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Hematology and serology analysis of healthy and *Anaplasma phagocytophilum* infected equids in Minas Gerais, Brazil. Análise hematológica e sorológica de equídeos saudáveis e naturalmente infectados por *Anaplasma phagocytophilum* em Minas Gerais, Brasil.

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Abstract

Anaplasma phagocytophilum is an obligatory intracellular bacterium that causes equine granulocytic anaplasmosis (EGA), that is characterized by fever, anorexia, depression, limb oedema. Hematological and serological alterations are important findings during the disease. We analyzed whole blood and serum from 37 horses and mules from three locations in Minas Gerais, Brazil, for total blood cell count, leucocytes, platelets, renal and hepatic profiles. Equids infected by *A. phagocytophilum* had differences in blood cell count and renal and hepatic profiles. As a zoonosis, the study of equine *A. phagocytophilum* infection in areas with high population density and in breeding farms, is important to understand the disease dynamics and avoid human cases of anaplasmosis.
Keywords: Equine Granulocytic Anaplasmosis. Zoonosis. Horses. Tick-borne pathogen.

Resumo

O *Anaplasma phagocytophilum* é uma bactéria intracelular obrigatória que causa a anaplasmose granulocítica equina, caracterizada por febre, anorexia, edema de membros. Alterações hematológicas e sorológicas são importantes achados durante o curso da doença. Analisamos sangue total e soro de 37 cavalos e mulas de três localidades de Minas Gerais, Brasil, para contagem celular total, leucócitos, plaquetas e perfis renal e hepático. Equídeos infectados por *A. phagocytophilum* apresentaram diferenças na contagem celular sanguínea e nos perfis renal e hepático. Sendo uma zoonose, o estudo da infecção por *A. phagocytophilum* em áreas de alta densidade populacional e em haras de reprodução é importante para entender a dinâmica da doença e impedir casos humanos da doença.
Palavras-chaves: Anaplasmose Granulocítica Equina. Zoonose. Cavalos. Patógenos transmitidos por carrapatos.

1. Introduction

Equine granulocytic anaplasmosis is an infectious disease caused by *Anaplasma phagocytophilum*, an obligatory intracellular bacterium, which infects preferably neutrophils. It has long been renamed, based on genetic and morphological analysis and *A. phagocytophilum* is now the former *Ehrlichia equi* and *Ehrlichia phagocytophila* (DUMLER et al., 2001; MERINO CHARREZ et al., 2021; VON LOEWENICH et al., 2003). First description of the agent infecting equines occurred in 1969 in California state, and in 1994 it was also described causing disease in humans (BAKKEN et al., 1994; GRIBBLE, 1969).

Disease in animals and humans are characterized by fever, anorexia, depression, limb oedema. Other symptoms may be present due the aggravation of it and the sensibility of the host. Inclusion body in neutrophils are observed in blood stream smears. Also, leucopenia, platelets reduction, and mild anemia are described as hematological findings during the disease. In most cases, treatment with tetracyclines are needed to eliminate the pathogen from blood stream (DUMLER, 2012; DUMLER et al., 2005; PRADO; PALHARES; MIRANDA, 2017).

Inclusion body detection and PCR analysis are considered the definitive diagnostic method (DE LA FUENTE et al., 2007; GAVIÃO PRADO, 2014; PRADO; PALHARES; MIRANDA, 2017), although clinical signs may help clinicians and practitioners to decide what protocol to follow during hospital referral and treatment (DUMLER, 2012).

Close contact between wild life, domestic and peri domestic animals are essential for maintenance of the agent in animals and humans (LESICZKA et al., 2021). In recent years more researches have shown the importance of the agent as source of financial loss in livestock and a real public health problem since it has a zoonotic potential (ATIF, 2015; WOLDEHIWET, 2010).

As a potential zoonosis and the co infection with other bacteria that may aggravate symptoms, the study of equine *A. phagocytophilum* infection in areas with high population density as Belo Horizonte and in breeding farms, where there are a close contact of equids and people, such as veterinarians and care takers, is important to understand the disease dynamics and avoid human cases of anaplasmosis.

2. Material and Methods

This study was approved by Universidade Federal de Minas Gerais's Ethical committee in Animal Use number 163/2013.

2.1 Studied population

It was used 37 equids that were diagnosed for equine granulocytic anaplasmosis by buffy coat smears analysis or by PCR as the positive group and another 37 equids that tested negative for IFA and/or PCR as the negative group. Animals originated from three different location in the state of Minas Gerais, which was Belo Horizonte, Ataléia, and São Vicente de Minas.

Animals from Belo Horizonte are draft hoses that are used to collect and discard garbage and civil construction disposals. Animals from Ataléia and São Vicente de Minas are from breeding farms. In both cases there is a close relation between humans and animals, characterizing the risk of transmission of *A. phagocytophilum* from equids to humans.

2.2 Sample collection and analysis

2.2.1 Ticks

Ticks infecting animals at the time of the clinical analysis were collected and fixed in 70% alcohol and analysis was performed as described by Aragão; Fonseca (1961).

2.2.2 Blood samples

Blood collection was performed by venipuncture of external jugular after cleaning the area with 70% alcohol. Blood was kept in two different tubes containing EDTA and clot activator. All samples were processed within 2 hours after collection and, when possible, freezed at -20º C until the analysis.

2.2.3 Blood cell count and serum biochemistry

Within 2 hours after collection, blood smears were done using blood from the EDTA tube and stained with modified Romanowsky staining. At the same moment, buffy coat smears were done and stained with Romanowsky stain. Both slides were left to dry at room temperature and stored in proper plastic boxes until analysis.

Differential leucocyte count was performed in light microscope by counting 100 cells and calculating absolute values using the percentage of cells counted.

Fibrinogen was calculated after determination of total protein by refratometry and a new analysis after denaturation of the plasma at 56º C for three minutes.

After clot retraction, tubes were centrifuged at 1200 x g for 5 minutes and serum was collected and stored in microtubes at -20ºC until analysis.

Biochemistry was performed in semi-automatic machine using specific kits for renal profile (urea and creatinine) and hepatic profile (gama glutamil transferase [GGT], aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase, albumin, and total proteins).

3. Results and discussion

Determination of ticks' species were performed and as expected, in horses the most prevalent and important tick specie found was *Dermacentor* (*Anocentor*) *nitens* (Fig. 1A and B) and *Amblyomma cajennense*. Although the number of animals parasitized was almost the same in the three locations, there was a difference in the number of tick species, been *Dermacentor* (*Anocentor*) *nitens* (30 specimens) most prevalent then the *Amblyomma cajennense* (10 specimens). Another importat find was that in the breeding farms, the presence of *Rhipicephalus* (*Boophilus*) *microplus* parasitizing horses was common.

Figure 1 - A. Equine pina infested by *Dermacentor* (*Anocentor*) *nitens*. B. Perianal area infested by *Dermacentor* (*Anocentor*) *nitens* in another animal. Note that in both animals the number of ticks is high, with presence of different development stages of the ticks.

No tick belonging to *Ixodes persulcatus* complex was identified during the study. These species are related to *A. phagocytophilum* transmission in the United States and in European countries. So far, there is no description of these species in Brazil (ARAGÃO; FONSECA, 1961; SALVAGNI et al., 2010). Until now, the importance of the species found in the study in the transmission and maintenance of the disease in Brazil was not well established.

In Europe and North America, *Ixodes scapularis* and *Ixodes ricinus* are responsible for the transmission of the agent to animals and humans, and together with *Borrelia burgdorferi* they are considered important zoonosis and a public health problem (ASMAN et al., 2017; BEN; LOZYNSKYI, 2019; ELIAS et al., 2021; HAUCK et al., 2019; ROSEF; PAULAUSKAS; RADZIJEVSKAJA, 2009; SKARPHÉDINSSON et al., 2007; STAŃCZAK et al., 2004).

We compared hematological and serological profiles from healthy and naturally infected equids and most relevant results are presented in table 1. Among all the analyzed hematological and serological parameters, there was difference between healthy and infected equids in the erythrocytes, VCM, HCM, band neutrophils, albumin, globulin, and the albumin: globulin ratio. The number of platelets did not differ between groups, but p value was 0,0592.

Table 1. Hematological and serological values of healthy and *Anaplasma phagocytophilum* infected equids from three locations in Minas Gerais, Brazil.

Parameter	Healthy (X±SE)	Infected (X±SE)
Erythrocytes (x10^6/ µl)	8.19±0.29^a	7.42±0.27^b
MCV (fl)	38.3±0.92^b	45.91±4.7^a
MCH (pg)	13.8±0.4^b	16.5±1.42^a
Band neutrophils (%)	0.27±0.14^b	0.81±0.22^a
Band neutrophils (x10^3/µl)	31.96±16.2^b	133.6±60.36^a
Albumin (md/dl)	3.24±0.14^a	2.82±0.12^b
Globulin (mg/dl)	3.57±0.17^b	4.07±0.15^a
Albumin:Globulin ratio	1.19±0.19^a	0.77±0.08^b
ALT (IU/L)	8.56±0.65^a	7.1±0.6^b

AST (IU/L)	320.45±9.14[a]	295.59±9.94[b]
Platelets (cells/ µl)	180,726.38±19,010.18	116,687.89±16,593.15

X: mean value. SE: standard error. Mean values followed by different letters in the same line differ between each other ($p<0,05$), analyzed by Wilcoxon test.

During acute phase, the presence of the bacteria in the blood stream is easily detected by evaluation of buffy coat smears and animals present alterations in hematology and biochemistry. Anemia is one of the most observed signs in equine granulocytic anaplasmosis, and although both groups have normal erythrocyte count considered normal for the specie, the infected group have lower values when compared to the healthy group. This reduction is probably immune mediated, and red cells are destructed by monocytic phagocytic system (LEPIDI et al., 2000).

Band neutrophil increase both in absolute and relative numbers shows that infected animals presented neutropenia at some point of the infection course. Body response to neutropenia is the release of young forms of neutrophils to try to eliminate the infection (AGUERO-ROSENFELD, 2002; MERINO CHARREZ et al., 2021; PRADO; PALHARES; MIRANDA, 2017).

In concern of the protein profile, it was observed an increase in globulins concentration and a decrease in albumin concentration. The albumin: globulin ratio was also reduced. During acute phase there is a high immunoglobulins production and secretions, probably IgM and IgA, with a conversion to IgG after two weeks of infection. As they are responsible for bacteria recognition and opsonization, globulins increase is expected. It is important to highlight that even Ig in augmented during acute and chronic phases, since *A. phagocytophilum* is an obligatory bacterium, cellular defense, such as NK and $CD8^+$ cells, are the most important to eliminate infected cells (CONTRERAS et al., 2017; DUMLER et al., 2005; NAIMI et al., 2020; SCORPIO; CHOI; DUMLER, 2018; TORINA et al., 2020).

Albumin in the most important protein in the bloodstream, being responsible of maintenance of blood oncotic pressure. It is also responsible for transporting substances, such as drugs and hormones. In cases that there is a high protein consumption without increased intake, animals and humans may use it as a protein source, leading to a decrease in albumin levels in serum, in order to keep homeostasis (EL HAMIANI KHATAT et al., 2021; HUANG et al., 2020; SILAGHI et al., 2018; UEMURA et al., 2019; YIN et al., 2018). During EGA, due to fever and myalgia, animals tend to ingest less food, and, on the other hand, there is an increase in the need of protein intake, to maintain cell metabolism. In this scenario, the only protein source for these animals is serum albumin and, with that, we see a reduction in its values in infected horses.

AST and ALT values were lower in the positive group than in the infected one. Although both groups presented values within references, it was expected that those enzymes were elevated, since there must be a hepatic overload and direct lesion during acute phase (AGUERO-ROSENFELD, 2002; LEPIDI et al., 2000)

4. Conclusion

Equine granulocytic anaplasmosis is an important disease with high occurrence in Europe and in North America. In Brazil, seroprevalence of the disease is high, although animals presenting clinical signs are not related in the same rate. Mild anaplasmosis may be presented with only hematological and serological alterations, and that may be a challenge to clinicians to properly diagnose the disease.

With the potential of human infection and death, the study of the dynamics of animal disease in areas with high population density and in breeding farms is mandatory and with these results public policies can be directed to identify and control infected ticks and hosts.

References

AGUERO-ROSENFELD, M. E. Diagnosis of human granulocytic Ehrlichiosis: State of the Art. **Vector Borne and Zoonotic Diseases**, v. 2, n. 4, p. 233-239, 2002. https://pubmed.ncbi.nlm.nih.gov/12804164/

ARAGÃO, H.; FONSECA, F. Notas de Ixodologia. VIII. Lista e chave para os representantes da fauna ixodológica brasileira. **Memórias do Instituto Oswaldo Cruz**, v. 59, n. 2, 1961.

ASMAN, M. et al. *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi* sensu lato and *Toxoplasma gondii* in *Ixodes ricinus* (Acari, Ixodida) ticks collected from Slowinski National Park (Northern Poland). **Journal of Vector Ecology**, v. 42, n. 1, p. 200-202, 2017. https://pubmed.ncbi.nlm.nih.gov/28504439/

ATIF, F. A. *Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsiales pathogens of veterinary and public health significance. **Parasitology Research**, v. 114, n. 11, p. 3941-3957, 2015. https://link.springer.com/article/10.1007/s00436-015-4698-2

BAKKEN, J. S. et al. Human Granulocytic Ehrlichiosis in the Upper Midwest United States: A New Species Emerging? **JAMA: The Journal of the American Medical Association**, v. 272, n. 3, p. 212-218, 1994. https://pubmed.ncbi.nlm.nih.gov/8022040/

BEN, I.; LOZYNSKYI, I. Prevalence of *Anaplasma phagocytophilum* in *Ixodes ricinus* and *Dermacentor reticulatus* and Coinfection with *Borrelia burgdorferi* and Tick-Borne Encephalitis Virus in Western Ukraine. **Vector Borne and Zoonotic Diseases**, v. 19, n. 11, p. 793-801, 2019. https://pubmed.ncbi.nlm.nih.gov/31211655/

CONTRERAS, M. et al. Vaccinomics approach to the identification of candidate protective antigens for the control of tick vector infestations and *Anaplasma phagocytophilum* infection. **Frontiers in Cellular and Infection Microbiology**, v. 7, p. 1-15, 2017. https://www.frontiersin.org/articles/10.3389/fcimb.2017.00360/full

DE LA FUENTE, J. et al. Sequence analysis of the msp4 gene of *Anaplasma ovis* strains. **Veterinary Microbiology**, v. 119, n. 2-4, p. 375-381, 2007. https://pubmed.ncbi.nlm.nih.gov/17052866/

DUMLER, J. S. et al. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: Unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. **International Journal of Systematic and Evolutionary Microbiology**, v. 51, n. 6, p. 2145-2165, 2001. https://pubmed.ncbi.nlm.nih.gov/11760958/

DUMLER, J. S. et al. Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. **Emerging Infectious Diseases**, v. 11, n. 12, p. 1828-1834, 2005. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3367650/

DUMLER, J. S. The biological basis of severe outcomes in *Anaplasma phagocytophilum* infection. **FEMS Immunology and Medical Microbiology**, v. 64, n. 1, p. 13-20, 2012. https://pubmed.ncbi.nlm.nih.gov/22098465/

EL HAMIANI KHATAT, S. et al. Epidemiological and Clinicopathological Features of *Anaplasma phagocytophilum* Infection in Dogs: A Systematic Review. **Frontiers in Veterinary Science**, v. 8, p. 1-27, 2021. https://pubmed.ncbi.nlm.nih.gov/34250067/

ELIAS, L. et al. The Microbiota of *Ixodes ricinus* and *Dermacentor reticulatus* Ticks Collected from a Highly Populated City of Eastern Europe. **Microbial Ecology**, 2021. https://pubmed.ncbi.nlm.nih.gov/34767049/

GAVIÃO PRADO, L. **Avaliação clínica e laboratorial de equídeos sororreagentes para *Anaplasma phagocytophilum* (Rickettsiales: Anaplasmataceae) em Minas Gerais, Brasil**. 74p. Dissertação (Mestrado) - Escola de Veterinária, Universidade Federal de Minas Gerais, 2014. https://repositorio.ufmg.br/bitstream/1843/SMOC-9NUPM5/1/disserta__o_luan_v28_08_14.pdf

GRIBBLE, D. H. **Equine Ehrlichiosis**. [s.l.] University of California, 1969.

HAUCK, D. et al. *Ixodes inopinatus* in northern Germany: occurrence and potential vector role for Borrelia spp., Rickettsia spp., and *Anaplasma phagocytophilum* in comparison with *Ixodes ricinus*. **Parasitology Research**, v. 118, n. 12, p. 3205-3216, 2019. https://pubmed.ncbi.nlm.nih.gov/31720842/

HUANG, W. et al. Decreased serum albumin level indicates poor prognosis of COVID-19 patients: hepatic injury analysis from 2,623 hospitalized cases. **Science China Life Sciences**, v. 63, n. 11, p. 1678-1687, 2020. https://pesquisa.bvsalud.org/global-literature-on-novel-coronavirus-2019-ncov/resource/pt/covidwho-610883

LEPIDI, H. et al. Comparative pathology and immunohistology associated with clinical illness after *Ehrlichia phagocytophila*-group infections. **American Journal of Tropical Medicine and Hygiene**, v. 62, n. 1, p. 29-37, 2000. https://pubmed.ncbi.nlm.nih.gov/10761721/

LESICZKA, P. M. et al. The Role of Peridomestic Animals in the Eco-Epidemiology of *Anaplasma phagocytophilum*. **Microbial Ecology**, v. 82, n. 3, p. 602-612, 2021. https://pubmed.ncbi.nlm.nih.gov/33547531/#:~:text=Our%20findings%20underline%20the%20role,material%20for%20monitoring%20zoonotic%20pathogens.&text=phagocytophilum%20in%20municipal%20areas%3B%20however,greatest%20anaplasmosis%20risk%20for%20humans

MERINO CHARREZ, O. et al. Detección molecular de *Ehrlichia canis* y *Anaplasma phagocytophilum* y alteraciones hematológicas de perros infectados. **Abanico Veterinario**, v. 13, p. 1-16, 2021. http://www.scielo.org.mx/scielo.php?pid=S2448-61322021000100119&script=sci_arttext

NAIMI, W. A. et al. Immunization against Anaplasma phagocytophilum Adhesin Binding Domains Confers Protection against Infection in the Mouse Model. **Infection and Immunity**, v. 88, n. 10, 2020. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7504939/

PRADO, L. G.; PALHARES, M. S.; MIRANDA, A. L. S. DE. Clinical and laboratory follow up of *Anaplasma phagocytophilum* naturally infected equines. **Revista Brasileira de Higiene e Sanidade Animal**, v. 11, n. 3, p. 314-321, 2017. http://www.higieneanimal.ufc.br/seer/index.php/higieneanimal/article/view/405

ROSEF, O.; PAULAUSKAS, A.; RADZIJEVSKAJA, J. Prevalence of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in questing *Ixodes ricinus* ticks in relation to the density of wild cervids. **Acta Veterinaria Scandinavica**, v. 51, art. 47, 2009. https://actavetscand.biomedcentral.com/articles/10.1186/1751-0147-51-47

SALVAGNI, C. A. et al. Serologic evidence of equine granulocytic anaplasmosis in horses from central West Brazil. **Revista Brasileira de Parasitologia Veterinaria**, v. 19, n. 3, p. 135-140, 2010. https://www.scielo.br/j/rbpv/a/tGwMmFvdg93FNPBq6LPMDQn/?lang=en

SCORPIO, D. G.; CHOI, K. S.; DUMLER, J. S. *Anaplasma phagocytophilum*-related defects in CD8, NKT, and NK lymphocyte cytotoxicity. **Frontiers in Immunology**, v. 9, p. 1-8, 2018. https://www.frontiersin.org/articles/10.3389/fimmu.2018.00710/full

SILAGHI, C. et al. Epidemiology, genetic variants and clinical course of natural infections with *Anaplasma phagocytophilum* in a dairy cattle herd. **Parasites and Vectors**, v. 11, art. 20, p. 1-13, 2018. https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-017-2570-1

SKARPHÉDINSSON, S. et al. Detection and identification of *Anaplasma phagocytophilum*, *Borrelia burgdorferi* and *Rickettsia helvetica* in Danish *Ixodes ricinus* ticks. **Apmis**, v. 115, n. 3, p. 225-230, 2007. https://pubmed.ncbi.nlm.nih.gov/17367468/

STAŃCZAK, J. et al. *Ixodes ricinus* as a vector of *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum* and *Babesia microti* in urban and suburban forests. **Annals of Agriculture and Environmental Medicine**, v. 11, n. 1, p. 109-114, 2004. https://pubmed.ncbi.nlm.nih.gov/15236507/

TORINA, A. et al. Innate immune response to tick-borne pathogens: Cellular and molecular mechanisms induced in the hosts. **International Journal of Molecular Sciences**, v. 21, n. 15, p. 1-23, 2020. https://pubmed.ncbi.nlm.nih.gov/32751625/

UEMURA, K. et al. Sarcopenia and Low Serum Albumin Level Synergistically Increase the Risk of Incident Disability in Older Adults. **Journal of the American Medical Directors Association**, v. 20, n. 1, p. 90-93, 2019. https://pubmed.ncbi.nlm.nih.gov/30056011/

VON LOEWENICH, F. D. et al. A case of equine granulocytic ehrlichiosis provides molecular evidence for the presence of pathogenic *Anaplasma phagocytophilum* (HGE agent) in Germany. **European Journal of Clinical Microbiology and Infectious Diseases**, v. 22, n. 5, p. 303-305, 2003. https://pubmed.ncbi.nlm.nih.gov/12740667/

WOLDEHIWET, Z. The natural history of *Anaplasma phagocytophilum*. **Veterinary Parasitology**, v. 167, n. 2-4, p. 108-122, 2010. https://pubmed.ncbi.nlm.nih.gov/19811878/#:~:text=phagocytophilum%2C%20was%20first%20described%20in,USA%20in%201971%20and%201982

YIN, M. et al. Predictive Value of Serum Albumin Level for the Prognosis of Severe Sepsis Without Exogenous Human Albumin Administration: A Prospective Cohort Study. **Journal of Intensive Care Medicine**, v. 33, n. 12, p. 687-694, 2018. https://pubmed.ncbi.nlm.nih.gov/28013574/

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