Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v25e-78756E

e-ISSN 1809-6891
Veterinary Medicine | Research article

Epidemiological characteristics and risk factors in focal properties of equine infectious anemia in Rio Grande do Sul State, Brazil

Características epidemiológicas e fator de risco em propriedades foco de anemia infecciosa equina no Estado do Rio Grande do Sul, Brasil

Rafaela de Carvalho Machado[1], José Conrado do Santos Jardim[1], Carolina Kist Traesel[1], Paula Fonseca Finger[1], Rogério Oliveira Rodrigues[2], Mario Celso Sperotto Brum[1]

1 Universidade Federal do Pampa (UNIPAMPA), Uruguaiana, Rio Grande do Sul, Brazil
2 Instituto de Pesquisas Veterinárias Desidério Finamor (IPVDF), Eldorado do Sul, Rio Grande do Sul, Brazil.
*corresponding author: mariobrum@unipampa.edu.br

Abstract: In Brazil, equine infectious anemia (EIA) infection is widespread in various regions, with variable prevalence levels. The objective of this work was to determine the epidemiological characteristics that could contribute to its dissemination on the western border of Rio Grande do Sul, Brazil. To accomplish this, an epidemiological questionnaire was administered to equine owners who had experienced EIA-positive animals on their properties from 2009 to 2019. The interviewees were contacted via phone calls, and the questions aimed to identify patterns among properties, on animal management, health, and owner's knowledge about the infection. The primary information was acquired from the data of the Official Veterinary Service (OVS) through data compilation and tabulation of the official forms. Additionally, the same questionnaire was applied to properties without records of the infection, as control. The results were analyzed using forward logistic regression to explore potential risk associations. Out of a total of 123 focal properties, 28 interviews were completed, six were interrupted or declined, 55 did not respond to phone calls, and 34 had outdated contact information. Among the 30 control properties, 15 agreed to participate in the survey. The results suggest that breeding, management, and sanitary control practices are Similar between focal and control properties. Only two related risk factors could be distinguished for properties that have never engaged in these practices, which were animal transit outside the property and contact with other horses. In addition, it was observed that the official register is outdated for a significant number of properties.

Keywords*:* disease control; horse; probability; retrovirus; relative risk

Resumo: No Brasil, a infeção por anemia infecciosa equina (AIE) está disseminada por diversas regiões, com níveis de prevalência variáveis. O objetivo deste trabalho foi determinar as características epidemiológicas que poderiam contribuir para a disseminação da AIE na região oeste do Rio Grande do Sul, Brasil. Para isso, foi aplicado um questionário epidemiológico aos proprietários de equinos que tiveram animais positivos para AIE em suas propriedades no período de 2009 a 2019. Os entrevistados foram contatados por meio de ligações telefônicas, e as perguntas visavam identificar padrões entre as propriedades, sobre o manejo dos animais, a sanidade e o conhecimento do proprietário

Received: March 14, 2024. Accepted: May 07, 2024. Published: October 03, 2024.

sobre a infeção. As informações primárias foram obtidas nos arquivos do Serviço Veterinário Oficial (SVO), compiladas e tabuladas. Adicionalmente, o mesmo questionário foi aplicado a propriedades sem registo de infeção, designadas controle. Os resultados foram analisados por meio de regressão logística *forward* para explorar potenciais associações de risco. De um total de 123 propriedades focais, 28 entrevistas foram completadas, seis foram interrompidas ou recusadas, 55 não responderam aos telefonemas e 34 tinham informação de contato desatualizada. Entre as 30 propriedades controle, 15 concordaram em participar do inquérito. Os resultados sugerem que as práticas de criação, gestão e controle sanitário são semelhantes entre as propriedades foco e as controle. Apenas dois fatores de risco puderam ser distinguidos das propriedades positivas e negativas, sendo estes o trânsito de animais da propriedade e o contato com outros cavalos. Além disso, observou-se que os registos oficiais dos produtores está desatualizado para um número significativo de propriedades.

Palavras-chaves: controle de doenças; cavalo; probabilidade; retrovírus; risco relativo.

1. Introduction

Equine infectious anemia (EIA) is a viral infection that affects members of the *Equidae* family (horses, donkeys, mules, and zebras) [1,2]. The virus (EIAV) belongs to the *Retroviridae* family, in the *Lentivirus* genus; it has a lipidic envelope, conic capsid, and the genome is formed by two copies of single-stranded RNA [3,5]. The monocyte-macrophage cell lineage is infected by the virus, followed by the reverse transcription of genomic RNA, and the insertion of the proviral DNA into the cellular genetic material. This process enables the development of permanent infection and the carrier condition, which is considered the main source of virus dissemination [6,7]. The transmission occurs mainly through blood-sucking vectors (*Tabanus* spp. and *Stomoxys* spp.) or iatrogenically (syringes, needles, and surgical equipment), and blood transfusion. Moreover, there is the possibility of transplacental transmission in cases of infected mares experiencing febrile episodes [9].

The EIAV has a global distribution, and between the years 2007 and 2014, the infection emerged in several European countries [2]. In Brazil, the first diagnosis of the infection was conducted in 1968 in the states of Rio de Janeiro, Rio Grande do Sul, and Minas Gerais [6,10,11]. Since then, the infection has been present in all regions of the country with varying levels of occurrence, associated with breeding practices, climate, vector presence, and control measures adopted [13,14]. The national control of the disease is governed by the National Equine Health Program (*Programa Nacional de Sanidade Equina - PNSE*), instituted by the Ministry of Agriculture, Livestock and Food Supply (*Ministério da Agricultura, Pecuária e Abastecimento - MAPA)* [15,16].

Each State has specific legislation to control the infection according to its levels and relevance for equine husbandry. In the state of Rio Grande do Sul, the State Equine Health Program (*Programa Estadual de Sanidade Equina – PESE*) coordinates the actions for *Equidae* health. EIA is subject to mandatory reporting, and control is performed due to transit permission of seronegative animals and euthanasia of positive ones [14,15]. The confirmatory diagnosis of the virus is conducted through the serological test by agar gel immunodiffusion (AGID), performed in accredited private laboratories [16].

The challenges regarding clinical identification are associated with the non-specificity of the symptoms, making the infection primarily diagnosed through passive surveillance. This is due to the necessity of performing serological tests for transporting these animals and emission of the Animal Transport Certificate (ATC) (*Guia de Transporte Animal - GTA*) [17,18]. In Rio Grande do Sul, seroprevalence has always been considered low, and in 2013 was estimated at 0.3% [17]. Despite these low prevalence data, in 2015 an increase was observed in the notifications [18]. The western border of Rio Grande do Sul has a significant equine population, and a large number of the focus of EIA were reported in the years 2018 and 2019 [19].

The aim of this study was to characterize the equestrian properties on the western border of Rio Grande do Sul, evaluate the management practices adopted and, thus, identify possible risk factors that contribute to dissemination of EIAV. Hence, the study intended to assess the level of knowledge among equine owners regarding infection and disease.

2. Material and methods

2.1 Region of study

The cases of equine infectious anemia that occurred in the municipalities of São Borja, Maçambará, Itaqui, Uruguaiana, Barra do Quaraí and Quaraí were evaluated. This region is part of the western border of the State of Rio Grande do Sul, Brazil. The official notification forms (Form-IN and Form-COM) were kindly provided by the Agricultural Defense System, from the State Secretariat of Agriculture, Livestock, and Rural Development of the State of Rio Grande do Sul (DSA/SEAPDR-RS). These forms were reviewed, and properties that reported at least one animal diagnosed with equine infectious anemia between the years 2009 and 2019 were considered in the research.

2.2 Epidemiological questionnaire

In order to identify the main characteristics of the properties, the herd, the level of knowledge about the disease, and possible modes of transmission and dissemination of the agent, an epidemiological questionnaire was developed. The questionnaire, consisting of previously validated multiple-choice questions, was performed via telephone, with a minimum of three attempts at different times, and contact made by two trained interviewers [21]. For comparison purposes, 30 properties with horses that had never reported cases of EIA were randomly selected and also included in the study. All information regarding the identification of producers was omitted. The administration of the final questionnaire took place between May and September 2020. During the telephone contact, the interviewers identified themselves and provided information about the study, the terms of free consent and clarification (TLCE), and the confidentiality of the information was assured. All procedures were submitted and approved by the Research Ethics Committee of the Universidade Federal do Pampa (CEP/UNIPAMPA), under registration 24280119.9.0000.5323.

2.3 Analysis of responses

The responses of the interviewees were documented in a spreadsheet in Microsoft Office Excel (2016) and analyzed through descriptive statistics. A step-by-step logistic regression analysis was also performed to assess factors associated with EIA. A likelihood ratio test of the null hypothesis that the parameter vector of a statistical model satisfies some smooth restriction was applied to assess how effectively the model described the outcome variable using a Stepwise Forward method. For statistical analysis, the Stata/Data Analysis version 17.0 (Stata Corporation) program was used, and the descriptive map was elaborated using QGIS 3.6 (http://qgis.osgeo.org).

3. Results

3.1 Descriptive analysis

Between the years 2009 and 2019, 125 properties tested positive for equine infectious anemia, with 123 included in the study. Each property that had at least one positive animal was considered a focus. Two focal properties were excluded from the study, one originating from the apprehension of animals of unknown origin (smuggling) and another due to a lack of information. From 2009 to 2015, 24 focuses were recorded, and from 2016 to 2019, at least 99 focuses were diagnosed. The infection was identified in the municipalities of São Borja (n = 71), Itaqui (n = 25), Uruguaiana (n = 19), Barra do Quaraí (n = 4), Quaraí (n = 3) and Maçambará (n = 1). All these municipalities are located on the western border of Rio Grande do Sul, bordering Uruguay or Argentina (Figure 1).

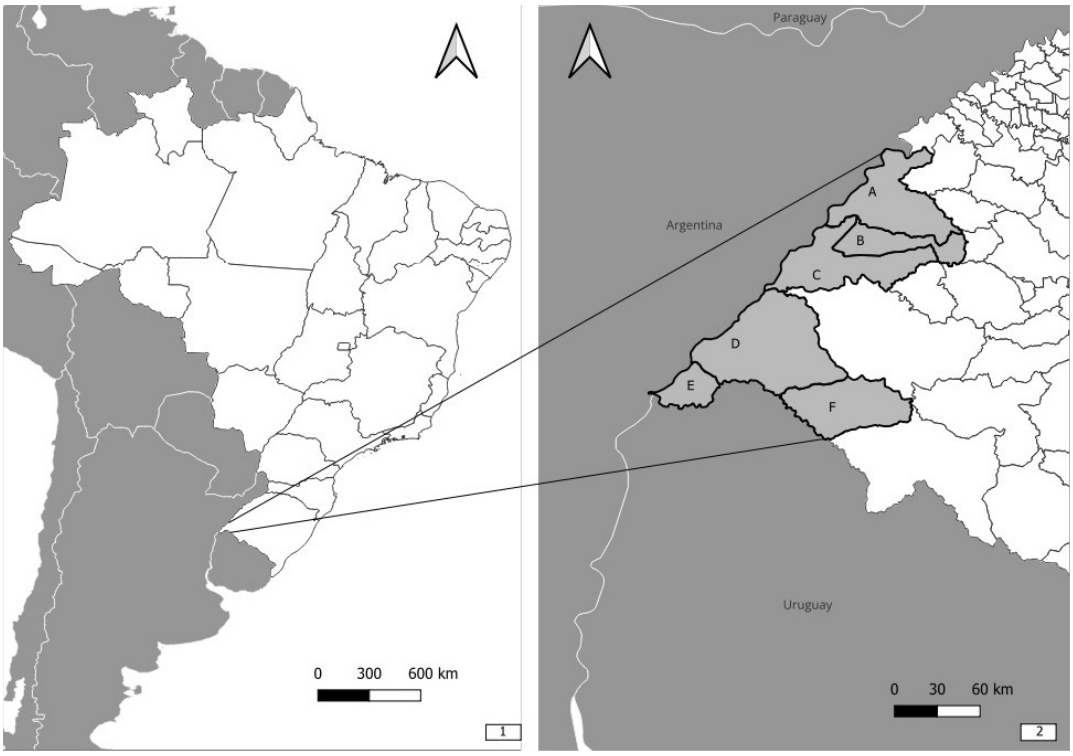

Figure 1. Western regions of Rio Grande do Sul State, Brazil. Municipalities of São Borja (A), Maçambará (B), Itaqui (C), Uruguaiana (D), Barra do Quaraí (E), and Quaraí (F) experienced an increase in the detection of equine infectious anemia (EIA) focal points between 2009 and 2019.

Among the focal properties (n = 123), only 28 (22.8%) completed the entire questionnaire. All 28 properties had at least one positive EIA diagnosis by the official service. Six producers (4.9%) refused or interrupted, 55 (44.7%) did not answer the phone, and for 34 producers (27.6%), the contact number was incorrect. For the 30 negative properties contacted, 15 (50%) completed the responses, three (10%) interrupted or refused to answer, and the remaining 12 (40%) did not answer the call. To assess the reliability of the responses, a question about the previous occurrence of EIA cases on the property was included. Two (7.1%) producers from the focal properties mistakenly answered that they had not recorded cases of the infection. As expected, all owners of negative properties indicated that they had never had cases of EIA.

For 50% of the focal properties (14/28), the first animal identified as positive was a horse that had a transit history and had undergone previous testing. For 12 focal properties (42.8%), the initially identified positive animal had no transit history or previous testing, suggesting that the infection occurred within the property, nearby properties, or through illegal transit. Additionally, two owners (7.1%) were unable to provide information about the index case of the property. All focal properties recorded positive cases only once, meaning that after sanitation measures were applied, the infection was not diagnosed again.

The characteristics of the properties and breeding practices are presented in Table 1. It is observed that horses are used for more than one purpose (working with cattle and sheep and participating in rodeos/parades). They are predominantly raised on rural properties, but a significant portion are also raised in peri-urban areas and alongside other horses. The presence of horseflies was reported in at least 60.1% of the focal properties, which also confirmed the presence of flooded areas and vegetation (streams, rivers, marshes) in 82.7% of the situations. These are epidemiological factors that facilitate the proliferation of these vectors. The control of these insects is carried out by only 2% of the properties characterized as focal.

Table 1. Characteristics of properties, origin, and utilization of animals on focal (n = 28) and negative properties - control (n = 15) for equine infectious anemia on the western border region of Rio Grande do Sul, Brazil.

		Focal (%)	Control (%)	OR	*p*	95% CI	
Herd size	1 to 10 animals	16 (57.1)	10 (66.7)	ref.			
	11 to 50 animals	11 (39.3)	3 (20.0)	1.09	0.723	-0.41	0.60
	51 to 100 animals	1 (3.6)	1 (6.7)	0.50	0.203	-1.75	0.37
	> 101 animals	0 (0.0)	2 (13.3)	0.01	0.005	-6.89	-1.21
Purpose of horses	Work in the field	19 (67.8)	7 (46.7)	ref.			
	Sports	16 (57.1)	11 (73.3)	0.50	0.224	0.16	1.52
	September 20th parade	18 (64.3)	9 (60.0)	0.69	0.512	0.22	2.08
	Reproduction	8 (28.6)	4 (26.6)	0.64	0.528	0.16	2.55
	Riding	6 (21.4)	2 (13.3)	1	1.000	0.15	6.25
Location	Rural area	19 (67.9)	12 (80.0)	ref.			
	Urban periphery	5 (17.9)	2 (13.3)	1.13	0.887	-1.56	1.81
	Urban zone	5 (17.9)	1 (6.7)	1.88	0.529	-1.30	2.60
	Urban stables	3 (10.7)	1 (6.7)	4.39	0.340	-1.55	4.52

Husbandry practices	Stall/tied	11 (39.3)	7 (46.7)	ref.			
	Loose by day/stable at night	7 (25.0)	3 (20.0)	1.33	0.732	0.25	6.93
	In the field	21 (75.0)	12 (80.0)	1.27	0.672	0.41	3.97
Where the horses lives has	Swamp	18 (64.3)	11 (73.3)	ref.			
	River/ditch	18 (64.3)	5 (33.3)	1.41	0.563	0.43	4.60
	Meadow	15 (53.6)	3 (20.0)	1.40	0.598	0.40	4.89
	No information	5 (17.8)	3 (20.0)	0.25	0.141	0.03	1.58
Vector presence	No	10 (35.7)	2 (13.3)	ref.			
	Yes	17 (60.7)	12 (80.0)	0.38	0.223	-2.52	0.58
	No information	1 (3.6)	0 (00.0)	0.08	0.172	-5.92	1.05
Vector control	No	26 (92.9)	15 (100.0)	ref.			
	Yes	2 (7.14)	0 (0.00)	1.69	0.752	-2.73	3.78
	No information	0 (0.00)	0 (0.00)	0.18	0.315	-4.93	1.58

**OR – odds ratio*
***95% CI - confidence interval*

Husbandry practices play a crucial role in contributing to the spread of the viral agent. Table 2 presents the responses related to the qualifications of the personnel responsible for animal treatment, as well as the sharing of riding, injectable, and surgical materials. All these procedures are associated with the transmission of the virus among animals. It was observed that riding equipment is shared, injectable equipment and medical care products are reused, and individuals without training work in the field. These procedures are common to all properties, reported by both positive and negative property owners.

Table 2. Characteristics of husbandry practices present in positive cases - focal (n = 28) and negative properties - control (n = 15) for equine infectious anemia on the western border of Rio Grande do Sul, Brazil.

		Focal (%)	Control (%)	OR	*p*	CI 95%	
Personnel responsible for the animals	Veterinary	20 (71.4)	13 (86.7)	ref.			
	Uncertified veterinarians	7 (25.0)	2 (13.3)	1.03	0.969	0.21	5.05
	Employees	9 (32.4)	4 (26.7)	1.23	0.743	0.34	4.43
Administration of injectable medication and vaccines	Do not use injectable medication	6 (21.4)	9 (00.0)	ref.			
	Disposable syringes	15 (53.6)	12 (80.0)	0.46	0.438	-2.71	1.17
	Re-usable syringes and needles	5 (17.9)	3 (20.0)	0.15	0.120	-4.26	0.48
	No information	2 (7.1)	0 (00.0)	0.81	0.915	-3.87	3.47
Riding equipment shared	Bridles	10 (35.7)	6 (40.0)	ref.			
	Halters	10 (35.7)	7 (46.7)	0.79	0.735	0.21	3.00
	Saddles	11 (39.3)	7 (46.7)	0.95	0.944	0.25	3.51
	Spurs	9 (32.1)	3 (20.0)	1.14	0.854	0.26	4.86
	Do not share	17 (60.7)	7 (46.7)	1.36	0.641	0.36	5.02

**OR – odds ratio*
***95% CI - confidence interval*

The results regarding the owners' knowledge level regarding EIAV, disease, and transmission methods are presented in Table 3. Despite the official control of the infection

requiring specific serological testing for transportation, it was observed that some producers (from both groups) are unaware of basic aspects of the disease. Another noteworthy factor is the disregard for the economic losses caused by the virus infection. A characteristic that deserves to be highlighted is the coexistence of healthy and infected animals, which occurred in 17 out of 28 (60.7%) of the focal properties.

Table 3. Horse owners' knowledge level about equine infectious anemia (EIA) on positive - focal (n = 28) compared to negative properties - control (n = 15).

		Focal (%)	Control (%)	OR	*p*	CI 95%
Suspected the infection of animals	Yes	4 (14.3)	0 (00.0)	ref.		
	No	24 (85.7)	15 (100.0)	0.22	0.328	-453 1.51
Know how EIAV is transmitted	No	1 (3.6)	6 (40.0)	ref.		
	Yes	27 (96.4)	9 (60.0)	35.28	0.018	0.60 6.52
Horses have come from properties with EIAV-infected animals	No	11 (39.3)	15 (100)	ref.		
	Yes	17 (60.7)	0 (00.0)	30	0.002	3.40 264.5
EIA causes losses	No	2 (7.1)	3 (20.0)	ref.		
	Yes	26 (92.9)	12 (80.0)	1.85	0.557	0.23 14.64

**OR – odds ratio*
***95% CI - confidence interval*

The movement of horses between properties and/or to equestrian events is important for the spread of the agent among herds. The ATC serves the purpose of controlling these movements, as well as preventing transport of animals that are seropositive for EIA. The owners' perception regarding the need for issuing ATCs is presented in Table 4. The analysis of the responses indicates a lack of awareness about the legal requirements for animal movement. Furthermore, it can be observed that the illegal movement of animals is a prevalent characteristic on the majority of properties, be they focal points or controls, including for commercial purposes.

Table 4. Ability to identify actions that contribute to the spread of equine infectious anemia between focal (n = 28) and control (n = 15) properties in the western region of Rio Grande do Sul, Brazil.

		Focal (%)	Control (%)	OR	*p*	CI 95%
When the horses should be tested	ATC emission	13 (46.4)	9 (60.0)	ref.		
	Routine	13 (46.4)	4 (26.7)	1.81	0.393	-0.77 1.96
	Transport	16 (57.1)	8 (53.3)	1.50	0.485	-0.74 156
	In case of sickness	2 (7.1)	2 (13.3)	0.77	0.838	-2.65 2.15
	No information	1 (3.6)	0 (00.0)	3.88	0.397	-1.78 4.49
Blood sampling responsible	Veterinary	27 (96.4)	15 (100.0)	ref.		
	Employee	3 (10.7)	2 (13.4)	1.31	0.790	-1.73 2.28
Horses' origin	Born on property	14 (50.0)	9 (60.0)	ref.		
	Acquired at fairs and auctions	10 (35.7)	4 (26.7)	1.20	0.794	-1.19 1.56
	Purchased (neighbor/breeder)	16 (57.1)	9 (60.0)	1.06	0.916	-1.02 1.14
	Other/donation	5 (17.8)	0 (00.0)	1.90	0.700	-2.64 3.93

ATC are emitted	No	2 (7.1)	2 (13.3)	ref.			
	Yes, for all horses	19 (67.9)	12 (80.0)	0.57	0.595	-2.58	1.47
	For some horses	5 (17.9)	1 (6.7)	1.28	0.853	-2.40	2.91
	None	1 (3.6)	0 (00.0)	1.28	0.895	-3.47	3.97
Horses from other countries	No	27 (96.4)	12 (80.0)	ref.			
	Yes	1 (3.6)	1 (6.7)	0.21	0.140	-3.54	0.50
	Argentina	0 (0.0)	1 (6.7)	0.10	0.150	-5.39	0.82
	Uruguay	0 (0.0)	1 (6.7)	0.16	0.287	-5.04	1.49
	Chile	1 (3.6)	1 (6.7)	0.50	0.575	-3.03	1.68
Horses share paddocks with other owners' animals	No	13 (46.4)	5 (3.3)	ref.			
	Yes	10 (35.7)	9 (60.0)	1.05	0.942	0.28	3.91
	Only some periods of the year	4 (14.3)	1 (6.7)	4.20	0.227	0.40	43.03
The frequency with which horses leave the property	Never	5 (17.8)	1 (6.7)	ref.			
	Once a week	5 (17.8)	2 (13.3)	0.86	0.891	-2.18	1.90
	Once a month	12 (42.8)	8 (53.3)	0.36	0.244	-2.67	0.68
	Once every six months	4 (14.3)	0 (00.0)	3.66	0.430	-1.93	4.52
	Less than once a year	0 (00.0)	4 (26.6)	0.03	0.047	-6.54	-0.04
	Just for September 20th parade	13 (46.6)	4 (26.6)	0.99	1.00	-1.78	1.78
When the ATC is emitted	Always	14 (50.0)	13 (86.7)	ref.			
	For monitored events	14 (50.0)	4 (26.7)	2.68	0.165	-0.40	2.38
	Just for September 20th parade	12 (42.8)	1 (6.7)	22.09	0.037	0.18	6.00
	Never	10 (10.7)	1 (6.7)	1.36	0.777	-1.83	2.45
Recognizes people who sell animals without health checks	No	15 (53.6)	7 (46.7)	ref.			
	Yes	13 (46.4)	8 (53.3)	0.64	0.491	0.18	2.23
Origin of the horses that are traded (bought and sold)	Same town	14 (50.0)	6 (40.0)	ref.			
	Another town	4 (14.3)	3 (20.0)	0.30	0.237	-3.14	0.77
	Argentina	0 (00.0)	0 (00.0)	1.28	0.884	-3.13	3.63
	Uruguai	0 (00.0)	0 (00.0)	1.28	0.884	-3.13	3.63
	Paraguai	0 (00.0)	0 (00.0)	1.28	0.884	-3.13	3.63
	No information	3 (10.7)	3 (20.0)	0.071	0.775	-2.64	1.96
Have you ever had cases of EIA in your animals?	No	2 (7.14)	15 (100.0)	ref.			
	Yes	26 (92.8)	(0.0)	1815.0	0.000	3.53	11.47
The first cases of EIA were diagnosed in animals that	Who had left the property	14 (50.0)	0 (00.0)	ref.			
	Who had never left the property	12 (42.8)	0 (00.0)	0.67	0.849	-4.38	3.60
	No information	2 (7.2)	0 (00.0)	0.29	0.550	-5.29	2.82
How many horses tested positive for EIA?	Quantos	134	0	ref.			
	No information	4	0	0.05	0.180	-7.15	1.34

**OR – odds ratio*

***95% CI - confidence interval*

3.2 Risk factor

In the multivariate analysis, only one variable showed significant results (p > 0.002) and was included in the final model (Table 5). Although the homogeneity of responses makes it more complicated to understand the categorization of risk factors among properties, it is implied that the illegal movement of these horses between different locations is the main cause of the occurrence of the infection. Meanwhile, the determining factor for the virus to infect the herd of a negative property is the introduction of an EIAV-infected animal.

Table 5. Multivariate model adjusted by the likelihood ratio test for potential risk factors for equine infectious anemia (EIA) in the western border region of Rio Grande do Sul, Brazil.

Variables		OR*	*p* value	95% CI**
Did the horses on the property come from places or stay together with horses infected with EIA?	Não	0.02	0.003	0.0014 0.2746
	Sim	30	0.002	3.40 264.5

**OR – odds ratio*
***95% CI - confidence interval*

4. Discussion

The epidemiological questionnaire that was applied made it possible to evaluate aspects of the husbandry, management, and sanitary care of horses on properties with a positive diagnosis of EIA on the western border of Rio Grande do Sul, Brazil. The municipalities in this region constitute a significant portion of the diagnosed EIA cases in the State [17,19]. This region of the State is characterized by the production of beef cattle and sheep, and the equestrian herd is predominantly composed of horses and some mules and donkeys. Horses are used for the management of cattle and sheep, assisting in covering large distances, as most properties practice extensive livestock farming. Besides, there is a cultural and emotional connection between the owners and the horses, and the locality is recognized for having several breeding facilities of high genetic standards. Another characteristic of this region is the Similarity of economic and cultural activities with the bordering regions of Argentina and Uruguay, which facilitates the movement of animals between countries, often in an illegal manner [18].

The epidemiological questionnaire was intended for administration through telephone contact to 153 producers (123 focal and 30 control), but only 43 answered. The low success of telephone contact may be attributed to various factors; however, it is believed that reluctance to answer calls from an unknown number is the main reason. The use of in-person questionnaires has proven to be more effective in obtaining information [9]. However, due to the pandemic caused by Covid-19, this option had to be discarded. It is mandatory for every rural producer to register in the DSA (Agriculture Defense System) system and keep personal and herd data updated [16]. Keeping the register up to date is crucial for the Official Veterinary Service (OVS) to contact the producer or in cases of sanitary emergencies [20]. In at least 27% of the focal properties, the producer's contact information was outdated, which should be a cause for concern in the veterinary service.

The type of breeding facility and location did not show significant differences between the two groups of evaluated properties (Table 1). A considerable number of them are situated in the city or in the peri-urban zone. These properties are associated with owners who use the animals for various purposes such as leisure, trail rides, parades, and/or sports competitions (rodeos). As previously demonstrated, these animals lack permanent veterinary assistance and are often transported without sanitary examinations [17]. This characteristic leads to the movement of animals to events, which is often done without the required sanitary examinations, including a negative serological test for EIA [17, 21]. The producers' responses show that many of them move their horses without issuing an Animal Transit Certificate (ATC). In addition, some producers who go to events with inspections only present the negative serological test for EIA without issuing the ATC. Furthermore, the extension of the validity period of the serological test of EIA that was approved by the State Equine Health Program (PESE) in 2014, from 60 to 180 days for interstate transit, is pinpointed as one of the factors that favored the spread of EIAV in RS herds [19]. Thus, since the primary infection usually produces nonspecific and transient clinical signs, the recently infected horse recovers and becomes a carrier. Consequently, this animal joins the herd or is transported without undergoing a new serological test [17].

The EIAV is primarily transmitted through the transfer of blood from an infected animal to a susceptible one. The main transmission methods include mechanical vectors, especially *Tabanus* spp. (horseflies) and *Stomoxys* spp. (stable flies), and iatrogenic transmission, including contaminated needles, syringes, surgical instruments, and riding equipment [1, 6]. The distribution and seasonality of vectors in the specific region have not been assessed. However, due to the climatic conditions throughout the year, it is suggested that the presence of vectors is concentrated between the months of December and April [24]. The geographical location of the studied region has environmental factors that favor the occurrence of vectors, such as periods of precipitation along with high temperatures, enabling vector proliferation and increased density [19]. Nevertheless, control measures are sporadically implemented, as demonstrated in this study. The transmission of EIAV by vectors is significant in regions where the environmental conditions favor insect multiplication. They are relevant in spreading the agent among animals within a herd and potentially to animals on nearby properties [25].

However, the iatrogenic route is the most important because it has the potential to transfer a larger volume of blood between animals [26]. The use of disposable injectable materials (needles and syringes) without sharing among animals is a measure that prevents the spread of the agent [23]. As answered in the present questionnaire, some producers share this type of material among horses, and this can be considered a favorable critical point for the transmission of the agent. Furthermore, factors contributing to this practice include a lack of knowledge and access to accurate information, veterinary assistance, and consequently, the administration of injectable medication by untrained individuals, as listed here [1, 10, 11].

During the analyzed period, the municipality of São Borja recorded 71 focal points, while Uruguaiana and Itaqui combined reported 44 focal points; yet, in all years, it was possible to diagnose at least one case. In these municipalities, cases occurred throughout the ten

evaluated years, with a higher number of records after 2015. The results obtained did not allow for the determination of specific causes favoring the occurrence. However, the maintenance of the endemicity level may be due to the association of various sanitary and management factors, as demonstrated.

The introduction of EIAV into a herd occurs preferably through the introduction of an infected animal with subclinical infection or in the incubation period [1]. This fact is exacerbated by the non-specificity and low intensity of clinical signs. Therefore, controlling animal movement through the ATC and with a negative serological test for EIA is an effective measure in passive surveillance [15,17]. In the studied region, animals are used for various purposes, many of which involve participation in equestrian events. These events are of short duration, often bringing together animals transported without documentation, and occur recurrently. Additionally, the validity of the EIA test is 180 days, allowing the same animal to move during this period with a single test, even when exposed to various occasions. All these factors create an environment where many animals from diverse localities congregate.

Equine breeding in Brazil is heterogeneous, and regional differences are determined by the breeds of animals, environmental characteristics, and the socio-cultural aspects of the breeders [27]. This diversity influences the spread of infectious agents and the manifestation of diseases. The risk factors associated with the occurrence of EIA in Brazil have been studied in various populations [6, 8, 10, 13]. In this study, the movement of animals and contact with EIA+ horses were considered important for spreading the infection. However, blood transfer between animals is necessary to transmit the agent. Despite not being considered a risk factor for the target population, the iatrogenic route is more efficient because horseflies are seasonal and less effective [24, 28]. The western region of Rio Grande do Sul borders Argentina, and illegal animal movement between the two countries is frequent. As such, the risk of cross-border transmission is high, as demonstrated previously [17].

5. Conclusion

Even though equine infectious anemia is at prevalence levels considered low, the infection is present in properties on the western border of Rio Grande do Sul. Furthermore, the transportation of these animals without the Animal Transit Certificate (ATC) and, consequently, without a negative diagnosis for EIA, also constitutes a factor that allows for its spread. Ultimately, the Similarity in the husbandry practices and sanitary management of animals in both the focal and control properties suggests that the introduction of a positive animal into the herd would be one of the most important coefficients for the dissemination of the infectious agent.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: Finger, P. F., Traesel, C. K., Brum, M. C. S. Formal Analysis: Machado, R. C, Jardim, J. C. S, Rodrigues, R. O. Funding acquisition: Brum, M. C. S. Investigation: Machado, R. C, Jardim, J. C. S. Project administration and Supervision: Brum, M. C. S. Writing (original draft): Machado, R. C. Writing (review and editing): Brum, M. C. S., Finger, P. F., Traesel, C. K., Oliveira, R. O. All authors approved the final version of the manuscript.

Acknowledgments

The authors express their gratitude to the rural producers and horse owners who collaborated with the study, as well as to the Veterinarians from the IDAs of São Borja, Itaqui, and Uruguaiana for sharing valuable information and to M.E.L. Martins for technical assistance with QGIs.

Availability of data and material

All datasets generated and/or analyzed in the current study are available and may also be made available by the corresponding author upon reasonable request.

Funding

RCM received a master's scholarship and JCJS received a doctoral scholarship from CAPES. This work was financed with resources from the Laboratório de Virologia/UNIPAMPA, PROPPI/UNIPAMPA (Pró-Reitoria de Pós-Graduação, Pesquisa e Inovação, Universidade Federal do Pampa), and financed in part by the "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" – Brasil (CAPES) – Finance Code 001.

References

1. Cook RF, Leroux C, Issel CJ. Equine infectious anemia and equine infectious anemia virus in 2013: a review. Veterinary Microbiology 2013;167(1-2):181–204. https://doi.org/10.1016/j.vetmic.2013.09.031

2. World Organisation for Animal Health. Equine Infectious Anemia. 2019:1–7. https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=glossaire.htm#terme_manuel_terrestre (accessed February 19, 2023).

3. Câmara RJF, Bueno BL, Resende CF, Balasuriya UBR, Sakamoto SM, Reis JKPD. (2020). Viral diseases that affect donkeys and mules. Animals. 2020;10(12):e2203. https://doi.org/10.3390/ani10122203

4. Scicluna MT, Issel CJ, Cook FR, Manna G, Cersini A, Rosone F, et al. Is a diagnostic system based exclusively on agar gel immunodiffusion adequate for controlling the spread of equine infectious anaemia? Veterinary Microbiology. 2013;165(1-2):123–34. https://doi.org/10.1016/J.VETMIC.2013.02.027

5. Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Alfenas-Zerbini P, et al. Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses (2022). Archives of Virology. 2022;167:2429–2440. https://doi.org/10.1007/s00705-022-05516-5

6. Bicout DJ, Carvalho R, Chalvet-Monfray K, Sabatier P. Distribution of equine infectious anemia in horses in the north of Minas Gerais State, Brazil. Journal of Veterinary Diagnostic Investigation 2006;18(5):479–482. https://doi.org/10.1177/104063870601800

7. Oliveira FG, Cook RF, Naves JHF, Oliveira CHS, Diniz RS, Freitas FJC, et al. Equine infectious anemia prevalence in feral donkeys from Northeast Brazil. Preventive Veterinary Medicine. 2017;140:30–37. https://doi.org/10.1016/j.prevetmed.2017.02.015

8. Barros ML, Borges AMCM, de Oliveira ACS, Lacerda W, de A, Aguiar DM. Spatial distribution and risk factors for equine infectious anaemia in the state of Mato Grosso, Brazil. Revue Scientifique et Technique. 2018;37(3):971–983. https://doi.org/10.20506/rst.37.3.2900

9. Bolfa P, Barbuceanu F, Leau SE, Leroux C. Equine infectious anaemia in Europe: time to re-examine the efficacy of monitoring and control protocols? Equine Veterinary Journal. 2016;48(2):140–142. https://doi.org/10.1111/evj.12466

10. Moraes DDA, Gonçalves VSP, Mota ALAA, Borges JRJ. Situação epidemiológica da anemia infecciosa equina em equídeos de tração do Distrito Federal. Pesquisa Veterinaria Brasileira. 2017;37(10):1074–1078. https://doi.org/10.1590/S0100-736X2017001000006

11. Rebelatto MC, Oliveira C, Welblen R, Silva SF, Oliveira LSS. Serological diagnosis of equine infectious anaemia virus infection in the central region of the Rio Grande do Sul State. Ciência Rural. 1992;22(2):179–196. https://www.scielo.br/j/cr/a/X34C957fvQ6d87687NWkSPt/?format=pdf&lang=en

12. Almeida VMA, Gonçalves VSP, Martins MF, Haddad JPA, Dias RA, Leite RC, et al. Anemia infecciosa eqüina: prevalência em eqüídeos de serviço em Minas Gerais. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2006;58(2):141-148. https://doi.org/10.1590/S0102-09352006000200001

13. Pádua BR, Dias RA, Fioravanti MCS, Borsanelli AC. Seroprevalence and risk factors associated with equine infectious anemia in the state of Goiás, Brazil. Preventive Veterinary Medicine. 2022;209:e105781. https://doi.org/10.1016/J.PREVETMED.2022.105781

14. Brasil. Ministério da Agricultura Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Instrução Normativa nº 17. Programa Nacional de Sanidade dos Eqüídeos (PNSE), de 8 de maio de 2018. https://www.gov.br/agricultura/pt-br/assuntos/sanidade-animal-e-vegetal/saude-animal/programas-de-saude-animal/INM00000017.pdf

15. Rio Grande do Sul. Secretaria Estadual de Agricultura, Pecuária, Produção Sustentável e Irrigação. Programa Estadual de Sanidade Equina. https://www.agricultura.rs.gov.br/pese

16. Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Instrução Normativa nº 45. Normas para Prevenção e Controle da Anemia Infecciosa Equina (AIE), de 15 de junho de 2004.: https://www.gov.br/agricultura/pt-br/assuntos/sanidade-animal-e-vegetal/saude-animal/programas-de-saude-animal/INM00000045.pdf

17. Barzoni CS, Nogueira DMP, Marques GD, Diehl GN, Pellegrini DCP, Brum MCS. Equine infectious anemia in the western region of Rio Grande do Sul, Brazil. Ciencia Rural 2018;48(6):e20170809. https://doi.org/10.1590/0103-8478cr20170809

18. Machado G, Corbellini LG, Frias-De-Diego A, Dieh GN, Santos DV, Jara M, et al. Impact of changes of horse movement regulations on the risks of equine infectious anemia: A risk assessment approach. Preventive Veterinary Medicine. 2021;190:e105319. https://doi.org/10.1016/j.prevetmed.2021.105319.

19. Cardenas NC, Sanchez F, Lopes FPN, Machado G. Coupling spatial statistics with social network analysis to estimate distinct risk areas of disease circulation to improve risk-based surveillance. Transboundary and Emerging Disease. 2022;69(5):e2757–2768. https://doi.org/10.1111/tbed.14627.

20. Rio Grande do Sul. Secretaria Estadual de Agricultura, Pecuária, Produção Sustentável e Irrigação. Decreto nº 52.434. Medidas de Defesa Sanitária Animal, de 26 de junho de 2015. https://www.agricultura.rs.gov.br/upload/arquivos/201708/21160316-novo-decreto-52-434.pdf

21. Costa E, Nogueira Diehl G, Santos DV, Silva APSP. Panorama da Equinocultura no Rio Grande do Sul. A Hora Veterinária. 2013;196:45-49. https://www.agricultura.rs.gov.br/upload/arquivos/201612/02101333-inftec-50-panorama-da-equinocultura-no-rio-grande-do-sul.pdf

22. Baldacchino F, Desquesnes M, Mihok S, Foil LD, Duvallet G, Jittapalapong S. Tabanids: Neglected subjects of research, but important vectors of disease agents! Infection, Genetics and Evolution. 2014;28:596–615. https://doi.org/10.1016/j.meegid.2014.03.029.

23. Issel CJ, Foil LD. Equine infectious anaemia and mechanical transmission: man and the wee beasties. Revue Scientifique et Technique. 2015;34(2):513–523. https://doc.woah.org/dyn/portal/index.xhtml?page=alo&aloId=32979

24. Lucas M, Krolow TK, Riet-Correa F, Barros ATM, Krüger RF, Saravia A, et al. Diversity and seasonality of horse flies (Diptera: Tabanidae) in Uruguay. Scientific Reports 2020;10:401. https://doi.org/10.1038/s41598-019-57356-0.

25. Barros ATM, Foil LD. The influence of distance on movement of tabanids (Diptera: Tabanidae) between horses. Veterinary Parasitology. 2007;144(3-4):380–384. https://doi.org/10.1016/j.vetpar.2006.09.041.

26. Williams D, Issel C, Steelman C, Adams WJ, Benton C. Studies with equine infectious anemia virus: transmission attempts by mosquitoes and survival of virus on vector mouthparts and hypodermic needles, and in mosquito tissue culture. American Journal of Veterinary Research. 1981;42(9):1469–1473.

27. Ministério da Agricultura, Pecuária e Abastecimento. Revisão do estudo do complexo do agronegócio do

cavalo. 2016. https://www.gov.br/agricultura/pt-br/assuntos/camaras-setoriais-tematicas/documentos/camaras-setoriais/equideocultura/anos-anteriores/revisao-do-estudo-do-complexo-do-agronegocio-do-cavalo/view

28. Resende CF, Santos AM, Cook RF, Victor RM, Câmara RJF, Gonçalves GP, Lima JG, Maciel e Silva AG, Leite RC, Reis JKP. Low transmission rates of equine infectious anemia virus (EIAV) in foals born to seropositive feral mares inhabiting the Amazon delta region despite climatic conditions supporting high insect vector populations. BMC Veterinary Research. 2022;18(1):286. https://doi.org/10.1186/s12917-022-03384-4.