

Equine infectious anaemia in equids of Southern Pantanal, Brazil: seroprevalence and evaluation of the adoption of a control programme¹

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ABSTRACT.- Nogueira M.F., Oliveira J.M., Santos C.J.S., Petzold H.V., Aguiar D.M., Juliano R.S., Reis J.K.P. & Abreu U.G.P. 2017. **Equine infectious anaemia in equids of Southern Pantanal, Brazil: seroprevalence and evaluation of the adoption of a control programme.** *Pesquisa Veterinária Brasileira* 37(3):227-233. Embrapa Pantanal, Rua 21 de Setembro 1880, Bairro Nossa Senhora de Fátima, Cx. Postal 109, Corumbá, MS 79320-900, Brazil. E-mail: marcia.furlan@embrapa.br

The working equid population in Corumbá, Southern Pantanal, is very large and has a crucial role in the main economic activity of the State of Mato Grosso do Sul, the beef cattle industry. The aim of the present study was to estimate the prevalence of equine infectious anaemia (EIA) in working equids of ranches in the municipality of Corumbá, by the official agar gel immunodiffusion (AGID) test, and evaluate the adoption of the Programme for the Prevention and Control of Equine Infectious Anaemia proposed by Embrapa Pantanal and official entities in the 1990s. From September to November 2009, forty ranches distributed through the area of the municipality were visited, and serum samples were obtained from 721 equines and 232 mules. According to previous publications and the present data, it was concluded that the prevalence of EIA in this population has increased from 18.17% to 38.60%, which represents at this time approximately 13,000 infected animals. There was no significant difference between the apparent prevalence of equines and mules. It was also verified that the control programme was not known by the greater part of the interviewed ranch owners, managers and foremen and, in their perception, EIA is not a primary threat to address. Among the studied variables, the serologic testing practice significantly reduced the risk for the presence of EIA seropositivity, as well as the separation of riding equipment and segregation of seropositives.

INDEX TERMS: Equine infectious anaemia, equids, Southern Pantanal, seroprevalence, control programme, EIAV, Equidae, AGID, Mato Grosso do Sul, prevalence, risk factor.

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RESUMO.- [Anemia infecciosa equina em equídeos do Pantanal Sul, Brasil: soroprevalência e avaliação da adoção de um programa de controle.] A população de equídeos de serviço em Corumbá, Pantanal Sul, é muito numerosa e tem um papel crucial na principal atividade econômica do estado de Mato Grosso do Sul, a pecuária de corte extensiva. O objetivo deste trabalho foi estimar a prevalência atual da anemia infecciosa equina (AIE) em equídeos de serviço em fazendas do município de Corumbá, pelo teste oficial de imunodifusão em gel de ágar (IDGA), e avaliar a adoção do Programa de Prevenção e Controle da Anemia Infecciosa Equina proposto pela Embrapa Pantanal e entidades oficiais nos anos 1990. De setembro a no-

vembro de 2009, quarenta fazendas distribuídas na área do município foram visitadas, e amostras de soro obtidas de 721 equinos e 232 muares. De acordo com publicações anteriores e os dados obtidos neste trabalho, concluiu-se que a prevalência da AIE nesta população aumentou de 18,17% para 38,60%, o que representa atualmente cerca de 13.000 animais infectados. Não houve diferença significativa entre as prevalências aparentes de equinos e muares. Verificou-se, também, que o programa de controle era desconhecido pela maior parte dos produtores, gerentes e capatazes entrevistados e, na percepção dos mesmos, a AIE não é uma ameaça importante a ser enfrentada. Dentre as variáveis estudadas, a prática da realização de testes sorológicos reduziu significativamente o risco para a presença de soropositividade para AIE, assim como a separação dos equipamentos de montaria e a segregação dos soropositivos.

TERMOS DE INDEXAÇÃO: Vírus da anemia infecciosa equina, equídeo, IDGA, Mato Grosso do Sul, prevalência, fator de risco.

INTRODUCTION

Corumbá (19° 0' 35" S, 57° 39' 17" W), State of Mato Grosso do Sul, Brazil, is 64,960.86 km² in size, and 95% of its territory is placed in the Pantanal biome, with Paiaguás and Nhecolândia representing the largest sub regions of Brazilian Pantanal (Fig.1). The whole area of Corumbá is occupied by ranches dedicated to extensive cattle raising (Abreu et al. 2010), resulting in a herd of 1.8 million head (IBGE 2013), and the second largest bovine herd in size among Brazilian municipalities. As all of this region is a floodplain submitted to annual cycles of floods and droughts, access to the fields is very often complicated, and the unique alternative to accomplish the cattle work is utilizing working equids. According to official statistics, Corumbá has the largest equine herd in Brazil, with 28,317 equines (IBGE 2013), and the fourth largest mule herd, with 4,075 animals (IBGE 2012), which means approximately one equid per 50 bovines. The Pantanal is considered an endemic zone for equine infectious anaemia (EIA), the most important viral disease of equids, which is also known as "swamp fever" (Silva et al. 1999a).

EIA is caused by a retrovirus of the Lentivirus genus and is distributed worldwide. The blood of an infected equid is a perennial source of infection, which can happen iatrogenically, through surgical instruments or riding equipment, or can be mediated by haematophagous insects as mechanical vectors. Transmission in utero, through the colostrum or during coitus can occur, but these mechanisms are not considered significant epidemiologically. EIA has three clinical stages: acute, with fever, weight loss and anaemia; chronic, when there are alternating periods of fever and weakness, with periods of normality; and asymptomatic, when the equid shows no sign of disease (OIE 2013). In the Pantanal, EIA seropositive equids are kept isolated but are not euthanatized, as the law dictates in the other parts of Brazil (Brasil 2004).

There is no data about the general prevalence of EIA in Brazil. The available data come from the results of the agar gel immunodiffusion (AGID) test, made in licensed laboratories, in equids subjected to transit around the country or

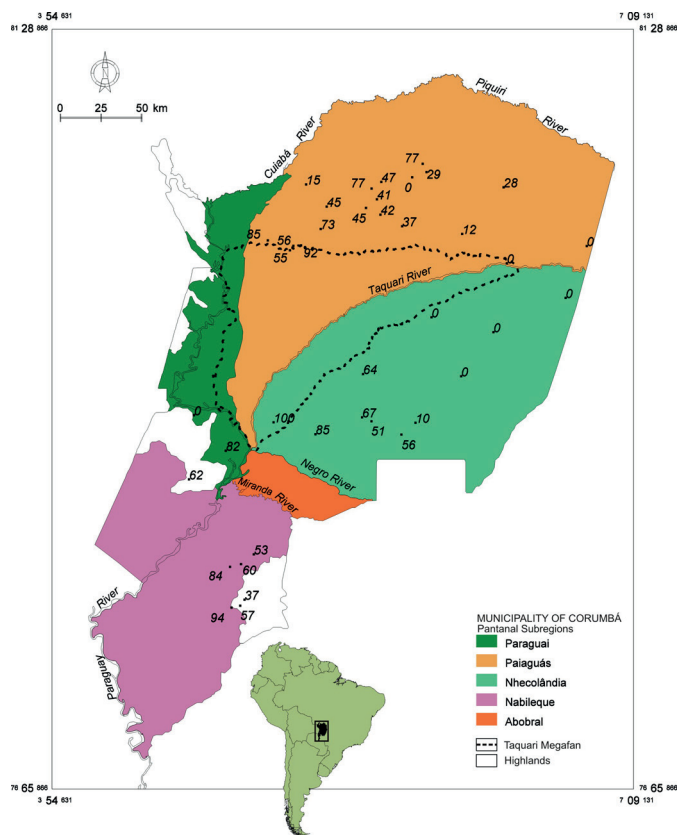


Fig.1. Corumbá territory, the subregions of Southern Pantanal and its main rivers, and the location of the ranches visited. Each dot represents a ranch sampled in this survey, and the number represents the equine infectious anaemia seroprevalence determined on that ranch by the official agar gel immunodiffusion test.

that have participated in events, as recommended by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA). In Pantanal, studies of the Research Center of Pantanal (Embrapa Pantanal) of the Brazilian Agricultural Research Corporation (Embrapa), from 1990 to 1995, showed a prevalence of EIA of 18.17% in horses used for work in the cattle ranches (Silva et al. 1999b). To revert this situation, Embrapa Pantanal, with governmental entities, created the Programme for Prevention and Control of Equine Infectious Anaemia in the Pantanal of Mato Grosso do Sul State (Silva et al. 2004). The Programme recommended serodiagnosis and segregation of the seropositive animals and their riding equipment, and it also recommended the early weaning of foals that were born from EIA seropositive mares, as a manner of still utilizing the infected animals but moving towards making the herds free of the disease.

The aim of the present work was to estimate the prevalence in 2009 of EIA in working equids of ranches in the municipality of Corumbá, by the official AGID test, and evaluate the adoption of the Programme for the Prevention and Control of Equine Infectious Anaemia proposed by Embrapa Pantanal and official entities in the 1990s.

MATERIALS AND METHODS

Ethics statement. All applicable national and institutional guidelines for the care and use of animals, in the years this research was planned and executed, were followed (Brasil 2008).

Study design. A cluster-sampling design was adopted to estimate the seroprevalence of EIA in working equines and working mules or donkeys of Corumbá (Bennett et al. 1991). Each ranch was considered a cluster, and the equids were considered the basic sampling units. Taking into account a previously determined EIA prevalence of 18% for working horses (Silva et al. 1999b), 10% of one-half length of confidence interval, a desired level of confidence of 99%, the number of individuals per cluster as 20 - of each species - and a rate of homogeneity of 0.20 (Otte & Gumm 1997), the minimum number of clusters (ranches to be visited) calculated by the CSurvey software version 1.5 was 25 (500 equines and 500 mules or donkeys).

On the occasion of sample collection, a questionnaire was used to interview the person who was in charge of the equids of the ranch, usually the manager or foreman. Questions were asked of the characteristics of the ranch, of knowledge about the Programme for the Prevention and Control of EIA in the Pantanal, a governmental programme promoted in the region from 1995 to 2005, if measures of control for EIA were adopted on that ranch and what these measures were, and about the perception of the effect of the disease.

Period and area of samples and data collection. This study was undertaken from September to November 2009. Forty ranches distributed through the area of Corumbá (Fig.1), selected from Embrapa Pantanal registers, were visited. Twenty ranches were located in the Paiaguás subregion, 11 in the Nhecolândia subregion, four in the Nabileque subregion and two in the Paraguai subregion. Three ranches were placed in the highlands. The selection of ranches was made according to the possibility to contact the owner or whomever was in charge, the possibility to have terrestrial access and the ranch's location relatively to the other ranches. The ranches were from 453 to 49,000 hectares in size, an average of 14,398 ha and median of 8,500 ha, covering 575,919 ha, or 5,759.19 km², of the municipality territory. All of them had equines to work with cattle, and 25 also had mules or donkeys, the numbers of the first ranging from 11 to 250 individuals in each ranch, totalling a population of 2858 equines, and the second from 1 to 41, totalling 401 animals. The mean and median numbers of equids on the ranches were 81 and 64, respectively.

Blood samples were taken by venipuncture of the jugular vein using disposable vacuum blood collection needles and tubes. The latter were centrifuged, and the serum was placed into cryotubes, which were stored in nitrogen containers until laboratory processing. Serum samples were obtained from 721 equines and 232 mules and donkeys and questionnaires from 34 premises.

Because just two donkeys were sampled, the group will be called "mules".

Serology. Serum samples were tested with an agar gel immunodiffusion test (AGID) utilizing a commercially available kit (Laboratórios Bruch, São Paulo/SP, Brazil) according to the manufacturer's instructions.

Statistical analysis. Descriptive statistics were employed to summarize results from serology and data obtained from questionnaires. The epidemiological parameters were estimated according to the related literature (Martin et al. 1987, Bennett et al. 1991, Martin et al. 1992, Dohoo et al. 2009). The accepted specificity (Sp) and sensibility (Se) for AGID are 100% and 98%, respectively, according to Coggins & Norcross (1970). To calculate the prevalence of EIA, each animal was weighted according to the herd size. The weighting factor was calculated as (# animals in the *i*th ranch/# sampled in the *i*th ranch)*(# animals in the municipality/# animals in all ranches visited). The number of equines in the municipality was, at the time the samples were taken, 30,032, and the number of mules and donkeys was 4,684 (IBGE 2009). The chi square test was used to explore associations between positivity and species; $\alpha = 0.05$ was used to ascribe statistical significance.

The association of risk factors raised in the questionnaires with the number of positive and negative equids was firstly examined by Correspondence Analysis, an exploratory multivariate technique (Fávero et al. 2009), using Statistical Analysis Systems (SAS) version 9.2. The association of each risk factor with presence of seropositive equids in the property was also verified by Fisher's Exact Test, and the strength of association was measured by calculating the odds ratio with a 95% confidence interval. EpiInfo 3.5.2 for Windows was utilized in these last analyses.

RESULTS

The total number of equids at each ranch, including both the sampled and seropositive ones, is presented in Table 1. In Figure 1, the number beside each dot represents the apparent simple prevalence of EIA in the equids of that ranch.

The estimates for the epidemiological parameters are shown in Table 2. The apparent animal prevalence considering all the equids was 38.60% (FC95% = 26.04 to 51.15), just the equines, 40.10% (FC95% = 26.46 to 53.74), and for mules, 33.30% (FC95% = 19.17 to 47.43). There was no significant difference between the apparent weighted prevalence of equines and mules ($\chi^2=3.241$; $P=0.072$).

Table 1. Total number of equines and mules, sampled and seropositive animals at each ranch

Ranches		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Equines	Total	30	60	66	15	55	11	29	70	80	50	50	25	53	150	80	21	100	60	80	13
	Sampled	14	16	34	8	18	5	17	4	23	29	22	17	18	6	21	20	10	23	24	12
	Positives	9	16	23	3	16	3	14	1	10	12	17	8	9	3	6	0	8	9	0	0
Mules	Total	22	1	38	0	13	25	0	20	23	0	0	0	2	41	2	0	13	2	0	13
	Sampled	16	1	20	0	13	14	0	20	23	0	0	0	2	14	0	0	10	1	0	13
	Positives	9	0	8	0	10	7	0	14	12	0	0	0	1	0	0	0	1	0	0	0
Prevalence* (%)		60.0	94.1	57.4	37.5	83.9	52.6	82.4	62.5	41.7	41.4	77.3	47.1	45.0	15.0	28.6	0.0	45.0	37.5	0.0	0.0
Ranches		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Equines	Total	166	150	100	60	70	120	20	15	50	60	34	34	30	80	70	250	79	250	110	12
	Sampled	21	25	25	20	14	20	15	13	21	20	17	16	22	20	20	19	20	20	20	12
	Positives	7	2	23	11	8	17	11	10	13	17	17	9	16	2	0	0	0	0	11	0
Mules	Total	18	31	21	2	0	26	0	25	15	0	4	0	6	0	24	0	1	0	13	0
	Sampled	18	24	0	0	0	0	0	0	4	0	3	0	2	0	20	0	1	0	13	0
	Positives	4	4	0	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	0	6
Prevalence* (%)		28.2	12.2	92.0	55.0	55.6	85.0	73.3	76.9	64.0	85.0	100.0	56.3	66.7	10.0	0.0	0.0	0.0	0.0	51.5	0.0

* Percentage of positive equids (equines and mules) among those sampled in the numbered ranch.

Table 2. Epidemiological parameter estimates considering all equids, only equines and only mules

	Ap (FC95%)	Tp	D	roh	HAp / HTP (FC95%)	HSe / HSp
All	38.60% (26.04 - 51.15)	39.39%	16.50	0.68	80.00% (67.60-92.40)	100%
Equines	40.10% ^a (26.46 - 53.74)	40.92%	14.53	0.79		
Mules	33.30% ^a (19.17 - 47.43)	33.98%	5.43	0.42	65.00% (44.10-85.90)	

Ap = apparent animal prevalence, FC = fiducial confidence, Tp = true animal prevalence, D = weighted design effect, roh = weighted rate of homogeneity, Hap = apparent herd prevalence, HTP = true herd prevalence, HSe = herd sensitivity, HSp = herd specificity, ^aEstimates with similar letter are not statistically different at 5% level.

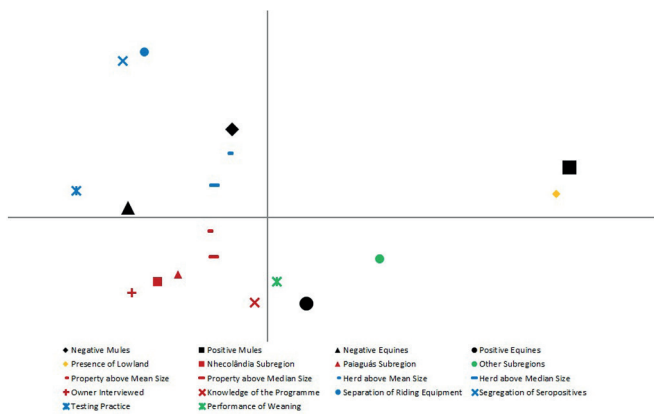


Fig.2. Graphical representation of the multidimensional relations of the chi square distances among the studied variables.

In Figure 2, it can be seen a graphical representation of the multidimensional relations of the chi square distances among the studied variables: positive mules, negative mules, positive equines, negative equines, presence of lowland, subregions (Nhecolândia, Paiaguás or other), property above mean and median size, herd above mean and median size, owner interviewed, knowledge of the Programme, separation of equipment (each equid has its own riding equipment), segregation of seropositives, testing practice and performance of weaning. Negative mules and negative horses are in the same quadrant, and we can verify that segregation of seropositives, separation of equipment, testing practice and herd above mean and median sizes are more aggregated to those two variables, demonstrating probable correspondence among them.

Table 3 summarizes the tested association of risk factors investigated in the questionnaires with the presence of seropositive equids on the premise. The variables that showed association with the frequency of positives ($P < 0.05$) were separation of riding equipment (Odds Ratio 0.076), segregation of seropositives (OR 0.038) and the testing practice (OR 0.024). Other responses obtained to the questionnaires are presented in Table 4.

DISCUSSION AND CONCLUSIONS

The number of premises visited was higher than initially planned due to the difficulty in finding sufficient numbers of donkeys and mules on the ranches because these equids are utilized in moving herds from one location to another

Table 3. Association of the investigated variables with the frequency of EIA-positive animals in the ranches

Variables	Description	Total	Positives	Frequency of positives (%)	OR	Inf. FC	Sup. FC	P
Area	highlands	3	3	100.0	2.017	0.094	43.042	1.000
	lowlands	37	29	78.4				
Pantanal subregion	Nhecolândia	11	7	63.6	0.308	0.054	1.754	0.209
	Paiaguás	20	17	85.0				
	Nhecolândia	11	7	63.6				
	other regions	9	8	88.9				
	Paiaguás	20	17	85.0				
Property size	above mean	13	11	84.6	1.571	0.270	9.126	1.000
	below mean	27	21	77.8				
	above median	20	16	80.0				
	below median	20	16	80.0				
	above median	20	15	75.0				
Herd size	above mean	14	11	78.6	0.873	0.175	4.354	1.000
	below mean	26	21	80.8				
	above median	20	15	75.0				
	below median	20	17	85.0				
Interviewed	owner	6	4	66.7	0.333	0.045	2.465	0.281
	other	28	24	85.7				
Knowledge of the Program	yes	13	11	84.6	1.294	0.201	8.309	1.000
	no	21	17	81.0				
Separation of riding equipment	yes	5	2	40.0	0.076	0.008	0.661	0.028
	no	29	26	89.7				
Segregation of seropositives	yes	6	2	33.3	0.038	0.004	0.356	0.004
	no	28	26	92.9				
Testing practice	yes	8	3	37.5	0.024	0.002	0.280	0.001
	no	26	25	96.2				
Weaning	yes	14	11	78.6	0.647	0.110	3.804	0.672
	no	20	17	85.0				

OR = odds ratio, Inf. FC = inferior fiducial confidence, Sup. FC = superior fiducial confidence.

Table 4. Answers obtained to the questionnaires

Questions	# Observations (%)	Answers
Which was the employment of the equids in the ranch?	34 (100)	Equids were used to work in cattle raising (daily practices and transportation from ranches to livestock markets).
Was weaning performed on the ranch?	20 (59) 7 (21) 7 (21)	No, it was not. Yes, and it occurred from seven months to less than one year of age. Yes, and it occurred from one year onward.
Were the practices the same for equines and mules?	17 (50) 17 (50)	(There was no answer or there were no mules in the ranch). Yes, all the practices were the same for equines and mules.
Was there perception of the effects of the disease in the equid?	10 (29) 10 (29) 14 (41)	(There was no answer). Yes, it was perceived when the equid was infected. No, it was not possible to know when the equid was infected.
Did the infected equids have a decreased work capacity when compared to the non-infected ones?	17 (50) 10 (29) 5 (15) 2 (6)	(There was no answer). No, they did not. Yes, they had a decreased working capacity. It was not known.
Were there diseases that could be confounded with EIA?	8 (24) 13 (38) 13 (38)	(There was no answer). No, there were not. Yes, there were some.
Mention examples of diseases that could be confounded with EIA.	4 (44) 3 (33) 1 (11) 1 (11)	Strangles. Trypanosomiasis. Encephalitis. Rabies.
What kills equids in the Pantanal?	27 (66) 4 (10) 3 (7) 3 (7) 3 (7) 1 (2)	Ophidian accidents. EIA. Strangles. Pythiosis. Trypanosomiasis. Puma predation.

and so were almost always on the road (as seen in Table 1, ranches #15, 23, 24, 26 and 28). However, besides the larger number of clusters selected, the precision of the estimated prevalence was much lower than was expected (Table 2). An explanation is that the encountered prevalence of 38.60% turned out to be higher than that previously reported by Silva et al. (1999b) of 18%, as were the estimated intraclass correlation coefficients (ρ) of 0.68. This, considering all animals, or only equines, was very similar to the ρ value of 0.7 found by Borges et al. (2013) in Northern Pantanal, reinforcing the idea that variance in infection rates within equid herds is similar to that between herds from different farms.

The EIA prevalence found for the municipality of Corumbá can be considered very high, having in mind surveys from other parts of Brazil. There is no reason to think this situation has changed, from 2009 to the present data, because very little has been done towards this direction. Heinemann et al. (2002) described a seroprevalence of 17.71% for working equids from the municipality of Uruará, State of Pará; Almeida et al. (2006), also for working equids, found 7.4% in the North and Northeast of Minas Gerais State; Aguiar et al. (2008) reported a seroprevalence of 9.6% in the municipality of Monte Negro, in the State of Rondônia; and Guimarães et al. (2011), 5.9% in the South of Bahia State. Matching the present data with those obtained by Borges et al. (2013) in Northern Pantanal, from samples collected at approximately the same time, in the North, 65% and 31.5% prevalence rates were observed for properties and animals correlated to 80% and 39.4% (true animal prevalences) in the South. On the other hand, Braga (2000) stated it was seen, through a serological survey in the State of Roraima, that about 40% to 60% of those

equines were EIA seropositive, and Freitas et al. (2015) reported a prevalence of 46.26% EIA positive cases in puruca and marajoara horses on the Marajo Island.

Borges et al. (2013) stated, about Northern Pantanal, that farms and equids in flooded areas had a greater chance of being EIA seropositive, but this association between presence of lowlands and positivity was not found in the Southern. It is known that flooded areas generally have a larger number of tabanids but, more importantly, that flooded areas are much more difficult to work in and to disseminate sanitary information to compared with dry areas. In agreement with this point of view is the conclusion obtained by Borges et al. (2013) that, even in wetlands, a negative spatial correlation was found between farms in regard to EIA prevalence, which would suggest that other factors, such as management practices, probably play a significant role in the transmission of EIAV. Almeida et al. (2006) and Freitas et al. (2015) also emphasized the importance of poor management practices in the maintenance of high prevalence rates.

Comparing the animal prevalence found here with the rate reported by Silva et al. (1999b) of 18% for working equines, it could be said that the prevalence of the disease may have doubled in 20 years. It reveals that the Programme for the Prevention and Control of Equine Infectious Anaemia proposed by Embrapa Pantanal and official entities in the 1990s did not accomplish the intended effect. Indeed, just 38% of the interviewees knew about the Programme, and this variable showed no association with the frequency of positives, as demonstrated in Table 3. Despite this fact, in Figure 1, it can be observed that five ranches in the Nhecolândia subregion had no positive equids, and that location was the one where the Programme had more

diffusion and adoption. Considering that less than half of the interviewees knew about this public policy and that the area of ranches without seropositives was in the region of higher policy diffusion, perhaps a limiting factor for the policy's adoption has been poor diffusion of the programme, besides low investment in credit, incentives, governmental infrastructure, technical assistance and monitoring, among others.

The statistical methods used to explore association between the studied variables showed agreement in their results. As primary seen in the multivariate technique among the investigated risk factors, three were shown to make equids less likely to be seropositive: the separation of riding equipment, the segregation of seropositives and testing practices. These measures are taken by the ranchers that are interested in preventing and controlling EIA, which premises already have no seropositives or a low prevalence of EIAV infection. The separation of riding equipment can be performed by everyone, but the segregation is dependent on the testing practice. Through our data, it can be stated that ranches where serodiagnosis is performed have an approximately 41-fold lower chance of having EIA-seropositive equids. The reasons for the low number of ranches that perform the serological tests are their high costs, mainly due to the laws which regulate the testing, and the absence of a licensed laboratory in the municipality.

Regarding the supplementary information provided by the questionnaire, 59% of the interviewees reported that weaning was not performed on the ranch and 21% that it occurred from one year onward, reinforcing the idea that poor management practices prevailed. The ranches with mules to move the herds reported to employ the same practices used with equines and, indeed, the equids EIA apparent prevalence of 33.30% and 40.10%, respectively, showed no statistically significant difference. Furthermore, it would be worthwhile to emphasize the perception of the effect of the disease by the equid owners. The data revealed that the majority of those who answered these questions could not distinguish an EIAV-infected equid from an uninfected one nor differences in working performance. Simultaneously, opinions about which diseases could be confounded with EIA were divided, but the vast majority agreed that the primary cause of death of equids in Southern Pantanal was snake bites, not an infectious disease.

The EIA-seropositive equids in the Pantanal region are still sold, but their prices are not uncommonly lower, and these individuals are not allowed to leave the high-risk area. Moreover, as demonstrated by the answers to our questionnaire, a lower performance of the infected equids is also observed. Having in mind that, by our data, there are nearly 13,000 EIAV-infected equids, these facts suggest significant economic losses. There is also the stigmatization of equids from Pantanal, which is a major problem for financial valuation of the Pantaneiro horse breed. Education on sanitary practices, emphasizing the benefits that would come from preventing EIA, and the facilitation of the diagnostic testing practice are urgently needed to control EIA in Southern Pantanal.

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Conflict of interest statement.- The authors declare that they have no conflict of interest.

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