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SCIENTIFIC ARTICLE
Animal Infectious Diseases

Prevalence and main risk factors of equine infectious anemia in the southern of Bahia Coast Identity Territory, Brazil

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ABSTRACT
The state of Bahia has the third largest equine herd in Brazil, with the horse industry overtaking several sectors of the economy currently. However, animals may be exposed to several pathogenic agents, especially the equine infectious anemia (EIA) virus. EIA is a persistent viral disease with a worldwide distribution and is considered the main infectious disease in horses. Therefore, this study sought data from serological survey EIA in Itapé and Barro Preto municipalities, inserted in the southern Coastal Identity Territory of Bahia, by using the agar gel immunodiffusion test. A total of 1,880 blood serum samples from Equidae older than 6 months were analyzed from 172 properties. The general prevalence of outbreaks was 25.58% (44/172), presenting 40.47% (34/84) in Barro Preto (cocoa zone) and 11.36% (10/88) in Itapé (livestock zone). The prevalence of positive animals in Itapé was 1.01% and in Barro Preto 12.15%. Each assessed property received a questionnaire with emphasis on sanitary management and a booklet containing basic disease information. It was concluded the EIA is endemic in two epidemiologically distinct areas in the Southern Coastal Identity Territory of Bahia, one with a high prevalence in the cocoa zone for both animals and properties, and the other, in livestock zone, with a significantly low prevalence for animals, however also high for properties. Regarding the risk factors, there was a correlation between the species and age of the animals. Mules and the elderly showed a significant association, probably due to the habitat they live in and longer exposure to the virus.

Keywords: anemia; agar gel immunodiffusion; mules; equine; lentivirus.

INTRODUCTION

The state of Bahia has the third-largest herd of horses in the country, with 481.869 heads (IBGE, 2016). In the southern Coastal Identity Territory of Bahia (SBCIT), the estimated headcount is 59.461 heads of Equidae (IBGE, 2010; MDA, 2008) in 26 municipalities, of which 3.71% are in the municipality of Itapé and 2.94% in Barro Preto. The horse industry has grown in recent years, but the economic loss due to equine infectious anemia (EIA) is still considerable (SILVA et al., 2013).

EIA is a persistent viral disease with worldwide distribution and affects all species of Equidae. Transmission occurs through the contaminated blood of affected animals by hematophagous insects or iatrogenically (CONSTABLE et al., 2017). It is characterized by recurrent fever, thrombocytopenia, anemia, rapid weight loss, and edema of the lower parts of the body. If death does not occur, a chronic phase develops, and the infection tends to become silent (OIE, 2013). This disease still has no cure, treatment, or vaccine, and the sacrifice of reactive animals is mandatory by law without

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indemnity. Thus, EIA has economic relevance due to mortality, decreased work capacity, sacrificed animals, traffic embargoes, and interference in equestrian events.

Official reports from the State Agency for Agricultural Defense of Bahia (ADAB, 2015) and SFA/BA and MAPA (2015) confirm outbreaks reported in 24 of the 26 municipalities (92.30%) of SBCIT (ADAB, 2015). The SBCIT is an agricultural region whose economy is based on cocoa production and livestock, both areas with essential use of Equidae. Thus, the study aimed to carry out the EIA serological survey, inferring the prevalence and spatial distribution in the municipalities of Itapé and Barro Preto.

MATERIAL AND METHODS

Description of municipalities

An epidemiological serum survey was carried out in the municipalities of Itapé and Barro Preto, with a territorial extension of 660.93 km^2 inserted in the 15.886 km^2 of SBCIT, in an estimated equine herd of 3,956 heads (Table 1).

Table 1. Herd of Equidae by species in Southern Coastal Identity Territory of Bahia (SBCIT), Brazil.

Municipalities	Hinnies	Equine	Mules	Total
Barro Preto	1,623	123	-	1,746
Itapé	1,250	850	110	2,210
Other municipalities in the SBCIT	36,815	16,343	2,347	55,505
SBCIT	39,688	17,316	2,457	59,461

Source: IBGE (2012).

The municipality of Itapé has an area of 459.36 km^2, geographic coordinates: 14°53'51.33"S, 39°25'41.02"W, population of 10,995 inhabitants, human development index of 0.653 (average), and forest area of 1,086 ha (IBGE, 2012). The transition climate between the coast and the hinterland is practically uniform, with a dry period in September and February and temperatures ranging between 16 °C in winter and 32 °C in summer. The rainfall was more than 2,000 mm, but nowadays it is no more than 1,500 mm. The relative humidity of the air varies between 60 % and 85% (PREFEITURA DE ITAPÉ, 2014).

The municipality of Barro Preto has an area of 201.57 km^2, geographic coordinates: 14°48'35"S, 39°28' 17"W, population of 6,453 inhabitants, and a human development index of 0.602 (IBGE, 2012). It constitutes a conservation unit of the Atlantic forest, inserted in the environmental protection area of Lagoa Encantada and Almada River. According to data from the Executive Committee of the Cacao Plantation Plan (ATEFFA-BA, 2013), there is approximately 8,130 ha in the municipality occupied with cocoa cultivation, producing an average of 13 arrobas (190.97 kg)/hectare, 90% of which is shaded with native tree species.

EIA serological survey

In the sample design, the primary units (rural properties or companies) were extracted from the ADAB register (ADAB, 2014), complemented by those from Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC, 2014). The number of properties drawn was determined by the formula for simple random sampling using the Epitools R program (SERGEANT, 2009). The following parameters were defined for the calculation: 95% confidence level, estimated prevalence of 20%, and absolute error of 5%. Thus, 172 properties of the study universe of 611 were randomly distributed proportionally by the predefined municipalities, according to their representativeness, with 88 properties in Itapé and 84 in Barro Preto (Table 2). If it was impossible to locate the property owner/responsible for the drawn property to sample the animals, or in the absence of animals, there was a substitution for another property with similar characteristics in the surroundings.

Table 2. Properties amount, sampled properties, and representativeness per municipality were included in the serological survey of equine infectious anemia.

Municipality	N of properties	Sampled properties	Representativeness (%)
Itapé	322	88	27.32
Barro Preto	289	84	29.06
Total	611	172	28.15

Source: ADAB (2015); CEPLAC (2014).

Each property visited was identified by geographic coordinates by the global positioning system to map the studied flocks in the municipalities worked on. For the definition of secondary samples for analysis of the study, Equidae (equines, donkeys, and mules) were selected, aged from 6 months, used for work, sports, and leisure, without distinction of species, race, sex, or function. Considering the homogeneous profile of the properties in the studied area, and because they mostly have small Equidae herds, all animals of this family in the properties represented secondary units of analysis.

Blood samples in the amount of 10 mL were collected from the puncture of the equine jugular vein using the vacuum blood collection system in a tube without anticoagulant. The serum obtained was stored in 2-mL microtubes at 20° C until sent to the ADAB Animal Health Laboratory to perform the serological test through the agar gel immunodiffusion test (AGID), "Coggins Test" (OIE, 2013).

Statistical analysis

The prevalence of outbreaks and animal rates were calculated using a database created using the Epi Info program (DEAN et al., 1994). The geographical variables obtained in the properties were related to the prevalence information for mapping outbreaks and geographic distribution in the study area using the ArcMap 9.3 program. For assessing possible risk factors (species, breed, age, and sex) for the spread of EIA in the study area, an exploratory analysis of the data (univariate) was performed for the χ^2 test or Fisher's exact test and subsequent offering of them to logistic regression. The calculations were performed with the aid of the Statistical Package for the Social Sciences program, version 9.0.

RESULTS

The researched properties that presented at least one AGID-positive serum animal were considered AGID. The overall prevalence of EIA outbreaks was 25.58 (44/172), 40.47% (34/84) in the municipality of Barro Preto (cocoa zone), and 11.36% (10/88) in Itapé (livestock area), as shown in Table 3. The prevalence of serologically positive animals was higher in the municipality of Barro Preto, at 12.15%, than in Itapé, with only 1.01% (Table 4). In addition, the tabulated data referring to the outbreaks were associated with geographical data of the properties under study, characterizing the spatial distribution of the EIA in the cities studied (Fig. 1).

Table 3. Frequency of focus properties for equine infectious anemia in the municipalities of Itapé and Barro Preto, Bahia, Brazil.

Municipalities	Focus properties	Negative properties	Total	(%)
Itapé	10	78	88	11.36
Barro Preto	34	50	84	40.47
Total	44	128	172	25.28

Source: Elaborated by the authors.

Table 4. Agar gel immunodiffusion test positive animals, negative animals, total sampled, and general prevalence from the municipalities of Itapé and Barro Preto, Bahia, Brazil.

Municipalities	Positive animals	Negative animals	Total	(%)
Itapé	15	1,462	1,477	1.01
Barro Preto	49	354	403	12.15
Total	64	1,816	1,880	3.40

Source: Elaborated by the authors.

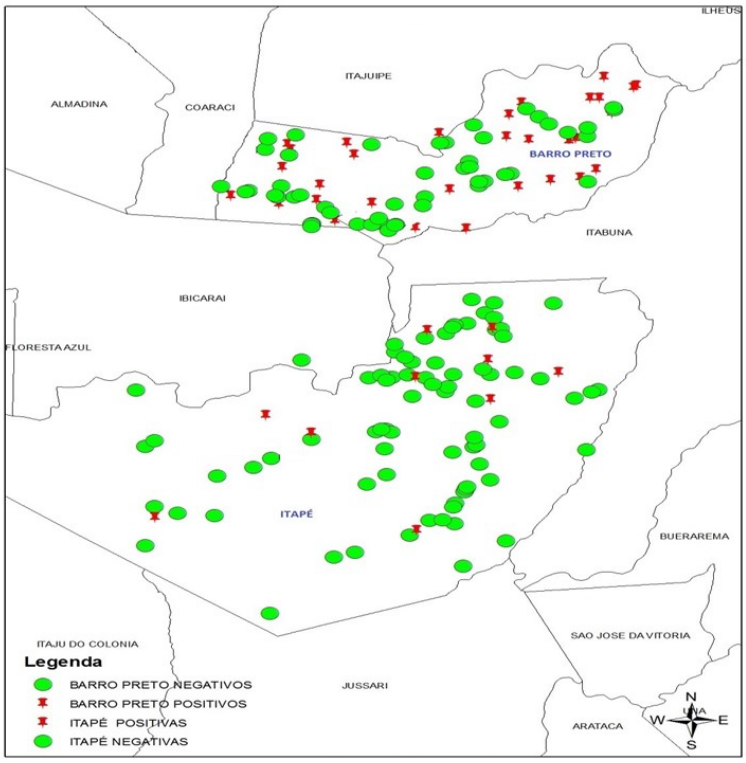

Figure 1. Spatial distribution of equine infectious anemia in the municipalities of Itapé and Barro Preto, Bahia, Brazil.
Source: Elaborated by the authors.

When comparing the EIA virus (EIAV) prevalence in horses and mules, there was a significant difference between these species. There was an association between positivity for the EIA when comparing the equine and mule species. As for donkeys, it was impossible to assess the association of positivity because the research did not present animals of this species that were reactive to the AGID test for this study universe. In total, 1,365 horses and 471 mules were sampled, 1.97% (27) of the horses and 7.85% (37) of the mules (Table 5). The $\chi 2$ calculation highlighted an association between mules and EIA positivity ($p < 0.001$). The analysis of the breeds of positive animals pointed out that the animals belonging to the SRD group (without a defined breed) showed a higher frequency of positivity (7.66%), which was statistically significant, $p < 0.001$ (Table 6).

Table 5. Frequency of seropositive Equidae for equine infectious anemia by agar gel immunodiffusion test, according to species in the municipalities of Itapé and Barro Preto, Bahia, Brazil*.

Species	Positive animals	Negative animals	Total	(%)
Equine	27	1,338	1,365	1.97
Mule	37	434	471	7.85
Total	64	1,772	1,836	3.48

*$x^2 = 35.96$; $p < 0.05$; $p < 0.001$.
Source: Elaborated by the authors.

Table 6. Frequency of seropositive Equidae for equine infectious anemia by agar gel immunodiffusion test, according to breed in the municipalities of Itapé and Barro Preto, Bahia, Brazil*.

Breed	Positive animals	Negative animals	Total	(%)
No defined breed	43	518	561	7.66
Pure / mixed	21	1,298	1,319	1.59
Total	64	1,816	1,880	3.40

*$x^2 = 44.14$; $p < 0.05$; $p < 0.001$.
Source: Elaborated by the authors.

Among the 911 males, 38 (4.17%) were positive, and among the 969 females, 26 (2.68%) were positive (Table 7).

Table 7. Frequency of sera positive for equine infectious anemia according to sex in Itapé and Barro Preto, Bahia, Brazil*.
*x2 = 3.16; p > 0.05; p = 0.07538.

Sex / gender	Positive animals	Negative animals	Total	(%)
Male	38	873	911	4.17
Female	26	943	969	2.68
Total	64	1,816	1,880	3.40

Source: Elaborated by the authors.

There was no statistical significance in analyzing a possible association between the frequency of animals reactive to EIAV and sex (p = 0.075). Regarding age, three categories of analysis were created: young horses (up to 5 years old), adult horses (between 5 and 10 years old), and elderly horses (over 10 years old). The data highlighted that 13.64% (58) of the animals over 10 years old (367) reacted positively to EIAV, presenting, therefore, a significant difference, indicating an association between this age group and positive cases (Table 8).

Table 8. Frequency of seropositive Equidae for equine infectious anemia by agar gel immunodiffusion test, according to age in the municipalities of Itapé and Barro Preto, Bahia, Brazil*.

Age (years old)	Positive animals	Negative animals	Total	(%)
Up to 5	2	749	751	0.26
5 to 10	4	700	704	0.56
Over 10	58	367	425	13.64
Total	64	1,816	1,880	3.40

*x^2 = 175.30; p < 0.05; p < 0.001.
Source: Elaborated by the authors.

EIAV circulation differed statistically between properties by the way animals are used. The cocoa stratum had a prevalence of 39.29% instead of 12.50% of the livestock stratum (Table 9). Regarding risk factors for the prevalence of EIA in the properties, the variables related to the origin, use of animals, individual or collective equipment, and entry of Equidae sequence of outbreaks in neighboring properties in the last 12 months were defined for statistical association (Table 10). Among the risk factors analyzed, for the 172 properties surveyed, only the variable related to the animals' use displayed a statistically significant difference.

Table 9. Focus on the predominant aptitude of properties in the municipalities of Itapé and Barro Preto, Bahia, Brazil*.
*x^2 = 16.20; p < 0.05; p < 0.001.

Aptitude (years)	Focus properties	Negative properties	Total	(%)
Up to 5	33	51	84	39.29
5 to 10	11	77	88	12.50
Total	44	128	172	25.58

Source: Elaborated by the authors.

Table 10. Risk factors assessment with their respective x2 values and the probability of occurrence at random (p).

Factors	Variables	x^2	p-value
Origin of animals	raise/buy	0.01	0.93632
Use of animals	cocoa/cattle	16.20	0.00006*
Use of collective equipment	yes/no	0.03	0.85804
Equine entry in last 12 months	yes/no	1.97	0.15999
Focus on neighboring properties	yes/no	0.38	0.85804

*p < 0.05.
Source: Elaborated by the authors.

DISCUSSION

The significant difference between the general prevalence of EIA in municipalities is probably caused by the concentration of vector insects, geographical diversity, and the different management practices of the properties. The geographical distribution of EIA outbreaks in the studied cities points to a dispersion model of this disease, possibly representing an endemic character in this territory.

In the state of Rio de Janeiro, Brazil, a temporal analysis of EIA cases between 2007 and 2011 showed a low prevalence of 0.43%, considering it endemic (BATISTA et al., 2016). Other states such as Paraná (PEROTTA et al., 2015), Distrito Federal (MORAES et al., 2017), and Minas Gerais (ALMEIDA et al., 2017) were also characterized as endemic zones because of their low prevalence: 1.03, 1.81, and 5.3%, respectively. Properties in the southern region of Bahia had a prevalence of 13.43% (36/268) (GUIMARÃES et al., 2011), similar to what was determined by this study in the livestock area, but lower than that observed in the cocoa area. Prevalences higher than those observed in this research were reported for herds in the same municipalities of Itapé and Barro Preto, where they inferred an overall prevalence of 33.89% (20/59), and per municipality of 16.66 (7/42) and 76.47% (13/17), respectively, when stratified by livestock and cocoa zone (ARAÚJO et al., 2007). This reduction in prevalence shown in the properties is possibly related to the educational actions (disease concept, control, and good management practices) carried out by the 2007 seroepidemiological survey and the active surveillance of ADAB in these municipalities.

The prevalence of serum antibodies against EIAV observed in the present study was lower than those detected in other regions of the country, such as 31.5% in Poconé (MT, Brazil) (BORGES et al., 2013), 46.24% in Ilha de Marajó (PA, Brazil) (FREITAS et al., 2015), 18.2% in Pantanal (MS, Brazil) (SILVA et al., 2001), and 17.71% Uruará (PA, Brazil) (HEINEMANN et al, 2002). This superiority in both the general prevalence and in the strata of this study is due to the high rate of infection with a favorable climate for developing hematophagous insects, which are a primary factor in determining disease endemicity. This condition is consistent with that observed in the cocoa zone, where the population of insect vectors is large. Furthermore, in other municipalities in Bahia (ROSA et al., 2012), the percentage of seropositive people of 4.3% (9/205) was higher than in the cattle-raising zone, but much lower than in the cocoa-tree region and similar to the general prevalence of this study.

This index can be associated with the collective use of syringes, and needles, carrying out tests only for transit, and lack of vector control. When stratified by epidemiological profile, the reductions are statistically relevant mainly in the cocoa stratum, probably because they follow the same trend shown for properties in these municipalities due to the impact of educational actions carried out in 2007 and active surveillance by ADAB. The higher prevalence detected in the cocoa zone by this study may be associated with the producer's resistance to the test-sacrifice model of the reactors, the lower zootechnical value of the animals, illegal trade, and the high concentration of insect vectors.

In this study, none of the 1,880 animals sampled presented clinical symptoms of EIA, consistent with the report that 94.5% (517/547) of the animals did not show any apparent signs of the disease, featuring asymptomatic carriers (BORGES et al., 2013).

The highest significance of an association between the mules observed in this study was probably due to the habitat related to the concentration of vector insects, the significant presence of asymptomatic animals, the failure of breeders to observe the legislation, and the manner in which animals are used. The most significant association between the mules observed in this study was probably due to the habitat related to the concentration of insect vectors, the significant presence of asymptomatic animals, non-compliance with legislation by breeders, and how the animals are used. This fact is consistent with the prevalence observed in Distrito Federal (MORAES et al., 2017), where there was a superiority in seroreactivity mules (11.11%) compared with horses (1.46%).

Regarding breed, the higher prevalence of the disease in SRD animals without statistically significant differences in the study is consistent with results observed in Acre, Brazil (SANTOS et al., 2001), and other regions of Bahia (GUIMARÃES et al., 2011). This is probably because these animals being used in various activities in the field, with greater exposure to disease than other breeds intended for exhibitions. The lack of EIA correlation with sex observed in the study was similar to previous surveys in other states (ALMEIDA et al., 2006; CHAVES et al., 2015), except Maranhão, Brazil, where there was a higher frequency in females (58.03%) (CHAVES et al., 2015).

Age was considered a risk factor for EIA in this study, given the higher prevalence of the disease in elderly animals (13.24%). Similar results were observed in other regions (BORGES et al., 2013; GUIMARÃES et al., 2011; SILVA et al., 2001). This relationship is probably due to prolonged exposure to the virus, with the persistence of the asymptomatic form of the disease, associated with the restriction of serological tests.

EIAV circulation statistically differs between properties by the way animals are used. The highest probability of EIA infection was observed in horses used in cocoa production, probably because of the higher concentration of insect vectors,

the difference between management practices, the lack of attention to legislation, and the zootechnical value of the animals. Although the variable use of collective equipment does not have a significant statistical result, the observations made in the field indicate that the sharing of syringes and needles contributes substantially to disseminating EIA in the cocoa stratum.

CONCLUSIONS

The EIA is endemic in two epidemiologically distinct areas in the SBCIT, one with a high prevalence in the cocoa zone for both animals and properties, and the other, livestock, with a significantly low prevalence for animals, but also high for properties. It is characterized in a dispersed way throughout the municipalities of Itapé and Barro Preto, in the state of Bahia, and its occurrence is more associated with the mule species, animals over 10 years old and of mixed breed, as well as in the properties that use these animals for services in the cocoa crop.

AUTHORS' CONTRIBUTIONS

Conceptualization: Araújo Júnior, J.M.; Costa, J.N.; Ferrão, I.S.; Ribas, R.L. **Investigation:** Araújo Júnior, J.M.; Ferrão, I.S.; Ribas, R.L.; Torres, P.E. **Methodology:** Araújo Júnior, J.M.; Costa, J.N.; Ferrão, I.S.; Ribas, R.L.; Nunes, B.Q.; Torres, P.E. **Data analysis:** Araújo Júnior, J.M.; Costa, J.N.; Ferrão, I.S.; Ribas, R.L.; Nunes, B.Q.; Torres, P.E. **Writing – original draft:** Araújo Júnior, J.M.; Mendonça, A.P.A. **Writing – review & editing:** Mendonça, A.P.A.; Costa, J.N.

AVAILABILITY OF DATA AND MATERIAL

All data generated or analyzed during this study are included in this published article.

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest

ETHICAL APPROVAL

This study was approved by the Ethics Committee on the Use of Animals of Universidade Federal do Recôncavo da Bahia, Campus Cruz das Almas, in 2015, under opinion No. 23007.004226/2015-18.

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