal, Southeastern Brazil, 51 dogs with uveitis tested positive to *E. canis* infection by IFA (66.6%) and dot-ELISA (86.2%). Anemia and thrombocytopenia were the most common hematological abnormalities found. A high association between clinical uveitis and positive serology for *E. canis* was demonstrated in this study (ORIÁ et al., 2008). In another study in the city of Jaboticabal, 52 dogs suspected of naturally acquired ehrlichiosis were selected at the VTH and tested by a commercial ELISA. A high prevalence of seropositive dogs (92.3%) was found. The most common clinical and laboratorial findings were inappetence, apathy, anemia and thrombocytopenia (OLIVEIRA et al., 2000).

Seroprevalence by IFA was determined in three rural areas of Minas Gerais State, Southeastern Brazil using 226 dogs living in these areas. *Ehrlichia canis* prevalence in this study ranged from 24.7-65.6% (COSTA JR et al., 2007). In this study, male dogs >2 years of age and those infested by ticks were at high risk of being seropositive to *E. canis* (COSTA JR et al., 2007). In a survey conducted in urban areas of Minas Gerais State, blood samples were collected from 51 dogs that were restricted to house backyards and 50 dogs that lived in apartments. *E. canis* was found by blood smear examination in 16% of dogs from houses with grassy yards, but in none of the dogs restricted to apartments. *R. sanguineus* was the only tick found in this study (SOARES et al., 2006). Differences on *E. canis* prevalence between these two studies may be due to different detection methods.

Rhipicephalus sanguineus can also transmit other hemoparasites (DANTAS-TORRES, 2008) and since it is widespread in Brazil (LABRUNA; PEREIRA, 2001), it is relatively common to find *E. canis*-infected dogs co-infected with *Anaplasma* sp. (MOREIRA et al., 2003; DAGNONE et al., 2003, 2009), *Babesia* sp. (MOREIRA et al., 2003; SOARES et al., 2006; TRAPP et al., 2006; BORIN et al., 2009), *Bartonella* sp. (DINIZ et al., 2007), *Hepatozoon* sp. (O'DWYER et al., 2006; MUNDIM et al., 2008), *Leishmania* sp. (DINIZ et al., 2007) and *Mycoplasma* sp. (MOREIRA et al., 2003, 2005).

Blood smear examination is not an effective diagnostic method as morulae are visualized only during the acute phase and the percentage of infected cells is usually less than 1% (CADMAN et al., 1994). Diagnostic sensitivity between cytological methods was assessed in 50 dogs naturally infected by *E. canis*. During the acute phase of the disease, the highest sensitivities were found in buffy coats (66%) and lymph nodes (60.4%) compared to peripheral

blood (8%) examinations (MYLONAKIS et al., 2003). Using 35 samples collected from dogs suspected of being infected with *E. canis*, based on clinical signs and presence of thrombocytopenia, 17 dogs (48.6%) showed intracytoplasmatic morulae in spleen aspiration and only two (5.7%) showed in buffy coat, however *E. canis* DNA was isolated in 29/40 (72.5%) spleen samples and 30/40 (75%) whole blood samples (FARIA et al., 2010). Moreover, 51 blood samples from dogs with both clinical signs consistent with ehrlichiosis and the presence of intracytoplasmatic inclusion bodies or morulae-like forms in white blood cells were submitted to molecular analysis. Thirty-two (64%) dog samples were positive for *E. canis* by nested-PCR for the 16S rRNA gene (DAGNONE et al., 2009). Thus, an absence of parasites in blood smears does not rule out the possibility of infection (HOSKINS, 1991).

4. *Ehrlichia ewingii* infection in dogs

In Minas Gerais State, Southeastern Brazil, 5/100 (5%) dogs tested positive by nested-PCR for the 16S rRNA gene of *E. ewingii*. These same dogs were also positive by a second PCR assay targeting a fragment of the ehrlichial *dsb* gene. Four animals had anemia and one of them also had thrombocytopenia. This was the first study to provide evidence of canine infection caused by *E. ewingii* in Brazil (OLIVEIRA et al., 2009c), however species confirmation by DNA sequencing was not reported. Given that a previous study documented false-positive amplifications, especially when targeting the 16S rRNA by PCR (SUKSAWAT et al., 2001), other genes should be analyzed to confirm *E. ewingii* infection and thus the initial results from Oliveira et al. (2009c) remain to be confirmed.

5. Ehrlichiosis in Cats

Antibodies against *E. canis* antigens have been described in domestic cats in a few countries (MATTHEWMAN et al., 1996; PEAVY et al., 1997; ORTUÑO et al., 2005; SOLANO-GALLEGU et al., 2006) and documented clinical cases of ehrlichiosis in these animals are rare (BREITSCHWERDT et al., 2002). *Ehrlichia* organisms have not been cultured from feline samples; evidence of infection came exclusively from serological and molecular studies (BREITSCHWERDT et al., 2002; OLIVEIRA et al., 2009b). Nevertheless, *Ehrlichia canis*-like organisms were detected by PCR and DNA sequencing in cats from Brazil and North America (BREITSCHWERDT et al., 2002; OLIVEIRA et al., 2009b). The first molecular detection of *E. canis* in Brazilian cats was reported in a study at a VTH of Minas Gerais State, Southeastern Brazil, in which blood samples from 3/15 cats tested positive by nested-PCR for the 16S rRNA gene. The *E. canis* sequence, a fragment of the 16S rRNA gene, showed 100% identity with the *E. canis* sequence obtained from dogs from the same study area (OLIVEIRA et al., 2009a, 2009b). Further studies are needed to better characterize the *Ehrlichia* spp. involving other genes, transmission, pathogenesis and clinical presentation in cats.

Wildlife Animals

Cervid species may be infected with *Ehrlichia* organisms (YABSLEY et al., 2002; MACHADO et al., 2006; KAWAHARA et al., 2009; LEE et al., 2009). In the United States, white-tailed deer (*Odocoileus virginianus*) is considered the main reservoir of *E. chaffeensis* and possibly of *E. ewingii* (YABSLEY et al., 2002; KAWAHARA et al., 2009). The first molecular detection of *E. chaffeensis* in Brazil was reported on the border of São Paulo and Mato Grosso do Sul States, between Southeastern and Central-Western regions of Brazil. In this study, 3/7 captured marsh deers (*Blastocercus dichotomus*) tested positive by nested-PCR for *E. chaffeensis* infection (MACHADO et al., 2006). Sequence analysis from positive samples showed 97% identity with sequences deposited in GenBank. Two out of three positive *E. chaffeensis*-positive marsh deer samples were also positive for *Anaplasma marginale* by nested-PCR.

Ehrlichia ruminantium infection in ruminants has been reported only in Africa and Caribbean region. Some non-African cervids are also known to be susceptible to this agent, including the white-tailed deer, the Timor deer (*Cervus timorensis*) and chital (*Axis axis*) (PETER et al., 2002). Recently, an *Ehrlichia* sp. closely related to *E. ruminantium* was detected in white-tailed deer from the United States, suggesting that the range of *Ehrlichia* species infecting these animals is broader than first thought (YABSLEY et al., 2008). To date, there are no reports of other *Ehrlichia* species rather than *E. chaffeensis* in wild ruminants from Brazil; monitoring of these animals is highly desired.

An *E. canis* serological survey by IFA of 20 free-ranging felids (18 pumas [*Puma concolor*], one ocelot [*Leopardus pardalis*] and two spotted cats [*Leopardus tigrinus*]) from different parts of Brazil was conducted and antibodies against *E. canis* were detected only in one puma (FILONI et al., 2006). Since IFA has high cross-reactivity with members of Anaplasmataceae family, definitive diagnosis of *E. canis* infection cannot be ensured. In another study, 72 blood samples from wild captive felids (9 pumas [*P. concolor*], 29 oncolots [*L. pardalis*], 6 jaguarondis [*P. yagouaroundi*], 2 margays [*L. wiedii*], 14 little spotted cats [*L. tigrinus*], 3 pampas cats [*L. pajeros*] and 9 jaguars [*Panthera onca*]) were tested for *Ehrlichia* spp. infection. Using IFA 5/72 (7%) animals tested positive for *E. canis* antibodies and 11/72 (15.3%) animals were positive for *E. canis* by nested-PCR based on 16S rRNA gene. Nested-PCR positive samples were submitted to another omp-1 gene based nested-PCR and only four samples tested positive. Sequencing of the 16S rRNA gene obtained showed 97% identity to *E. canis* strain Jaboticabal. However, based on omp-1 sequences, *Ehrlichia* sp. detected from Brazilian felids may be a novel *Ehrlichia* species. It was the first study of molecular detection of *Ehrlichia* sp. in Brazilian wild felids (ANDRÉ et al., 2010).

Public Health

There are to date two recognized diseases caused by *Ehrlichia* species: human monocytic ehrlichiosis (HME) caused by *E. chaffeensis*; and human granulocytic ehrlichiosis (HGE) due to *E. ewingii* (OLANO; WALKER, 2002). Other rickettsial agents, *A. phagocytophilum* and *N. sennetsu* also cause disease in humans.

HME and HGE have been described worldwide (OTEO et al., 2000; GUILLAUME et al., 2002; GARDNER et al., 2003; RUSCIO; CINCO, 2003; MASTRANDREA et al., 2006). Three human ehrlichiosis cases have been serologically identified as HME in Brazil since 1980 (CALIC et al., 2004; COSTA et al., 2005, 2006) but molecular confirmation has not been performed. The disease in humans has been suggested serologically in other South American countries including Argentina (RIPOLL et al., 1999), Chile (LÓPEZ et al., 2003) and Peru (MORO et al., 2009). The lack of molecular characterization of the organism precludes any conclusion regarding the pathogenic agent in these cases.

An *E. canis* isolate has been successfully obtained in cell culture from blood of an asymptomatic person in Venezuela (PEREZ et al., 1996). The genetic sequence from this isolate was identical to *E. canis* isolates infecting dogs and *R. sanguineus* ticks in the same area of Venezuela, suggesting that human infection may be transmitted by *R. sanguineus* ticks (UNVER et al., 2001). *Ehrlichia canis* DNA was also amplified from blood of six human patients with clinical signs of HME in Venezuela, suggesting that *E. canis* can be associated with clinical manifestation in humans (PEREZ et al., 2006). In addition, the genetic characterization of the entire 16S rRNA gene of two strains of *E. canis* in Botucatu region, São Paulo State, Brazil, showed that one strain naturally-infecting dogs was identical to the Venezuelan strain infecting humans (DINIZ et al. 2007). Thus, *E. canis* strains from Brazil may be capable of infecting humans.

Infestations by the brown dog tick, *R. sanguineus*, in humans have been reported worldwide (MANFREDI et al., 1999; GUGLIELMONE et al., 1991; FELZ et al., 1996; VENZAL et al., 2003; DEMMA et al., 2005). They were observed twice in Brazil, in the city of Goiania, Goiás State, Central-Western region where larvae, nymph and adult stages of *R. sanguineus* were found (LOULY et al. 2006), and in the city of Recife, Pernambuco State, Northeastern region, where only adult ticks were found parasitizing humans (DANTAS-TORRES et al., 2006). In the city of Londrina, Paraná State, Southern Brazil, owners of dogs with ticks were more likely to have been exposed to ticks themselves. Only 10% of the owners who occasionally interacted with their dogs while more than 25% of the owners who frequently or very frequently interacted reported past tick infestations (TRAPP et al., 2006). Thus, interaction between human beings and *R. sanguineus* is likely to be more common than is usually recognized (DANTAS-TORRES, 2008).

Ehrlichia ewingii, an agent known to cause granulocytic ehrlichiosis in dogs, was recognized in 1998 to cause infections in humans (BULLER et al., 1999), but it has not been identified as an infecting agent of humans in Brazil. A single case of ehrlichiosis caused by an *Ehrlichia ruminantium*-like bacterium, called the Panola Mountain *Ehrlichia*, has been identified in a 31-year-old man from Georgia, United States (REEVES et al., 2008).

Human exposure to tick vectors is seasonal and occurs predominantly in rural and suburban areas involving recreational, peridomestic, occupational, and military activities (DEMMA et al., 2005). Both forms of human ehrlichiosis (monocytic and granulocytic) have common clinical and laboratory manifestations that include fever, headache, myalgia and malaise, thrombocytopenia, leukopenia, and elevated liver enzymes (OLANO et al., 2003;

STONE et al., 2004). Infection by *E. chaffeensis* can cause a severe form of HME that can be life-threatening in HIV-infected patients (PADDOCK et al., 2001). Central nervous system infection is found rarely in HGE, whereas rashes are common in HME cases. The key for HME or HGE diagnosis is the identification of fever and thrombocytopenia, leukopenia, and elevated serum alanine-amino transferase in a patient exposed to ticks in endemic areas during times of tick activity (STONE et al., 2004; OLANO et al., 2003).

The first case of HME diagnosed in the United States was in a 51-year-old man who became ill in April 1986, 12 to 14 days after bitten by ticks in rural Arkansas (MAEDA et al., 1987). The disease was first thought to be caused by the canine pathogen *E. canis*. However, *E. chaffeensis* was shown to be the main causative agent of HME in the US in the 1990's (BAKKEN et al., 1994).

The first study using IFA was carried out in 1998 for *E. chaffeensis* infection in Minas Gerais State, Southeastern Brazil, in which no reactivity was found in 473 students from four schools (GALVÃO et al., 2002). The two first suspected cases of human ehrlichiosis in Brazil occurred in 2001, in Minas Gerais State, Southeastern Brazil (CALIC et al., 2004). The first patient was a 39-year-old man suspected of harboring the Brazilian Spotted Fever (BSF) agent. He had fever, headache, nausea, vomiting, myalgia, conjunctivitis, respiratory and renal failure. IFA testing for BSF and murine typhus, microagglutination testing for leptospirosis, and ELISA testing for yellow fever and dengue were performed and they were all negative. However, IFA detected antibodies against *E. chaffeensis*. Antibodies against *A. phagocytophilum* were not present. The second patient was a 20-year-old man presenting similar clinical signs. Antibodies were detected against *E. chaffeensis*, but not against *A. phagocytophilum*. Based on clinical and serologic results a suggestive diagnosis of HME was established.

Nine of 771 (1.2%) febrile patients had antibodies against *E. chaffeensis* by IFA in a study conducted in Minas Gerais, Southeastern Brazil, from 2001 to 2005. This case series resulted from a specific protocol to search for rickettsial agents as a cause of fever (COSTA et al., 2006). In the study, all patients reported tick bite prior to the disease. Based on the accepted criteria for diagnosis (WALKER, 2000), all cases had epidemiological and serologic findings consistent with HME (COSTA et al., 2006).

In 2001, another IFA serosurvey for rickettsial agents conducted in healthy individuals from a rural community in Minas Gerais State showed that 46/437 (10.5%) had antibodies against *E. chaffeensis* (COSTA et al., 2005). The infection rate was higher among people living in farms when compared to those living in the village. History of tick exposure was widespread affecting roughly 100% of this population.

IFA is considered the gold standard for clinical diagnosis of HME. However, it is important to note the cross-reactivity between *E. canis* and *E. chaffeensis*. There is only one description of *E. chaffeensis* (from a deer) confirmed by sequencing in Brazil, however *E. canis* in dogs is common and widespread. The authors' hypothesis is that some, if not all, human cases attributed to *E. chaffeensis* in Brazil are actually caused by *E. canis* (DINIZ et al., 2007).

The first molecular surveillance for tick-borne diseases on humans was recently conducted in rural areas of Rondônia and São Paulo States, Northern and Southeastern Brazil, respectively

(LABRUNA et al., 2007). No *Ehrlichia* DNA was detected by real-time PCR in 75 blood samples from febrile patients with history of tick exposure.

In summary, suspected human ehrlichiosis has been serologically suggested in Brazil since 2001. The surveillance studies are limited and restricted to only a few geographic areas. Comprehensive epidemiological studies using both serological and molecular methods are needed to fully establish the extent and importance of human ehrlichiosis in Brazil.

Conclusion

In conclusion, two *Ehrlichia* species, *E. canis* and *E. chaffeensis*, have been confirmed to occur in Brazil to date. Only *E. canis* has been successfully isolated in cell cultures from clinical samples of dogs. The disease caused by *E. canis* is considered endemic in dogs from several regions of Brazil, whereas the infection by *E. chaffeensis* has been found in a wild reservoir. Although human ehrlichiosis has been serologically suggested in Brazil, the extent of the disease and its causative agent remain unknown.

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