Frequency of Equine Monocytic Ehrlichiosis (EME) in Brazil[1]

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ABSTRACT.- Moreira L.M.C., Cardoso K.M., Aboud-Dutra A.E., Ferrão C.M. & Gazêta G.S. 2013. **Frequency of Equine Monocytic Ehrlichiosis (EME) in Brazil.** *Pesquisa Veterinária Brasileira 33(6):697-699.* Laboratório Referência Nacional em Vetores das Riquetsioses, Instituto Oswaldo Cruz, Avenida Brasil 4365, Rio de Janeiro, RJ 21040360, Brazil. E-mail: leonardo.moerbeck@ioc.fiocruz.br

From a cross-sectional observational study with convenience samples, 347 blood samples from horses were collected from different physiographic regions, as follows: Santa Catarina Plateau (Santa Catarina State - SC), Médio Paraíba do Sul (São Paulo State - SP and Rio de Janeiro State - RJ), Mountainous and Metropolitan regions (Rio de Janeiro State - RJ). Samples were tested for the presence of antibodies (IgG) anti *Neorickettsia risticii* by indirect immunofluorescence assay (IFA). The frequency obtained in this study corroborates with the ones obtained in the U.S.A., which refers to endemic regions. Fisher's exact test showed significant differences in the number of positive animals between regions, indicating that the probability of an animal becoming infected varies depending on the area. The CI 95% revealed no association between infection and geopolitical space. Moreover, Odds ratio test showed differences of an animal getting infected in different regions. This event could be influenced by the type of treatment used in each area, as the seasonal frequency of injury or even potential vectors. Therefore, there are seropositive animals for *N. risticii* in the studied areas, suggesting that this agent may be circulating in those regions. Future studies mainly based on molecular analyzes are needed to confirm these serological findings.

INDEX TERMS: EME, Equine Monocytic Ehrlichiosis, Potomac horse fever, frequency, IFA, Equidae.

RESUMO.- [**Relato epidemiológico da Erlichiose monocítica equina (EME) no Brasil.**] A partir de um delineamento observacional transversal com amostras de conveniência, 347 amostras de sangue foram coletadas de diferentes regiões fisiográficas: Planalto de Santa Catarina (Estado de Santa Catarina - SC), Região do Médio Paraíba do Sul (Estados de São Paulo - SP e Rio de Janeiro - RJ), Região Serrana e Metropolitana (ambas do Estado do Rio de Janeiro - RJ). As amostras foram testadas para a presença de anticorpos (IgG) anti-*Neorickettsia risticii* por imunofluorescência indireta (IFI). A prevalência obtida no presente estudo corrobora com demais resultados obtidos nos Estados Unidos da América. O Teste Exato de Fisher demonstrou diferença significativa no número de animais positivos entre as regiões, indicando assim que a probabilidade de um animal se infectar varia dependendo da região. O intervalo de confiança (IC 95%) revelou não haver associação entre a infecção e o espaço geopolítico, este evento pode ser influenciado pelo tipo de tratamento em cada área, como sazonalidade do agravo ou frequência de potenciais vetores. Assim, a soropositividade ora encontrada sugere a circulação de *N. risticii* nas áreas estudadas. Estudos futuros baseados, principalmente, em análises moleculares serão importantes para a confirmação dos achados sorológicos no presente trabalho.

TERMOS DE INDEXAÇÃO: EME, Erlichiose monocítica equina, Febre do Potomac, IFI, Equidae.

INTRODUCTION

Riquetsial diseases are characterized as emerging and re-emerging infections caused by intracellular and obligate

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bacteria, associated with Rickettsiales order. Equine Monocitic Ehrlichiosis (EME), also known as Potomac Horse Fever (PHF) is a Riquetsial disease and its causative agent is *Neorickettsia risticii* (Rikihisa & Perry 1985, Dutra et al. 2001, Ferrão et al. 2007).

Described by the first time in the U.S.A. in 1979, EME has been increasing its registers in new areas, such as, France, Italy, India, Australia, Canada, Venezuela, Uruguay and Brazil, EME is now considered endemic in different regions of the Americas featuring a large geographic scale (Rikihisa & Perry 1985, Jones 1990, Dutra et al. 2001, Ferrão et al. 2007, Coffman et al. 2008).

EME is characterized by the presence of various symptoms such as fever, anorexia, depression, tachycardia, gastrointestinal hyper motility, acute diarrhea or not, dehydration, leukopenia, ventral and limbs edema and laminitis, showing the importance of a differential diagnosis to reduce the mortality rate (5-30%) (Rikihisa & Perry 1985, Jones 1990, Dutra et al. 2001, Ferrão et al. 2007, Coffman et al. 2008).

The epidemiologic known course features common characters such as low-lying areas near to rivers and lakes, proving high incidence during the summer time. The transmission of *N. risticii* is not yet fully known, nevertheless, experimental studies have shown that horses are sensitive to transmission by blood transfusion, as well as by oral, subcutaneous and intradermal means (Coimbra et al. 2005).

Studies about the cycle of EME showed the presence of DNA of *N. risticii* in trematodes parasitizing snails, highlighting those invertebrates as a possible reservoir and/or vector (Barlough et al. 1998, Pusterla et al. 2000, Park et al. 2003, Coimbra et al. 2005). Aquatic insects are also mentioned as likely involved in this cycle (Park et al. 2003, Ferrão et al. 2007).

The objective of this study is to analyze the epidemiological aspects of EME in different regions from Brazil.

MATERIALS AND METHODS

Blood samples were acquired from horses in different physiographic regions, such as: Santa Catarina Plateau (samples from Lages city in Santa Catarina State), Médio Paraíba do Sul region (including samples from Queluz city in São Paulo State, Valencia and Barra do Piraí cities from Rio de Janeiro State), Mountainous region from Rio de Janeiro State (featuring Itaipava and Trajano de Morais cities) and The Metropolitan region from Rio de Janeiro State (including Queimados, Mangaratiba and Rio de Janeiro cities). The sampling and processing of blood occurred during routine activity of the Center for Zoonosis Control in Lages/SC, and from investigations of outbreaks of rickettsial diseases by the Department of Health and Civil Defense from Rio de Janeiro State or just from the spontaneous demand sent to Photochart laboratory tests/RJ. From a cross-sectional observational study with convenience samples, three hundred forty seven samples of sera were sent for analysis and maintained at -20°C until testing.

The antibody (IgG) anti *Neorickettsia risticii* was performed by indirect immunofluorescence assay (IFA), using the diagnostic kit from the Fuller Laboratories, containing *N. risticii* as the antigen, hyperimmune horse serum as the positive control, phosphate buffered saline (PBS) as negative control and anti-IgG conjugated equine.

Positive sera were separated and given minimum titration of 1:50. Reactive samples were tested in serial dilutions (1:100, 1:200, 1:400, 1:800) and titrated.

The strength of association between the acquired results and the surveyed areas were analyzed with the assistance of software BioEstat 5.0, with Fisher's exact test (α bilateral =0.01), odds ratio (OR), probability of occurrence (p), confidence interval 95% (CI 95%), Rri (Relative rick increase) and Number needed to harm (NNH). The metropolitan region of Rio de Janeiro State was considered as a control when calculating the odds ratio, not only because its greater sampling, but also because there has already confirmed the presence of positive animals (Ferrão et al. 2007). Frequency was also calculated.

Table 1. Distribution and prevalence of equine serum samples, according to States and Cities of collection, tested by indirect immunofluorescence assay for the presence of antibodies against *Neorickettsia risticii*

State	Cities	Samples				P[c] (%)
		Collected		Positives		
		N[a]	(%)[b]	N	(%)	
Santa Catarina	Lages	32	9.22	03	7.69	9.38
	Total	32	9.22	**03**	**7.69**	**9.38**
São Paulo	Queluz	07	2.02	01	2.56	14.29
	Total	07	2.02	**01**	**2.56**	**14.29**
Rio de Janeiro	Valença	12	3.46	03	7.69	25.0
	Barra do Piraí	06	1.73	01	2.56	16.67
	Rio de Janeiro	265	76.37	26	66.67	9.81
	Queimados	15	4.32	02	5.13	13.33
	Mangaratiba	03	0.86	01	2.56	33.33
	Itaipava	06	1.73	02	5.13	33.33
	Trajano de Morais	01	0.29	00	00	00
	Total	07	2.02	02	5.13	33.33
TOTAL		347	100	39	100	11.24

[a] Absolute numbers, [b] percentage, [c] prevalence.

Table 2. Analysis about the strength of association between the different results obtained and the regions investigated, considering the metropolitan region of Rio de Janeiro State as control

Region	*OR*[b]	P[c]	CI 95%[a]		RRI[f]	NNH[g]
			≥[d]	≤[e]		
Planalto Catarinense	0.961	0.8777	0.2598	3.1601	0.87%	115
Médio Paraíba	2.1897	0.2466	0.7642	6.2736	9.75	11
Serrana	3.5034	0.3519	0.6502	18.8781	18.32	6

[a] Confidence interval of 95%, [b] *Odds ratio*, [c] probability, [d] greater or equal to, [e] smaller or equal to, [f] relative risk increase, [g] number needed to harm.

RESULTS

The prevalence for the analyzed regions was 11.24% and the prevalence, according to physiographic regions distribution shows rates above 9.38% for all regions. In relation to the states of Santa Catarina, São Paulo and Rio de Janeiro, the prevalence obtained was 9.38% (3/32), 14.29% (1/7) and 11.36% (35/308) respectively (Table 1). From the 39 results that were positive for *Neorickettsia risticii*, all of them showed titers equal to or less than 1:200.

The strength of association between the different results obtained and the areas surveyed showed that an animal in the region of Santa Catarina Plateau, has a lower chance of becoming infected, whereas in the regions of Médio Paraíba do Sul and Mountainous the chance of infection is higher than in the metropolitan area (Table 2).

DISCUSSION

Although IFA test is commonly used for studies involving Rickettsiales, specific antibodies become detectable in about

eight to eighteen days after infection (Ferrão et al., 2007). Epidemiological data suggest that subclinical evolution is relatively common (Chaichanasiriwithaya et al. 1994, Long et al. 1995), and antibody titers can be detected for extended periods after infection or vaccination (Rikihisa & Perry 1985, Long et al. 1995). Surveys involving antibodies were made in non-endemic regions resulting in the absence of positive samples, indicating a high specificity from the test applied, the same used in this study (Füller Laboratories). Nevertheless, *N. risticii* shows cross reaction with *Neorickettsia helminthoeca*, *N. sennetsu* and *Escherichia canis*. Thus, endpoint titers amongst *N. risticii*, *N. helminthoeca* and *E. canis* lower than 1:50, are here used as a minimal titration. (Rikihisa & Perry 1985, Rikihisa 2000, Ferrão et al. 2007).

Even though, *N. sennetsu* and *N. helminthoeca* may not be pathogenic for horses, they can affect these mammals, producing sensitivity to high titers (1:320) when seropositive by IFA using *N. risticii* as the antigen. (Berguey's Manual 2005). On the other hand, infection in horses by *N. sennetsu* has not yet been reported in Brazil and most of the other regions of the globe, except the East and Southeast Asia, where it has been reported since 1954 (Newton et al. 2009). *N. helminthoeca* has already been reported in Brazil. (Headley et al. 2006, Headley 2009)

Prevalence rates obtained in this study corroborate the prevalence rates of Potomac Horse Fever (EME) previously obtained in studies in the USA, where there is a variation of 7.5% to 76% depending on the region (Ak et al. 1998).

The calculated *p*-values were lower than *p*-tabulated (p=6.6349) in Fisher's exact test, indicating significant differences in the number of positive animals between regions. Therefore, the probability of an animal becoming infected varies depending on the area. Although the 95% CI includes the value one (1) in all tests, indicating no statistically significant association at the 5% level, between the geopolitical space as a whole, and the infection by *N. risticii*. The probability of an animal getting infected in Médio Paraíba do Sul and Mountainous regions is higher than getting infected in the Metropolitan region (OR>1), whereas in the Santa Catarina Plateau this probability is lower than in the Metropolitan region (OR<1), justifying the significant difference found in Fisher's exact test. (Table 2). This event could be influenced by specific factors such as the type of treatment used in each area, as disorder's seasonality or frequency of the potential vectors. The NNH from Santa Catarina Plateau indicates that 115 animals are needed of free exposure to risk factors for an ill, whereas for the Metropolitan and Mountainous regions are needed eleven and six exposed animals, respectively, to get sick (Table 2).

CONCLUSION

Therefore, there are seropositive animals for *Neorickettsia risticii* in the studied areas, suggesting that this agent may be circulating there. Future studies based, mainly, on molecular analyzes are needed to confirm these serological findings.

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