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Seroepidemiology Surveys of Equine *Arteritis virus* in Equids Population of Center-West Region of São Paulo State, Brazil

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Abstract: In the present study were analyzed 554 serum samples from equids originated from 82 farms located in nine municipalities in the center-west region of São Paulo state, Brazil, with the objective of diagnostic animals serum reactive to *Equine Arteritis Virus* (EAV), applying the microneutralization test. The sampling revealed that 1.99% of animals showed soroconversion examined to microneutralization test anti-EAV. The frequencies for municipalities ranged from 1.29% (Bauru), 3.74% (Agudos), 7.69% (Piratininga) to 10% (Duartina) of equine serum-reagents to EAV. The races affected were older than two years. Between sexes analyzed, the major frequency animal's serum reagents for EAV - 2.72% - was observed in the groups of males versus 1.62% for female. In the region studied, stallions have detachable importance epidemiological in the chain of EAV carriers via the semen or natural mating. A significant positive result for females (6/370), mainly responsible for transmission via excretions of abortions and male (transmission via semen) revealed the importance of diagnostic microneutralization test for the control of epizootic outbreaks and inapparent carriers to EAV.

Key words: *Equine arteritis virus* · Mare · Microneutralization test · Stallion · Viruses

INTRODUCTION

Equine Viral Arteritis (EVA) is a viral infection of equids characterized by transient and variables clinical signs [1] can range from an asymptomatic, persistent or acute infection to abortion or hemorrhagic fever in naturals outbreaks epizootic. The EVA is a infectious-contagious disease that affects the respiratory system of horses, so-called by inflammatory lesions features induced by the causative virus on the small blood vessels, especially in the arterioles of the animal with an acute infection. The panvasculitis determined by the virus of EVA has varied form of manifestation. Staying in the depending mainly of the intensity of the which can result in hemorrhage, edema or be as severe conditions, resulting in prominent systemic vascular necrosis and abortion favoring the spread of the virus. Tissue blood vessels are the main targets however, depending on the intensity of infection by the EAV, the virus can replicate in tissues of the respiratory mucosa [2-5], reproductive tract [6], lung, intestine and kidney [7]. Occasionally, the placenta are important viral replication sites, which favor the spread of the virus. In extensive surveys of field sampling conducted in different populations and states of Brazil by Souza [8], Souza *et al.* [4,9] and Lara *et al.* [10,11,12] were observed prevalence rates variables. The authors ascertain that EVA can cause great economic losses for the equine industry, being an important parameter to be taken into account as epizootic outbreaks and the impracticability of reproductive cycles linked to abortions in pregnant females and the need for exclusion of the reproductive process the stallions carrying the virus in the plenitude of the stage of reproduction.

The family *Arteviridae* was reclassified by the International Committee on Taxonomy of Viruses in [13] and curently comprises four enveloped, positive-stranded RNA viruses *Equine Arteritis Virus* (EAV), lactate dehydrogenase-elevating virus of mice, porcine reproductive and respiratory syndrome vírus and simian

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hemorragic fever virus. Macrophages appear to be the primary target cell for all arteriviruses. The infection may persist in the semen of infected males, which makes sexual transmission an important secondary route of infection. The virus is able to escape immune surveillance and establish a largely asymptomatic persistent infection [14, 15].

Since its first description the more than 50 years passed by Doll *et al.* [16] to reporting focus in Ohio in the U.S. as virulent Bucyrus strain (VBS53) when they described that if referred to particle with ability to pass through filters, other strains KY77 and KY84 were isolated from new outbreaks in 1984 in the north American, in the Kentucky state. Subsequently, new strains have been identified in new outbreaks being the IL93 isolated in the Midwestern USA in 1993 and CA95 in California [17-20].

Arteriviruses are small, enveloped, animal viruses with an icosahedral core containing a positive-strand RNAvirus genome [21].

The genomic RNA of EAV comprises nine known open reading frames (ORFs) flanked by a 59 untranslated region (59UTR) and a 39UTR [22-24, 25].

The vast majority of EAV infections are inapparent or subclinical, but occasionally outbreaks of EVA occur that are frequently characterized by an influenza-like illness in adult horses, abortion in pregnant mares and interstitial pneumonia in very young foals [15, 26, 27].

A variable percentage, from 10 to 70 %, of stallions acutely infected with EAV can become persistently infected carriers, shedding the virus constantly in semen [27, 28]. Carrier stallions transmit EAV venereally to susceptible mares following natural breeding or artificial insemination.

Infected mares can then disseminate the virus to susceptible cohorts with which they have close contact [20, 27, 29].

In accordance with the classification system of Baltimore [30], these viruses belong to group IV with virus replication cycle ssRNA(+) in the cytoplasm cell, where the chain of ssRNA (+) copy tape sequence complementary ssRNA (-) to the following synthesis the viral genome ssRNA (+). RNA-dependent RNA polymerase, or RNA replicase, is an enzyme that catalyzes the replication of RNA from an RNA model.

Equine Viral Arteritis is an acute, contagious, viral disease of equids caused by *Equine Arteritis Virus*. It is characterized by fever, depression, dependent edema in especially of the limbs, scrotum and prepuce in the stallion, conjunctivitis, nasal discharge, abortion and infrequently, death in young foals.

In the pathogenesis of the disease is involved several cellular defense mechanism. A macrophage-restricted molecule, found to mediate the internalization of the virus by macrophages.

The output of EAV virus from persistently infected stallions is testosterone-dependent [31]. The carrier state has only been found in the stallion, not in the mare, gelding or sexually immature colt. Carrier stallions are seropositive so tests for serum antibody can be used to screen for potential carriers. Seropositive mares and geldings are those that have recovered from infection and are no longer shedders of virus. Acutely infected horses may transmit virus by aerosol [32].

Similarly to the first outbreak of equine viral arteritis registered in the U.S. in 1953 on a farm in Bucyrus, Ohio-USA, when a virus was characterized as being responsible for cases of abortion and frame respiratory disease [16], in Brazil the first outbreak of equine viral arteritis, which has been recorded, occurred in Ibiúna County of São Paulo State, in a property of livestock race Mangalarga Paulista, where abortion was observed in a mare in the fifth month of pregnancy. Other eleven animals had nasal discharge and blefaroedema, some of them with edema of abdomen and members, besides orchitis in two males who made up the group [9]. Surveys seroepidemiological indicate that the virus is widespread among the population of equids, but only rarely causes disease [33-35].

This research has as objective to evaluate the frequency of seropositive equids to EAV in a population of animals raised in the region Center-west of São Paulo state, Brazil.

MATERIALS AND METHODS

In this research, were sampled different ages, sexes and races of equines during the years 2007 and 2008 in Center-West region of São Paulo state. The criterion for the sampling of animals in areas of nine counties was independently of the classic symptoms of Equine Viral Arteritis (EVA), history epizootic outbreaks, or clinical signs such as fever frames followed by acute respiratory disease and abortion in females.

Blood samples to obtain sera, were collected from horses through the external jugular vein puncture, using Vacutainer® in siliconized glass tubes without anticoagulant, provided of rubber stopper and vacuum for aspire a volume of 10 mL.

Laboratory analyses were conducted in the Laboratory of Rabies and Encephalitis of Instituto Biológico, São Paulo, Brazil. Serum from 554 equines

female and male sex, of different races and age rang groups in the rurals counties of Agudos, Arealva, Avaí, Bauru, Cabrália Paulista, Duartina, Iacanga, Lucianopólis and Piratininga were tested. The microneutralization test was utilized for being able to detect neutralizing antibody. The plates are read microscopically for nonviral cytopathic effect CPE after 12–18 hours and again for viral CPE after 48–72 hours' incubation. A serum dilution is considered to be positive if there is an estimated 75% or preferably a 100% reduction in the amount of viral CPE in the serum test wells compared with that present in the wells of the lowest virus control dilution. A titre of ¼, or major than, is considered to be positive. A negative serum should only have a trace (less than 25%) or no virus neutralisation at the lowest dilution tested.

At the moment of the realization of a set of proofs, possible factors for variability of results were controlled: the cell suspension, the virus titration and serum control of the reaction. For this purpose sera negative and positive patterns with titers previously determined were used [35].

RESULTS

Tables 1-5 and Figures 1-2 represent the results obtained from nine municipalities studied.

Among the nine municipalities studied for 554 analyzed samples, the sampling revealed that 1.99% of the animals examined showed seroconversion for micro-serum neutralization technique, anti-*Equine Arteritis Virus* (EAV). The frequencies serum-reagents ranged from 1.29% [Bauru: 0.78% (1 male equine), 1.54% (4 female equine)], 3.70% [Agudos: 2.44% (1 female) and 7.70% (1 male)], 7.69% [Piratininga: 5.88% (1 female) and 11.11% (1 male)] to 10% in Duartina (2 male) according to the sex of the population of municipalities studied. The region of Bauru, numerically had the highest concentration of serum-reagents presenting 1.54% (4 F/259 F; 66.93% of F) females and 0.78% (M 1M/128; 33.07% M) of males and total 1.29% (5 positives/387) serum reagents in all animals examined in the municipality of Bauru (Table 1; Figure 1 and 2).

Among the ten races studied-Mules (*Equus caballus* x *Equus asinus*); Paint horse, Brazilian of Equestrian, Quarter Horse, Thoroughbred, Mangalarga, Anglo-Arab, Asinine, Creole and Without Defined Race (SRD), the major prevalence of serum reagents *Equine Arteritis Virus* 90.91% (10/11 positive) as observed in the Quarter Horse and only 9.09% (1/11 positive) serum reagent for the mangalarga race (Table 2). The other races resulted negative to the microneutralization test for EAV.

Among the age groups studied (for one year until 25 years of age),the major frequency mean of 5.66% [(6 / 106) (2M: 4F), (> 2-4 years)] decreased significantly ($P<0.05$) to 1.52% [(3 / 198), (1M: 1F), (> 4-8 years)] and 1.28% [(2 / 156) (2M: 1F), (> 8 years)] of reagents the *Equine Arteritis Virus* in the population total studied (Table 3; Figure 1).

Between animals serum reagents, for sexes analyzed, the major frequency ($P>0.05$) of animals serum reagents for EAV-2.72% (5/184)-was observed in the group of males versus 1.62% (6/370) for female according to the sex of the study population (Table 4; Figure 1).

Table 1: Number of reagents and not reagents animals to equine viral arteritis in the municipalities studied in the mesoregion of Bauru

	Examineds by sex (%)			Reagents by sex(%)		
Counties	Female	Male	Total examined	Female	Male	subtotal by Counties (%)
Agudos	41 (75.93)	13 (24.07)	54	2.44 (1/41)	7.70 (1/13)	3.70 (2/54)
Arealva	7 (100)	0	7	0	0	0
Avaí	5 (83.33)	1 (16.67)	6	0	0	0
Bauru	259 (66.93)	128 (33.07)	387	1.54 (4 /259)	0.78 (1/128)	1.29 (5/387)
C. Paulista	5 (100)	0	5	0	0	0
Duartina	13 (65)	7 (35)	20	0	10 (2/20)	10 (2/20)
Iacanga	12 (48)	13 (52)	25	0	0	0
Lucianopólis	11 (45.83)	13 (54.17)	24	0	0	0
Piratininga	17 (65.38)	9 (34.62)	26	5.88 (1/17)	11.11 (1/9)	7.69 (2/26)
Totals	370 (66.79)	184 (33.21)	554	1.62 (6/370)	2.72 (5/184)	1.99 (11/554)

Table 2: Percent of reagents and not reagents animals to Equine Viral Arteritis according with the races studied in the region of Bauru

	Race											Reagents	
Municipality	SRD*	M	PH	BH	QM	PSI	ML	A-A	A	C	Subtotal	Female[a]	Male[b]
Agudos	2	0	5	0	45	0	2	0	0	0	54	1 QM (7a)	1 QM (25a)
Avaí	2	0	0	0	0	3	1	0	0	0	6	0	0
Arealva	0	0	0	0	7	0	0	0	0	0	7	0	0
Bauru	27	4	21	45	283	1	1	0	5	0	387	4 QM (3:4a; 1:16a)	1 QM (14a)
C. Paulista	2	3	0	0	0	0	0	0	0	0	5	0	0
Duartina	0	0	0	0	14	0	3	0	0	3	20	0	2 QM (4a; 7a)
Iacanga			0	0	25	0	0	0	0	0	25	0	0
Lucianopólis	8	13	0	0	3	0	0	0	0	0	24	0	0
Piratininga	3	4	0	0	19	0	0	0	0	0	26	1 ML (5a)	1 QM (3a)
Total	49	24	26	45	396	4	7	0	0	3	554	6	5

SRD*=Without Defined Race; M=Mules (*Equus caballus* x *Equus asinus*); PH=Paint Horse; BH=Brazilian of Equestrian; QM=Quarter Horse; PSI=Thoroughbred; ML=Mangalarga; AA=Anglo-Arab; A=Asinine; C=Creole; [a] – female reagent: 5 QM and 1 ML; [b]– male reagent: 5 QM.

Table 3: Percent of reagents and not reagents animals to Equine Arteritis Virus according with the age groups (months) studied in the mesoregion of Bauru

	Reagents by age range*										
Municipality	0-12	%	13-24 (> 1-2)	%	25-48 (>2-4)	%	49-96 (>4-8)	%	97 (>8)	%	Total
Agudos	1	0	3	0	13	7.69 (1 F: 7a)	22	0	15	6.67 (1 M: 25a)	54
Arealva	0	0	1	0	0	0	3	0	3	0	7
avai	1	0	0	0	0	0	3	0	2	0	6
C. Paulista	0	0	0	0	0	0	3	0	2	0	5
Bauru	18	0	66	0	62	4.84 [3 F:4a]	125	0	116	1.72 [1 M: 14a; 1 F:16a]	387
Duartina	0	0	0	0	4	25 (1M: 4a)	7	14.29 (1 M :7 a)	9	0	20
Iacanga	0	0	2	0	7	0	14	0	2	0	25
Lucianópolis	1	0	0	0	5	0	16	0	2	0	24
Piratininga	0	0	1	0	15	6.66 (1 M: 3a)	5	20 (1 F:5 a)	5	0	26
Sub total: reagents (%)						5.66 (6/106)	(3/198)	1.52	(2/156)	1.28	1.99 (11/554)
Sex	21	0	73	0	106	2M:4F	198	1 M: 1F	156	2M:1F	5 M: 6 F

F= female; M= male; a=years. *P<0.05.

Table 4: Frequency of all animals reagents and not reagent according studied sex

	Female	Reagent%	Male	Reagent%	Total
Reagent	6	1.62	5	2.72	11
Nonreagent	364	98.38	179	97.28	543
Subtotal	370		184		554

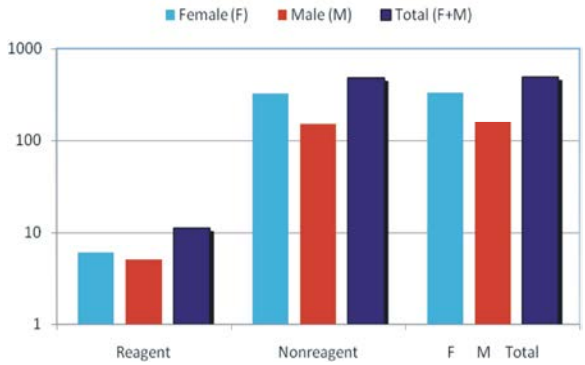

Fig. 1: Distribution of frequency (log base 10) of reactive and non reactive to infection by the Equine Viral Arteritis virus according to sex study

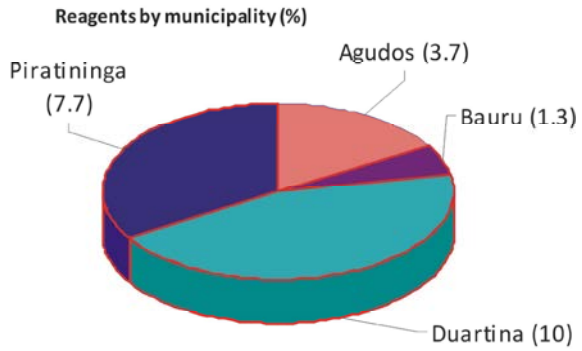

Fig. 2: Distribution of frequency of serum-reagents animals to infection for virus of Equine Viral Arteritis of according mesoregion of Bauru

DISCUSSION

In this study, average of 1.99% Equine Viral Arteritis for the nine cities studied are very much lower to those obtained by Souza [8], Souza *et al.* [4, 9], Lara *et al.* [10,12] studying in populations of equids the answers to microneutralization test in several regions of São Paulo state, Brazil (Table 1). The oscilations in the prevalence and frequency of the sero-reactive to Equine viral arteritis (EAV) has also been observed in other studies, which will be mentioned the follow. According with Souza [8], MacLachlan *et al.* [36] and Lara *et al.* [10] the microneutralization test-universally accepted methodology-which through analysis of seroconversion detected the animals serum-reactives, to that is to analyze seroconversion in different age groups enabling monitoring the prevalence rate of infection of *Equine Arteritis Virus* (EAV). In animals raised in São Paulo of State, it was possible to verify the lack of equivalence of the frequency distribution of seropositivity in groups inhere to different age groups.

The microneutralization have proven effective when compared to other tests to detect infection of equine viral arteritis.

Thus, with the aim of detecting antibodies anti-EVA, the reaction microneutralization, indirect immunofluorescence and complement fixation, Nosetto *et al.* [37] in the Argentina analyzed 250 blood serum samples of horses from different areas of country, with some of the animals showed symptoms similar to those described for this disease and others had no such symptoms. Paradoxically, among the animals who exhibited symptoms consistent with infection by the VAE, only 1.5% were sero-reagents and among the horses 11.9% were asymptomatic serum reagent. The authors also mention that these results were similar, considering-if the responses obtained from three serological techniques used.

Similarly, Lieberman [38] studied the EAV infection by detecting antibodies, through and trial, the serum neutralization test in 908 serum samples from asymptomatic horses bred in different parts of the former German Democratic Republic 128 animals found sero-reagents, totalizing the prevalence of 14.0%.

In contrast, in the Federal Republic of Germany, Kaaden *et al.* [39], in a survey, seroepidemiological similarly found a lower prevalence rate, as in 739 blood serum samples obtained from clinically normal horses during a period of two years, detected, only 3.8% animal serum-reactive to antigens of the *Equine Arteritis Virus*, using also the proof of micro-serum- neutralization and also in Germany, their republic Western investigating the presence of antibodies anti-LAV, in serum-sanguineous, horses.

Schützler *et al.* [40] evaluated 352 blood samples collected from asymptomatic animals by proving micro-serum neutralization detected prevalence rate equal to 17%.

The efficiency of the proof of micro-serum neutralization was also confirmed by McCue *et al.* [41], with the objective to determine the prevalence of anti-EAV equine population in the State of California, United States, examined 1063 samples the blood serum collected between April 1988 and December 1989, of animals created in 35 farms of the mentioned state, an average rate of prevalence equal to 13.6%. In the cited research, was also used microneutralization test considered standard for detection of antibodies in the serum anti-EVA.

Results similar to those of this research showing variations in the frequency of serum reagents was also obtained by Maasommeh [42] in Iran when analyzing 199 blood serum samples of horses and mules collected on 28 properties, found 1.0% carriers of antibody anti-LAV using the microneutralization test. In the cited work, pointed out that these animals serum reagents had symptoms similar to those described for Equine Viral Arteritis, detaching-if: nasal and ocular discharge, conjunctivitis, edema of hind limbs, diarrhea and abortion of pregnant and Kölbl *et al.* [43], which for the purpose of determining the distribution of the infection of *Equine Arteritis Virus* in horses bred in Austria, evaluated 944 blood serum samples collected between 1988 and 1989 and using proof of the microneutralization, had obtained a prevalence of 10.9%. These researchers

have also shown a significant variation in the results, according to regional distribution, as in the Salzburg region of the prevalence rate was 4.6% and in the lower region of Austria was 15.7%.

In this research, the results showed a higher frequency of serum-reactive aged > 2 to 4 years (54.54%) > 4-8 (27.27%) years and > 8 (18.18%) years for EAV. The means decreasing by age group of 33.33% (> 2-4 years), 16.67% (> 4-8 years) and the lowest average of 1.75% (> 8 years) were significantly influenced by the incidence of seropositivity to EVA, between ages (Table 3). Still, was possible to analyze the proportionality of the average was 43.80% (233 animals analyzed) serum-reactive for the age groups studied in the animal population and of the 1.59% (554 animals analyzed) for the set of age groups reagent and non-reactive in all the districts studied (Table 2).

Approach to a similar situation can be seen in the results reported by Cavirani *et al.* [44], Italy, to examine the means of proof of microneutralization 148 blood serum samples of horses of different ages and belonging to seven properties, taken between July and December 1988, had a prevalence of 20.9%. According to the authors, the sample was distributed over the classification of horses, according to age in four age groups, had higher prevalence rate in older animals, because the group of foals aged between 3 and 6 months, the prevalence was 23.52%; in the second group, consisting of foals with 7 to 24 months of age, the prevalence was equal to 2.12%; in the other two experimental groups consisting of adult horses, with aged between 25 and 60 months and another with horses over 60 months of age, the prevalence rates were, respectively, equal to 18.75% and 45.75%.

According Moraillon *et al.* [34], many have been the serological methods described for the diagnosis of EAV infection, however, recommended if that the serum-neutralization test as standard to easily detect the strong response neutralizing antibodies developed within 1 to 2 weeks after initiation of infection, antibody titers that persist in the blood of convalescent animals during many years.

The antibodies found in blood serum of horses infected with EAV, both in the acute phase, as well as convalescent, were considered as good indicators immunology infection, being the microneutralization test according with Senne *et al.* [35], the reference test used for demonstration of antibodies anti-virus equine arteritis.

For supply some supposed and techniques described disadvantages the detection of serum antibodies by microneutralization, some researchers have developed and devised other methods of immuno-serological diagnosis among which stood out: the plaque reduction test [45, 46], complement fixation [37, 45, 47, 48], the reaction immunodiffusion [49], indirect immunofluorescence [37, 50, 51], the immunoenzymatic ELISA (Enzyme linked immunosorbent assay) [34, 52] and PCR (polymerase chain reaction) [53]; However, some authors have reported difficulties in reading and interpreting evidence of microneutralization for detection of serum antibodies, anti-AVE by the techniques previously used and on that basis, with the objective standardized using a for the serological diagnosis of *Equine Arteritis Virus*, Senne *et al.* [35] developed and standardized a method recommending the use of complement at a concentration of 10%. After evaluations comparative with the other recommended techniques the authors concluded to be the micro-serum-neutralization serological proof more sensitive and widely used for the detection of antibodies anti-AVE.

Considering the above observations of the authors, in this research the frequency of detected serum reagents how much to groups studied separately for sex, age or race and the overall population analyzed for evidence of microneutralization was evident the effectiveness of the technique of for detection of the virus in the population studied. So being, in this present research, we analyzed 554 samples originating from 82 different properties, being that for the 10 most frequently studied race was 90.91% for quarter horse and only one (9.09 %) sero-reactive for the Mangalarga race (Table 2) and the absence of reagents in the other races in the test microneutralization for EAV. On the other hand, for a total of 11 animal serum reagents was found a higher frequency of seropositivity of 2.72% (5 / 184) of males versus 1.62% (6 / 370) of females in the population of the nine studies municipalities (Table 4; Figure 2).

Results similar to those found in this study were reported by several authors in prevalence studies. Therefore, Ceccarelli *et al.* [47], used the serum-neutralization reaction in serological survey to evaluate the occurrence of infection of equine arteritis virus in 1093 serum samples, collected in 79 properties located in different regions of Italy and found that only 0.9% of the sera tested by serum neutralization proof contained anti-EVA; Akash *et al.* [54], in Japan between 1973 and 1974 studied the prevalence of *Equine Arteritis*

Virus, using the reaction of microneutralization in horses imported from different countries, noting that among 140 samples tested, 12 (8.6%) had antibodies to *Equine Arteritis Virus* in animals coming from Germany, France and the Soviet Union; In Japan, Konishi *et al.* [55], researching for proof of microneutralization and plaque reduction anti-EAV antibodies in 107 blood serum samples collected from apparently healthy horses, obtained only negative results; Moraillon *et al.* [56], examined by microneutralization test 4,280 equine serum samples, being 3,324 from France and the remaining (956) from animals raised in other European and African countries, obtaining as a result of an average prevalence rate of serum-reactive animals equal to 18.5%; Testing samples of blood serum of horses, for if estimate prevalence rate of *Equine Arteritis Virus*, of the Equine Infectious Anemia and equine rhino-pneumonitis in animals raised in Morocco, Moraillon *et al.* [56] obtained a result average equivalent to 38% in a population of equines whose sera were evaluated for proof of micro-serum neutralization; In the State of Ontario, Canada, Lang *et al.* [57], with the intent to perform an epidemiological survey to assess the rate of prevalence of anti-virus equine arteritis, tested 742 serum samples from horses through of microneutralization test and ELISA method and obtained a prevalence rate equal to 16.2% through of the two tests; Examining 564 samples from a serum bank of blood of equines, collected in 89 properties located in the State of Quebec, Canada, Elazhary *et al.* [58] observed a frequency of 8.5% of anti-EVA antibodies in serum samples equine blood evaluated. Moreover, they could to see that 28% of the farms studied had seropositive animals, demonstrating the spread of infection by EVA virus in Canada. In that research the authors used the microneutralization test for demonstrate the presence of antibodies anti-EVA in blood serum; Huntington *et al.* [33], utilizing the microneutralization test, studied blood serum samples collected from 770 mares raised in Australia finding a prevalence of anti-LAV equal to 28.8%; In the Poland, Golnik *et al.* [59], researching blood serum samples of 711 horses, collected in 1989 and 1990, had a prevalence of 29.5% animal serum reagents, front to antigens of the EAV, utilizing the reaction of microneutralization. In Germany, actually reunified, Herbst *et al.* [60] found significantly higher prevalence, because to examine 1081 serum samples from horses utilizing the microneutralization test found a prevalence rate of 8.7%, indicating the rate of infection of horses in the Germany, by the equine arteritis virus; and in the period 1988-90, Fukunaga *et al.* [61] examined through of microneutralization test with the EVA antigens, 1656 samples of blood serum collect from horses bred in several properties located in regions eastern and western Japan, obtaining a prevalence of 0.5%.

In this research, analysing by sex the frequency of 2.72% in males higher than in females (1.62%), emphasizes the importance of male reproductives in the epidemiology of EAV that second Timoney *et al.* [17 - 19, 29] and Little *et al.* [31] Timoney *et al.* [62], the carrier stallion appears to have played a major epidemiologic role in the dissemination and perpetuation of the virus. Has been shown the persistence of stallions infected with EAV, probably involving the predisposition hormal testorone-dependent.

In the genome replication, the arterivirus replication cycle is presumed to occur entirely in the cytoplasm of the infected cell, despite the fact that at least two viral proteins are targeted to the nucleus. Following uncoating, the incoming genome is translated into the two large replicase polyproteins pp 1a and pp 1ab.

Cole *et al.* [6] examined the transmissibility and abortogenic effect of Equine Viral Arteritis in group of pregnant mares, the which were exposed via contact group control with mares bred to stallions infected with the virus of *Equine Arteritis Virus*. Febrile response was noted in each control group mare and in the pregnant mare. Seroconversion was recorded in 18 mares. EAV was isolated from the nasopharynx of pregnant mares. In the pregnant mares aborted EAV was isolated from fetal organs and placenta.

After respiratory transmission, EAV initially replicates in lung macrophages and endothelial cells. The virus then spreads to draining lymph nodes, from where it becomes disseminates throughout the body. By the third day of infection, a viremia has developed and virus can be isolated from practically all tissues. The effect of EAV infection in the natural host ranges from subclinical to flulike symptoms in adult animals, abortion in pregnant mares and interstitial pneumonia in neonates. Clinical features are characteristic vascular lesions, necrosis of small muscular arteries, acute anorexia and fever.

Apart from mortality in young foals, the case fatality rate in outbreaks of EVA is very low. Affected horses almost invariably make complete clinical recoveries. A long-term carrier state can occur in a high percentage of infected stallions, but not in mares or non-breeding horses [18, 19].

Clinical symptom (features) and pathogenesis: Virulence is strain dependent, but the genetic basis for this has not yet been determined. Very typical for natural EAV infections is the persistence that occurs in about 35% of the infected stallions. The virus persists in the reproductive tract of these "carrier stallions" and is continuously shed into semen. However, it is testosterone-dependent and high serum titers of neutralizing antibodies are unable to clear the virus from persistently infected animals [31]. Nevertheless, according to Souza [8] the study of seroconversion through of the micro-neutralization technique, universally accepted method, by easily detect the strong neutralizing antibody response developed between 1 to 2 weeks after the onset of infection, has if constituted as an indispensable tool to allow through of this proof, the monitoring of several variables that may influence the onset of this viral infirmity of epizootic character on the prevalence of infection by equine arteritis virus in animals raised in the State of São Paulo, in the which if verified there is no equivalence of the frequency distribution of seropositivity in groups of animals belonging to different age groups.

In outbreak of equine viral arteritis in the United Kingdom, Wood *et al.* [32] reported that EAV was isolated from semen and heparinised blood samples and seroconversions were demonstrated by using the EAV neutralization test. Titers of antibodies that persist in the blood of convalescent animals for many years [34, 35].

CONCLUSION

This study allowed to affirm that the Equine viral arteritis virus is present in the equine population of the mesoregion of Bauru. The frequency of serum-reactive in 554 animals of the nine cities studied analyzed the proof of the micro-serum- neutralization was 1.99%.

Among nine municipalities studied in the mesoregion of Bauru, four showed the existence of the virus in animals in animals in age groups above of two years old. The highest frequency of 5.66% for those aged more than 2 years decreased to 1.28% in animals with more than 8 years old.

Between the sexes of horses studied, the highest frequency of the EVA was 2.72% for males versus 1.62% for females. Stallions have very important role in the epidemiological chain of equine viral arteritis as carriers via semen or natural mating.

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