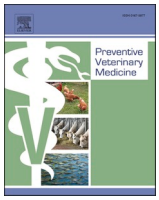
Preventive Veterinary Medicine 209 (2022) 105781

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed



Seroprevalence and risk factors associated with equine infectious anemia in the state of Goiás, Brazil

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ARTICLE INFO

Keywords:
Equids
Agar gel immunodiffusion
Equine infectious anemia
Relative risk
Probability
Prevalence

ABSTRACT

Equine infectious anemia (EIA) is an infectious disease affecting equine in most countries and represents a notifiable disease with compulsory euthanasia of positive animals. The present study aimed to determine the prevalence of EIAV infected equines in herds of the state of Goiás (Central Brazil) and to evaluate the risk factors associated with the occurrence of the disease. Blood samples were collected from 1170 equids from 332 randomly selected farms divided into three different strata according to their herd characteristics. Also, an epidemiological questionnaire was applied during the visit to the farm. Of the 332 farms evaluated, 12 (3.1%; 95% CI: 1.24 – 6.00) had at least one positive equine for EIA, and of the 1170 evaluated equines, 14 (2%; 95% CI: 0.31–3.00) were positive in agar gel immunodiffusion. Multivariate analysis revealed that the use of a vaccination pistol ($p < 0.001$) and the presence of water bodies inside the farm ($p < 0.01$) were risk factors associated with the occurrence of EIA. Thus, the present study demonstrated a low but widespread prevalence of EIAV infected animals in the herds of Goiás state and that iatrogenic and environmental risk factors were associated with the occurrence of the disease.

1. Introduction

Equine infectious anemia virus (EIAV), belonging to the genus *Lentivirus*, is the etiologic agent of equine infectious anemia (EIA), a disease present in almost all countries. According to the World Organization for Animal Health (WOAH, 2021), EIA is a disease of mandatory notification to the local veterinary service (Agrodefesa) (Costa et al., 2022).

Infected equines represent the main source of infection within the farm, and transmission of the virus occurs by the transfer of blood from an infected to a healthy animal. In nature, EIAV is most often transmitted between hosts through interrupted feeding of blood-sucking vectors of the order Diptera (Cook et al., 2013; Foil et al., 1983; Issel et al., 1988). However, iatrogenic transmission of EIAV is epidemiologically important and can occur through the use of shared needles among several animals, common use of surgical materials, and blood transfusion (Cook et al., 2013; Ramachandran and Sakkubai, 1989). Thus, the importance of human participation in the transmissibility of EIAV among equines reinforces the need for caution during sanitary practices.

In Brazil, EIA is of great importance for equine health, and the disease causes significant economic losses resulting from the progressive weakening of the animal and the compulsory euthanasia of positive animals (Santos et al., 2016). The National Equid Health Program (PNSE) was instituted by Ministério da Agricultura, Pecuária e Abastecimento (Ministry of Agriculture, Livestock and Food Supply), in order to prevent, diagnose, control, and eradicate health threats to equine industry (MAPA, 2008).

Specifically, regarding EIA, the strategies established by the PNSE include health education, transit control, registration, inspection and certification, immediate intervention in suspected cases, and conducting epidemiological studies in different Brazilian states to determine the epidemiological situation of the disease in the country (MAPA, 2008). However, the national prevalence of EIA remains unknown. Some studies that evaluated the prevalence of EIA in equine breeding establishments in Brazil reported a wide variation in the results obtained,

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https://doi.org/10.1016/j.prevetmed.2022.105781
Received 8 June 2022; Received in revised form 15 September 2022; Accepted 16 October 2022
Available online 18 October 2022

with the prevalence of positive animals ranging from 0% to 52% (Almeida et al., 2006; Borges et al., 2013; De Andrade Almeida et al., 2017; Guiraud et al., 2017; Machado et al., 2021; Moraes et al., 2017; Pena et al., 2006).

In Goiás state, horse industry has significant relevance for socioeconomic development as it generates thousands of direct and indirect jobs, and EIA is considered an endemic disease in equine herds. However, the only study that evaluated the prevalence of the disease in the state dates from the 1970s, in which a prevalence of 3.7% of positive animals was found (Jardim, 1978). Thus, the present study aimed to determine the prevalence of EIAV infection in equine herds in Goiás state and to evaluate the risk factors associated with the occurrence of the disease.

2. Material and methods

2.1. Study area and equine population

The study of EIAV seroprevalence was carried out in the state of Goiás, located in the central Brazil. The target population included equids (donkeys, mules, and horses) from farms registered with the local veterinary service, called Goiás Agricultural Defense Agency (Agrodefesa). The study region had a geographic area of 340,242 km^2 and an equine population of 382,523 animals (IBGE, 2017), representing 7.3% of the national herd. The Agrodefesa staff performed the study, traveling throughout the state to perform the survey and sample collection.

2.2. Sampling

To determine the seroprevalence of herds and equines seropositive for EIA, a two-stage sampling was performed, the first stage being the farms and the second, the equids within the farms. To improve understanding of the EIA epidemiology, the herds were stratified according to the type of equine raising system: farms with equids only (Stratum 1), farms with equids and cattle (Stratum 2), and urban area equine farms (Stratum 3), as described in Table 1. Stratum 1 and 3 comprised farms with equid breeding with various purposes, such as leisure, sport and traction force (carthorses), Stratum 1 included farms located in rural areas and Stratum 3 included farms located in urban areas. The choice of the farms was random based on the list of equine farms registered in Agrodefesa, and the number of animals to be sampled was calculated to allow at least 90% specificity at the herd level. The sample size was simulated for herds of 5–10 horses, since the average herd size in each stratum was approximately five. The expected prevalence (in animals) was 10% and the cut-off point (i.e., number of seropositive animals necessary to assign positive status to a farm) was simulated from 1 to 3. For calculations, the Epitools computer program was used (https://epitools.ausvet.com.au/ herdplusthree), considering the sensitivity and specificity of the agar gel immunodiffusion assay (AGID; Bruch Laboratory, São Paulo, Brazil) diagnostic test, 98% and 100%, respectively (Coggins et al., 1972).

Thus, in farms that had up to nine equids, blood serum was obtained from all animals, whereas in those with herds $\geq$ 10 animals, samples were collected from nine animals. In this case, the inclusion criterion was the longest time in the farm. Table 1 provides information related to the sampled herds and number of individual samples per stratum.

The farms selected in Strata 1 and 2 that did not meet the requirements for sample collection (i.e., those that did not have equids) were replaced by the closest farm within the stratum and in the same municipality. No substitution was carried out in Stratum 3, as all farms in urban areas were selected.

Table 1
Number of farms with equine breeding registered with Agrodefesa, number of equines per stratum, number of farms selected for sample collection and number of animals selected within each stratum for serological survey of EIA in state of Goias, Brazil.

Stratum	Number of registered farms	Number of registered Equines	Number of selected farms	Number of animals to be sampled
1	11,256	55,355	139	504
2	80,525	400,984	139	489
3	99	450	54	174
Total	**91,880**	**456,789**	**332**	**1167**

2.3. Blood sample collection

Blood collection was performed by 27 field teams from Agrodefesa staff, each including two veterinarians, from November 2020 to January 2021. Blood samples were collected aseptically by jugular venous puncture, centrifuged to obtain serum, identified, and stored in duplicate in 1.5 mL microtubes at − 20 °C until processing.

2.4. Serological tests

Laboratory analyses for the serological diagnosis of EIA were carried out in accordance with national legislation (MAPA, 2008, 2018). Serum samples were tested for the presence of antibodies against EIAV using the agar gel immunodiffusion (AGID) test according to the manufacturer's instructions. This serological test is the diagnostic method of choice recommended by World Organization for Animal Health for EIA since it can detect antibodies to the EIAV even in the case of asymptomatic animals (Espadandim et al., 2021; WOAH, 2021). All analyses were conducted by the Agrodefesa Veterinary Laboratory (LABVET) between December 2020 and January 2021.

2.5. Apparent prevalence in herd and animals

To calculate the prevalence of EIAV infection, the relative weight of each selected farm and each tested animal in the herd were calculated and recomposed to calculate the prevalences for Goiás state. The calculations were made in R software (R Core Team, 2019), using the package *survey*.

The sample size varied with the expected prevalence, but it turns out that the values of the three strata were the same, because even in Stratum 3, the sample universe (n = 99) was much larger than the calculated sample size.

2.6. Epidemiological survey and risk analysis

During visits to the farms, an epidemiological questionnaire was applied to the farmers to obtain information on aspects related to possible risk factors associated with EIA infection. Data were collected *in loco*, in face-to-face interviews, and the questions included information on the number of equids, age, breed and sex, geographical coordinates of the farm, socioeconomic factors, EIA prevention and control measures, environmental aspects, and sanitary management data of the farm.

Statistical analysis was performed using R software (R Core Team, 2019). The confidence intervals were determined using a binomial logistic regression model. Prevalence values were calculated separately for the farms (focus) and animals.

All potential risk factors were subjected to univariate analysis, using the Fisher Exact Test (*fischer.test* function of R) for categorical variables and Student t Test (*t.test* function of R) to compare (between positive and negative farms) the means of the continuous variables. The association between positive animals and the independent variables was analyzed. A value of $p \leq 0.2$ was considered as a criterion to select the variables to be submitted in the multivariate logistic regression (Abreu et al., 2009). A generalized linear model (glm) was used to determine the risk factors associated with EIA. To achieve that, we used the ISLR package of R. A stepwise model was used until the remaining variables had $p < 0.05$. Fit models were compared using the Akaike Information Criterion (AIC),

the chosen model being the one with the highest AIC.

3. Results

3.1. Descriptive analysis

Of the 332 farmers interviewed, 116 (35.1%) declared that they bought equines to expand their herd. Of these, 101 (87.1%) declared that they purchased animals only from other farms, 6 (5.2%) only at events, and 9 (7.7%) both from other farms and at events. Seventy-four (63.8%) farmers declared that they had animals acquired only from their municipality, 32 (27.6%) from another municipality, and 10 (8.6%) from other states.

Ninety-eight (29.5%) farmers declared that their equids participated in events. Of these, 15 (15.3%) declared that the animals participated in events every two years, 20 (20.4%) declared that their participation was annual, and 63 (64.3%) declared that their animals participated in events more than once a year.

The presence of veterinary assistance on the rural property was declared by 154 (46.4%) farmers of which 65 (42.2%) declared that this assistance was regular and 89 (57.8%) declared that it only occurred when there were some sick animals.

Regarding the preventive management of EIA on rural properties, 98 (34.2%) farmers declared that they carried out tests: 26 (26.5%) only for the acquisition of animals, 43 (43.8%) to participate in events, 24 (24.4%) to monitor flocks, and 5 (5.1%) in all three situations mentioned. In relation to the application of medicines or vaccines, 200 (61.7%) farmers reported using disposable syringes; 31 (9.6%), reusable syringes; 93 (28.7%), pistols; and 8 did not respond. Of these, 98 reported the use of the same needle when administering drugs or vaccines to different equines. Of the 332 farmers, 251 (75.6%) declared that they did not separate equipment by horse. Of the 81 farmers who declared that they separated the equipment, 76 (93.8%) separated the bridles, 59 (72.8%) separated the spurs, and 74 (91.4%) separated the harnesses. Three hundred twenty-eight (98.8%) farmers declared that they did not carry out any specific management for the prevention of EIA, such as weaning newborn foals or using screened stalls.

When asked about the transit of animals, 115 (34.6%) farmers declared that they required an animal transport guide for transport, and 232 (69.9%) declared that they did not require a negative certificate for EIA to receive an animal on the farm. Of the 332 farmers interviewed, five (1.5%) declared that they had already had animals positive for EIA on their farm, and all declared that the animal was euthanized, and the property cleaned up by Agrodefesa and subsequently released.

Regarding the environmental aspects associated with the epidemiology of EIA, 245 (73.8%) farmers reported having water collections on their farm. Most farms included rivers, lakes, swamps, and dams. One hundred seventy-seven (53.3%) farmers declared that there was vegetation or cattail on the banks of the water collections, and 236 (71%) declared that there were fragments of forest (native or secondary) on the farm. Two hundred eighty-five (85.8%) farmers declared that during the day, the equines were loose in the pasture, and 185 (55.7%) reported the presence of tabanids on the farm. Two hundred twenty-seven (68.4%) reported having heard of EIA; however, 147 (44.2%) reported knowing how the horse was infected. Among the responses, the following stood out: stinging tabanids; contaminated needles, harnesses, and spurs; contaminated surgical material and dental equipment and natural mating.

One hundred-thirteen (34%) owners declared that they did not know about legislation on EIA, and 227 (68.4%) declared they knew that EIA is a disease that causes economic losses. Among the reasons stated, the following stand out: sacrifice of the positive animal and difficulty in replacing an animal of the same genetic pattern, prohibition of transit of animals on that far, interdiction of the farm, debilitated animals, and costs with tests for sanitation.

3.2. Prevalence and risk factors

Samples were collected from 1170 horses, from 143 municipalities of Goiás state, of which 1027 (87.8%) were horses, 9 (0.8%) were donkeys, and 134 (11.5%) were mules. Of the 332 farms sampled, 139 (41.9%) belonged to Stratum 1, 139 (41.9%) to Stratum 2, and 54 (16.2%) to Stratum 3.

The apparent prevalence of farms with EIAV positive animals by stratum for the Goiás state was described in Table 2 and showed in the Fig. 1. Of the 332 farms sampled, 12 (3.1%; 95%CI: 1.24 – 6.00) had one or more animals that were positive for EIA.

The apparent prevalence of EIAV infection in the equines sampled from the three strata of the study was shown in Table 3. Of the 1170 equines sampled, 14 (2%; 95%CI: 0.31 – 3.00) were seropositive for EIA. When evaluating the apparent prevalence of EIAV infection in the farms and sampled animals, no evident differences were observed.

As a result of the univariate analysis (Supplementary Table 1), the following variables presented with $p \leq 0.2$, when compared with the result of positive farms for EIAV infection, and were included in the multivariate analysis: altitude ($p = 0.003$), number of female donkeys aged up to 6 months ($p = 0.16$), production systems ($p = 0.14$), herd density ($p = 0.004$), participation in events ($p = 0.12$), participation in events more than once a year ($p = 0.13$), veterinary assistance ($p = 0.04$), frequency of veterinary assistance ($p = 0.1$), use of disposable syringes ($p = 0.07$), use of vaccination pistol ($p = 0.006$), use of the same needle when administering medication or vaccine ($p = 0.007$), separate equipment by equid ($p = 0.04$), separate bridle by equid ($p = 0.07$), separate spurs by equid ($p = 0.13$), separate harnesses by equid ($p = 0.07$), presence of rivers on the farm ($p = 0.02$), distance from water collections to equids ($p < 0.001$), and distance from forest fragments to equids ($p = 0.008$) (Table 4).

In the multivariate analysis, only two variables showed significant results and were included in the final model: the use of a vaccination pistol ($p < 0.001$) and the presence of rivers on farm ($p < 0.01$) (Table 5). Farms that used vaccination pistol were 5.2 more likely to have EIAV infection than farms that did not use. In addition, farms with rivers were 3.0 more likely to have EIAV infection than those without rivers in their territory.

4. Discussion

The prevalence of EIAV infection found both on farms and in animals was low, with no significant difference between the evaluated strata.

Table 2
Apparent prevalence of farms with a focus of equine infectious anemia in three strata evaluated in the state of Goiás, in 2021.

Stratum	Sampled farms	Positive farms	Prevalence	CI (95%)
1	139	7	5.037	2.16 – 9.60
2	139	4	2.86	0.88 – 6.58
3	54	1	2.02	0.091 – 8.42
Total	**332**	**12**	**3.12**	**1.24 – 6.00**

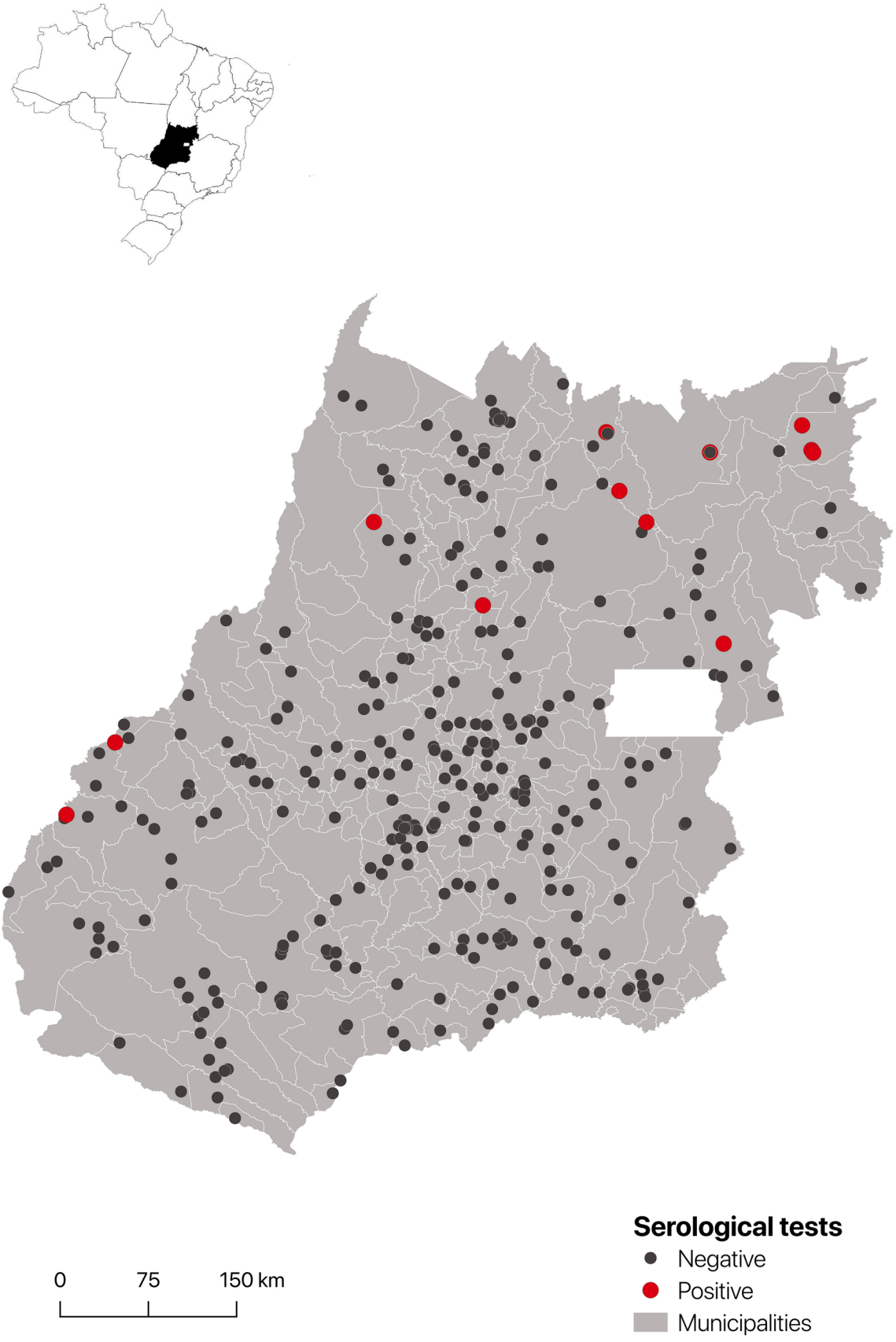

Fig. 1. Geographical location of the surveyed farms of the state of Goiás and the positives and negatives farms to EIAV infection.

Table 3
Apparent prevalence of seropositive equines for equine infectious anemia in three strata in Goiás state, 2021.

Stratum	Sampled equines	Positive equines	Prevalence	CI (95%)
1	507	8	1.58	0.38 – 2.18
2	484	5	2.07	0.26 – 3.37
3	179	1	2.73	0.14 – 6.89
Total	**1170**	**14**	**2.01**	**0.31 – 3.00**

Table 4
Variables that presented $p \leq 0.2$ in the univariate analysis, when compared with the result of positive farms for EIA and were included in the multivariate analysis.

Variables	*p*
Altitude of farm	0.003
Type of raising system	0.14
Herd density	0.004
Equine participation in events	0.12
Equine participation in events more than once a year	0.13
Presence of veterinary assistance	0.04
Frequency of veterinary assistance	0.1
Use of disposable syringes when administering medication or vaccine	0.07
Use of vaccination pistol when administering medication or vaccine	0.006
Use of the same needle when administering medication or vaccine	0.007
Separate equipment by equid	0.04
Separate bridle by equid	0.07
Separate spurs by equid	0.13
Separate harnesses by equid	0.07
Presence of rivers on the farm	0.02
Number of female donkeys aged up to 6 months	0.16
Distance from water collections to equids	0.001
Distance from forest fragments to equids	0.008

Table 5
Final model of the multivariate analysis of the association of risk factors with equine infectious anemia in farms of Goiás state, 2021.

Variables	Estimate	Odds Ratio	CI (95%)	*p*
Use of vaccination pistol	1.651	5.215	1.580 – 20.142	0.0089**
Presence of rivers within the farms	1.383	3.987	1.112 – 13.384	0.025*

*p<0.001;**p<0.01.

Despite the low prevalence, EIAV infection is spread across Goiás state. Thus, it is extremely important that surveillance measures for agricultural defense recommended by the PNSE are maintained and intensified (MAPA, 2004).

The present study represents the first seroepidemiological survey to assess the prevalence of EIA in Goiás State herds, considering the particularities of each farm category. In Brazil, available data only refer to studies that searched for the prevalence of the disease in a given region, with variations in prevalence from 0% in animals in Rio Grande do Sul state to 46.6% in Marajó Island, in Pará state (Bicout et al., 2006; Borges et al., 2013; Chaves et al., 2014; Freitas et al., 2015; Guiraud et al., 2017; Heidmann et al., 2012; Machado et al., 2021; Moraes et al., 2017; Silva et al., 2013).

Otherwise, data of the animal health defense services do not accurately report the prevalence of EIA in Goiás and in the other states of Brazil, since these data refer to samples collected for transporting animals, restricting only the category of animals that usually participate in events or competitions. Prevalence studies have been conducted at the state level only in the states of Minas Gerais and Mato Grosso (Almeida et al., 2006; Barros et al., 2018), in which a prevalence of 6.6% and 3.1% of positive animals was identified, respectively, indicating that EIA was still a sanitary problem in Brazil.

The results of the present study showed that from 12 farms with at least one animal positive for EIAV infection 11 (91.7%) were represented by farms that did not typically send animals to agricultural events. These results reinforce the hypothesis that official data regarding the occurrence of EIA do not reflect the real prevalence of the disease, since most tests are performed for intercity or interstate transit of animals to participate in events, and because there are few serological studies (Ribeiro and Freiria, 2018). Thus, most of the equine population tested belongs to farms of high zootechnical value, while the large number of animals in the field that have never been tested represents a potential risk of spreading the EIAV. These results also reinforce the importance of transit control and the importance of carrying out EIA exams for animal transit, since the vast majority of new cases are detected during routine laboratory testing (Barros et al., 2018; Cook et al., 2013).

Transmission of the EIAV can occur by iatrogenic route through the common use of materials contaminated with infected blood, such as needles, syringes, and surgical instruments (Coetzer et al., 1994). The use of a vaccination pistol was considered a risk factor for the occurrence of EIAV infection in herds by the multivariate analysis. Farms in which the vaccination pistol was used for equine vaccine management had a 5.21 times greater risk of EIAV infection than farms that did not. Therefore, there is a risk of an increase in the prevalence of EIAV infection through the iatrogenic route during the management of equines.

The results of the multivariate analysis indicated that equines at farms with rivers had a 3.987 times higher risk of EIAV infection than those at farms without watercourses. Ecological conditions and the population of hematophagous insects are factors that facilitate the spread of the disease in equine herds (Cook et al., 2013). Tabanids are the main vectors for the transmission of EIAV, and the presence of water collections makes it possible to increase the number of vectors in the environmental transmission of EIAV (Barros and Foil, 2007). Most owners declared that their farms included rivers, lakes, swamps, and dams. According to Silva et al. (2001), it is not possible to completely eliminate the transmission of EIAV by hematophagous insects in the field, and the risk of disease transmission increases with an increase in the prevalence of EIA.

Among the preventive measures instituted to reduce the prevalence of EIAV infection in a herd, the full testing of equines on a regular basis and the elimination of positive animals stand out, since if the source of infection (infected equine) does not exist, the risks of vector transmission will also not exist. In this context, health education can be a key factor in raising awareness among farms about the dangers of using shared veterinary equipment, the correct disposal of needles and syringes, and the benefits of serological monitoring, all of which directly assist with reducing the prevalence of EIA in herds (Barros et al., 2018).

The present study demonstrated the low, but widespread, prevalence of EIAV infection in farms and animals in Goiás State. Furthermore, iatrogenic and environmental risk factors were associated with the occurrence of EIAV infection. Thus, our results reinforce the importance of health education with a focus on preventing and controlling EIAV infection within the state, specifically regarding the correct use of vaccination pistols and other equipment.

Funding

This work was supported by the Goiás Agricultural Defense Agency (Agrodefesa).

Declaration of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2022.105781.

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