Rev. Salud Anim. Vol. 31 No. 1 (2009): 1-7

Review article

DIFFERENCES AND SIMILARITIES BETWEEN *Anaplasma marginale* AND *Anaplasma phagocytophylum*

Belkis Corona and Siomara Martínez

National Centre for Animal and Plant Health (CENSA), Apartado 10, San José de las Lajas, La Habana, Cuba. E-mail: bcorona@censa.edu.cu

***ABSTRACT:* *Anaplasma marginale* and *A. phagocytophilum* are intracellular rickettsial pathogens causing bovine anaplasmosis and human granulocytic anaplasmosis, respectively. Effective vaccines for the control of anaplasmosis are not available despite attempts using different approaches, such as attenuated strains, infected erythrocyte and tick cell-derived purified antigens. Recent reports demonstrated that *A. marginale* and *A. phagocytophilum* co-exist in geographic areas, that concurrent infections may occur in ruminants and ticks and that there are similarities and differences at molecular level between both species. The aim of this article is to make a comparison between the main characteristics of *Anaplasma marginale* and *Anaplasma phagocytophilum*.**

*(Key words: **Anaplasma marginale**; **Anaplasma phagocytophilum**; major surface proteins)*

DIFERENCIAS Y SIMILITUDES ENTRE *Anaplasma marginale* y *Anaplasma phagocytophilum*

***RESUMEN:* *Anaplasma marginale* y *A. phagocytophilum* son rickettsias intracelulares que causan anaplasmosis bovina y anaplasmosis granulocítica humana, respectivamente. Aún no existe una vacuna efectiva para el control de la anaplasmosis, a pesar de haber sido utilizados con este fin cepas atenuadas, eritrocitos infectados y antígenos purificados, derivados de células de garrapatas. Reportes recientes han demostrado que *A. marginale* y *A. phogocytophilum* coexisten en áreas geográficas, que infecciones concurrentes pueden ocurrir en rumiantes y garrapatas y que existen similitudes y diferencias a nivel molecular entre estos dos microorganismos. El objetivo de este trabajo es realizar una comparación entre las principales características de *A. marginale* y *A. phagocytophilim*.**

*(Palabras clave: **Anaplasma marginale**; **Anaplasma phagocytophilum**; proteínas principales de la superficie)*

Anaplasma marginale

A. marginale is a rickettsial organism causing bovine anaplasmosis in cattle with significant economic losses in tropical and subtropical regions worldwide. It invades the erythrocyte and leads to extravascular hemolysis. Ticks are biological vectors of *A. marginale* but the pathogen is often transmitted mechanically to susceptible cattle by blood-contaminated mouthparts of biting flies or fomites. These obligate intracellular organism replicates in membrane-bound parasitophorous vacuoles in bovine erythrocytes or tick cells. Both cattle and ticks become persistently infected with *A. marginale* and thus serve as reservoirs of infection (1).

Many geographic strains of *A. marginale* have been identified, which differ in biology, genetic characteristics and transmissibility by ticks. The genetic diversity of *A. marginale* strains have been characterized using major surface protein (MSP) genes involved in interactions with vertebrate host cells (2). These genes

may have evolved more rapidly than other genes because of selective pressures exerted by the host immune system (3). Some studies have demonstrated genetic variation among different *A. marginale* strains (4), by means of Random Amplified Polymorphic DNA (5); restriction fragment length polymorphism analysis (6), Repetitive Extragenic Consensus (REP/ERIC) PCR patterns (4), PCR assay based on specific sequences of MSP (7;8) and protein sequences (9).

de la Fuente *et al.* (10) results support the hypothesis that genetic heterogenicity observed among strains of *A. marginale* within endemic regions could be explained by cattle movement and maintenance of different genotypes by independent transmission events, due to infection exclusion of *A. marginale* in cattle and ticks, which commonly results in the establishment of only one genotype per animal. However, when distantly related genotypes exist in the same region, infections of a single host with multiple *A. marginale* strains are possible (11).

The presence of different *A. marginale* genotypes in different countries (12) suggests that MSP1a sequences, although conserved during multiplication of the parasite in the bovine host and after transmission by ticks (13), are rapidly changing, resulting in genotype variation within *A. marginale* populations. For this reason the DNA sequence of the *msp*1α gene does not provide a distinct phylogeographical resolution, because of its high variability (7;8). In contrast, msp4 sequences may provide useful phylogeographical information (9).

Anaplasma marginale can persist in ruminants host for the animal whole life (14). In cattle persistently infected with *A. marginale*, there are cyclic peaks of rickettsemia every 2 to 6 weeks containing different variants of the immunoprotective major surface protein MSP2 (15). MSP2 is encoded by a multigene family and sequence variation is achieved by segmental gene conversion of a single polycistronic expression site by different pseudogenes. These pseudogenes contain a hypervariable region and portions of flanking 5´and 3´conserved sequences but they are otherwise truncated and cannot encode full-length MSP2 (16).

Membrane surface proteins can be useful as microbial identifiers and therefore act as antigens eliciting an immune response. Safe vaccine possibilities currently include testing if recombinant major surface protein antigens produce an effective immune response to protect the animal from future infections (17).

Major surface protein 5 (MSP5) of *Anaplasma marginale* was determined to be antigenic and highly conserved among various isolates of *A. marginale* (5;18). Antibodies to MSP5 of *A. marginale* were recognized in both acute stages of infection and chronically infected carrier cattle, a highly sensitive and specific competitive ELISA was developed using this antigen and a monoclonal antibody to MSP5 (19); for these reasons the msp5 gene and the MSP5 protein are the best candidates for *Anaplasma marginale* diagnosis (20).

Anaplasma phagocytophilum

The order Rickettsiales represents an obligate intracellular bacteria that reside in vacuoles of eukaryotic cells, with the potential to cause fatal tick-transmitted diseases in humans and several mammalian species. Recent genetic studies reorganized some species within the order *Rickettsiales*, between the families *Rickettsiaceae* and *Anaplasmataceae* (21). Based on these studies, three organisms, formerly known as *Ehrlichia phagocytophila*, *Ehrlichia equi*, and the HGE (human granulocytic ehrlichiosis) agent, were unified as a single species and now reclassified as *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis, an emerging tick-borne disease in the United State and Europe (22). Closely related to *Ehrlichial* and *Rickettsial* organisms, *A. phagocytophilum* is a small, fragile, Gram-negative bacterium presenting unique challenge for culture, isolation, enumeration, and labelling (23;24). *A. phagocytophilum* has been worldwide detected, particularly in North America and Europe as well as in South Africa, South America, and Asia (25).

Infection with *A. phagocytophilum* has been recognized in a variety of mammalian hosts, including humans, cats, dogs, horses, ruminants, and wildlife species (26). Clinical disease ranges from mild to fatal, and associated findings include fever, anorexia, weight loss, polyarthritis, and possibly meningitis. Laboratory findings most often include thrombocytopenia and/or lymphopenia and neutropenia. Current methods of serologic diagnosis primarily involve the use of an indirect immunofluorescent antibody test (IFA).

The organisms are transmitted by ticks and their life cycle varies with the type of tick species, tick population density, and the wildlife that are indigenous to an specific climate and geographic location. Wildlife animals such as deer, rodents, and other small mammals maintain *A. phagocytophilum* and serve as the reservoir hosts, with transmission to domestic animals and man as a result of tick bites (27).

The outer membrane proteins of *A. phagocytophilum* have not been characterized

systematically. The Omp-1/P44/Msp2 superfamily is the most studied outer membrane protein family of *A. phagocytophilum*. The genome has three *omp-1*, one *msp*2, two *msp*2 homolog, one *msp*4, and 113 *p44* loci encoding proteins belonging to this superfamily (28).

Recent *A. phagocytophilum* genome sequencing data have provided a wealth of new genetic information. However, there is no experimental evidence demonstrating *A. phagocytophilum* surface-exposed proteins in addition to P44. Furthermore, almost one-half of the predicted open reading frames of *A. phagocytophilum* encodes conserved or novel hypothetical proteins that have never been characterized in any bacterium, some of which may be surface proteins. Therefore, it is imperative to use new approaches, including proteomics, to generate a more complete picture of the expression and function of *A. phagocytophilum* surface proteins (28).

The PCR detection of *A. phagocytophilum* DNA is both sensitive and specific for the acute phase diagnosis of anaplasmosis (29). With the advent of real-time PCR and with the increased availability of molecular diagnostic testing, veterinarians should be able to obtain rapid confirmation of the diagnosis of anaplasmosis.

Differences and similarities between *Anaplasma marginale* and *Anaplasma phagocytophilum.*

Anaplasma marginale and *A. phagocytophilum* are closely related, obligate intracellular, tick-borne rickettsial animal pathogens that parasitize two very different host cell types. Only ruminants are known to be susceptible to *A. marginale*. Whereas the spectrum of species infected with the zoonotic *A. phagocytophilum* includes small rodents, ruminants, dogs, horses and humans (21).

It is thought that *A. marginale* exclusively infect bovine erythrocytes. Acute bovine erythrocytic anaplasmosis is characterized by severe anemia, icterus and hemoglobinuria due to removal of infected cells. Animals recovered remain chronically infected for life, and experience regular, but low level of parasitemia (30). Each rickettsemia peak is composed of new antigenic variants that are selected following a specific antibody responses by the host. By contrast, *A. phagocytophilum* infections produce an acute, febrile illness accompanied by appearance of characteristic colonies of the microbes in peripheral blood neutrophil granulocytes as well as their precursors in the bone marrow (31) with concomitant impairment of resistance to secondary infection.

A. phagocytophilum can be cultured *in vitro* in a human promyelocytic cell line, HL-60, as well as in cell lines ISE6 and IDE8 from the North American tick vector, *Ixodes scapulari* (32;33). *A. marginale* can likewise be propagated in Ixodes tick cell lines. However, a continuous mammalian culture system has been lacking for *Anaplasma marginale*, and the corresponding cell surface receptors are unknown. Moreover, no nucleated host cells of *A. marginale* have been identified, and none of the cell lines that support *A. phagocytophilum* could be infected with *A. marginale* (34).

There are similarities at the molecular level between *A. margianle* and *A. phagocytophilum*. Similar to *A. marginale*, *A. phagocytophilum* uses combinatorial mechanisms to generate a large array of outer membrane protein variants. Such gene polymorphism has profound implications for the design of vaccines, diagnostic test and therapy (30).

In *Anaplasma marginale*, persistence is associated with antigenic variation of the immunoprotective outer membrane protein MSP2. Extensive diversity of MSP2 is achieved by combinatorial gene conversion of a genomic expression site by truncated pseudogenes. The major membrane protein of *A. phagocytophilum*, MSP2 (P44), homologous to MSP2 of *Anaplasma marginale*, has a similar organization of conserved and variable regions, and it is also encoded by a multigene family containing some truncated gene copies (16). This suggests that the two organisms could use similar mechanisms to generate diversity in outer membrane proteins from their small genomes.

As in *A. marginale* infections, a dominant antibody response in patients infected with *A. phagocytophilum* is expressed against a variable 40 kDa outer membrane protein MSP2 (P44) (35). The gene encoding MSP2 (P44), like *msp*2, is a member of a cross-hybridizing multigene family and it is homologous to *A. marginale msp*2 (60 to 66% similarity and 40 to 53% identity), depending of the gene and the strain. Sequence alignment of different *msp*2 (p44) variants and *A. marginale msp*2 reveals significant variation in the same central hypervariable region (36). As in *A. marginale*, the *A. phagocytophilum* genome contains incomplete msp2 (p44) genes with a unique central hypervariable region and conserved 5´and 3´ flanking sequences (37) that could be a source of diversity for combinatorial recombination mechanisms. The results obtained by Barbet *et al.* (30), related with MSP2 (P44) from *A. phagocytophilum* and MSP2 from *A. marginale* suggest that similar mechanisms for generating outer membrane protein diversity and

establishing infections are available to the two organisms.

A. marginale St. Maries is reported to have 56 genes that have been placed into this superfamily, including eight *msp*2, eight *msp*3, one *msp*4, three *opag,* 15 *omp-1,* 12 *orfX,* seven *orfY,* and two *msp*3 remnants. These genes are scattered throughout the genome with a bias in location towards the origin of replication. MSP2 and MSP3 are the immunodominant proteins. The *msp2* and *msp* gene subsets each include one full-length expression locus and seven reserve/silent sequences that are thought to recombine into the expression locus to generate antigenic variation (38).

The *A. phagocytophilum* genome has three *omp-1,* one *msp2,* two *msp2* homolog, one *msp*4, and 113 *p44* loci belonging to the OMP-1/MSP2/P44 superfamily. Although both *Anaplasma* spp. *msp*2 genes are members of PF01617 and the OMP1/MSP2/P44 superfamily, the *A. marginale msp*2 gene is distinct from the *A. phagocytophilum msp*2 gene. In addition, the previously identified *omp-1N* is not a member of this Pfam, but it is homologous to *E. chaffeensis omp-1N* and the *msp*2 operon-associated gene 3 of *A. marginale* (39).

Other outer membrane proteins have been reported in *A. marginale,* including *msp*5, *msp*1α, and *msp*1β. The *msp*5 gene (a SCO1/SenC family protein) is found in all the Rickettsiales, whereas *msp*1α and *msp*1β are unique to *A. marginale*. Sequence homologies have also been shown for the MSP4 gene of the two pathogens (2).

Even before the recent reclassification within the family *Anaplasmataceae*, the *msp*5 gene was known to be highly conserved among all *Anaplasma* species, (11) which, at that time, included *A. marginale*, *A. centrale*, and *A. ovis* (18). Based on 16S rRNA gene sequence similarity, *A. phagocytophilum* and *A. platys* were placed within the same family (21). Strik *et al.* (25) demonstrated the high conservation of MSP5 of *A. phagocytophilum* among various isolates in the United State and Europe. Because of the cross-reactivity between the MSP5 orthologs of *A. phagocytophilum* and *A. marginale*, the commercial available cELISA should be used in epidemiological studies where distinctions between these two infectious agents in cattle are necessary. However, MSP5 or MAP2 might serve as a screening tool for the rapid clinical diagnosis of ehrlichiosis and/or anaplasmosis in various species.

Recombinant MSP5 of *A. marginale* has been used as a diagnostic-test antigen in an indirect-ELISA format for surveying *A. marginale* infection in cattle in several countries. The results obtained by Alleman *et al.* (40) indicate that an indirect ELISA using rMSP5 of *A. marginale* cannot distinguish between infections with *A. phagocytophilum* and infections with *A. marginale*. Cattle infected with either organism will likely be seropositive, giving rise to false representation of disease incidence in a particular area.

A major protein antigen(s) is expressed on the outer membrane of *A. phagocytophilum*, and some of the immunodominant major surface proteins (MSPs) share sequence similarity with *Anaplasma marginale* MSP2 and MSP4 (41;42) and *Ehrlichia ruminantium* MAP1 (43). Although the biological function of *A. marginale* MSP4 is unknown, this MSP is probably involved in host-pathogen interactions and may evolve more rapidly than other nuclear gene proteins because of selective pressures exerted by host immune systems. Furthermore, the analysis of *msp4* sequences provided phylogeographic patterns for *A. marginale* strains (9).

Although *A. phagocytophilum* has a broad geographic distribution, all strains identified thus far appear to have considerable serological cross-reactivity and a minor degree of variation in the nucleotide sequences of the 16S rRNA, *groESL*, *gltA*, *ank*, and *msp*2 genes (44), with the exception of some *ank* sequences from infected German ticks that are different from other *ank* sequences of human and animal strains (21). However, the clinical and host tropism diversity of *A. phagocytophilum* suggests the presence of genetic differences among these bacteria that have not been characterized.

REFERENCES

1. Kocan KM, de la Fuente J, Blouin EF, Garcia-Garcia JC. *Anaplasma marginale* (Rickettsiales: Anaplsmataceae): recent advances in defining host-pathogen adaptations of a tick borne rickettsia. Parasitol. 2004;129:285-300.

2. de la Fuente J, Massung RF, Wong SJ, Chu FK, Lutz H, Meli M, et al. Sequence analysis of the *msp4* gene of *Anaplasma phagocytophilum* strains. J Clin Microbiol. 2005;43:1309-1317.

3. de la Fuente J, Rubial P, Mtshali MS, Naranjo V, Shuqing L, Mangold AJ, et al. Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequence. Vet Microbiol. 2006;119:382-390.

4. Ferreira AM, Suzart S, Vidotto O, Knowles DP, Vidotto MC. Use of the repetitive DNA elements to define genetic relationships among *Anaplasma marginale* isolates. FEMS Microbiol Lett. 2001;197:139-143.

5. Corona B, Muñoz ML, Hernández M, Martínez S. Use of random amplified polymorphic DNA markers for the detection of genetic variation in *Anaplasma marginale* isolates. Rev Salud Anim. 2004;26(1):29-34.

6. Krueger C and Buening GM. *Isolation and restriction endonuclease cleavage of Anaplasma marginale* DNA in situ I agarose. J Clin Microbiol. 1988;26:906-910.

7. Lew AE, Bock RE, Minchin CM, Masaka S. A msp1 polymerase chain reaction assay for specific detection and differentiation of *Anaplasma marginale* isolates. Vet Microbiol. 2002;86:325-335.

8. Corona B, Minet C, Albina E, Vega A, Martínez, S. Sequence of *msp1á* gene of *Anaplasma marginale* Havana isolate and expression in eukaryotic cells. Spanish J Agricul Res. 2005;3(3):275-280.

9. de la Fuente J, Passos LM, Van Den Bussche RA, Ribeiro MF, Facury-Filho EJ, Kocan KM. Genetic diversity and molecular phylogeny of *Anaplasma marginale* isolates from Minas Gerais, Brazil. Vet Parasitol. 2004;121(3-4):307- 316.

10. de la Fuente J, Ruybal P, Mtshali MS, Naranjo V, Shuqing L, Mangold AJ, et al. Analysis of world strains of *Anaplasma marginale* using major surface protein 1a repeat sequences. *Vet Microbiol.* 2007;119:382-390.

11. Palmer GH, Knowles DP, Rodríguez JL, Gnad DP, Hollis LC, Marston T, et al. Stochastic transmission of multiple genotypically distinct *Anaplasma marginale* strain in a herd with high prevalence of Anaplasma infection. J Clin Microbiol. 2004;42:5381-5384.

12. Palmer GH, Rurangirwa FR, McElwain TF. Strain composition of the ehrlichia *Anaplasma marginale* within persistently cattle, a mammalian reservoir for the tick transmission. J Clin Microbiol. 2001;39:631-635.

13. Bowie MV, de la Fuente J, Tocan KM, Blouin EF, Barbet AF. Conservation of major surface protein 1 genes of the ehrlichial pathogen *Anaplasma marginale* during cyclic transmission between ticks and cattle. *Gene*. 2002;282:95-102.

14. Palmer GH, Brown WC, Rurangirwa FR. Antigenic variation in the persistence and transmission of the ehrlichia *Anaplasma marginale.* Microbes Infect. 2000;2(2):167-76.

15. French DM, McElwain TF, McGuire TC, Palmer GH. Expression of *Anaplasma marginale* major surface protein 2 variants during persistent cyclic rickettsemia. Infect Imun. 1998;66:1200- 1207.

16. Brayton KA, Palmer GH, Lundgren A, Yi J, Barbet AF. Antigenic variation of *Anaplasma marginale* msp2 occurs by combinatorial gene conversion. Molecular Microbiology. 2002;43:1151-1159.

17. Kawasaki PM, Kano FS, Tamekuni K, Garcia JL, Marana ER Vidotto O, et al. Immune response of BALB/c mouse immunized with recombinant MSPs proteins of *Anaplasma marginale* binding to immunostimulant complex (ISCOM). Res Vet Sci. 2007;1(3):347-354.

18. Knowles DS, Torioni de Echaide S, Palmer GH, McGuire T, Stiller D, McElwain TF. Antibody against *Anaplasma marginale* MSP5 epitope common to tick and erythrocyte stages identifies persistently infected cattle. J Clin Microbiol. 1996;34:2225-2230.

19. Torioni de Echaide S, Knowles DP, McGuire TC, Palmer GH. Suarez CE, McElwain FF. Detection of cattle naturally infected with *Anaplasma marginale* in a region of endemicity by nested PCR and a competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5. J Clin Microbiol. 1998;36:777-782.

20. Corona B. Utilization of *msp5* gene and MSP5 recombinant protein of *Anaplasma marginale* Havana isolate in the detection of bovine anaplasmosis. PhD thesis. 2004.

21. Dumler JS, Barbet AF, Bekker CP, Dasch GA, Palmer GH, Ray SC, et al. Reorganization of genera in the families *Rickettsiaceae* and *Anaplasmataceae* in the order *Rickettsiales:* unification of some species of *Ehrilichia* with

Anaplasma, *Cowdria* with *Ehrlichia* and *Ehrilichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and HE agent as subjective synonyms of *Ehrlichia phagocytophila*. Int J Syst Evol Microbiol. 2001;51:2145-2165.

22.Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. J Clin Microbiol. 1994;32: 589-595.

23.Borjesson DL. Culture, Isolation, and Labeling of *Anaplasma phagocytophilum* for Subsequent Infection of Human Neutrophils. Book Title: Bacterial Pathogenesis: Methods and Protocols. Series: Methods in Molecular Biology. 2008;431.

24.McQuiston JH, McCall CL, Nicholson WL. Ehrlichiosis and related infections. J Am Vet Med Assoc. 2003;223:1750-1756.

25.Strik NI, Alleman AR, Barbet AF, Sorenson HL, Wamsley HL, Gaschen FP, et al. Characterization of *Anaplasma phagocytophilum* major surface protein 5 and the extent of its cross reactivity with *Anaplasma marginale*. Clinic Vaccine Immunol. 2007;14(23): 262-268.

26.Foley JE, Foley P, Madigan JE. Spatial distribution of seropositivity to the causative agent of granulocytic ehrlichiosis in dogs in California. Am J Vet Res. 2001;62:1599-1605.

27.Preziosi DE, Cohn LA. The increasingly complicated story of Ehrlichia. Compend Contin Educ Pract Vet. 2002;24:277-288.

28.Hotopp JC, Lin M, Madupu R, Crabtree J, Angiuoli SV, Eisen J, et al. Comparative genomics of emerging human ehrlichiosis agents. PLoS Genet. 2006;2:e21.

29.Vancouver I, Lester SJ, Breitschwerdt EB, Collis CD, Hegarty BC. *Anaplasma phagocytophilum* infection (granulocytic anaplasmosis) in a dog from Vector-Borne and Zoonotic Diseases. Can Vet J. 2005;46(9):825-827.

30.Barbet AF, Meeus PF, Belanger M, Bowie MV, Yi J. Expression of multiple outer membrane protein sequence variants from a single genomic locus of *Anaplasma phagocytophilum*. Infect Immun 2003;71:1706-1718.

31.Klein MB, Miller JS, Nelson CM, Goodman JL. Primary bone marrow progenitors of both granulocytic and monocytic lineages are susceptible to infection with the agent of human granulocytic ehrlichiosis. J Infect Dis. 1997;176:1405-1409.

32. de la Fuente J, Almazán C, Van Den Bussche RA, Bowman J, Yoshioka JH, Tocan KM. Characterization of genetic diversity in *Dermacentor andersoni* (Acari: Ixodidae) with body size and weight polymorphism. Exp. Parasitol. 2005;10(1):16-26.

33.Munderloh UG, Jauron SD, Fingerle V, Leitritz L, Hayes SF, Hautman JM, et al. Invasion and intracellular development of the human granulocytic ehrlichiosis agent in tick cell culture. J Clin Microbiol. 1999;37:2518-2524.

34.Munderloh UG, Lymch MJ, Herron MJ, Palmer AT, Kurtti TJ, Nelson RD, et al. Infection of endothelial cells with *Anaplasma marginale* and *A. phagocytophilum*. Vet Microbiol. 2004;101:53-64.

35.Ijdo JW, Zhang Y, Hodzic E, Magnarelli LA, Wilson ML, Telford SR, et al. The early humoral response in human granulocytic ehrlichiosis. J Infect Dis. 1997;176:687-692.

36.French DM, Brown WC, Palmer GH Emergence of *Anaplasma marginale* antigenic variants during persistent rickettsemia Infect Immun. 1999;67:5834-5840.

37.Zhi N, Ohashi N, Rikihisa Y. Multiple p44 genes encoding major outer membrane proteins are expressed in the human granulocytic ehrlichiosis agent. J Biol Chem. 1999;274: 7828-17836.

38.Brayton KA, Kappmeyer LS, Herndon DR, Dark MJ, Tibbals DL. Complete genome sequencing of *Anaplasma marginale* reveals that the surface is skewed to two superfamilies of outer membrane proteins. Proc Natl Acad Sci USA. 2005;102:844-849.

39.Barbet AF, Lundgren AM, Alleman AR, Stuen S, Bjöersdorff A, Brown RN, et al. Structure of the expression site reveals global diversity in MSP2/P44 variants in *Anaplasma phagocytophilum*. Infect Immun. 2006;74: 6429-6437.

40. Alleman AR, Barbet AF, Sorenson HL, Strik NI, Wamsley HL, Song S, Munderloh E, Ramswamey C, Gaschen FP, Luckschander N, Bjoersdorff A. Cloning and expression of the gene encoding the major surface protein 5 (MSP5) of *Anaplasma phahogocytophilum* and potential application for serodiagnosis. Vet Clin Pathol. 2006;35:418-425.

41. Lin Q, Rikihisa Y, Felek X, Wang S, Massung RF, Woldehiwet, Z. *Anaplasma phagocytophilum* has a functional *msp2* gene that is distinct from p44. Infect Immun. 2004;72:3883-3889.

42. Vidotto MC, Kano SF, Gregori F, Headley SA, Vidotto O. Phylogenetic analysis of *Anaplasma marginale* strains from Paraná state, Brazil, using the msp1α and msp4 genes. J Vet Med. 2006;53:404-411.

43. van Vliet AH, Jongejan F, van Kleef M, van der Zeijst BA. Molecular cloning, sequence analysis, and expression of the gene encoding the immunodominant 32-kilodalton protein of *Cowdria ruminantium*. Infect Immun. 1994;62:1451-1456.

44. Chae JS, Foley JE, Dumler JS, Madigan JE. Comparison of the nucleotide sequences of 16S rRNA, 444 *Ep-ank*, and *groESL* heat shock operon genes in naturally occurring *Ehrlichia equi* and human granulocytic ehrlichiosis agent isolates from Northern California. J Clin Microbiol. 2000;38:1364-1369.

(Recibido 2-8-2008; Aceptado 21-12-2008)