Tropical Biomedicine 32(4): 726–735 (2015)

Molecular detection of *Anaplasma* sp. and *Ehrlichia* sp. in ticks collected in domestical animals, Colombia

Jorge Miranda[1] and Salim Mattar[1*]
[1]Instituto de Investigaciones Biológicas del Trópico, Facultad de Medicina Veterinaria y Zootecnia, Universidad de Córdoba, Montería, Colombia
http://www.unicordoba.edu.co/index.php/investigacion/inst-investigacion/instituto-de-investigaciones-biologicas-del-tropico
[*]Corresponding author e-mail: smattar@correo.unicordoba.edu.co; mattarsalim@hotmail.com
Received 13 March 2015; received in revised form 12 August 2015; accepted 7 September 2015

Abstract. *Anaplasma* and *Ehrlichia* species are important tick-borne pathogens that cause disease in cattle, dogs, horses and humans; with little information available about these agents in Colombia. The aim of this study was to provide molecular evidence for the presence of *Anaplasma* sp. and *Ehrlichia* sp. species in ticks collected from horses, dogs and cattle in Cordoba, Colombia. In this study, 1.105 ticks were removed from 226 zebu cattle (*Bos indicus*), 87 dogs (*Canis lupus familiaris*) and 19 horses (*Equus caballus*), from different localities of Cordoba. Ticks were identified taxonomically and PCR assays were used for the amplification of *Anaplasma* sp. and *Ehrlichia* sp. DNA. The amplification products were sequenced and analyzed. From the 1,105 ticks examined, 679 (61.5%) were *Rhipicephalus microplus*, collected from cattle, 353 (32%) were *Rhipicephalus sanguineus* from dogs and 73 (6,6%) *Dermacentor nitens* collected on horses; 332 pools were formed to develop the PCR assay. *Anaplasma* sp. and *Ehrlichia* sp. DNA was detected in 5.7% (19/332 pools). Direct sequencing of amplicons showed that seven sequences had similarities between 99–100% with *Anaplasma marginale*, one sequence showed 100% identical with *Anaplasma phagocytophilum*, seven sequences showed 100% identity with *Ehrlichia ewingii*, one sequence had 100% identity with *E. chaffensis* and three sequences showed similarities of 99% and 95% with *Ehrlichia mineirensis* and *Ehrlichia canis* respectively. In conclusion, several tick-borne pathogens identified in this survey suggests that there is a risk for the emergence of tick-borne diseases in domestic animals and humans in Colombia. Our data provides evidence of *Ehrlichia* and *Anaplasma* species circulating in ticks from Cordoba.

INTRODUCTION

Anaplasma and *Ehrlichia* are small, Gram-negative, intracellular bacterial parasites, which belong to the family Anaplasmataceae and cause a variety of infections in humans as well as wild and domestic animals. *Anaplasma* and *Ehrlichia* species are maintained in nature through an enzootic cycle involving ticks and vertebrate hosts (Rar *et al.*, 2010).

Ticks of the family Ixodidae transmit *Anaplasma* and *Ehrlichia* genera naturally to vertebrate hosts. The pathogens are divided into various distinct genogroups. The genus *Anaplasma* comprises six species: *A. centrale*, *A. marginale*, *A. bovis*, *A. ovis*, *A. phagocytophilum* and *A. platys*. The genus *Ehrlichia* consists of five species, which include *E. canis*, *E. chaffeensis*, *E. muris*, *E. ewingii* and *E. ruminantium* (Aktas, 2014; Dumler, 2005). Most of these species are pathogens of veterinary importance in dogs, horses and cattle. They also are responsible for large economic losses estimated at millions of dollars per year in various countries (Oliveira *et al.*, 2011).

Human granulocytic anaplasmosis (HGA) and human monocytic ehrlichiosis (HME) are zoonotic tick-borne diseases

caused by *A. phagocytophilum* and *E. chaffeensis* respectively. The clinical presentation of HGA is nonspecific and usually consists of fever of ≥38.5°C, headache, malaise, myalgia, and/or arthralgia, and is often accompanied by laboratory abnormalities such as leukopenia, thrombocytopenia, and increased activity of hepatic enzymes (Brouqui *et al.*, 2004; Koebel *et al.*, 2012). Clinical presentation of HME includes a flu-like syndrome with varying degrees of anemia, thrombocytopenia, and leukopenia, and elevated liver enzymes (Blanco & Oteo, 2002). However, many clinical suspicions of these diseases are not investigated, have no proven etiology, and are subsequently not reported.

In Colombia, only few studies on *Anaplasma* sp. and *Ehrlichia* sp. in animals, ticks and humans have been reported by serological and molecular methods (Hidalgo *et al.*, 2009; Vargas-Hernandez *et al.*, 2012). Seroepidemiological data suggest that many human infections go unrecognized in Cordoba and the Caribbean (Máttar & Parra, 2006). The aim of this study was to provide molecular evidence for the presence of *Anaplasma* sp. and *Ehrlichia* sp. in ticks collected from horses, dogs and cattle in Cordoba in the Northern region of Colombia.

MATERIALS AND METHODS

Study areas. The department of Córdoba is an area mostly used for agriculture and livestock, is a tropical dry forest with temperatures ≥24°C and from 20 to 100 m above sea level. The ticks were collected from municipalities of Planeta Rica (08° 24' 53" N. 75° 13' 18" W), Los Córdoba (08° 54' 00" N. 76° 21' 35" W), Ciénaga de Oro (08° 52' 41" N. 75° 37' 27" W), Sahagún (08° 56' 58" N. 75° 26' 5" W), San Carlos (08° 48' 02" N. 75° 42' 08" W), San Pelayo - Pelayito (08° 57' 37" N. 75° 50' 31" W) and Montería (08° 45' 27" N. 75° 53' 25" W) in the North West of Colombia.

Sample collection. From October 2011 to Jun 2012, a total of 1.105 ticks were removed from 226 zebu cattle (*Bos indicus*), 87 dogs (*Canis lupus familiaris*) and 19 horses (*Equus caballus*), from the different localities listed above. Only adult ticks were collected and preserved in 96% ethanol and transported to the Instituto de Investigaciones Biologicas del Tropico, University of Córdoba, for laboratory analyses.

Tick identification and nucleic acid extraction. All ticks were observed under a stereomicroscope (Leica EZ4HD, *CH-9435*, Heerbrugg, Switzerland) and identified taxonomically following the scheme described by (Barros-battesti *et al.*, 2006). Ticks were grouped in pools of 3 or 5 individuals from the same host, the total number of pools was 332. The pools were preserved in ethanol at 96°C for subsequent laboratory procedures.

Each pool of ticks were dried and dissected using sterile scalpel blades and transferred into 2 ml sterile microcentrifuge tubes. The ticks were crushed after 200µl of phosphate buffered saline were added. DNA was extracted using a QIAamp DNAMini-Kit (QIAGEN, Valencia, CA) and eluted in a final volume of 100 µl.

PCR amplification. Two conventionals PCR assays were used for the amplification, one for Anaplasmataceae and other for *Ehrlichia* specific genus *dsb* gene. For Anaplasmataceae assay the PCR reactions contained a final concentration of 0.2µM of the primers GE2 5'- GTTAGTGGGAGAC GGGTGAGT-3' and HE3 5'-TATAGGTACC GTCATTATCTTCCCTAT-3' for forward and reverse primers respectively, these primers amplifies a 360 bp fragment of the 16S rRNA (Aguiar *et al.*, 2008). PCR cycling conditions were performed in automated MJ Research PTC-100TM thermal cyclers as follows: 1 cycle at 95°C for 2 min, followed by 40 cycles of 10 s at 95°C, 15 s at 55°C and 1 min at 70°C.

For the amplification of genus-specific *Ehrlichia*, the assay amplifies a 409 bp fragment of the *dsb* gene which encodes a protein involved in disulfide bond formation. The PCRs contained a final concentration of 0.2µM of the primers Dsb 330 5'-GATGATG TCTGAAGATATGAAACAAAT-3' and Dsb 728

5'-CTGCTCGTCTATTTTACTTCTTAAAGT-3' for forward and reverse primers respectively (Widmer *et al.*, 2011). PCR cycling conditions of 1 cycle at 95°C for 2 min, followed by 40 cycles of 15 s at 95°C, 30 s at 55°C and 1 min at 72°C. In both assays a recombinant *Taq*DNA Polymerase (Invitrogen, Brazil) was used; made of 2.5 U and 5 µl of template DNA in a final volume of 50 µl.

Positive control of *Ehrlichia canis*, DNA was kindly donated by Professor Marcelo Bahia Labruna from University of São Paulo (USP) Brazil, positive control for *Anaplasma* sp. DNA was obtained from blood samples obtained from a naturally infected bovine that was diagnosed clinically and by microscopic visualization by veterinarians from the University of Córdoba from the department of Córdoba. Negative deionized water and extraction controls were included in each PCR. Electrophoresis on agarose gel of 1.5% was used to separate PCR products, stained with ethidium bromide and examined using an ultraviolet (UV) transilluminator (ImageQuant 100, Uppsala, Sweden). The PCR products were cleaned using a PureLink Quick Gel Extraction kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, both strands of each fragment were sequenced directly in an automatic sequencer (model ABI-PRISM 3130XL, Applied Biosystems, Foster City, CA).

Sequence analysis

The nucleotide sequences were identified and compared with others sequences using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) database website (http://www.ncbi.nlm.nih.gov/BLAST). All analyzed sequences had the primer region sequence removed before analysis.

RESULTS

Tick taxonomic identification

Of the 1,105 ticks collected directly from animals, 679 (61.5%) were identified as *Rhipicephalus microplus*, collected from 226 zebu cattle (*Bos indicus*), 353 (32%) *Rhipicephalus sanguineus* from 87 dogs (*Canis lupus familiaris*) and 73 (6,6%) *Dermacentor nitens* collected on 19 horses (*Equus caballus*). The total number of pools was 332.

PCR amplification

Anaplasma sp. and *Ehrlichia* sp. DNA was detected in 5.7% (19 out of 332) pools of ticks by conventional PCR. The prevalence of *Anaplasma* sp. and *Ehrlichia* sp. in ticks was expressed as a percentage and minimum infection rate (MIR) or the minimum percentage of ticks in a pool with detectable *Anaplasma* sp. or *Ehrlichia* sp. (Table 1).

For the primers GE2 and HE3 (which amplify a fragment of the 16S rRNA gene of Anaplasmataceae family), 16 samples yielded the expected amplicon size. All PCR products were successfully sequenced and BLAST analysis was performed to compare the nucleotide sequences of the PCR products with the sequences in GenBank. Direct sequences of amplicons showed that 2.4% (8/332) had more genetic similarity with *Anaplasma* species; In seven samples the nucleotide sequences were 100% identical

Table 1. Identified tick species, pools which tested positive for *Anaplasma* sp. and *Ehrlichia* sp. DNA, percentage of infection in pools and minimum infection rate (MIR) from each tick species

Tick species	Number of ticks	Number of pools	Positive pools	N pools (%)	% Anaplasma positive pools	% Anaplasma MIR	% Erhlichia positive pools	% Erhlichia MIR
Rh. microplus	679	226	15	226 (6,6%)	3% (7/226)	1% (7/679)	3,5% (8/226)	1,2% (8/679)
Rh. sanguineus	353	87	3	87 (3,4%)	0 (0/87)	0% (0/353)	3,4% (3/87)	0,8% (3/353)
D. nitens	73	19	1	19 (5,2%)	5,2% (1/19)	1,3% (1/73)	0 (0/19)	0% (0/73)
Total	1105	332	19	332 (5,7%)	2,4% (8/332)	0,75% (8/1105)	3,3% (11/332)	1% (11/1105)

to each other and showed an identity of 99–100% with the specie *Anaplasma marginale* (GenBank: NR_074556, JQ839012 and JQ839011). Only the partial nucleotide sequence of *Anaplasma* CPY 31 was upload in the GenBank database under the accession numbers KM009068. These seven pools of *Rh. microplus* ticks were collected from cattle. The eighth sequence showed 100% identity with the species *Anaplasma phagocytophilum* (EU287434) and 99% similarity with others *A. phagocytophilum* (GU111741, EF547934, JN656381). The partial nucleotide sequence of *Anaplasma* CPY-35 was deposited in GenBank database under the accession numbers KM009069. This pool of *D. nitens* ticks was collected from a horse.

The other eight sequences 2.4% (8/332) were detected in *Rh. microplus* collected from cattle. This sequences have more genetic similarity with *Ehrlichia* sp. Seven *Ehrlichia* sp. showed a nucleotide sequences that were 100% identical to each other and 100% identical with *Ehrlichia ewingii* (DQ365880, AF497628, AY093441). Only the partial nucleotide sequence of *Ehrlichia* sp. PB91 was deposited in the GenBank database under the accession number KM009067. The eighth sequence showed 100% identity with *E. chaffensis* (NR074500, JQ085940, GQ499971), this sequence of *Ehrlichia* sp. ES-113 was deposited in GenBank under the accession number KM009066.

For Dsb 330 and Dsb 728 primers (which amplify a fragment of *dsb* gene), 3 samples yielded the expected amplicon size (which not were tested by Anaplasmataceae family primers), all three pools from *Rh. sanguineus* were collected from dogs. These three sequences were 100% identical to each other and showed 99% similarity with the recently proposed new species *Ehrlichia mineirensis* UFMG-EV (GenBank JX629808) and 95% similar with *Ehrlichia canis* (DQ460715, GU586135, AF403710) as shown in Figure 1. One of the sequence *Ehrlichia* sp. 1E, was deposited in GenBank under the accession number KM015219.

DISCUSSION

Ehrlichiosis and anaplasmosis are tick-borne diseases with great veterinary importance; however, in the last few years there has been growing concern about the increase in human cases of these diseases in different parts of the world (Cruz *et al.*, 2012).

Several studies have been carried out on *Ehrlichia* species from several countries, maily in dogs, and few in ticks. Ndip, *et al.* in Cameroon found that 30% (28/92) of *Rhipicephalus sanguineus* ticks collected from dogs were infected by three species, *E. canis* (68%), *E. ewingii* (21%) and *E. chaffeensis* (14%) (Ndip *et al.*, 2007). Murphy *et al.*, 1998, in Oklahoma, USA, reported nine pools of ticks positive for *E. ewingii*, seven pools from *Rh. sanguineus*, one pool from *Amblyomma americanum* and one pool from *Dermacentor variabilis* all collected from dogs. Two pools of *Rh. sanguine*us were positive for *E. canis* (Murphy *et al.*, 1998).

In the present study an *Ehrlichia* species with a sequence, 100% similar to *E. ewingii* was the most commonly detected specie. The tick *Rhipicephalus microplus* is probably the main vector (7 pools), however this hypothesis needs to be confirmed in Colombia. One explanation to the high numbers of *Rh. microplus* positives is because the areas where ticks were collected are mostly used for livestock. In contrast, to the studies of Ndipl 2007; Murphy *et al.*, 1998, none of *Rh. sanguineus* pool in this study contained nucleotide sequence similar to *E. canis*.

In south America, molecular evidence of *Ehrlichia*-infected dogs and others animals have been reported in Argentina (Eiras *et al.*, 2013), Venezuela (Gutierrez *et al.*, 2008), Peru (Vinasco *et al.*, 2007), Chile (Lopez *et al.*, 2012), Brazil (Santos *et al.*, 2009) and West Indies (Loftis *et al.*, 2013). Serological evidence of human exposure has been reported from Brazil, Argentina and Colombia (Calic *et al.*, 2004; Hidrón Botero *et al.*, 2014; Ripoll *et al.*, 1999). Tomassone *et al.* in Argentina, reported 9,2% of

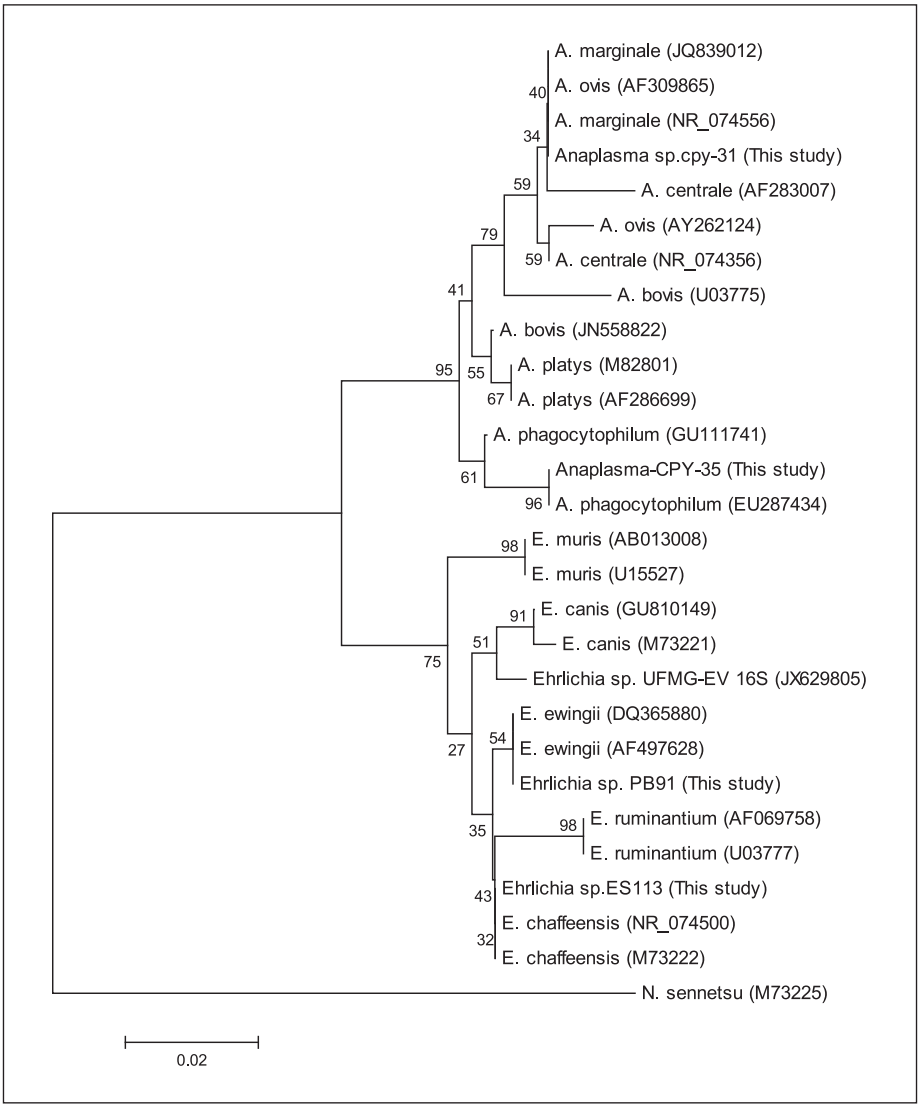

Figure 1. Dendrogram showing the phylogenetic relationships based on nucleotide partial sequences of the 16S rRNA gene (310 bp) of *Anaplasma* and *Ehrlichia* species detected in ticks. The sequences obtained in this study are labeled with parentheses (this study). The tree was constructed using the neighbor-joining method and numbers above internal nodes indicate the percentages of 1,000 bootstrap replicates that supported the branch. GenBank accession numbers are shown in parentheses. *N. sennetsu* sequence was used as outgroup. Evolutionary analyses were conducted using MEGA 6.

Amblyomma parvum were infected with *E. chaffeensis* (Tomassone *et al.*, 2008). In our study we did not analyze *Amblyomma* ticks; but we found an *Ehrlichia* sp. closely related to *E. chaffeensis* (nucleotide sequence 100% similar) in *Rh. microplus*. This results show that others species of ticks in addition to *Amblyomma americanum* (principal vector of *E. chaffeensis* in North America) could be responsible fo transmission in South America.

In Brazil, Widmer *et al.*, 2011 detected DNA of an unknown *Ehrlichia* in *A. triste* and *A. cajennense* ticks collected from free-living jaguars (*Panthera onca*). Partial sequences obtained from these samples resulted in a new ehrlichial strain, designated as *Ehrlichia* sp. strain Jaguar (Widmer *et al.*,

2011). In the same country Cruz *et al.*, 2012, isolated an *Ehrlichia* agent in IDE8 tick cell culture in *Rh. microplus* collected from infested cattle in the state of Minas Gerais. The molecular and phylogenetic analysis based on 16S rRNA, *groEL*, *dsb*, *gltA* and *gp36* genes showed that the *Ehrlichia* spp. isolated is a new species, named Candidatus *Ehrlichia mineirensis* (Cruz *et al.*, 2012). Three sequences detected in *Rh. sanguineus* collected from dogs in the present study were 99% identical to Candidatus *Ehrlichia mineirensis*. further sequence analysis (*gltA* and *gp36* genes) to establish the current status of this strain are necessary, as well as needed to clarify the pathogenic potential, geographic distribution or host range of this agent.

In Colombia, despite the few reports of ehrlichiosis in animals, only *E. canis* has been reported. In 2003, Benavidez *et al.*, diagnosed by serologic test and clinical compatibility a dog with ehrlichiosis (Benavides & Ramírez, 2003). In 2006, in Bucaramanga, 41% (33/80) of dogs were reported seropositives for *E. canis* based on IFA test (Contreras *et al.*, 2007).

In dogs from Villeta, Cundinamarca Hidalgo *et al.*, found seroprevalence of 31.8% to *Ehrlichia* sp. A study in dogs from three cities of Colombia during 2011, reported *E. canis* in 83%, 80% and 26% of dogs in Barranquilla, Cartagena and Medellin respectively (McCown *et al.*, 2014b). Vargas *et al.*, 2012, showed that 82,4% (75/91) of dogs were positives to *E. canis* by IFA test. This study also demonstrated for the first time in Colombia evidence of *E. canis* by molecular detection in the blood of 40.6% of dogs (37/91). Sequences from all positive dogs were 99% identical with *E. canis* isolated from around the world (Vargas-Hernandez *et al.*, 2012).

All reports show *E. canis* to be widely distributed in Colombia; however, other species of *Ehrlichia* genus are unknown in the country. On the other hand, *Anaplasma marginale* is one of the most prevalent tick-transmitted diseases of cattle worldwide. In the United States, economic losses resulting from morbidity and mortality of anaplasmosis are estimated to be over $300 million per year. The disease is the most prevalent hemoparasitoses in Latin America, responsible for economic losses between in $800 and $875 million in these countries (Kocan *et al.*, 2003; Oliveira *et al.*, 2011).

Bovine anaplasmosis is endemic in Central and South America, and the Caribbean Islands. It is enzootic in most Latin American countries, with the exception of desert areas or mountain ranges such as the Andes (Guglielmone, 1995). In this region *A. marginale* is considered to be transmitted by *Rh. microplus* ticks, as shown in our results (7 pools of *Rh. microplus* with *A. marginale*). However, other associations between *A. marginale* and others ticks are very common in different parts of the world. For example, in Tanzania Fyumagwa *et al.*, 2009 detected *A. marginale* in a wide range of tick species, including *Amblyomma gemma*, *Rhipicephalus appendiculatus*, *R. compositus*, *R. decoloratus*, *R. praetextatus* and *R. pulchellus* (Fyumagwa *et al.*, 2009). According to other authors, biological transmission of *A. marginale* is effected by ticks, and approximately 20 species of ticks have been incriminated as vectors worldwide (Kocan *et al.*, 2003).

In Colombia, bovine anaplasmosis is endemic in many areas; however, climatic conditions (high - low temperature), the altitude above sea level and the vector distribution affect it is prevalence. Some serologic studies indicated seroprevalences between 0 and 100% around the country (Kocan *et al.*, 2003). According to Patarroyo *et al.*, 1978, in Córdoba the prevalence of anaplasmosis was 90% (533/590) using an agglutination card test (the results obtained in this survey have confirmed that infection with *A. marginale* is widespread in Córdoba and suggest that anaplasmosis could be endemic in the whole region.

A. phagocytophilum also causes self-limiting, economically significant disease in Europe (Reinbold *et al.*, 2010). *A. phagocytophilum* is the most common tick-borne zoonotic bacteria of livestock and free-living ungulates, which has been found to persist in species such a horse (Franzen *et al.*, 2005). The *Ixodes ricinus* tick has been found to be the main vector of in this region

(Aktas, 2014). We found in our study a pool of *D. nitens* infected with an *Anaplasma* sp. That is closly related to *A. phagocytophilum* (sequence 100% similar), it is possible that the horse where *D. nitens* that was collected was persistently infected with this strain, however, the clinical status of the horse at the moment of tick collection could not be established.

In Europe, the rate of *A. phagocytophilum* infection in *I. ricinus* varies according to locations, from zero to 30% and it is usually higher in adults than nymphs (Blanco & Oteo, 2002; Rosef *et al.*, 2009; Strle, 2004). In the USA, approximately 10–50% of *Ixodes scapularis* ticks are infected (Thomas *et al.*, 2009). In South America, *A. phagocytophilum* has been found in *R. sanguineus* (4,2%) from Argentina (Oscherov *et al.*, 2011). In Brazil, *A. phagocytophilum* was detected in *A. cajennense* (6,6%) and *R. sanguineus* (2,5%) ticks (Santos *et al.*, 2013). There are no reports of *A. phagocytophilum* in ticks from Colombia. In our study *Anaplasma* sp. closed related to *A. phagocytophilum* was found at a very low rate (MIR 1,3%) in *Dermacentor nitens* collected from one horse. This result could be similar to the lowest result found in others locations in South America, North America and Europe.

McCown *et al.*, 2014 in a serology study in dogs from three cities of Colombia during 2011, reported *A. phagocytophilum in* 51%, 40% and 12% in Cartagena, Barranquilla and Medellin cities respectively. However, the same author argues that the test used, could not differentiate between *A. phagocytophilum* and *A. platys* (McCown *et al.*, 2014b). A survey conducted in dogs from Barranquilla by serological and molecular methods showed 53.2% (116 dogs), and 16% (35 samples) were positive for the presence of *A. platys* DNA. In this study, only two samples were seropositive for *A. phagocytophilum*, but PCR negative (McCown *et al.*, 2014a).

Seroepidemiological data suggest that many human infections go unrecognized in Colombia. In Sucre (north of Colombia). A prospective study in people with occupational risk factors (farmers and day workers) found three persons 3,3% (3/90) with IgM antibodies specific for *Ehrlichia chaffeensis* by IFA. (Ríos *et al.*, 2008). A seroprevalence rate of 20% (15/75) *A. phagocytophilum* was reported in livestock farmers living in Cordoba and Sucre (Máttar & Parra, 2006). These data suggest that HME and HGA zoonotic cases may occur in Colombia with more frequency; however, such cases has been not published to date, and the possibility remains that infection is largely underdiagnosed.

In conclusion, little is known regarding the prevalence of *Anaplasma* and *Ehrlichia* species in different tick species in Colombia. Various possible tick-borne pathogens detected in this survey suggest a risk for the emergence of tick-borne diseases in domestic animals and humans in the country. Further surveys involving a larger number of ticks sampled and molecular techniques to confirm the prevalence of tick-borne pathogens of veterinary and medical importance must be carried out across the country. Our data provides the first evidence that *Ehrlichia* and *Anaplasma* species are circulating in ticks collected in the Cordoba north Caribbean region of Colombia.

REFERENCES

Aguiar, D.M., Hagiwara, M.K. & Labruna, M.B. (2008). *In vitro* isolation and molecular characterization of an Ehrlichia canis strain from Sao Paulo, Brazil. *Brazilian Journal Microbiol*ogy **39**: 489-493.

Aktas, M. (2014). A survey of ixodid tick species and molecular identification of tick-borne pathogens. *Veterinary Parasitology* **200**: 276-283.

Barros-battesti, D., Arzua, M. & Bechara, G. (2006). Carrapatos de importância médico veterinária da região Neotropical. Um guia ilustrado para identificação de espécies.

Benavides, J. & Ramírez, G. (2003). Ehrlichiosis canina. *Revista Colombia Cienc Pec* **16**: 268-274.

Blanco, J.R. & Oteo, J.A. (2002). Human granulocytic ehrlichiosis in Europe. *Clinical Microbiol ogy Infection* **8**: 763-772.

Brouqui, P., Bacellar, F., Baranton, G., Birtles, R.J., Bjoersdorff, A., Blanco, J.R., Caruso, G., Cinco, M., Fournier, P.E., Francavilla, E., Jensenius, M., Kazar, J., Laferl, H., Lakos, A., Lotric Furlan, S., Maurin, M., Oteo, J.A., Parola, P., Perez-Eid, C., Peter, O., Postic, D., Raoult, D., Tellez, A., Tselentis, Y. & Wilske, B. (2004). Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. *Clinical Microbiology Infection* **10**: 1108-1132.

Calic, S.B., Galvao, M.A., Bacellar, F., Rocha, C.M., Mafra, C.L., Leite, R.C. & Walker, D.H. (2004). Human ehrlichioses in Brazil: first suspect cases. *Brazilian Journal Infectious Diseases* **8**: 259-262.

Contreras, S., Sanabria, E., Arcila, V., Casteñanos, V. & Bedoya, J. (2007). Validacion diagnostica de pacientes con sintomatologia clínica compatible con Ehrlichiosis mediante evaluacion en frotis sanguíneo, prueba de IFI con antígeno de Ehrlichia canis y cuadro hematico con recuento de plaquetas. *Revista Spei Domus* **3**: 17 - 21.

Cruz, A.C., Zweygarth, E., Ribeiro, M.F., da Silveira, J.A., de la Fuente, J., Grubhoffer, L., Valdes, J.J. & Passos, L.M. (2012). New species of Ehrlichia isolated from Rhipicephalus (Boophilus) microplus shows an ortholog of the *E. canis* major immunogenic glycoprotein gp36 with a new sequence of tandem repeats. *Parasitogy & Vectors* **5**: 291.

Dumler, J.S. (2005). Anaplasma and Ehrlichia infection. *Annals New York Academy Sciences* **1063**: 361-373.

Eiras, D.F., Craviotto, M.B., Vezzani, D., Eyal, O. & Baneth, G. (2013). First description of natural Ehrlichia canis and Anaplasma platys infections in dogs from Argentina. *Comparative Immunology Microbiology Infecioust Disease* **36**: 169-173.

Franzen, P., Aspan, A., Egenvall, A., Gunnarsson, A., Aberg, L. & Pringle, J. (2005). Acute clinical, hematologic, serologic, and polymerase chain reaction findings in horses experimentally infected with a European strain of Anaplasma phagocytophilum. *J Vet Intern Med* **19**: 232-239.

Fyumagwa, R.D., Simmler, P., Meli, M.L., Hoare, R., Hofmann-Lehmann, R. & Lutz, H. (2009). Prevalence of Anaplasma marginale in different tick species from Ngorongoro Crater, Tanzania. *Veterinary Parasitology* **161**: 154-157.

Guglielmone, A.A. (1995). Epidemiology of babesiosis and anaplasmosis in South and Central America. *Veterinary Parasitology* **57**: 109-119.

Gutierrez, C.N., Martinez, M., Sanchez, E., De Vera, M., Rojas, M., Ruiz, J. & Triana-Alonso, F.J. (2008). Cultivation and molecular identification of Ehrlichia canis and Ehrlichia chaffeensis from a naturally co-infected dog in Venezuela. *Veterinary Clinical Pathology* **37**: 258-265.

Hidalgo, M., Vesga, J.F., Lizarazo, D. & Valbuena, G. (2009). A survey of antibodies against Rickettsia rickettsii and Ehrlichia chafeensis in domestic animals from a rural area of Colombia. *American Journal Tropical Medicine & Hygiene* **80**: 1029-1030.

Hidrón Botero, A., Muñoz Ramirez, F. & Vega Miranda, J. (2014). Primer caso de ehrlichiosis monocítica humana reportado en Colombia. *Infection* **18**, 162-166.

Kocan, K.M., de la Fuente, J., Guglielmone, A.A. & Melendez, R.D. (2003). Antigens and alternatives for control of Anaplasma marginale infection in cattle. *Clinical Microbiology Review* **16**: 698-712.

Koebel, C., Kern, A., Edouard, S., Hoang, A.T., Celestin, N., Hansmann, Y., Jaulhac, B., Brouqui, P. & De Martino, S.J. (2012). Human granulocytic anaplasmosis in eastern France: clinical presentation and laboratory diagnosis. *Diagnostic Microbiology Infectious Diseases* **72**: 214-218.

Loftis, A.D., Kelly, P.J., Freeman, M.D., Fitzharris, S., Beeler-Marfisi, J. & Wang, C. (2013). Tick-borne pathogens and disease in dogs on St. Kitts, West Indies. *Veterinary Parasitology* **196**: 44-49.

Lopez, J., Abarca, K., Mundaca, M.I., Caballero, C. & Valiente-Echeverria, F. (2012). Molecular identification of Ehrlichia canis in a dog from Arica, Chile. *Rev Chilena Infectol* **29**: 527-530.

Máttar, S. & Parra, M. (2006). Detection of antibodies to Anaplasma, Bartonella and Coxiella in rural inhabitants of the Caribbean area of Colombia. *Revista MVZ Córdoba* **11**: 781-789.

McCown, M.E., Alleman, A., Sayler, K.A., Chandrashekar, R., Thatcher, B., Tyrrell, P., Stillman, B., Beall, M. & Barbet, A.F. (2014a). Point prevalence survey for tick-borne pathogens in military working dogs, shelter animals, and pet populations in northern Colombia. *Journal Spec Oper Med* **14**: 81-85.

McCown, M.E., Monterroso, V.H. & Cardona, W. (2014b). Surveillance for Ehrlichia canis, Anaplasma phagocytophilum, Borrelia burgdorferi, and Dirofilaria immitis in Dogs From Three Cities in Colombia. *J Spec Oper Med* **14**: 86-90.

Murphy, G.L., Ewing, S.A., Whitworth, L.C., Fox, J.C. & Kocan, A.A. (1998). A molecular and serologic survey of Ehrlichia canis, E. chaffeensis, and E. ewingii in dogs and ticks from Oklahoma. *Vet Parasitol* **79**: 325-339.

Ndip, L.M., Ndip, R.N., Ndive, V.E., Awuh, J.A., Walker, D.H. & McBride, J.W. (2007). Ehrlichia species in Rhipicephalus sanguineus ticks in Cameroon. *Vector Borne Zoonotic Diseases* **7**: 221-227.

Oliveira, J.B., Montoya, J., Romero, J.J., Urbina, A., Soto-Barrientos, N., Melo, E.S., Ramos, C.A. & Araujo, F.R. (2011). Epidemiology of bovine anaplasmosis in dairy herds from Costa Rica. *Veterinary Parasitology* **177**: 359-365.

Oscherov, E.B., Milano, A.M.F., Lobo, B., Anda, P. & Escudero, R. (2011). Detection of Anaplasma platys and other pathogens in ectoparasites from urban hosts in Northeast Argentine. *Revista. Ibero-Latinoam. Parasitology* **70**: 42-48.

Patarroyo, J.H., Villa, O. & Diazgranados, H. (1978). Epidemiology of cattle anaplasmosis in colombia: I. Prevalence and distribution of agglutinating antibodies. *Tropical Animal Health and Production* **10**: 171-174.

Rar, V.A., Livanova, N.N., Panov, V.V., Doroschenko, E.K., Pukhovskaya, N.M., Vysochina, N.P. & Ivanov, L.I. (2010). Genetic diversity of Anaplasma and Ehrlichia in the Asian part of Russia. *Ticks Tick Borne Dieases* **1**: 57-65.

Reinbold, J.B., Coetzee, J.F., Sirigireddy, K.R. & Ganta, R.R. (2010). Detection of Anaplasma marginale and A. phagocytophilum in bovine peripheral blood samples by duplex real-time reverse transcriptase PCR assay. *Journal Clinical Microbiology* **48**: 2424-2432.

Ríos, R., Franco, S., Mattar, S., Urrea, M. & Tique, V. (2008). Seroprevalencia de Leptospira sp., Rickettsia sp. Ehrlichia sp. en trabajadores rurales del departamento de Sucre, Colombia. *Infection* **12**: 90-95.

Ripoll, C.M., Remondegui, C.E., Ordonez, G., Arazamendi, R., Fusaro, H., Hyman, M.J., Paddock, C.D., Zaki, S.R., Olson, J.G. & Santos-Buch, C.A. (1999). Evidence of rickettsial spotted fever and ehrlichial infections in a subtropical territory of Jujuy, Argentina. *American Journal Tropical Medicine Hygiene* **61**: 350-354.

Rosef, O., Radzijevskaja, J., Paulauskas, A. & Haslekas, C. (2009). The prevalence of Anaplasma phagocytophilum in host-seeking Ixodes ricinus ticks in Norway. *Clinical Microbiology Infeciont* **15 Suppl 2**: 43-45.

Santos, F., Coppede, J.S., Pereira, A.L., Oliveira, L.P., Roberto, P.G., Benedetti, R.B., Zucoloto, L.B., Lucas, F., Sobreira, L. & Marins, M. (2009). Molecular evaluation of the incidence of Ehrlichia canis, Anaplasma platys and Babesia spp. in dogs from Ribeirao Preto, Brazil. *Veterinary Journal* **179**: 145-148.

Santos, H.A., Thome, S.M., Baldani, C.D., Silva, C.B., Peixoto, M.P., Pires, M.S., Vitari, G.L., Costa, R.L., Santos, T.M., Angelo, I.C., Santos, L.A., Faccini, J.L. & Massard, C.L. (2013). Molecular epidemiology of the emerging zoonosis agent Anaplasma phagocytophilum (Foggie, 1949) in dogs and ixodid ticks in Brazil. *Parasitogy Vectors* **6**: 348.

Strle, F. (2004). Human granulocytic ehrlichiosis in Europe. *Intestious Journal Medical Microbiology* **293 Suppl 37**: 27-35.

Thomas, R.J., Dumler, J.S. & Carlyon, J.A. (2009). Current management of human granulocytic anaplasmosis, human monocytic ehrlichiosis and Ehrlichia ewingii ehrlichiosis. *Expertimental Review Anti Infectious Ther* **7**: 709-722.

Tomassone, L., Nunez, P., Gurtler, R.E., Ceballos, L.A., Orozco, M.M., Kitron, U.D. & Farber, M. (2008). Molecular detection of Ehrlichia chaffeensis in Amblyomma parvum ticks, Argentina. *Emergency Infectiouss Disease* **14**: 1953-1955.

Vargas-Hernandez, G., Andre, M.R., Faria, J.L., Munhoz, T.D., Hernandez-Rodriguez, M., Machado, R.Z. & Tinucci-Costa, M. (2012). Molecular and serological detection of Ehrlichia canis and Babesia vogeli in dogs in Colombia. *Veterinary Parasitology* **186**: 254-260.

Vinasco, J., Li, O., Alvarado, A., Diaz, D., Hoyos, L., Tabachi, L., Sirigireddy, K., Ferguson, C. & Moro, M.H. (2007). Molecular evidence of a new strain of Ehrlichia canis from South America. *Journal Clinical Microbiology* **45**: 2716-2719.

Widmer, C.E., Azevedo, F.C., Almeida, A.P., Ferreira, F. & Labruna, M.B. (2011). Tick-borne bacteria in free-living jaguars (Panthera onca) in Pantanal, Brazil. *Vector Borne Zoonotic Diseases* **11**: 1001-1005.