Communication

[*Comunicação*]

Equine Monocytic Ehrlichiosis (EME) in Rio de Janeiro State, Brazil

[*Ehrliquiose Monocítica Eqüina (EME) no estado do Rio de Janeiro, Brasil*]

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Equine monocytic ehrlichiosis (EME), also known as equine ehrlichial colitis or Potomac fever, is a disease caused by *Neorickettsia risticii*, a biological agent with affinity for monocytes (Holland et al., 1984; Rikihisa et al., 1985). This is a disease of an enzootic type that presents a mortality rate varying between 5% and 30% (Palmer, 1993). This disease was already described in the United States, Canada, European Union, India, Uruguay and the South of Brazil (Whitlock et al., 1985; Vidor et al., 1988; Dutra et al., 2001). The clinical signs are non-specific, mainly the clinical and sub-clinical forms of the disease (Ristic et al., 1986). The animals may present fever of 38.9ºC to 41.7ºC, as well as, depression, anorexia, diarrhea from moderate to severe, accompanied of abdominal discomfort, laminitis, toxemia, and dehydration. In pregnant mares, it may cause abortion associated with placentitis and even retained placenta (Knowles et al., 1983; Long et al., 1995).

Up to recent times, the vector of transmission of *N. risticii* was unknown (Kahler, 1989). It was speculated that it would be a blood-sucking arthropod. However, the recent researches did not testify the evidence that such arthropods, such as ticks, are involved in the transmission cycle (Barlough et al., 1997). However, with the aid of the polymerase chain reaction (PCR) technique, the etiologic agent was located in metacercariae and aquatic insects (Gibson et al., 2005). This last topic means an important intermediary host and also works as an infected prey for host animals, which transport the final stage of the trematodes, or serve as vehicle for the disease in the terrestrial environment. Likewise, helminth vectors, that are parasites in the intestines of bats and fowls, were detected by the presence of this biological agent (Chae et al., 2000). Irrespective of the involvement of intermediate hosts in the cycle, the contamination of the animal was also observed from animal to animal during the ehrlichemia phase of the infectious cycle, when the pathogen is eliminated with the stool, contaminating water and feed (Biswas et al., 1994).

The diagnosis of EME is done by means of clinical symptomatology and laboratorial tests, such as cell culture, indirect fluorescence assay (IFA) and PCR (Biswas et al., 1994; Mott et al., 1997). Blood smear technique is not included as diagnosis methodology, because the inclusion bodies are not observed. The IFA test is used to monitor exams in field diagnosis and research laboratory, not only concerning its practicity, but also because it is very specific (Ristic et al., 1986).

Blood samples were collected (10ml each) from 27 equines. Seventeen participated in two collections, with intervals between 30 to 180 days. The collections occurred in different

periods of time, as suspicious clinical cases were observed. The first and the second sampling were taken in the metropolitan area of the city of Rio de Janeiro, while the third sampling was collected in a mountainous area of the State of Rio de Janeiro.

The first sampling was taken from 19 equines with blood samples collections in June, and November 2004. In the period between the two collections, an animal died, and four animals were removed from the property after the first collection.

The second sampling was taken from seven equines with collections in November 2004 and March 2005. Between these collections, an equine died, and four equines left the property after the first collection.

The third sampling was taken from an equine and the collections were made in February and March 2005. During the first collection, the animal presented colic with wathery diarrhea, let-like, anorexia and reoccurring fever (39,8°C at the evening). The animal was given a treatment based in sulfa, 10% enrofloxacin, flumexin meglumine and therapy fluid. The animal remained stable. After the 12th day, oxitetracycline was introduced LA once a day, for seven days. In the first 24 hours after the change of the treatment, the fever ceased and the stools became mushy. Starting on the second day of the new treatment, scylalous stool were formed. After the treatment, the animal presented edema in the left hindlimb. Also, during the second blood collection, the clinical status was stable.

Blood was collected by means of puncturing the jugular vein in a vacuum flask without anticoagulant, with a 25x8mm needle for multiple collection. After the collection, the vial was placed in the shade by one hour at 45°, for clot retration. Once the material was delivered to the laboratory, it was centrifuged at 3000rpm by 5min for aseptic separation of the serum. The serum obtained was stored in 1,5ml microtubes and frozen at -20°C, with proper identification, until completion of the test.

On May 12th and 28th 2005, *N. risticii* serological tests were accomplished[1]. The sera were diluted in series (1:50, 1:100, 1:200, 1:400, and 1:800) in buffered saline and, soon after, they were distributed in the wells and incubated, allowing the antigen-antibody reaction occur. The results were visualized by fluorescence microscopy and 400nm wavelength with magnifications varying between 400x and 2000x.

Fifteen of the 19 equines examined (79.0%) of the first sampling group presented titers of 1:50. The four remaining equines, identified as 1, 3, 4, and 6 showed titer results of 1:100, 1:400, 1:400, and 1:800, respectively. In the second group of collection, 42.9% (6/14) maintained the titer of 1:50, and 28.6% (4/14) were negative. The animals identified as 1 and 3 presented titer of 1:200, and the animals identified as 4 and 6 showed titers of 1:400 and 1:800.

In the second sampling, first collection, 85.7% (6/7) of the equines presented titers of 1:50 and 14.3% (1/7) 1:400. Also in the second sampling, second collection, blood was collected of just two animals. One of these animals, that titered 1:50 in the first collection, became non-reagent. The animal that titered 1:400 in the first collection showed a titer of 1:800. This animal was identified as number 5.

The animal of the third sampling presented titers of 1:200 and 1:800 in the first and second collection, respectively, and was identified as number 2.

Even though the IFA test method be normally used for the diagnosis of *N. risticii*, a false-negative result can be observed when the sera samples were collected in the beginning of the course of the disease. This may occur during the period when the class of detectable immunoglobulins is IgM. The class IgG is only detectable eight to 18 days after the infection (Ristic et al., 1986; Madigan et al., 1995). Also, a false-positive result can be produced when the animals are immunized with vaccines containing bovine fetal serum among its components, which can adhere to the surface of the membrane and can stimulate the antibody synthesis. Therefore, the serum tested by a method that includes bovine fetal serum can produce a positive

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reaction. Similarly, a false-positive result can result when specific IgG non-*N risticii* of the tested serum connects to components non-*N. risticii* of the cell culture (Barlough et al., 1984). In spite of the possibility of false-positive and false-negative results, studies revealed that the test of IFA for *N. risticii* presented high specificy in groups of equines that were experimentally inoculated with this bio agent. In this research, an animal was used as negative control, which had been inoculated with non-infected canine monocytes (Rikihisa et al., 1990).

A comparative study verified that titers of up to 1:20 could present crossed reaction with *Ehrlichia canis*, the canine monocytic ehrlichiosis agent, while titers up to 1:320 may be experienced from the crossed reaction with *Ehrlichia sennetsu*. It is also verified that *Anaplasma phagocytophila*, of ehrlichiose granulocytic, does not show crossed reaction with *N. risticii* (Ristic et al., 1986).

Regardless of titers between 1:50 and 1:320, which suggests EME, the result should be associated to the clinical treatment. Therefore, in the initial course of the infection, the rate of circulating IgG is low, making it necessary the use of paired sera within the first four weeks, when an increase of the rate circulating IgG is observed, confirming the diagnosis. Such situation can be exemplified in the case of the animal, described here, submitted to a third sampling. Its first collection happened eight days after the beginning of the clinical synthomatology, with initial titer of 1:200. Twenty-eight days later, the sampling resulted in a titer of 1:800, when the serum conversion occurred and the EME diagnosis was confirmed. The change in therapeutics to EME resulted in clinical improvement (Ristic et al., 1986).

Among the animals of the first sampling, there was horse number 6, which was in final stage of the pregnancy. This animal, 17 days before the accomplishment of the second collection, showed edema in the right knee, claudication, and edema of the mammary gland. After delivering, it was observed retention of the placenta, which was solved by an application of oxytocin.

Unfortunately, 24 hours later, the animal was prostrated, anorexic, with a 41ºC fever, showing signs of great amount of foul-smelling blood secretion in the vagina and endometritis. On the following day, a vaginal secretion was observed with yellow stripes and the right forelimb (RF) presenting increased pulse and claudication. Lastly, in the day of the collection, the animal presented claudication of the second degree and pulse in RF and left forelimb (LF) with light sinking of the hoof crown of the RF. The edema in the ventral portion of the abdomen continued. Thirty days after the collection, the sinking of the hoof crown of the RF, and LF was observed. In addition, a greenish reflux and malabsorption syndrome were observed. Finally, after twenty-eight days, the animal died.

Also, during the second collect, animal number 3 was showing symptoms of acute abdomen with impactation of the pelvic flexure at palpation and a laparotomy was performed. After six days, it showed mushy stool, fever, edema in the abdomen, and peritonitis, what caused death. In conclusion, these animals, numbers 3 and 6, presented clinical findings compatible with EME and titers in agreement with the presented clinical phase.

The animal number 4 did not present suggestive symptomatic clinical conditions during the study period, but the titers in the two collections alerted to the presence of healthy carriers, what seems to be common in endemic areas (Rikihisa et al., 1990).

The animal number 5, which presented titers of 1:400 and 1:800 during the two collections, respectively, showed a discreet swelling in the limb, without fever, pain or claudication that spontaneously reverted to its normal condition. Therefore, it was assumed as subclinical case of EME.

The group of presented symptomatic clinical conditions associated to the titer results obtained from the tested samples confirming the clinical or subclinical existence of EME in the Rio de Janeiro state, Brazil.

Keywords: equine, equine monocítica ehrlichiosis, abdominal discomfort

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RESUMO

Realizaram-se testes de imunofluorescência indireta (IFI) para Ehrliquiose Monocítica Eqüina (EME) em soros de 27 eqüinos, provenientes de duas propriedades do estado do Rio de Janeiro (regiões Metropolitana e Serrana) onde ocorreram manifestações clínicas sugestivas de EME. Coletaram-se duas amostras de sangue de cada animal. Os intervalos entre as coletas variaram entre 30 e 180 dias. Vinte e um animais (77,8%) foram reagentes à IFI com título 1:50, nos testes realizados nas duas amostras coletadas. Os demais (22,2%) – animais 1 a 6 – foram reagentes à IFI, com títulos sorológicos de 1:100, 1:200, 1:400, 1:400, 1:400 e 1:800 na primeira coleta e 1:200, 1:800, 1:200, 1:400, 1:800 e 1:800 na segunda coleta, respectivamente. Três animais (1, 4 e 5) apresentaram manifestações subclínicas. Um animal (2) recuperou-se após o tratamento. Dois animais (3 e 6) evoluíram para óbito. Títulos sorológicos maiores que 1:320 são conclusivos para o diagnóstico específico. Este é o primeiro relato de EME no estado do Rio de Janeiro, Brasil.

Palavras-chave: eqüino, ehrliquiose monocítica eqüina, desconforto abdominal

REFERENCES

BARLOUGH, J.E.; JACOBSON, R.H.; PEPPER, C.E. Role of recent vaccination in production of false-positive coronavirus antibody titers in cats. *J. Clin. Microbiol.*, v.19, p.442-445, 1984.

BARLOUGH, J.E.; RIKIHISA, Y.; MADIGAN, J.E. Nested polymerase chain reaction for detection of *Ehrlichia risticii* genomic DNA in infected horses. *Vet. Parasitol.*, v.68, p.367-373, 1997.

BISWAS, B.; VEMULAPALLI, R.; DUTTA, S.K. Detection of *Ehrlichia risticii* from feces of infected horses by immunomagnetic separation and PCR. *J. Clin. Microbiol.*, v.32, p.2147-2151, 1994.

CHAE, J.S.; PUSTERLA, N.; JONSON, E.; DEROCK, E. et al. Infection of aquatic insect with trematode metacercariae carrying *Ehrlichia risticii*, the cause of Potomac horse fever. *J. Med. Entomol.*, v.37, p.619-625, 2000.

DUTRA, F.; SCHUCH, L.F.; DELUCCHI, E. et al. Equine monocytic ehrlichiosis (Potomac horse fever) in horses in Uruguai and southern Brazil. *J. Vet. Diagn. Invest.*, v.13, p.433-437, 2001.

GIBSON, K.E.; RIKIHISA, Y.; ZHANG, C. et al. *Neorickettsia risticii* is vertically transmitted in the trematode *Acanthatrium oregonense* and horizontally transmitted to bats. *Environ. Microbiol.*, v.7, p.203-212, 2005.

HOLLAND, C.J.; RISTIC, M.; COLE, A.L. et al. Causative agent of Potomac Horse Fever. *Vet. Rec.*, v.24, p.554-555, 1984.

KAHLER, R.C. Transmission is unsolved mystery of equine monocytic ehrlichiosis. *J. Am. Vet. Med. Assoc.*, v.194, p.1681-1687, 1989.

KNOWLES, R.C.; ANDERSON, D.W.; SHIPLEY, W.D. et al. Acute equine diarrhea syndrome (AEDS): a preliminary report. *Proc. Am. Assoc. Equine Pract.*, v.29, p.353-357, 1983.

LONG, M.T.; GOETZ, T.E.; KALOMA, I. et al: Evaluation of fetal infection and abortion in pregnant ponies experimentally infected with *Ehrlichia risticii*. *Am. J. Vet. Res.*, v.56, p.1307-1316, 1995.

MADIGAN, J.E.; RIKIHISA, Y.; PALMER, J.E. et al. Evidence for a high rate of false-positive results with the indirect fluorescent antibody test for *Ehrlichia risticii* antibody in horses. *J. Am. Vet. Med. Assoc.*, v.207, p.1448-1453, 1995.

MOTT, J.; RIKIHISA, Y.; ZHANG, Y. et al. Comparison of PCR and culture to the indirect fluorescent-antibody test for diagnosis of Potomac horse fever. *J Clin Microbiol.*, v.35, p.2215-2219, 1997.

PALMER, J.E.; WHITLOCK, R.H.; BENSON, C.E. Equine erlichial colitis (Potomac horse fever): Recognition of the disease in Pennsylvania, New Jersey, New York, Ohio, Idaho, and Connecticut. *J. Am. Vet. Med. Assoc.*, v.189, p.197-199,1986.

PALMER, J.E. Potomac horse fever. *Vet. Clin. N. Am.: Equine Pract.*, v.9, p.399-410, 1993.

RIKIHISA, Y.; PERRY, B.D.; CORDES, D.O. Ultrastrutural study of ehrlichial organisms in the large colons of ponies infected with Potomac horse fever. *Infect Immun.*, v. 49, p.505-512, 1985.

RIKIHISA, Y.; REED, S.M.; SAMS, R.A. et al. Serosurvey of horses with evidence of equine monocytic ehrlichiosis. *J. Am. Vet. Med. Assoc.*, v.197, p.1327-1332, 1990.

RISTIC, M.; HOLLAND, C.J.; DAWSON, J.E. et al. Diagnosis of equine monocytic ehrlichiosis (Potomac horse fever) by indirect immunofluorescence. *J. Am. Vet. Med. Assoc.*, v.189, p.39-46, 1986.

VIDOR, E.; BISSUEL, G.; MOREAU, Y., et al. Serologie positive a *Ehrlichia risticii* chez une jument presentant un tableau d'ehrlichiose equine. *Prat. Vet. Equine.*, v.20, p.5-10, 1988.

WHITLOCK, R.H.; PERRY, B.; JENNY, A. et al. Potomac horse fever: update and research priorities. *Proc. Annu. Meet. US Anim. Health Assoc.*, v.86, p.23-29, 1985.

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