

Retrospective study of Equine Infectious Anemia in the Southern Amazonia region, Brazil, 2013-2018.

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Abstract. The Equine Infectious Anemia (EIA) is a viral disease caused by a retrovirus that leads to a persistent infection, most of the times without relevant clinical symptoms, thus being an illness of great relevance for the Brazilian equideoculture due the obligatory sacrifice of positive animals, according to local legislation. The aim of this study was to analyze the frequency of animals positive for EIA from cities of Mato Grosso and Pará states of the Southern Amazonia region in Brazil during the years 2013 to 2018. Data from 31.097 exams performed in an accredited laboratory by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) were analyzed. The cumulative frequency found on the evaluated period was 0,73%, with annual frequencies of 0,81%, 0,67%, 1,15%, 0,63%, 0,64% and 0,65% respectively. The cumulative frequency of positive animals of Mato Grosso state found was 0,59%, a result that possibly indicates more efficiency of the animal health surveillance service in the region. On the other hand, in Pará state, the cumulative frequency found was 2,49%, demonstrating more occurrence of this disease in the equine herd of this territory. In that way, according to the results obtained, it was possible to verify the circulation of the EIA virus in Mato Grosso and Pará states, mostly in this last one, and to determine that public politics of animal health surveillance should be increased for a greater control of the epidemiological indices of this disease.

Keywords. Retrovirus, Mato Grosso state, Pará state, epidemiology.

1. Introduction

The Equine Infectious Anemia (EIA), also known as swamp fever, is a viral disease caused by a *Lentivirus* of the family *Retroviridae* that infects horses, donkeys and mules, with worldwide distribution and highest occurrence in tropical or subtropical areas [1].

The viral transmission between susceptible animals occurs mainly by bloodsucking insects, especially flies of the genera *Tabanus*, that transfers the virus from an infected animal to a healthy one, acting as a mechanical vector of the agent. An iatrogenic infection can also be established due sharing of contaminated objects, such as surgical equipment and spurs, or even by contact with secretions of infected animals, like milk and semen [2].

The EIA leads to a persistent infection. According to Maia et al. [3], the main factor that contributes for the viral persistence is the ability of the virus to insert a copy of his DNA in the chromosomal DNA of the host, forming a pro-virus, that may not manifest itself during a long time. There are no available vaccines for this disease prevention commercialized in Brazil. As this agent is an RNA virus, the high failure rates of RNA polymerase allows antigenic variation among viral progenies. The control of the infection is mostly based on identification and restriction of transit and trade of positive animals [1].

According to the Instituto Brasileiro de Geografia e Estatística (IBGE) data, the Brazilian equine herd is made up of more than 5 million animals [4]. The EIA presents itself as a great obstacle for the development of the local equideoculture, leading to losses to owners that need the work of these animals and professionals interested on breed improvement,

also blocking the access to the international market [5], because according to the Brazilian law, any animal positive for EIA must be sacrificed in certain units of the federation [6].

In the last years, fewer studies for the investigation of positive animals for EIA were conducted within the equine herd from the Southern Amazonia region. In that way, considering the social and economical importance of equids in Brazil and the lack of information about the prevalence of EIA in this territory, the aim of this study was to do a report about the occurrence of positive animals for EIA in cities of Mato Grosso and Pará states located in the Southern Amazonia region during 2013 to 2018.

2. Research methods

During January 2013 to December 2018, an accredited laboratory by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA), located in Sinop, received 31.097 equids samples from different cities of Mato Grosso and Pará states. According to the normative instruction nº45 of 2004, established by MAPA, these samples were submitted to the Agar Gel Immunodiffusion assay (AGID) [6].

For this study, information from the records of all animals submitted to the AGID test during the 72 months period was gathered. From these files, we collected the information of exam date, animal origin city and the test result, totalizing in 31.097 exams analyzed.

All data was transferred to the SPSS 2.0 software for descriptive analysis and association test between the independent and the dependent variable (test result). The analysis was made through Fisher test using the association chi-square with 5% significance level.

3. Results and discussion

After analysis of data obtained from the accredited laboratory, in which 31.097 exams were performed using samples of equids from 53 cities of Mato Grosso state and 14 cities of Pará estate, it was possible to determine that the number of seronegative animals submitted to the Agar Gel Immunodiffusion assay (AGID) was superior to the number of seropositive animals.

In this studied period, the accumulated prevalence of positive cases in both stated evaluated was 0,73%, with annual frequencies of 0,81%, 0,67%, 1,15%, 0,63%, 0,64% and 0,65%, from 2013 to 2018 respectively, with significant association between the analyzed years ($p < 0,05$), as shown in Table 1.

The cumulative prevalence of positive animals for EIA from Mato Grosso state found was 0,59%, with annual rates of 0,81%, 0,60%, 0,68%, 0,56%, 0,58% and 0,51 during the evaluated years of this study, respectively (Table 2). There was no significant

association between the variables in Mato Grosso state during 2013 to 2018 ($p > 0,05$).

Tab. 1 - Frequency of positive equids from Southern Amazônia region for Equine Infectious Anemia by Agar Gel Immunodiffusion assay, evaluated by accredited laboratory during 2013 to 2018.

| YEAR | TESTED ANIMALS | POSITIVE ANIMALS | FREQUENCY (%) |
|-------|----------------|------------------|--------------------|
| 2013 | 2.086 | 17 | 0,81% ^b |
| 2014 | 3.097 | 21 | 0,67% ^b |
| 2015 | 4.840 | 56 | 1,15% ^a |
| 2016 | 6.299 | 40 | 0,63% ^b |
| 2017 | 6.959 | 45 | 0,64% ^b |
| 2018 | 7.816 | 51 | 0,65% ^b |
| TOTAL | 31.097 | 230 | 0,73% ^b |

$p < 0,05$.

Tab. 2 - Frequency of positive equids from Mato Grosso state for Equine Infectious Anemia by Agar Gel Immunodiffusion assay, evaluated by accredited laboratory during 2013 to 2018.

| YEAR | TESTED ANIMALS | POSITIVE ANIMALS | FREQUENCY (%) |
|-------|----------------|------------------|--------------------|
| 2013 | 2.086 | 17 | 0,81% ^a |
| 2014 | 2.999 | 18 | 0,60% ^a |
| 2015 | 4.260 | 29 | 0,68% ^a |
| 2016 | 5.661 | 32 | 0,56% ^a |
| 2017 | 6.524 | 38 | 0,58% ^a |
| 2018 | 7.203 | 37 | 0,51% ^a |
| TOTAL | 28.733 | 171 | 0,59% ^a |

$p > 0,05$

As is shown in Figure 1, there was a reduction tendency in the frequency rates of seropositive animals in the Mato Grosso state during the evaluated period. The annual frequencies did not show a significant fluctuation, which indicates a greater stability in the number of positive cases for EIA in the Mato Grosso region.

In a similar study, Santos et al. [7] observed the frequency of seropositive equids in the mid-northern Mato Grosso state during January 2006 to November 2011. The annual frequency of positive animals for EIA obtained by authors in this research was 3,76%, 4,26, 2,89%, 3,11%, 2,89% and 2,40% during the evaluated period, a result that also indicates a reduction tendency of positive cases in the region. According to the authors, this reduction can be due to a higher monitoring of susceptible animals and because of periodic performance of the AGID test in the equine population, allowing the planned actions guaranteed by legislation to be carried out for the

control and prophylaxis of this disease. This information can also be supported by the fact that the number of equids tested for EIA in Mato Grosso during the period studied was 28,733 animals, which demonstrates that more actions regarding animal surveillance were taken in the region.

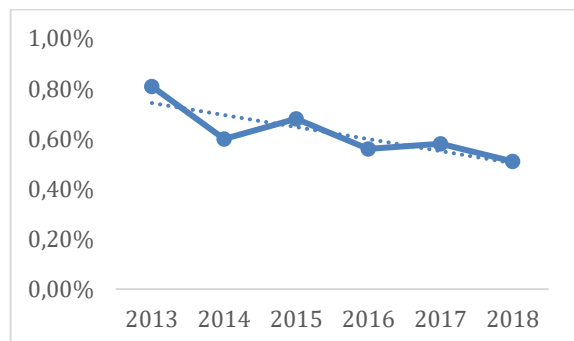


Fig. 1 – Frequency of positive equids from Mato Grosso state for Equine Infectious Anemia by Agar Gel Immunodiffusion assay, evaluated by accredited laboratory during 2013 to 2018.

Regarding the analysis of positive animals from Pará state, the cumulative frequency found during the evaluated period was 2,49%. The distribution of positive animals during 2014 to 2018 resulted in 3,06%, 4,65%, 1,25%, 1,60% and 2,28% frequencies, respectively. The results showed significant association between the variables ($p < 0,05$) (Table 3). During 2003, no ADIG test was performed in equids from Pará state.

Tab. 3 - Frequency of positive equids from Pará state for Equine Infectious Anemia by Agar Gel Immunodiffusion assay, evaluated by accredited laboratory during 2014 to 2018.

| YEAR | TESTED ANIMALS | POSITIVE ANIMALS | FREQUENCY (%) |
|-------|----------------|------------------|--------------------|
| 2014 | 98 | 3 | 3,06% ^c |
| 2015 | 580 | 27 | 4,65% ^a |
| 2016 | 638 | 8 | 1,25% ^b |
| 2017 | 435 | 7 | 1,60% ^c |
| 2018 | 613 | 14 | 2,28% ^c |
| TOTAL | 2.364 | 59 | 2,49% ^c |

$p < 0,05$

As is shown in Figure 2, despite a reduction tendency in the frequency rates of seropositive animals for EIA by AGID assay, it is noticed that in the Pará state more fluctuations between the frequencies of positive animals during the evaluated period did occur. Besides that, the frequency of positive equids were superior than the observed in Mato Grosso state, but the number of tested animals was inferior ($N = 2.364$), indicating a lower concern with the animal's health in this region.

In a study made during 2014, Freitas [8] observed a

24,05% prevalence of seropositive equids for EIA in the cities of Cachoeira do Arari, Salvaterra, Santa Cruz do Arari e Soure, of Ilha de Marajó in Pará state. For this analysis, 349 equines were tested, resulting in 84 positive animals. In a similar study in the following year (2015), Freitas et al. [9] observed a 46,26% frequency of positive equines for EIA in the same cities of the state (136 positives, $N = 294$).

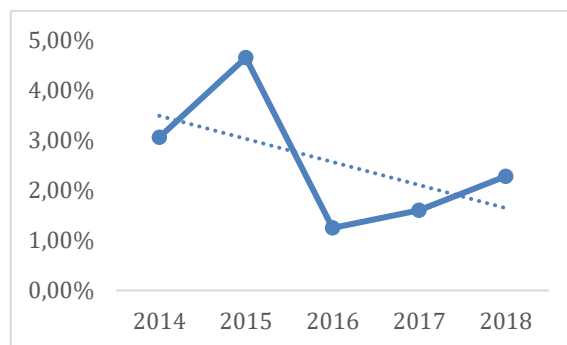


Fig. 2 – Frequency of positive equids from Pará state for Equine Infectious Anemia by Agar Gel Immunodiffusion assay, evaluated by accredited laboratory during 2014 to 2018.

Freitas [8] describes that the high positivity rates in the region can be due to the animal management characteristics. It is said that in the Pará state, the horses are kept in poor conditions and that the sanitary control adopted is still deficient, allowing the permanence of seropositive animals within the herd, which increases the chances of a healthy animal to get infected, mainly by the vector insect. About these ones, it is also said that the Ilha de Marajó has favorable temperature and humidity conditions for the insect multiplication. On the other hand, the animals in this study are generally athletes that, in order to participate in equestrian events, need negative tests for EIA, thus being frequently tested.

About the monthly distribution of positive cases of EIA during the analyzed years, the months with higher cumulative frequencies of positive animals from both evaluated states were October (1,28%), February (1,16) and September (1,13%) (Table 4). During this analysis, it was possible to observe that significant association between the months of the year and the test result occurred ($p < 0,05$).

Regarding the monthly distribution of positive EIA cases in Mato Grosso state during 2013 to 2018, the months with higher frequencies of seroreactive animals were September, February and October, with respectively rates of 0,96% (27 positives, $N = 2.791$), 0,94% (11 positives, $N = 1.162$) and 0,88% (19 positives, $N = 2.140$). There was a significant association between the months of the year and the AGID test result of equids from Mato Grosso state ($p < 0,05$).

The portion of Mato Grosso state evaluated in this study is part of the Amazon biome. In this region, the period of highest rainfall occurs between November

to March, while the dry period runs from May to September. The months of April and October are known as transition months between one season to another [10].

Tab. 4 - Frequency of positive equids from Southern Amazônia region for Equine Infectious Anemia by Agar Gel Immunodiffusion assay, evaluated by accredited laboratory during 2013 to 2018 according to the month.

| MONTH | TESTED ANIMALS | POSITIVE ANIMALS | FREQUENCY (%) |
|-----------|----------------|------------------|--------------------|
| January | 801 | 07 | 0,87% ^c |
| February | 1.284 | 15 | 1,16% ^a |
| March | 2.306 | 15 | 0,65% ^c |
| April | 2.651 | 12 | 0,45% ^b |
| May | 3.420 | 25 | 0,73% ^c |
| June | 3.524 | 33 | 0,93% ^c |
| July | 4.692 | 20 | 0,42% ^b |
| August | 3.756 | 20 | 0,53% ^b |
| September | 2.999 | 34 | 1,13% ^a |
| October | 2.332 | 30 | 1,28% ^a |
| November | 2.059 | 12 | 0,58% ^c |
| December | 1.273 | 07 | 0,54% ^c |

p<0,05

As it is shown in Figure 3, it is possible to notice a peak in the frequency of positive equids for EIA in Mato Grosso state during September and October, a period that goes with the dry season in the region. According to Santos et al. [7], the peak in the prevalence of seroreactive animals occurs because of equestrian events that take advantage of the lowest rainfall rates. Because of the agglomeration of susceptible animals, is it possible that the transmission of the virus from an infected animal to a healthy one occurs during the blood meal of the vector or even through the shared use of objects contaminated with the virus.

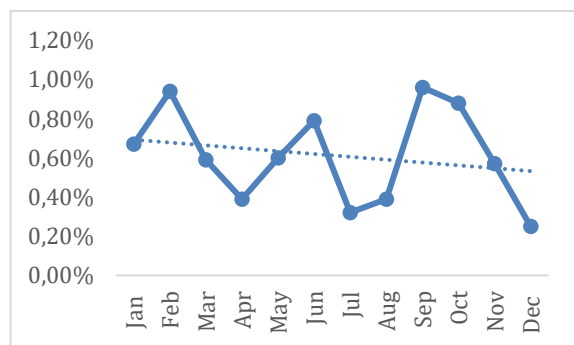


Fig. 3 – Frequency of positive equids from Mato Grosso state for Equine Infectious Anemia by Agar Gel Immunodiffusion assay, evaluated by accredited laboratory during 2013 to 2018 according to the month.

In addition, the increase of positive cases for EIA

during February can be justified due a higher occurrence of the vector in the environment because of the rainy season, which according to Santos et al. [7], this season lead to an increase in the insect population. In a study made in the Pantanal region of Mato Grosso state with 547 equids of 25 properties from Poconé, Borges [11] observed that the farms located in swampy areas obtained 60 times more chances of having positive animals for EIA than farms located in dry areas. The author also reported that equids living in areas subject to flooding are 146.4 times more likely to be positive for EIA than animals that live in dry areas.

As about the monthly distribution of positive EIA cases in Pará state, the months with the highest rates were October (11 positives, N=192), December (4 positives, N=103) and January (2 positives, N=56), with frequencies of 5,72%, 3,88% and 3,57% respectively. There was no significant association between these variables (p>0,05).

In this region, the variation in the rainfall rates is marked by a rainy season, occurring from December to May, and by a dryer season, that corresponds to the period from June to November [12]. As it is shown in Figure 4, there was a gradative increase in the positive EIA cases between August to October, a period that coincides with the moment of higher prevalence of the vector due to the higher rainfall rates, which contributes to the maintenance of this insect in the environment.

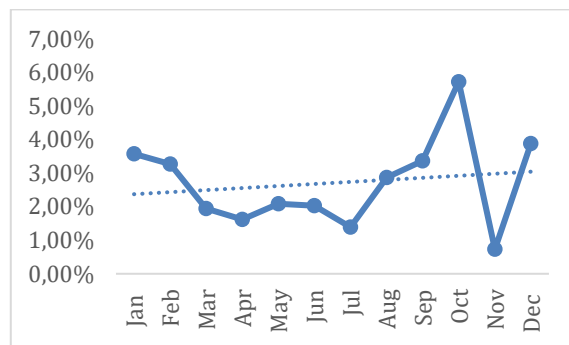


Fig. 4 – Frequency of positive equids from Pará state for Equine Infectious Anemia by Agar Gel Immunodiffusion assay, evaluated by accredited laboratory during 2014 to 2018 according to the month.

In a study with equids from southern cities of Pará state, Heidmann et al [13] obtained a result similar to this one. The authors observed that during the period of January 2007 to Dezember 2010, the peaks of diagnosed animals for EIA occurred during the start of the dry season (May to June) and end of the rainy season (October to November).

4. Conclusions

In this study, it was possible to verify that the Equine Infectious Anemia is present in the equid herd from both Brazilian states evaluated during the period from 2013 to 2018, but according to the data

obtained, it was observed that the frequency of this disease is higher among animals from Pará state.

This high prevalence is possibly due to low sanitary surveillance of animals in the region and to the climatic conditions that make the place favorable for multiplication of the vector insect, which allows the spread of the agent in the environment. In Mato Grosso state, thanks to the actions of the local surveillance service, the EIA positive frequencies have been getting smaller and stable, indicating greater control of the disease among this state herd.

5. References

- [1] Flores EF. *Virologia Veterinária*. UFMS, Santa Maria; 2007. 888 p.
- [2] Megid J., Ribeiro MG., Paes AC., *Doenças Infecciosas em Animais de Produção e de Companhia*. Roca, São Paulo; 2015. 1296.
- [3] Maia CA., Garcia CA., Rossi DA., Melo RT., Mendonça EP., Coelho LR., Monteiro GP., Nalevaiko PC. Anemia Infecciosa Equina – Revisão de literatura. *PUBVET*. 2011; 5(11).
- [4] Brasil. Pesquisa da Pecuária Municipal. Brazil (BR): Instituto Brasileiro de Geografia e Estatística – IBGE; 2021.
- [5] Almeida VMA., Gonçalves VSP., Martins MF., Haddad JPA., Dias RA., Leite RC., Reis JKP. Anemia infecciosa equina: prevalência em equídeos de serviço em Minas Gerais. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 2006; 58(2):141-148.
- [6] *Instrução Normativa n° 45 2004* (Jurisdiction). s IV and VI.
- [7] Santos JD., Lourenço FJ., Castro BG. Estudo retrospectivo da anemia infecciosa equina na região médio-norte mato-grossense, Brasil, 2006-2011. *Revista Brasileira de Medicina Veterinária*. 2016; 38(1):79-85.
- [8] Freitas NFQR. Prevalência da anemia infecciosa equina na Ilha de Marajó, estado do Pará, Brasil [master's thesis]. Belém (BR): Universidade Federal do Pará; 2014.
- [9] Freitas NFQR., Oliveira CMC., Leite RC., Reis JKP., Oliveira FG., Bomjardim HA., Salvarani FM., Barbosa JD. Equine infectious anemia on Marajo Island at the mouth of the Amazon river. *Pesquisa Veterinária Brasileira*. 2015; 35(12):947-950.
- [10] Fisch G., Marengo JA., Nobre CA. Uma revisão sobre o clima da Amazônia. *Acta Amazonica*. 1998; 28(2):101-126.
- [11] Borges AMCM., Soroprevalência da anemia infecciosa em equídeos do pantanal, município de Poconé, estado de Mato Grosso, Brasil [master's thesis]. Cuiabá (BR): Universidade Federal de Mato Grosso; 2012.
- [12] Secretaria Estadual de Meio Ambiente – SEMA. Macrozoneamento Ecológico Econômico do Estado do Pará. Pará (BR): Diretoria de Meio Ambiente 2004.
- [13] Heidmann MJ., Fischer VL., Manhezzo TG., Santos JD., Castro BG. Estudo retrospectivo da anemia infecciosa equina na região centro-sul do Pará, Brasil, 2007-2010. *Revista Brasileira de Medicina Veterinária*. 2012; 34(3):192-197.