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Spatial distribution and risk factors for equine infectious anaemia in the state of Mato Grosso, Brazil

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Summary

The prevalence of equine infectious anaemia (EIA) in the three biomes of the state of Mato Grosso (Amazonia, Cerrado and Pantanal) was estimated. Serum samples were collected from 3,858 equines in 1,067 herds between September and December 2014. The agar gel immunodiffusion (AGID) assay was used to detect EIA virus antibodies, and if a herd contained a seropositive animal it was classified as a focus. The prevalence rates were 17.2% (95% confidence interval [CI]: 14.9–19.8%) for herds and 6.6% (95% CI: 5.8–7.5%) for animals. The Pantanal region showed the highest prevalence rates: 36.1% (95% CI: 30.8–41.7%) for herds and 17.0%

(95% CI: 14.7–19.6%) for animals. The spatial distribution of relative risk was calculated according to the kernel density, which revealed three major clusters with the highest prevalence rates occurring in the north-western (Amazon biome), north-eastern (Cerrado biome) and southern (Pantanal biome) regions. A high spatial correlation was found among ranches, with high intra-herd prevalence rates located in the Amazon and Cerrado biomes, but the highest spatial correlation with EIA foci was found in the Pantanal biome. Variables related to ranch management, reflecting human influence, were associated with positive equines. Based on the results, it can be concluded that EIA is present in all biomes of the state, and that the risk factors are associated with human interference in the transmission process. Given this situation, the EIA control programme should be re-evaluated and more prophylactic measures should be adopted to control the disease.

Keywords

Agar gel immunodiffusion – AGID – Disease – Epidemiology – Equine – Infection – *Retroviridae*.

Introduction

Brazil, which ranks among the world’s major horse producers, has the largest equine herd in Latin America and the third largest in the world. The country has a total population of eight million horses, mules and donkeys, annually generating revenues of over 7.3 billion Brazilian real and employing 3.2 million people in the various agribusiness sectors involved in equine breeding. Equines in Brazil are destined mostly for work in cattle ranch activities such as cattle herding, vaccination, deworming, etc. (1).

For this reason, the maintenance of equine herds in the state of Mato Grosso, which is emerging as one of Brazil’s largest cattle producing states (2), is extremely important for local cattle ranchers. According to the database of the Mato Grosso Institute of Agricultural Defence (*Instituto de Defesa Agropecuária do Estado de Mato Grosso* – INDEA-MT), the population of equines in Mato Grosso has been estimated at approximately 307,000 animals, distributed among

53,710 ranches (INDEA). The health of equines on these ranches must be closely monitored, given that outbreaks of infectious diseases could cause serious losses for local farmers.

Equine infectious anaemia (EIA) is an important disease in horses in Brazil. Its aetiological agent is the equine infectious anaemia virus (EIAV), an enveloped ribonucleic acid (RNA) virus of the family *Retroviridae* and the genus *Lentivirus* (3). The virus infects all members of the family Equidae; infected individuals may develop fatal viraemia, while those that survive remain viraemic for life, acting as a reservoir (4, 5). The chronic form of EIA is characterised by recurring cycles of anaemia, oedema, weight loss and lethargy in EIA-positive horses, which indirectly affects animal husbandry in general (5). In addition, a decrease of almost 40% in the performance of EIA-positive horses when compared with horses testing negative for EIA has been reported (6).

Equine infectious anaemia is sometimes referred to as ‘swamp fever’ because it is most prevalent in warm, wet regions where large haematophagous flies, *Stomoxys calcitrans* (stable fly) and *Tabanus* species (spp.) (horse fly), are responsible for the natural transmission of the disease among animals (7). The disease can also be transmitted iatrogenically, which occurs through blood-contaminated instruments such as syringes, needles, surgical instruments and equine accessories (8). Equine infectious anaemia virus can also be transmitted via semen from stallions to mares, and from mares to their foals *in utero* and via suckling, although this route is of minor epidemiological importance (8, 9, 10).

In Brazil, EIAV was first reported in the states of Rio Grande do Sul and Rio de Janeiro (11). Since then, different prevalence rates in herds have been reported in Brazil, ranging from 3% in the south-east to 23% in the north and 52% in the central west (12, 13, 14). The National Equine Health Plan (NEHP) requires the euthanasia of seropositive equines. In order to reduce the significant losses to farmers, this requirement does not apply to areas where EIA is endemic (15).

In spite of this long-standing NEHP requirement, EIA continues to have negative impacts on horse breeding in Brazil. Official data are out of date and do not reflect the country's current situation, given the increase in the number of livestock in recent decades. This study aimed to support Brazil's NEHP by conducting a survey to estimate the prevalence of EIAV antibodies in the state of Mato Grosso, and to evaluate the possible risk factors and spatial distribution of infected herds. Few studies have investigated the occurrence of EIA in the context of associated risk factors and the spatial distribution patterns of the disease. The results of this official survey may give rise to new control measures and possibly lead to adjustments in the equine health programme of the state of Mato Grosso. An autologistic regression model was built to determine the spatial autocorrelation effect of variables in order to identify the risk factors with odds ratio (OR) values and to model the spatial distribution of EIAV throughout the state of Mato Grosso.

Materials and methods

The study was designed by experts from INDEA-MT and the Federal University of Mato Grosso (UFMT). The fieldwork was carried out from September to December 2014 by inspectors and technicians from INDEA-MT. To ensure the official status of the survey, this study was approved by the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) and the sampling procedures were approved by the UFMT Ethics Committee on Animal Use, under Protocol No. 23108.128034/2016-31.

Study area and equine population

For this study, the state of Mato Grosso was divided into three regions: the Amazonia (hot and humid tropical climate with forest vegetation predominating), Cerrado (tropical climate, hot and rainy in the spring–summer months and cool and dry in the autumn–winter, with predominantly forest and savannah vegetation) and Pantanal biomes (tropical–continental climate, typically hot and rainy in the spring–summer months and cool and dry in the autumn and winter). The region is flooded annually during the rainy season and vegetation is inhomogeneous, ranging from predominantly grassland in lower areas to savannah at intermediate altitudes (Fig. 1). Within each of these regions, a predetermined number of ranches where equines are raised (primary sampling units) were randomly chosen. This process was based on the register of ranches kept by INDEA-MT and was completed using data reported by the farmers in an annual compulsory declaration. In 2014, the total equine population in the state of Mato Grosso was 242,501 horses, 59,873 mules and 4,557 donkeys (totalling 306,931 animals). These animals were distributed among 32,069 (59.7%) ranches in the Amazon biome, 18,114 (33.7%) in the Cerrado biome and 3,527 (6.6%) in the Pantanal biome, making a total of 53,710 ranches in the entire state. The number of ranches to be studied in each biome was determined using Epi Info™ 7 (Centers for Disease Control and Prevention [CDC], Atlanta, GA, United States of America), with an estimated prevalence of 50%, absolute target precision of 6% and confidence interval of 95%. All the municipalities (*n* = 141) of the state were included in the investigation, and were classified as belonging to a given biome when the latter predominated in the municipality. The investigation did not include nature and indigenous reserves. In this scenario, farms were requested to collaborate with the sampling procedure if their ranch was chosen according to the abovementioned procedures. The ranches were chosen using a systematic random sampling method, considering a constant difference interval between numbers of units.

Please insert Figure 1 here

To compose the secondary sampling units, a minimum number of equines to be examined on each ranch was estimated in order to identify at least one seropositive equine in the herd, allowing the herd to be classified as either infected (focus) or non-infected with EIAV. The diagnostic test was based on a herd-level sensitivity and specificity (16) of 98% and 100%, respectively (14, 17), and on an estimated intra-herd prevalence rate of 10%. This calculation was performed using HerdAcc version 3 software (http://epitools.ausvet.com.au/content.php?page=HerdSens4) (18) and the sample size was chosen to enable herd-level sensitivity and specificity greater than or equal to 90%. Thus, all the animals were sampled on ranches with up to ten equines older than six months, while ten animals were sampled on ranches that had more than ten animals older than six months, and on those ranches, samples were chosen by systematic random sampling, considering a constant difference interval between numbers of animals.

Blood sampling

Blood samples were collected by jugular venepuncture, using 21 G needles suitable for vacuum tubes. At the same time, ranch owners were asked to answer a questionnaire about health variables such as the location of the ranch (geographical coordinates), individual characteristics of each animal, type of ranch management, and measures adopted to prevent infectious diseases (Table I). This information was entered into a database created with Microsoft Excel™ software.

Please insert Table I here

Laboratory analysis

Serum samples were tested for antibodies against core protein p26 of EIAV by the agar gel immunodiffusion (AGID) test (19), following the instructions of a commercial kit manufacturer (Bruch Laboratories, Brazil). The procedure follows the standard protocol of the NEHP for EIA testing, as specified by Directive No. 378, 17 December 2014, of MAPA, Brazil (20).

Prevalence and statistical analysis

According to the NEHP, herds containing a seropositive animal are classified as foci. Prevalence rates for animals and ranches (herds) were calculated separately. Since ranches with different herd sizes were evaluated, the apparent prevalence rate among animals was adjusted on the basis of the herd size of each ranch and biome under analysis, according to Formula [1]. The apparent prevalence rate and standard error, at a 95% confidence interval (95% CI), were subjected to a complex sample analysis using IBM SPSS® statistical software, version 16.0. True prevalence (T_{Prev}) was calculated by considering the sensitivity (0.98) and specificity (1.0) values of the AGID tests (14, 17), according to Formula [2]. Predictive values of the diagnostic test for animal and herd prevalence rates were calculated as described by Thrusfield (21).

Formula [1]

$$Weight = \frac{n.\ of\ equines\ on\ ranches}{n.\ of\ sampled\ equines} \times \frac{n.\ of\ equines\ in\ the\ biome}{n.\ of\ equines\ on\ sampled\ ranches}$$

Formula [2]

$$True\ animal\ prevalence = \frac{apparent\ prevalence + specificity - 1}{sensitivity + specificity - 1}$$

Kernel density estimation (KDE) was used to create a relative risk map based on spatial data, according to Formula [3] (22), where $\lambda_1(s)$ is the positive kernel estimator and $\lambda_0(s)$ is the negative kernel estimator. Spatial risk was considered to be constant, according to Formula [4], where n_1 represents the total positive area and n_0 the total negative area. The significance of the kernel estimate was determined by the Monte Carlo test (23), and the marked correlation function was applied to check for the presence of a spatial pattern (24).

Formula [3]

$$R(s) = \frac{\lambda_1(s)}{\lambda_0(s)}$$

Formula [4]

$$R_0(s) = \frac{n_1}{n_0}$$

An autologistic regression model was developed, and adjusted to determine the spatial autocorrelation effect of variables, identify the risk factors, with OR values, and draw the spatial distribution model of the risk of EIAV in the entire state of Mato Grosso. The model introduces a spatial autocorrelation term in the form of weighting coefficients and explains the problem of spatial autocorrelation effects in the process of statistical analysis. To evaluate the spatial relationship of the occurrence of EIA according to intra-herd prevalence and positive herds (focus) and the independent variables (Table I), an autologistic regression model was created using Formula [5], as proposed by Besag (25) and Bo *et al.* (26), where P denotes the probability of an event occurring for every geographical unit, y represents the presence of disease, X corresponds to the independent variables, W is the spatial proximity matrix: ρ represents the regression coefficients of the covariates and β is the coefficient of autocovariate regression.

Formula [5]

$$P(y=1)=\frac{e^{\rho W+X\beta}}{1+e^{\rho W+X\beta}}$$

The variable 'number of cattle per ranch' was included in the model in order to evaluate the presence of bovines on the ranches. Each variable was tested individually (univariate) and in combination (multivariate) to obtain a summarised model considering only significant variables.

The association between positivity for EIAV antibodies and the individual independent variables was analysed by considering values for herd size adjusted by chi-square (χ^2) or Fisher exact tests, with $p \leq 0.05$ considered significant. The analysis was performed using IBM SPSS® statistical software, version 16.0. The regression analysis was performed using R software, and the spatial analysis used the spatstat application of the software created by the R Core Team (27).

Results

Samples collected from 3,858 equines, comprising 3,230 horses, 602 mules and 26 donkeys on 1,067 ranches in the state of Mato Grosso, were evaluated. The sampling involved 426 ranches (39.9%) in the Amazon biome, 347 (32.5%) in the Cerrado biome and 294 (27.5%) in the Pantanal wetland biome (Table I; Fig. 1). The equine populations on these ranches ranged from 1 to 89 animals, with an average of 5.5 equines per ranch in the whole state, and 4.9, 4.7 and 5.6 equines per ranch in the Amazon, Cerrado and Pantanal biomes, respectively.

Prevalence of equine infectious anaemia in the state of Mato Grosso

At least 232 ranches had positive samples, which resulted in an adjusted prevalence value of 17.2%. Sixty-one (14.3%) ranches in the Amazon, 65 (18.7%) in the Cerrado and 106 (36.1%) in the Pantanal were classified as positive. Among the 141 municipalities in the state, 63 (44.6%) presented positive herds. Positive equines totalled 386 (10.0%), and, per biome, 89 (5.9%) were from the Amazon, 86 (6.9%) from Cerrado and 211 (18.6%) from the Pantanal biomes.

Table II lists the non-weighted and weighted samples and the apparent prevalence rates for herds and animals according to their biomes. Positive predictive values per herd and per animal were 100%; the negative predictive value per herd was 99.59% and per animal 99.86%. The estimated herd prevalence in the Amazon biome was 14.3% (95% CI: 11.3%–18.0%; T_{Prev}: 14.6%]; that in the Cerrado biome was 18.7% (95% CI: 15.0%–23.2%; T_{Prev}: 19.1%); and that in the Pantanal biome was 36.1% (95% CI: 30.8%–41.7%, T_{Prev}: 36.8%]. The estimated prevalence among animals in the Amazon biome was 5.3% (95% CI: 4.2%–6.6%; T_{Prev}: 5.4%); in the Cerrado biome it was 5.8% (95% CI 95%: 4.6%–7.1%; T_{Prev}: 5.9%); and in the Pantanal biome it was 17.0% (95% CI: 14.7%–19.6%; T_{Prev}: 17.4%). Estimated prevalence rates for the entire state were 17.2% (95% CI: 14.9–19.8%; T_{Prev}: 17.6%) for herds and 6.6% (95% CI: 5.8%–7.5%; T_{Prev}: 6.7%) for animals.

Please insert Table II here

Relative risk of equine infectious anaemia foci and spatial distribution of the intra-herd prevalence rates

Prevalence rates were calculated per ranch and ranged from 0 to 100% (Fig. 2). The calculated relative risk encompassed areas from the three biomes (Fig. 3). The disease risk was found to range from 0.2 to 1.4, but was not constant in space (p = 0.04). Figure 4, which illustrates the spatial distribution according to the KDE, shows that the highest intra-herd prevalence rates occurred in three major clusters, in the north-western (Amazon biome), north-eastern (Cerrado biome) and southern (Pantanal biome) regions, ranging from 0.05 to 0.3 (5–30%). Two other small clusters with high intra-herd prevalence rates were also identified, in the south-western and central regions of the state of Mato Grosso.

Please insert Figures 2, 3 and 4 here

A correlation was found between ranch location and prevalence rate, illustrating a clustering process, i.e. the ranches with the highest prevalence rates are in close proximity, as are the ranches with the lowest prevalence rates.

Risk factor analysis

Tables III and IV describe the results of the autologistic regression models relating intra-herd prevalence and EIA foci to the variables studied, respectively. Considering intra-herd prevalence, the autologistic regression model showed a high spatial correlation (ρ = 1.9; OR = 6.5). The variables of ranch location in the Amazon and Cerrado biomes, working equines on ranches, and herds composed of equines purchased from other farms with no requirement for AGID testing prior to equines being received on the premises were associated with the spatial distribution of the highest EIA intra-herd prevalence rate in the state. The autologistic regression model considering EIA foci showed the highest spatial correlation (ρ = 3.4; OR = 31.2). The variables associated with the spatial distribution of foci were represented by ranches located in the Pantanal biome, external working equines, the use of shared riding gear (saddles, bits, etc.), no requirement for AGID testing prior to receiving equines, presence of flooded areas, and number of cattle on the ranch.

Please insert Tables III and IV here

According to the adjusted χ^2 tests, the chance of horses testing positive for EIAV was 1.641-fold higher than for mules and donkeys, and males and older animals had a 1.412- and 1.801-fold higher chance of being associated with seropositivity than females and younger animals, respectively. Furthermore, equines used for work and domesticated equines, respectively, were 6.66 and 3.47 times more likely to have EIAV antibodies than equines used for sport, leisure, reproduction and those that had not been domesticated (unbroken horses). Table V lists the results of the adjusted association and risk factor analysis by chi-square testing of seropositive equines and individual variables.

Please insert Table V here

Discussion

This survey revealed that 15 to 20% of the ranches in the state of Mato Grosso had at least one equid seropositive for EIAV. Under current NEHP regulations, each of these properties is classified as a separate EIA focus. The AGID test was employed because its use is still endorsed by the World Organisation for Animal Health (OIE). It is currently the only official NEHP recognised diagnostic assay for EIA in Brazil and the survey was conducted as an integral part of this government programme. However, it is recognised that recent published reports have suggested that AGID may be less sensitive than certain enzyme-linked immunosorbent assay (ELISA) based tests for detection of EIAV-specific antibodies, particularly when these tests are combined with confirmatory immunoblot assays (28, 29). Consequently, the results presented here may underestimate the prevalence of EIA in Mato Grosso state. Nonetheless, the survey provides a valuable contemporary overview of EIAV seroprevalence in this region of Brazil, where equids are used extensively for livestock production and/or transportation. Moreover, analysis of the differences in the prevalence rate per site enabled the creation of a map highlighting the relative risk for each of the three ecosystems, thereby providing a visual interpretation of the regions with the highest and lowest prevalence of infection. The Pantanal biome presented indices of foci exceeding 30%, while those in the Amazon and Cerrado biomes were close to 14 and 18%. As for the number of individual seropositive animals, equines raised in the Pantanal stood out compared to those in the other biomes, reaching prevalence rates of close to 20%, while equines from the Amazon and Cerrado biomes showed prevalence rates of close to 7%.

Our findings indicate that the EIA prevalence rates in this state are high, notwithstanding the NEHP launched in Brazil in 2004 (15). Prior to this survey, estimates of EIAV seroprevalence in Brazil were based entirely on a small number of scientific studies, most without any form of statistical analysis (12, 13, 14, 30), and on results obtained from routine samples submitted to official testing laboratories. However, because the latter mainly involved applications for inter-state transportation permits, these samples are likely to have been derived from a very restricted equid population (breeding and/or competition) and hence are of limited value (15).

Despite the high prevalence rates, the distribution of foci was not uniform, and most of the cases were grouped in three areas with a high relative risk index. These areas are distributed in the three biomes, as follows: in the north-western region of the Amazon biome, the north-eastern region of the Cerrado biome, and the southern region of the Pantanal biome. Identifying these regions will be helpful for the implementation of control measures to be applied by the animal defence service of the state of Mato Grosso. To ensure the effectiveness of prophylactic actions, this state-run service should adopt rules for controlling the transit of equines between the regions. Brazil’s National Programme for Equine Health (PNSE) recommends animal health education of horse owners and the euthanasia of all EIAV-positive animals. Although attempts to control the movement of

EIAV-seropositive equids by means of animal health education are undoubtedly useful, the euthanasia of virus-infected working equids is likely to be met with considerable resistance, because these animals require extensive, time-consuming training and are vital to the running of cattle ranching operations, particularly in the state of Mato Grosso. Moreover, as described above, there is a provision in Brazil's current laws that precludes euthanasia in areas where EIAV is endemic. Furthermore, asymptomatic carrier equids may present a low risk in terms of natural insect-mediated transmission (31, 32, 33), so intensive educational efforts to explain the dangers of using shared veterinary equipment may represent a more politically viable option.

The findings of this study demonstrate a negative association between EIA foci and veterinary assistance. Unfortunately, only approximately 5% of ranches reported utilising this type of professional assistance (Table I). This fact may account for the results observed in several Brazilian studies (14, 34, 35, 36, 37) indicating a generalised lack of technical information among many ranch owners concerning the correct disposal of needles or syringes, riding equipment hygiene and the benefits of serological monitoring. As an example of the latter, our study found that ranches that did not require AGID testing before allowing equids onto the premises were more likely to constitute an EIA focus and have a high intra-herd prevalence of the disease.

The spatial autologistic regression model revealed that ranches with higher intra-herd prevalence rates tend to cluster together in the Amazon and Cerrado biomes. The proximity between these ranches also presented a high spatial relationship and OR (Table III). According to data from the sample population, the regions of the Amazon and Cerrado have smaller herds ($\bar{x}$ = 4.9 and 4.7) than the Pantanal ($\bar{x}$ = 5.6), which is why they may present higher intra-herd prevalence rates. Moreover, these two biomes cover a more extensive area than that of the Pantanal. This distribution was also related to ranches that use equines for work, those that purchase equines, and those that do not require EIA testing prior to the purchase. All these variables may influence intra-herd prevalence.

The distribution of EIA foci showed the greatest spatial relationship with the Pantanal biome, and the probability of a ranch being considered a focus of infection in this biome was 2.1-fold greater than in the other biomes. Ranches that use animals from other ranches to work, ranches that share riding gear (saddles, bits, spurs), those that do not require EIA tests and those that have flooded areas are also associated with the distribution of foci. All the variables are involved in the introduction and/or maintenance of the virus in herds (38, 39) and must be considered in future EIA control programmes.

In addition to the historical importance of wetland areas in the prevalence of the disease – in Brazil, this is especially true of the Pantanal biome – these areas have predominantly large cattle ranches, which are often difficult to reach during floods, and, in certain areas, equines offer the only feasible mode of transportation. Most ranches located in flood-prone areas keep their horses together at a single site during the flood season, and this may favour the movement of tabanid flies among their hosts and, hence, the transmission of EIAV (40). These characteristics indicate that equines are gathered together seasonally and, because they are used as a means of transport, in certain situations they travel through several ranches, thus favouring contact between infected and susceptible animals.

These observations, allied to the fact that EIAV infection is persistent and characterised by the maintenance of asymptomatic carriers (33), result in owners deciding not to participate in actions to combat the disease, especially when they involve the immediate euthanasia of seropositive animals and a ban on the transit of equines on the ranch until the focus of the disease has been completely eradicated. Moreover, this decision is supported by a loophole in the current law (15), which allows seropositive animals to be kept alive in areas endemic for EIA. Thus, given the difficulty of controlling the movement of animals between regions inside the state, EIAV-positive animals are often transported to the Pantanal to prevent them from becoming the target of disease control actions.

The distribution of EIA foci was also associated with the number of cattle on a ranch, with the odds of at least one seropositive equine being greater than 1.0 for each bovine kept on the premises. Therefore, the probability of a ranch having an infected equine increases in proportion to the number of cattle. This is not surprising, since the demand for working horses increases proportionally with cattle herd size.

A significant finding from this study is that EIAV infection rates in unbroken horse populations are significantly lower (~5%) than those among horses that are in the process or have completed their training (>20%) (37, 40). This suggests that while transmission of EIAV in unbroken horses is almost entirely insect mediated (7, 33), the higher prevalence in working horses is likely influenced by humans. Human action is considered an important component in the chain of infection of the virus through poor management techniques that expose healthy animals to contaminated veterinary supplies, utensils and riding equipment (14).

As expected, males along with older animals had higher rates of seropositivity than younger equids or females. In addition to time itself being a factor in the probability of exposure to EIAV, younger equines exhibit more intense defensive behaviour in repelling bloodsucking tabanids, leading to the redistribution of vectors among older animals in the herd (33). Moreover, male equines are used as working animals more often than females, which means they are more exposed to viral infection (37, 40).

Lastly, EIAV antibodies were more often detected in horses than in mules and donkeys. This finding differs from that of a survey carried out by Borges *et al.* (14) in the Pantanal biome, where no difference was found among these equine species. However, little is actually known about the role of mules and donkeys in the epidemiology of the infection, although experimental infections have resulted in clinical, pathological and laboratory findings similar to those observed in horses (41, 42). Nevertheless, this issue deserves further attention, because detectable virus-specific antibody responses may take longer to develop and be produced at lower levels in donkeys (42). Furthermore, during the Italian national surveillance programme for EIAV, a comparison between horses and mules demonstrated that a disproportionate number of the latter gave test-negative results in AGID but were positive in ELISA, and confirmatory immunoblot tests suggested that mules may be serologically less responsive than horses after exposure to this virus (29).

Conclusions

Based on the findings of this survey, it can be stated that EIA is present in the three biomes of Mato Grosso, and that its presence stands out in the Pantanal biome. A spatial analysis indicated an inconstant spatial distribution characterised by the formation of three large clusters associated with variables related mainly to human influence. In view of the results, the animal health defence service should re-evaluate the disease control plan at the state level and implement stricter measures in order to reduce infection to acceptable levels or even eradicate the disease entirely. Measures based on the sanitation of foci and their surroundings must be viewed as an adjunct to health education, and testing should be made mandatory before equids can be transported anywhere within the state of Mato Grosso.

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Table I
Independent variables adopted in the questionnaire completed by 1067 ranch owners, in the three biomes (Amazon, Cerrado and Pantanal) in the state of Mato Grosso, Brazil

Independent variables	Number of ranches (%)							
	Amazon		Cerrado		Pantanal		Total	
Equine function on ranches								
Work	416	(97.6)	339	(97.7)	287	(97.6)	1,042	(97.6)
Others [(a)]	10	(2.4)	8	(2.3)	7	(2.4)	25	(2.4)
Density of equines								
Median ≤5 equines/ha	222	(52.1)	205	(59.1)	199	(67.7)	626	(58.6)
Median >5 equines/ha	204	(47.9)	142	(40.9)	95	(32.3)	441	(41.3)
Origin of equines								
Born on the ranch	135	(31.7)	80	(23.0)	47	(15.9)	262	(24.5)
Purchased	291	(68.3)	267	(77.0)	247	(84.1)	805	(75.5)
Participate in events (fairs or agricultural shows)	41	(9.6)	36	(10.3)	23	(7.8)	100	(9.3)
External working equines	137	(32.1)	92	(26.5)	127	(43.2)	356	(33.3)
Use of injectable medicines	74	(17.3)	114	(32.8)	85	(28.9)	273	(25.6)
Sharing saddles, etc. (riding gear)	76	(17.8)	52	(14.9)	71	(24.1)	199	(18.6)
Requires AGID test before receiving equines	58	(13.6)	47	(13.5)	57	(19.4)	162	(15.1)
Performs routine AGID test	27	(6.3)	19	(5.5)	29	(9.8)	75	(7.0)
Flooded areas on the ranch	105	(24.6)	120	(34.6)	136	(46.2)	361	(33.8)
Veterinary assistance	12	(2.8)	17	(4.9)	22	(7.4)	51	(4.7)

a) Leisure, sports practices and reproduction
AGID: agar gel immunodiffusion

Table II
Values of non-weighted and weighted samples and prevalence rates of equine infectious anaemia, with 95% confidence intervals, in the different biomes of the state of Mato Grosso

Biomes	Category	Non-weighted count		Weighted count		95% confidence interval	
		Samples	Positive	Samples	Prevalence	Lower	Upper
Amazon	Herd	426	61	32,069	14.3%	11.3%	18.0%
	Animal	1,496	89	165,413	5.3%	4.2%	6.6%
Cerrado	Herd	347	65	18,114	18.7%	15.0%	23.2%
	Animal	1,233	86	110,656	5.8%	4.6%	7.1%
Pantanal	Herd	294	106	3,527	36.1%	30.8%	41.7%
	Animal	1,129	211	30,862	17.0%	14.7%	19.6%
Mato Grosso	Herd	1,067	232	53,710	17.2%	14.9%	19.8%
	Animal	3,858	386	306,931	6.6%	5.8%	7.5%

Table III
Autologistic regression model estimate considering intra-herd prevalence and all the independent variables listed in the questionnaire applied during sampling procedures

	Estimate	*p*	OR	95% CI
ρ	1.877	<0.0001	6.537	4.544–9.403
Amazon biome	0.291	<0.0001	1.338	1.262–1.420
Cerrado biome	0.240	<0.0001	1.271	1.208–1.338
Equine function on ranches (work)	0.281	<0.0001	1.324	1.182–1.484
Origin of equines (purchased)	0.024	0.0424	1.024	1.000–1.049
No prior AGID test required before entrance	0.147	<0.0001	1.159	1.098–1.223

AGID: agar gel immunodiffusion
CI: confidence interval
OR: odds ratio
ρ : regression coefficients of covariates

Table IV
Autologistic regression model estimate considering equine infectious anaemia foci and all the independent variables listed in the questionnaire applied during sampling procedures

	Estimate	*p*	OR	95% CI
ρ	3.439	0.0071	31.164	2.496–407.696
Pantanal biome	0.762	<0.0001	2.142	1.476–3.105
External working equines	0.369	0.0242	1.447	1.047–1.993
Shared saddle and tack	0.585	0.0102	1.795	1.163–2.850
No prior AGID test required before entrance	0.570	0.0214	1.768	1.105–2.930
Flooded areas on the ranch	0.561	0.0005	1.752	1.276–2.402
Number of cattle on ranch	0.000	0.0003	1.000	1.000–1.000

AGID: agar gel immunodiffusion
CI: confidence interval
OR: odds ratio
ρ: regression coefficients of covariates

Table V
Results of adjusted association and risk analysis by chi-square (χ^2) test of individual variables and seropositive equines in the state of Mato Grosso, Brazil

Variables	Non-weighted samples			Adjusted chi-square			
	Tested	Positive	%	%	OR	95% CI	*p*
Equine species							
Horses	3,230	338	10.4	7.1	1.641	1.620–1.663	
Mules	602	48	7.9	4.5	0.636	0.628–0.644	
Donkeys	26	0	0.0	0.0	-	-	<0.0001
Sex							
Female	1,573	130	8.2	5.0			
Male	2,285	256	11.2	6.9	1.412	1.396–1.429	<0.0001
Age							
≥6 months to 3 years	503	10	1.9	1.0	0.140	0.135–0.145	
>3 years to 10 years	2,282	223	9.7	6.0	0.922	0.912–0.932	
>10 years	1,073	153	14.2	8.8	1.801	1.781–1.822	<0.0001
Equine function							
Other [(a)]	250	3	1.2	1.1			
Work	3,608	383	10.6	6.7	6.660	6.379–6.952	<0.0001
Broken in							
No	632	23	3.6	2.1			
Yes	3,226	363	11.2	6.9	3.468	3.384–3.555	<0.0001

a) Sports, leisure and reproduction
CI: confidence interval
OR: odds ratio
p: probability value

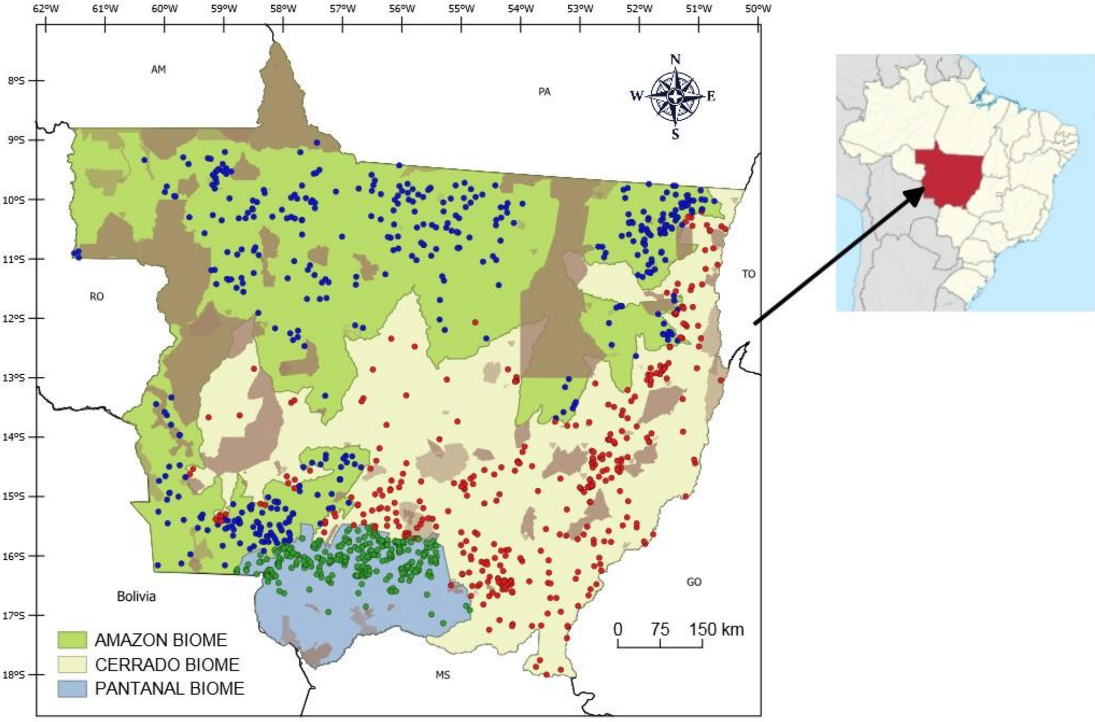

Fig. 1

Map of the state of Mato Grosso, Brazil, divided into three biomes

Dots represent ranches involved in the survey
Brown areas represent parks, and nature and indigenous reserves

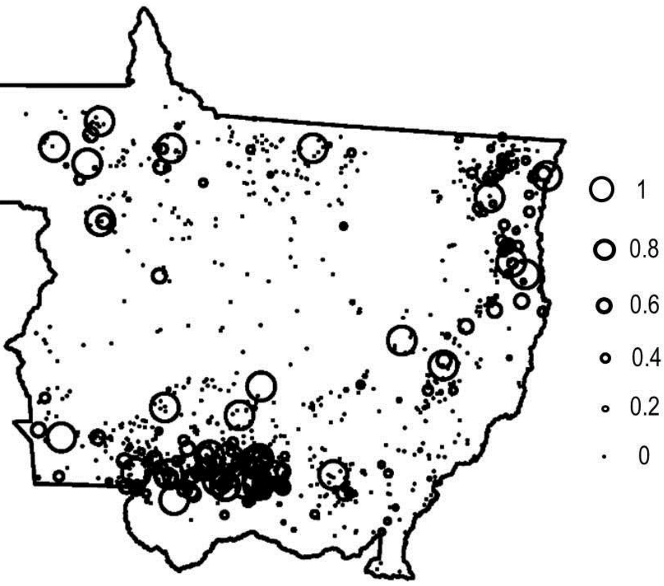

Fig. 2

Spatial prevalence distribution of equine infectious anaemia virus on ranches sampled in the state of Mato Grosso, Brazil

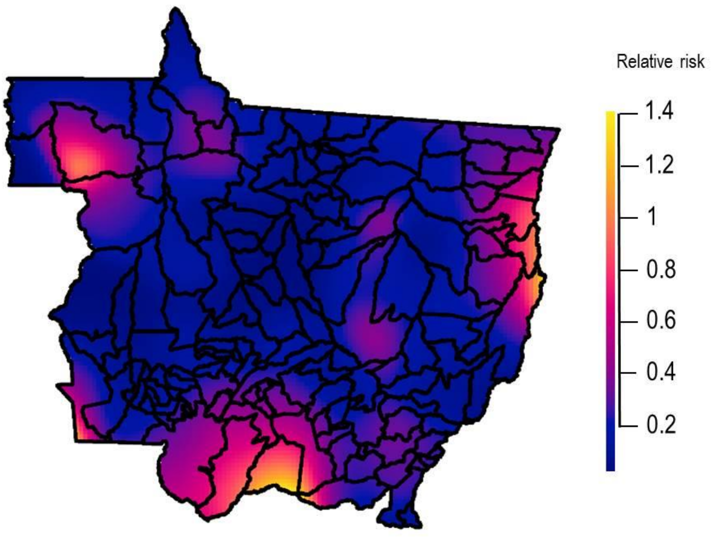

Fig. 3

Estimated relative risk of equine infectious anaemia in the state of Mato Grosso, Brazil

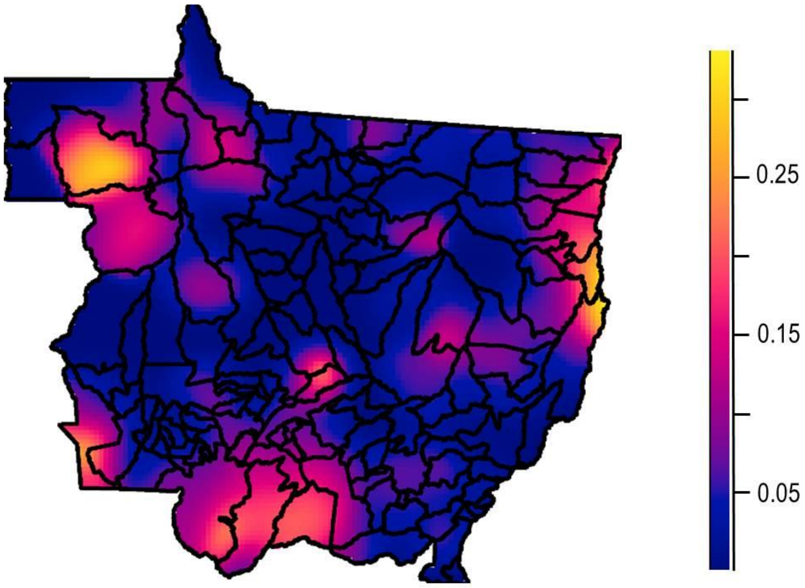

Fig. 4

Kernel density estimation of the spatial distribution of the intra-herd prevalence in the state of Mato Grosso, Brazil