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Epidemiological situation of Equine Infectious Anemia in the state of Paraná, Brazil

Situação epidemiológica da Anemia Infecciosa Equina no estado do Paraná, Brasil

Ricardo Gonçalves Velho Vieira[1]; Rafael Gonçalves Dias[2]; Fernando Ferreira[3]; Ricardo Augusto Dias[4]; José Henrique Hildebrand Grisi Filho[5]; Marcos Bryan Heinemann[6]; Evelise Oliveira Telles[7]; José Soares Ferreira Neto[8]*

Highlights

The prevalence of infected farms in the state was 1.55% [0.92; 3.00].
The prevalence of seropositive animals in the state was 0.55% [0.27; 1.00].
Introduction of equids was risk factor for EIA in the state (OR=5.5 [1.9; 15.9]).

Abstract

To assist decision making regarding the National Equine Health Program in the state of Paraná, a study was conducted to estimate the prevalence of infected farms and seropositive animals for Equine Infectious Anemia (EIA) and also identify possible risk factors for the disease. The state was divided into three regions, within which about 300 farms were randomly selected. On the selected farms, a minimum number of animals aged 6 months or older were examined to characterize them as infected or free of EIA. In the sampled farms, an epidemiological questionnaire was applied to investigate possible associations between the disease and its characteristics, including sanitary practices and livestock management. The test used was the Agar Gel Immunodiffusion Test. A total of 2818 equids from 889 farms were tested.

[1] Veterinary, Agência de Defesa Agropecuária do Paraná, Curitiba, PR, Brazil. Master Student of the Experimental and Applied Epidemiology to Zoonoses Program, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, USP, São Paulo, SP, Brazil. E-mail: ricardovieira@adapar.pr.gov.br

[2] Veterinary, Agência de Defesa Agropecuária do Paraná, Curitiba, PR, Brasil. E-mail: rafaeldias@adapar.pr.gov.br

[3] Full Professor, Faculdade de Medicina Veterinária e Zootecnia, USP, São Paulo, SP, Brasil. Researcher. WOAH-Collaborating Centre for Economics of Animal Health in the Americas Region. E-mail: fferreir@usp.br

[4] Associate Professor, Faculdade de Medicina Veterinária e Zootecnia, USP, São Paulo, SP, Brasil. Researcher. WOAH-Collaborating Centre for Economics of Animal Health in the Americas Region. E-mail: ricardodias@usp.br

[5] Assistant Professor, DVM, Faculdade de Medicina Veterinária e Zootecnia, USP, São Paulo, SP, Brasil. Researcher. WOAH-Collaborating Centre for Economics of Animal Health in the Americas Region. E-mail: jgrisi@usp.br

[6] Associate Professor, Faculdade de Medicina Veterinária e Zootecnia, USP, São Paulo, SP, Brasil. Researcher. WOAH-Collaborating Centre for Economics of Animal Health in the Americas Region. E-mail: marcosbryan@usp.br

[7] Full Professor, Faculdade de Medicina Veterinária e Zootecnia, USP, São Paulo, SP, Brasil. Researcher. WOAH-Collaborating Centre for Economics of Animal Health in the Americas Region. E-mail: bufalo@usp.br

[8] Full Professor, Faculdade de Medicina Veterinária e Zootecnia, USP, São Paulo, SP, Brasil. Researcher. WOAH-Collaborating Centre for Economics of Animal Health in the Americas Region. E-mail: jsoares@usp.br

* Author for correspondence

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In the state, the prevalence of infected farms was 1.55% [0.92; 3.00] and the prevalence of seropositive animals was 0.55% [0.27; 1.00]. Introduction of equids showed an association with EIA (OR=5.5 [1.9; 15.9]). Paraná equine owners should be alerted to the need to observe health precautions regarding EIA when introducing animals to their herd. In 2018, the sensitivity of the Surveillance System for EIA in Paraná was only 1.36%, probably insufficient to change the endemic balance of the disease, therefore, needs to be re-evaluated involving in the process all public and private agents interested in the subject.
Key words: Equine Infectious Anemia. Prevalence. Risk factors. Paraná. Brazil.

Resumo

Para auxiliar a tomada de decisões em relação à execução do Programa Nacional de Sanidade dos Equídeos no estado do Paraná, foi realizado um estudo para estimar a prevalência de focos e de animais para Anemia Infecciosa Equina (AIE) e também identificar os possíveis fatores de risco para a doença. O estado foi dividido em três regiões, dentro das quais cerca de 300 propriedades foram aleatoriamente selecionadas. Nas propriedades sorteadas, foi examinado um número mínimo de animais com idade igual ou superior a 6 meses para caracterizá-la como foco (pelo menos um animal soropositivo) ou não foco de AIE. Nas propriedades amostradas foi aplicado um questionário epidemiológico para averiguar as possíveis associações entre a doença e as características da propriedade, incluindo suas práticas sanitárias e zootécnicas. O teste utilizado foi a Imunodifusão em Gel de Ágar (IDGA). Foram testados 2818 equídeos oriundos de 889 propriedades. No estado, a prevalência de focos foi de 1,55% [0,92; 3,00] e a de animais 0,55% [0,27; 1,00]. A introdução de equídeos apresentou associação com a AIE (OR=5,5 [1,9; 15,9]). Os proprietários paranaenses de equídeos devem ser alertados para a necessidade de observar cuidados sanitários relativos à AIE ao introduzir animais nos seus plantéis. Em 2018, a sensibilidade do Sistema de Vigilância para AIE no Paraná foi de apenas 1,36%, provavelmente insuficiente para alterar o equilíbrio endêmico da doença, portanto, precisa ser reavaliado envolvendo no processo todos os agentes públicos e privados interessados no tema.
Palavras-chave: Anemia Infecciosa Equina. Prevalência. Fatores de risco. Paraná. Brasil.

Introduction

Equine infectious anemia (EIA) has no effective treatment; it is caused by a lentivirus that leads to persistent and debilitating infection in equids, but is most often asymptomatic (World Organization for Animal Heath [WOAH], 2019). Its transmission occurs due to the inoculation of blood from an infected animal through the bite of horseflies (*Tabanus* sp.) and stable flies (*Stomoxys calcitrans*) and may occur iatrogenically through the shared use of contaminated materials such as needles, surgical instruments, dental rasps, esophageal probes, trocars, hoof trimmers, harnesses, spurs, and other fomites. Transmission of this disease through the placenta is also possible (WOAH, 2019).

EIA is considered an obstacle to the development of the equine production chain, as it impairs the athletic performance of the animals and hinders the improvement of the breeds and the access to the international equestrian market (Almeida et al., 2006; Andrade et al., 2018). In Brazil, the fight against this disease is carried out within the scope of the National Equine Health Program (PNSE) coordinated by the Ministry of Agriculture, Livestock and Food Supply (MAPA). In the country, EIA is a notifiable disease, and serological testing is required for animal movement, with subsequent euthanasia of positives. The herds of origin of positive animals must be transformed into free herds by routine serological testing (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2004). This is the basis of the Brazilian Surveillance System (SS) for EIA. Until 2018 the serodiagnosis was made exclusively by agar gel immunodiffusion (AGID); since then the possibility of using the enzyme-linked immunoassay (ELISA) as a screening test for IDGA has been introduced (MAPA, 2018). The tests are carried out in official and private laboratories accredited by MAPA.

Although there are few well-designed studies on the epidemiological situation of this disease in Brazil, based on the available information, it can be stated that EIA is widely distributed throughout the country, with a heterogeneous epidemiological situation among the federative units (FUs) and a predominance of low prevalence rates. Studies with an adequate sample design, covering the entire territory of FUs were conducted only in the Federal District (Moraes et al., 2017), Minas Gerais (Almeida et al., 2006, 2017), Mato Grosso (Barros et al., 2018), Rio Grande do Sul (Machado et al., 2021) and Goiás (Pádua et al., 2022).

In the Federal District, fieldwork was carried out in 2010 with sampling aimed at draft animals. The prevalence of infected farms was 2.3 % [1.0; 4.2] and the prevalence of seropositive animals was 1.8 % [0.6; 3.1] (Moraes et al., 2017).

In Minas Gerais, the sampling was directed towards service animals, and the fieldwork was conducted in the years 2003 and 2004. The prevalence of infected farms was 5.3 % [4.3; 6.3] and the prevalence of seropositive animals was 3.1 % [2.2; 3.9] (Almeida et al., 2006). In the state, another study with sample planning was conducted from 2004 to 2006 on the horses kept in stud farms, and the prevalence of infected farms was 0.4 % [0.3; 1.4] and the prevalence of seropositive animals was 0.07 % [0.0; 0.3] (Almeida et al., 2017).

In Mato Grosso, the fieldwork was carried out in 2014, and the estimated prevalence of infected farms was 17.2 % [15; 20] and the prevalence of seropositive animals was 6.6 % [5.8; 7.5] (Barros et al., 2018).

In Rio Grande do Sul, no positive results for AGID were found in serum samples from 1010 animals from 341 farms (Machado et al., 2021).

In Goiás, the fieldwork was carried out from November 2020 to January 2021, and the estimated prevalence of infected farms was 3.12 % [1.24; 6.00] and the prevalence of seropositive animals was 2.01% [0.31; 3.00] (Pádua et al., 2022).

The other existing studies from FUs, regions, or country, reported simple proportions of "positive animals revealed by SS/livestock" (Macêdo, 2019; Ribeiro & Freiria, 2018; Costa, 2018), "positive animals revealed by SS/animals tested by SS" (Heidmann et al., 2012; Santos et al., 2016; Pena et al., 2006; Guimarães et al., 2011; Melo et al., 2012) or "number of positive animals or outbreaks in a given period" (Barzoni et al., 2018). High-quality data on the prevalence and risk factors for EIA in the FUs would be very useful, as they will facilitate the estimation of the positive and negative predictive values of the diagnostic protocol, improving the performance of SS and adapting the PNSE strategies to each epidemiological reality.

EIA is endemic in the state of Paraná; however, its prevalence and distribution are not adequately characterized. From 2005 to 2019, 344 EIA outbreaks were reported in the state, with a significant variation between years and without any clear evidence of a downward trend in the number of outbreaks in that period (Figure 1).

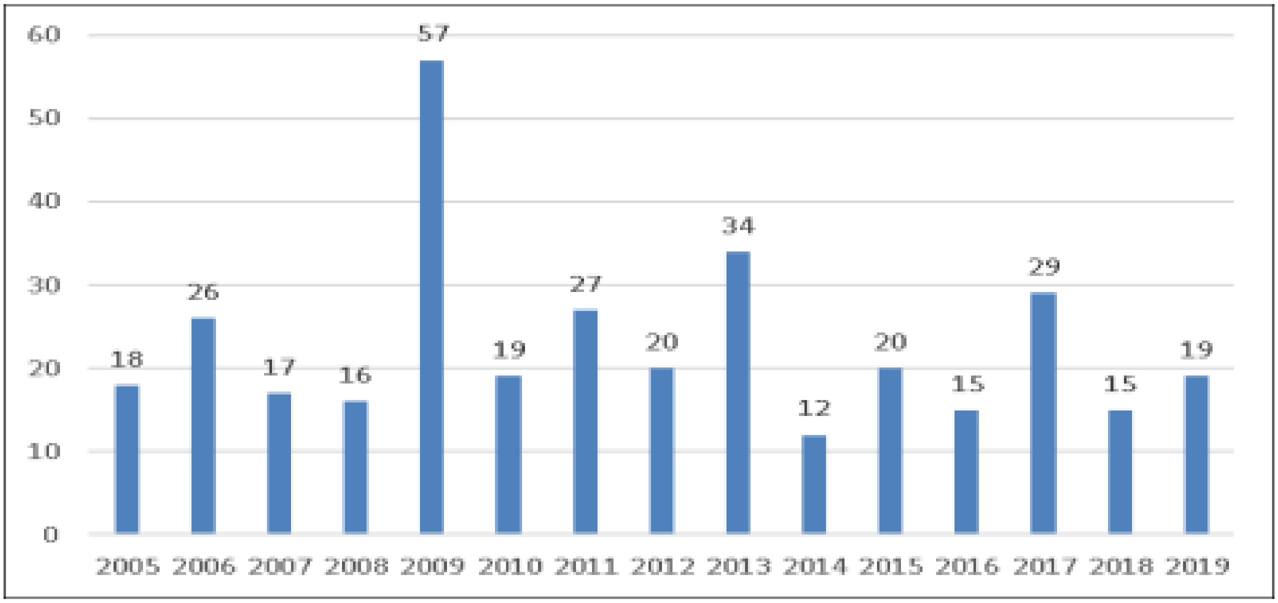

Figure 1. Number of equine infectious anemia outbreaks detected in Paraná by the Oficial Veterinary Service of the State (Agência de Defesa Agropecuária do Paraná-ADAPAR) from 2005 to 2019.
Source: http://indicadores.agricultura.gov.br/saudeanimal/index.htm

Thus, the objective of this study was to estimate the prevalence of infected farms and seropositive animals for EIA in the state of Paraná, in addition to checking the possible associations between the disease and the characteristics of farms and sanitary and management practices. The data generated in the present study served as a basis for proposing public policies within the scope of the PNSE.

Material and Methods

Study planning

The study was planned by the Oficial Veterinary Service of the Paraná State (Agência de Defesa Agropecuária do Paraná-ADAPAR) with the support of the Collaborating Centre for Animal Health of the Faculty of Veterinary Medicine and

Animal Science of the University of São Paulo (Centro Colaborador em Saúde Animal da Faculdade de Medicina Veterinária e Zootecnia (FMVZ) da Universidade de São Paulo (USP)). The study design, data analysis, and interpretation of results were carried out by the Collaborating Centre for Animal Health, and the fieldwork was carried out by ADAPAR from March to April 2018.

Initially, the state was divided into regions based on the similarities of the production systems and the operational capacity of ADAPAR. In each region, a set of farms and animals were randomly selected, and the samples collected from them were submitted for the EIA serological diagnosis, which was carried out at the Marcos Enrietti Diagnostic Center in accordance with Normative Instruction No. 45 of MAPA (MAPA, 2004). For the selected farms, a questionnaire was administered regarding the characteristics of the property (location, number of horses, type and purpose of breeding, and presence of wetlands); animal management (reproduction system, introduction of animals, and participation in agglomerations); and sanitary practices (carrying out tests for EIA, use of repellents, sharing of fomites and needles, and veterinary assistance) to check for possible associations with the disease.

The results of the questionnaires and serology were entered into a database and analyzed at the Laboratory of Epidemiology and Biostatistics at the Collaborating Centre for Animal Health of the FMVZ-USP. The Ethics Committee on Animal Use of the FMVZ-USP approved this study (process CEUA 3910251018).

Sampling and diagnosis

For each region, a two-step sample was used. In the first step, a random selection was made from an established number of farms for the equines aged 6 months or more, based on the ADAPAR register. The farms that could not participate in the study were replaced by new drawing. For each selected farm, a minimum number of horses aged 6 months or more were examined to classify the property as infected or free of EIA (second step of the sample). The animals were randomly selected, and a 10 mL blood sample was taken from each to perform AGID for the diagnosis of EIA.

The sample size in the first stage was calculated according to Thrusfield and Christley (2018), based on the following assumptions: estimated prevalence, 0.25; precision, 0.05; confidence level, 0.95; population size, 72,000; resulting in a sample of 288 farms.

AGID detects antibodies against the p26 viral antigen and the main core protein and is widely recommended for the serological diagnosis of EIA (Animal and Plant Health Inspection Service [APHIS], 2007; WOAH, 2019). It is the only serological test that has been statistically correlated with the presence of the EIA virus in the blood. False positive results by this test are rare; they may be caused by technical errors and are often corrected by repeating the test. The false negative results are usually due to misinterpretation of the test signal by the technician or low serum antibody levels (APHIS, 2007).

The 100 % sensitivity and specificity of AGID for EIA diagnosis is often mentioned in the package inserts of kits manufactured by specialized laboratories. However, Coggins and Norcross (1970) reported a sensitivity of 98 % and a specificity of 100 % for the test. Considering that in the present study, among the 2818 animals tested, only 14 were reported to be positive for AGID (0.50 %), it can be concluded that the specificity of the test is 99.50 % or more. Furthermore, for the calculation of aggregate sensitivity and specificity according to the second sampling step, conservative values of 98.00 % and 99.50 % were chosen for sensitivity and specificity, respectively, with an intra-herd prevalence of 20 %.

The number of animals examined in each property ensured minimum aggregate sensitivity and specificity values of 92 %. Operationally, on farms harboring up to 50 equines aged 6 months or more, 10 animals of the same age group were sampled, and on farms with 51 or more animals, 15. The sampled animals were chosen at random, and the farm were considered infected when at least one of the examined animals was positive in the AGID assay.

Calculations were performed using the Epitools program (Sergeant, 2018a).

Data processing

The prevalence of infected farms and seropositive animals was calculated for each region and state, along with the respective confidence intervals, as recommended by Dean et al. (1996). The prevalence of infected farms and animals in the state and the prevalence of animals within the regions were calculated in a weighted manner, as proposed by Dohoo et al. (2010). The weight of each property in the calculation of the prevalence of infected farms in the state was given by the following:

$$P_1 = \frac{\text{Existing farms in the region}}{\text{Farms sampled in the region}}$$

Similarly, the weight of each animal in the calculation of the prevalence of animals in the state was given by the following:

$$P_2 = \frac{\text{Equines} \geq 6 \text{ months in the farm}}{\text{Equines} \geq 6 \text{ months sampled in the farm}} \times \frac{\text{Equines} \geq 6 \text{ months in the region}}{\text{Equines} \geq 6 \text{ months sampled in the region}}$$

In the expression given above, the first term refers to the weight of each animal for the calculation of animal prevalence within regions.

Considering the results from the entire state, two groups of farms, namely infected and free of EIA were formed and were compared based on the variables addressed in the questionnaires to calculate the extent of the association between these variables and the disease. A first exploratory analysis of the data (univariate) was conducted by selecting the variables with $p \leq 0.20$ for the X^2 test and subsequently using these for multivariate logistic regression analysis (Hosmer et al., 2013).

The final multivariate model was built using the stepwise forward method with sequential inclusion of the most significant variables in the univariate analysis. The variables that improved the fit measured by the maximum likelihood ratio with the coefficient of the variable statistically different from 0 ($p < 0.05$, Wald test), were included in the model. All calculations were performed using the R program (R Core Team [R], 2016).

Results and Discussion

Figure 2 shows the state of Paraná divided into three regions for the present study, and Table 1 presents the registration data of the equine population of the state.

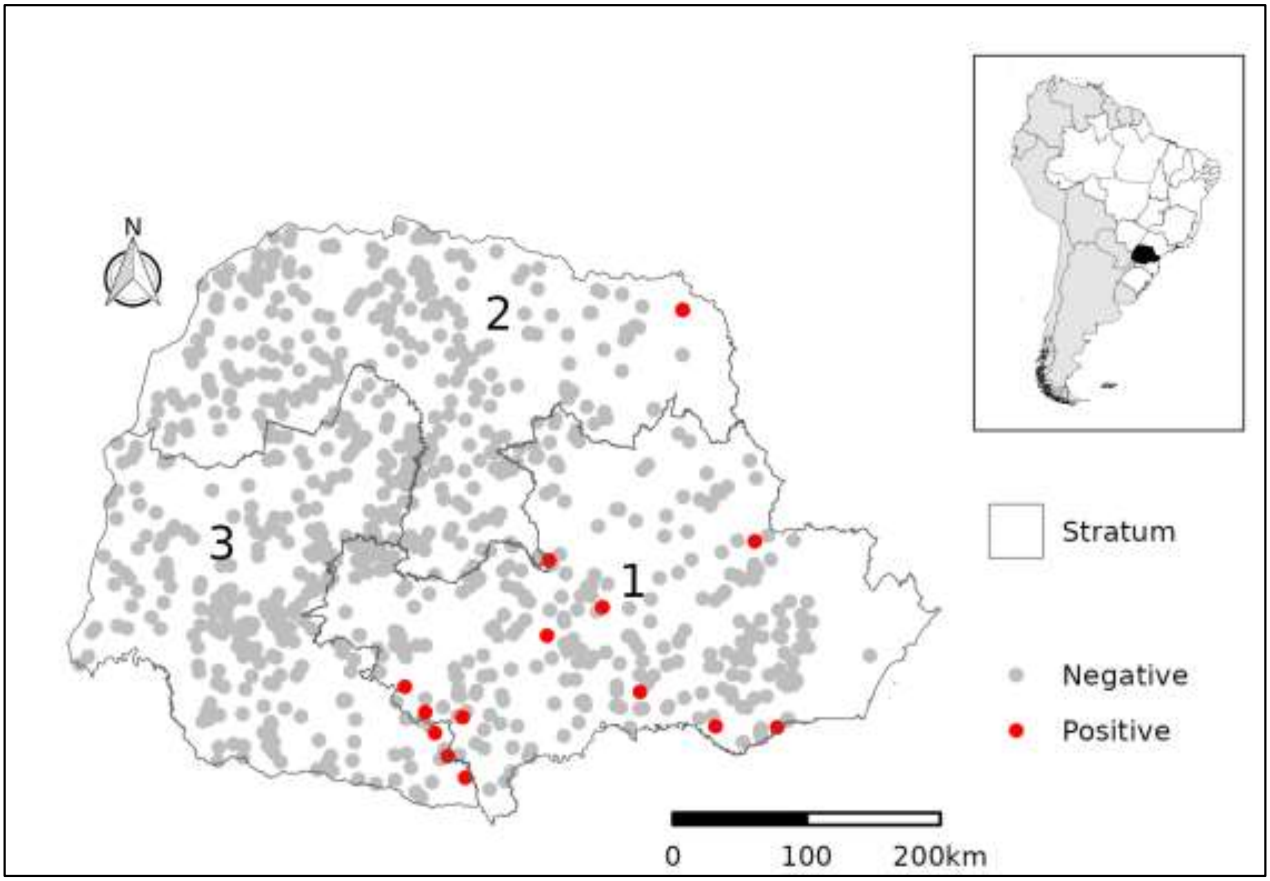

Figure 2. Geographic location and sanitary status for EIA of the farms sampled in three regions/ stratum of the state of Paraná, Brazil. The map on the right indicates the location of the state of Paraná in Brazil and South America.

Table 1
Registration and sample data from the equine infectious anemia study in the state of Paraná, Brazil. Source: ADAPAR

region	number of farms with equidae	number of equidae aged ≥6 months	sampled number of farms with equidae	sampled number of equidae aged ≥6 months
1	24,285	100,469	297	907
2	32,587	128,969	299	1,032
3	14,264	50,471	293	879
Paraná	**71,136**	**279,909**	**889**	**2,818**

Tables 2 and 3 show the prevalence of infected farms and seropositive animals in the state.

Table 2
Prevalence of farms infected with equine infectious anemia in the state of Paraná, Brazil, 2018

region	positive / examined	prevalence (%)	CI 95% (%)	
			lower	upper
1	11 / 297	3.70	1.55	5.86
2	1 / 299	0.33	0.00	0.99
3	2 / 293	0.68	0.00	1.63
Paraná	**14 / 889**	**1.55**	**0.92**	**3.00**

Table 3
Prevalence of seropositive animals for equine infectious anemia in the state of Paraná, Brazil, 2018

region	positive / examined	prevalence (%)	CI 95% (%)	
			lower	upper
1	11 / 907	1.37	0.30	2.43
2	1 / 1,032	0.06	0.00	0.19
3	2 / 879	0.19	0.00	0.44
Paraná	**14 / 2,818**	**0.55**	**0.27**	**1.00**

The results in Tables 2 and 3 show that the overall prevalence of infected farms and seropositive animals was low, with a difference only between the prevalence of infected farms in regions 1 and 2.

Compared to the epidemiological situation of Paraná with that of other FUs where the studies were conducted with a planned sample covering the entire territory, it is evident that the estimated prevalence of infected farms in the state (1.55 % [0.92; 3.00], Table 2) is equal to that reported in the Federal District for draft animals (2.3 % [1.0; 4.2]; Moraes et al., 2017) and Goiás (3.12 % [1.24; 6.00]; Pádua et al., 2022), and lower than that reported in Minas Gerais for service animals (5.3 % [4.3; 6.3]; Almeida et al., 2006) and Mato Grosso (17.2 % [15; 20]; Barros et al., 2018). This confirms the heterogeneity of disease occurrence patterns among Brazilian FUs, indicating the requirement to formulate coping strategies according to local situations. These differences among the FUs are associated with the favorable environmental conditions for the multiplication of vectors, sanitary standards, and management practices in each region.

Using the estimated prevalence value of seropositive animals in the state and its upper limit of the confidence interval (0.55 % and 1.00 %, Table 3), and the conservative values of sensitivity and specificity for the AGID test previously mentioned (S = 98.00 %; E = 99.50 %), the most likely positive predictive value (PPV) of the test was calculated to be 9.72 %, being at best 50.57 %. So, the epidemiological situation of EIA in Paraná indicates that diagnostic protocols with specificity very close to or equal to 100 % should be adopted.

Considering that as of 2014 the MAPA allowed the use of the ELISA technique as a screening test for IDGA (MAPA, 2014), the sensitivity and specificity of the serial testing protocol became 97.02% and 99.99%. The calculation was performed in the Epitools program (Sergeant, 2018b), considering the values of 99.00% and 97.00% for the sensitivity and specificity of the ELISA test (Diniz et al., 2020). Reproducing the reasoning of the previous paragraph, the most likely PPV of the test was calculated to be 98.19%, and in the best case scenario 99.01%, i.e, even using a testing protocol with very high specificity (99.99%), the epidemiological situation of EIA in Paraná indicates that, probabilistically, of every 100 animals that test positive for the serial ELISA+IDGA protocol, two will be false positives. This result must be considered for the case definition, because the classification of the animal as positive implies its sacrifice.

The calculation of PPV depends on the estimated prevalence; therefore, it is recommended that the Brazilian FUs must conduct epidemiological studies to clarify the EIA situation in their territories, facilitating the rational management of PNSE. However, it is fundamental that these studies must be standardized using the same methodology. Studies carried out in the Pantanal of Mato Grosso (MT) and Mato Grosso do Sul (MS) showed very different results for the same ecosystem and mode of production. Nogueira et al. (2018) reported prevalence of infected farms 80 % [68; 92] and prevalence of seropositive animals 39 % [26; 51] in the Pantanal of MS, whereas Barros et al. (2018) reported prevalence of infected farms 36 % [31; 42] and prevalence of seropositive animals 17 % [15; 20] in the Pantanal of MT. The study by Nogueira et al. (2018) covered only the Pantanal of MS, and that of Barros et al. (2018) covered the entire state of MT.

By comparing the estimated prevalence of infected farms (Table 2) to the existing number of farms with horses in

Paraná (Table 1), it was observed that in 2018, there were 1103 [654; 2134] EIA infected farms in the state, out of which only 15 were detected (Figure 1). The sensitivity of the Surveillance System for EIA in Paraná in 2018 ranged from 0.70 % – 2.29 %, with the most likely number being 1.36 %. This value is very low and is insufficient to change the endemic balance of the disease, indicating that PNSE is not able to achieve the expected objectives; therefore, a reassessment involving all public and private agents interested in this subject should be performed (Callefe & Ferreira, 2020). Analogous reasoning can be made using the annual average number of EIA outbreaks detected by Surveillance System from 2005 to 2019 (23) (Figure 1).

The key elements to lead this discussion are as follows: 1) take into consideration the results presented here; 2) a clear definition of the program's objective: control or eradication; 3) recognizing that it is difficult to gather public support in the fight against EIA, as it is not zoonotic and is significant only for the people interested in equestrian sports and in the improvement of breeds, a clear minority of the equine population of Paraná and Brazil; 4) take into consideration the existence of successful international experience for control/eradication programs with shared responsibilities and costs between the private and public sectors (Canadian Food Inspection Agency [CFIA], 2014); and 5) adopting the mechanisms to assess the effectiveness of the implemented actions.

Despite the small number of detected infected farms (n = 14; Table 2), responsible for the rather wide confidence interval of the only variable that remained in the final logistic regression model, the introduction of animals as a risk factor for EIA has great biological plausibility considering the etiopathogenesis and transmission mechanisms of the disease (OR=5.46 [1.87; 15.94], p=0.002). Therefore, breeders of horses in Paraná should be alerted regarding the necessity to observe sanitary precautions while introducing animals into their herd to prevent EIA.

Conclusions

The equine breeders in the state of Paraná should be informed that the spread of EIA in the state is associated mainly with the introduction of animals in their herds without prior serological test for the disease. The state should review its actions to combat the disease, considering that the strategies implemented so far have not changed the endemic balance of the disease. This process should involve all public and private agents interested in the subject.

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