


RETROSPECTIVE STUDY OF EQUINE INFECTIOUS ANEMIA IN THE MID-NORTH REGION OF MATO GROSSO STATE, BRAZIL, 2013-2018

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ABSTRACT

Equine Infectious Anemia (EIA) is a disease of great importance for the Brazilian equine economy, both due to its clinical manifestation in animals, which leads to progressive weight loss and anemia, as well as due to the need to sacrifice positive animals in some regions of the federation, in accordance with local law. Thus, this study aimed to analyze the prevalence of EIA in the mid-north region of the state of Mato Grosso, Brazil, during the period of 2013 to 2018. To this end, 28,020 tests conducted by an official laboratory, located in the city of Sinop (MT), were analyzed. Based on the results obtained, the total incidence of positive animals during the evaluated period was 0.55% and the annual frequencies were 0.81%, 0.60%, 0.69%, 0.46%, 0, 58% and 0.41%, from 2013 to 2018 respectively. Compared to studies on the subject carried out in the same region and in others, the current one showed a lower prevalence of the disease, a result that is probably due to the health surveillance activities conducted within the state, which act with the objective of maintaining the incidence of the disease increasingly controlled.

Keywords: Equine retrovirus. Mato Grosso state. Epidemiology.

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INTRODUCTION

Equine Infectious Anemia (EIA), also known as swamp fever or equine HIV, is a disease caused by an enveloped RNA virus of the genus *Lentivirus* of the *Retroviridae* family that infects horses, donkeys, and mules, with a worldwide distribution, although it is more prevalent in humid and mountainous regions in tropical or subtropical weather (DAMAZIO et al., 2022).

Viral transmission between susceptible animals occurs mostly by arthropods with hematophagous habits, such as the *Stomoxys calcitrans* fly, or by insects from the genus *Tabanus*, which act as mechanical vectors of the agent. The infection can also be established iatrogenically, owing to the sharing of contaminated objects, such as surgical or riding equipment. Transmission by sexual contact, in utero or by ingestion of colostrum from an infected mare can also occur but are not considered epidemiologically significant (NOGUEIRA et al. 2017).

During the reproductive cycle, agents of the *Retroviridae* family are recognized for inserting a DNA pro-virus inside the host cell genome, which is only possible in the presence of the reverse transcriptase enzyme that performs reverse transcription of the viral genome and allows for persistent infection of the animal. This disease can manifest itself in an acute or chronic manner, but most of the infected animals are asymptomatic; thus, the infected animals are kept in the herd for a long time, allowing the dissemination of the agent inside the property.

Vaccines for disease prevention are not commercialized in Brazil; therefore, infection control must be based mainly on the identification and transit restriction, or sale of animals diagnosed as positive (FLORES, 2007). The EIA is part of the list of diseases subject to the measures provided in the Regulamento do Serviço de Defesa Sanitária Animal (MAPA; Federal Decree 24.548/1934). Normative Instruction No. 45 of 2004 of the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) (BRASIL, 2004) contains the norms that must be followed for the prevention and control of the disease and determines that its notification is mandatory. Furthermore, EIA control and prophylaxis measures also follow what is determined by the Programa Nacional de Sanidade dos Equídeos (PNSE) established by Ordinance No. 200 of 1981 (BRASIL, 1981).

In recent years, few epidemiological studies have investigated EIA in equine herds in the state of Mato Grosso. Considering the social and economic importance of horses in Brazil and the lack of information on the prevalence of the disease in this territory, this study

aimed to report its occurrence in cities in the mid-northern region of Mato Grosso from 2013 to 2018.

MATERIAL AND METHODS

Between January 2013 to Dezember 2018, 28.020 equid samples from different cities of Mato Grosso state were received in a laboratory registered by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) located in Sinop. These samples were subjected to an Agar Gel Immunodiffusion Assay (AGID), a test recommended for the diagnosis of EIA according to Normative Instruction No. 45 of 2004 established by MAPA (BRASIL, 2004).

For this study, data from the identification files of all animals submitted to the AGID test were collected, including the examination date, species, sex, age, and test results of every animal, totaling 28.020 examinations evaluated. These data were then transferred to SPSS software (version 2.0) for statistical analysis and association tests between the independent and dependent variables. The analysis was conducted using the Fisher test and the association chi-square test with a 5% significance level.

RESULTS AND DISCUSSION

After data analysis obtained from the registered laboratory, it was possible to determine that the number of seronegative animals for Equine Infectious Anemia (EIA) submitted to the Agar Gel Immunodiffusion Assay (AGID) during the evaluated period was superior to the number of seronegative animals. During the study period, of the 28.020 samples, only 155 were positive. The incidence of positive animals found was 0,55% and the annual frequencies were 0,81%, 0,60%, 0,69%, 0,46%, 0,58% and 0,41%, from 2013 to 2018, respectively. There was no significant statistical association between the years evaluated and the test results ($p>0.05$), as shown in Table 1.

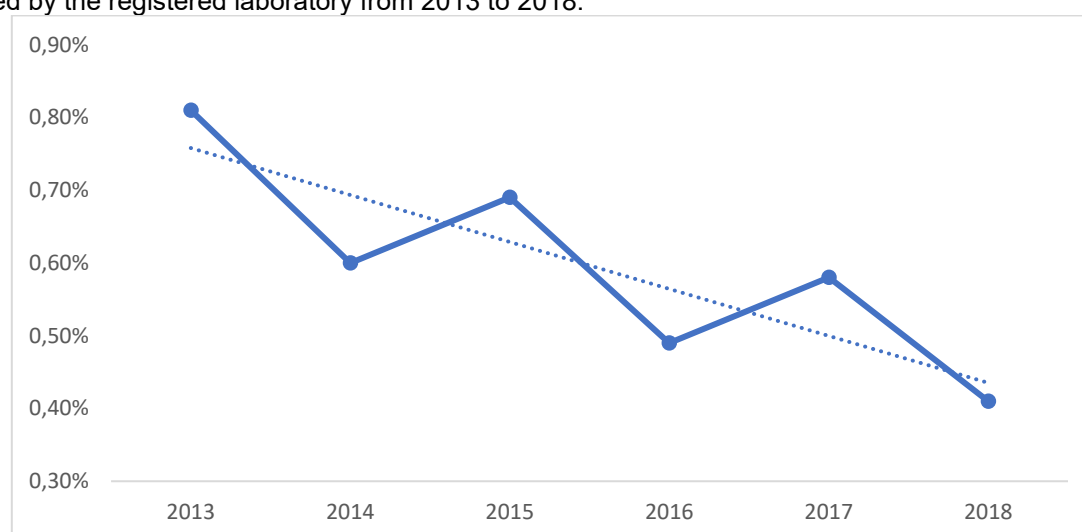
As shown in Figure 1, during the evaluation period, there was a tendency for a decrease in positive cases of EIA. In addition, it is also possible to observe that there was no significant fluctuation between the annual results, showing a greater stabilization of disease occurrence in the region.

Table 1. Frequency of positive equines for Equine Infectious Anemia by the Agar Gel Immunodiffusion Assay, evaluated by the registered laboratory from 2013 to 2018.

YEAR	TOTAL OF TESTED ANIMALS	TOTAL OF POSITIVE ANIMALS	FREQUENCY (%)
2013	2.086	17	0,81% ^a
2014	2.998	18	0,60% ^a
2015	4.178	29	0,69% ^a
2016	5.489	27	0,49% ^a
2017	6.474	38	0,58% ^a
2018	6.795	28	0,41% ^a
TOTAL	28.020	155	0,55%

(p>0.05)

Figure 1. Frequency of positive equines for Equine Infectious Anemia by the Agar Gel Immunodiffusion Assay, evaluated by the registered laboratory from 2013 to 2018.



Few studies have been conducted to determine the prevalence of EIA in equine herds in Mato Grosso. BARROS (2017) analyzed 3.858 equine samples from rural properties in different biomes of Mato Grosso. Of the total evaluated, 89 animals were seroreactive in the Amazon biome, resulting in a prevalence of 14.3%. In the Cerrado biome, 86 animals tested positive for EIA, with an observed prevalence of 18.7%. In the Pantanal biome, 211 animals tested positive, with a prevalence of 36.1%. MELO et al. (2012) analyzed 886 horse samples from the same biome. Serum samples from 59 horses from the Amazon biome, 772 from the Cerrado, and 55 from the Pantanal were analyzed. No positive animals were observed in the Amazon biome, whereas in the Cerrado, a frequency of 3.36% of positive animals was observed and 36.3% of the frequency in the Pantanal biome.

In a similar study, SANTOS et al. (2016) analyzed data from equids from mid-northern Mato Grosso State submitted to the AGID test by a certified lab by MAPA from January 2006 to November 2011. The cumulative prevalence found was 3,33%, with annual

prevalences of 3.76%, 4.26%, 2.89%, 3.11%, 2.89%, and 2.40% from 2006 to 2011, respectively. This study also highlighted the tendency for a decrease in the prevalence of EIA in the region over time.

According to the authors, this decrease can be explained by sanitary surveillance activities and periodic examinations of animals in the state, which allow the control and prophylaxis measures provided by legislation to be fulfilled. Concerns about animal health can also be evidenced by the number of animals evaluated in both studies. In the analysis conducted by SANTOS et al. (2016) between 2006 and 2011, 17,263 horses were subjected to the AGID test, and in this study, 28.020 equids were tested from 2013 to 2018.

Regarding the monthly distribution of the positive cases, the months with the highest frequencies of seroreagent animals for EIA in the studied region were September, October, and February, with values of 0,96%, 0,86% and 0,81%. During statistical analysis, an association between the AGID test results and the months of the year was found ($p < 0,05$), as shown in Table 2.

In this study, data from animals collected from cities in the Southern Amazon were analyzed. This is a region of Brazil that comprises the areas located between the south of the state of Amazonas, north of Mato Grosso, south of Pará and the state of Rondônia (SOUZA, 2018). In the Amazon biome, the period of the highest rainfall ranges from November to March, whereas the dry season occurs between May to September. The transition period between the rainy and dry regimes occurs in April and October (FISCH et al., 1998).

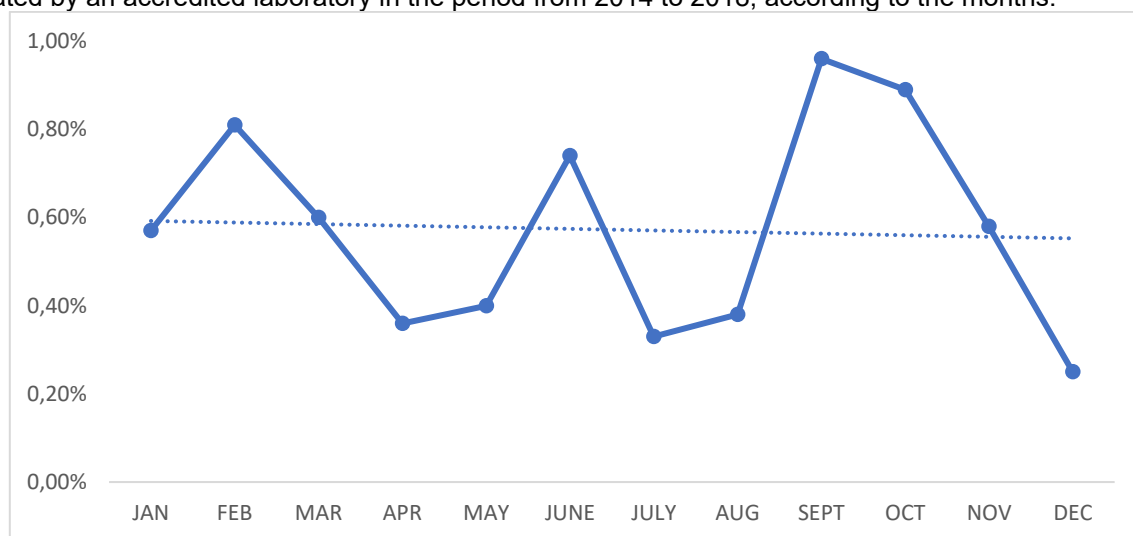
Table 2. Frequency of positive equines for Equine Infectious Anemia by the Agar Gel Immunodiffusion Assay, evaluated by the registered laboratory from 2013 to 2018, according to the months.

MONTH	TOTAL OF TESTED ANIMALS	TOTAL OF POSITIVE ANIMALS	FREQUENCY (%)
JANUARY	692	4	0,57% ^a
FEBRUARY	1.101	9	0,81% ^b
MARCH	2.166	13	0,60% ^a
APRIL	2.465	9	0,36% ^b
MAY	2.979	12	0,40% ^b
JUNE	3.085	23	0,74% ^a
JULY	4.183	14	0,33% ^b
AUGUST	3.337	13	0,38% ^b
SEPTEMBER	2.688	26	0,96% ^a
OCTOBER	2.120	19	0,89% ^a
NOVEMBER	1.870	11	0,58% ^a
DECEMBER	1.134	2	0,25% ^b

($p < 0,05$)

As shown in figure 2, the highest peak in the frequency of horses positive for EIA occurred in September, the month that runs with the dry season in the studied region. According to SANTOS et al. (2016), the increase in the prevalence of seroreactive animals during this period is due to agricultural fairs and other equestrian events that require a negative EIA test for any animal to participate. Owing to the larger sampling of animals performed at these times, the probability that an infected animal will be identified in the AGID examination is greater.

Figure 2. Frequency of positive equines for Equine Infectious Anemia by Immunodiffusion in Gel Agar evaluated by an accredited laboratory in the period from 2014 to 2018, according to the months.



The peak of positive cases for EIA observed in February may be related to the greater occurrence of the vector in the environment, owing to the rainy season, which provides adequate climatic conditions for the multiplication of these insects. In a study carried out in the Pantanal region of Mato Grosso with 547 horses from 25 farms in the city of Poconé, BORGES (2012) observed that farms located in swampy areas of the region were 60 times more likely to have animals positive for EIA than farms in dry areas. He also reported that horses living in areas subject to flooding were 146.4 times more likely to be positive than animals living in dry areas.

Regarding the gender of the animals, 95 seroreactive females were found, resulting in a frequency of 0.61%, and 60 positive males, resulting in a frequency of 0.47%. No significant differences were observed among the analyzed variables ($p > 0.05$), as shown in Table 3.

This result is like the one that found by SANTOS et al. (2016) and BORGES (2012) in Mato Grosso, HEIDMANN et al. (2012) in Pará, GUIMARÃES et al. (2011) in Bahia and FIORILLO (2011) in Minas Gerais, who also found no difference in the frequency of positive animals for EIA between males and females evaluated.

However, BARROS (2017) found a statistically significant difference in the analysis of gender variables and results from the test ($p < 0.05$) within the state of Mato Grosso. The author determined that for males, the chance of presenting the disease is 1.412 times in relation to females, since males are the most exploited category as a working animal, being more exposed to viral infection (SILVA et al., 1999).

A total of 219 donkeys, 24,045 horses, and 3,735 mules were evaluated in this study, resulting in frequencies of 0.91%, 0.48%, and 0.99%, respectively, for seroreactive animals. In the statistical analysis, a significant association was found between these variables ($p < 0.05$), as shown in Table 4.

Table 3. Frequency of positive equines for Equine Infectious Anemia by Immunodiffusion in Gel Agar evaluated by an accredited laboratory in the period from 2013-2018, according to gender.

GENDER	TOTAL OF TESTED ANIMALS	TOTAL OF POSITIVE ANIMALS	FREQUENCY (%)
FEMALE	15.397	95	0,61% ^a
MALE	12.605	60	0,47% ^a

($p > 0.05$)

Table 4. Frequency of positive equines for Equine Infectious Anemia by Immunodiffusion in Gel Agar evaluated by an accredited laboratory in the period from 2013-2018, according to the species.

SPECIE	TOTAL OF TESTED ANIMALS	TOTAL OF POSITIVE ANIMALS	FREQUENCY (%)
DONKEY	219	2	0,91% ^a
HORSE	24.045	116	0,48% ^b
MULE	3.735	37	0,99% ^a

($p < 0,05$)

According to Fisher's exact test using the chi-square association, in this study, the number of horses positive for EIA was lower than expected, whereas the number of seropositive mules and donkeys was higher than expected. The difference in disease frequency between species may be a reflection of the type of use each of these species is usually associated with.

Most horses are intended for recreational or reproductive activities, in addition to being animals that tend to have a greater associated economic value and receive greater health care from their owners or breeders. This can be justified by the high number of horses that were tested during the evaluation period of this study. Mules are generally

animals intended only for service and receive less sanitary handling. Also, they are animals that do not tend to be moved between properties, therefore, they do not undergo periodic examinations such as IDGA assay. As a result, there is a greater probability that once tested, a mule will be positive for EIA, as it spends a longer period exposed to all the risk factors associated with the disease.

BARROS (2017) observed that herds housed in properties with the main purpose was service are 22.162 times more likely to have positive animals when compared to properties whose main purpose was sport, leisure or reproduction. MORAES et al. (2017) found an association between mules and positivity for EIA ($p < 0.005$) in an analysis performed with draft horses from the Federal District. In this evaluation, 478 horses and 18 mules were sampled and 1.46% ($n=7$) of the horses and 11.11% ($n=2$) of the mules were positive.

Unlike those, donkeys are animals that normally have lower levels of viremia, which may explain why they usually do not show clinical signs of the disease (COOK et al., 2001), thus also contributing to the lower rate of diagnosis of truly positive animals.

Regarding the age, 7,850 of the animals evaluated were between 0 and 4 years old and the prevalence found was 0.35%. Among horses aged 4 to 8 years, a total of 9,897 AGID tests were performed, resulting in a prevalence of 0.45%. Out of animals older than 8 years, 10,716 IDGA tests were performed, resulting in a prevalence of 0.91%. As shown in table 5, there was a statistically association between the variables ($p < 0.05$) and it was also observed that there is a greater tendency of seropositivity for EIA in animals older than 8 years.

Table 5. Frequency of positive equines for Equine Infectious Anemia by Immunodiffusion in Gel Agar evaluated by an accredited laboratory in the period from 2013-2018, according to age.

AGE	TOTAL OF TESTED ANIMALS	TOTAL OF POSITIVE ANIMALS	FREQUENCY (%)
0 – 4 YEARS	7.699	26	0,33% ^a
4 – 8 YEARS	9.633	40	0,41% ^a
OVER 8 YEARS	10.459	89	0,85% ^b

($p < 0,05$)

SANTOS et al (2016) also reported a tendency towards increased seropositivity in equines aged over four years. According to authors, this situation is possibly related to the course of the disease, in addition to the fact that, in this age group, the animals are going through the taming process and end up becoming more exposed to the virus through the shared use of objects. Like these, in the study made by BARROS (2017), also carried out in the state of Mato Grosso, it was reported that horses older than 10 years are 1.801 times more likely to be positive for EIA when compared to other animals of lesser age.

During the period of this study, data from 52 cities of Mato Grosso were evaluated. Of this total, 26 municipalities had at least 1 animal positive for EIA during the analyzed years. The cities with the highest cumulative frequency of seroreactive animals were Confressa, Peixoto de Azevedo and Itaúba, with respective values of 8.33%, 3.14% and 1.93%.

In this study, it was verified that the incidence of Equine Infectious Anemia (EIA) in cities in the mid-northern region of Mato Grosso was below that was found in other studies carried out in the state and in other regions. This result is possibly due to the services of surveillance and sanitary defense carried out in this state, which allow the rates of positive animals to be controlled and to constantly decrease. However, other studies should still be guided so that the epidemiological statistics of the disease are better determined within the Mato Grosso region, thus allowing better rates of control and prevention of the disease throughout the state.

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