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Vesicular Stomatitis Alagoas Virus Outbreaks Expansion From 2016 To 2024 Within The Maranhão State, Brazil.

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Abstract

Background: *Vesicular stomatitis (VS) is one of the most relevant viral enzootic diseases of the livestock in the tropical and subtropical regions of the Americas. The VS disease in Brazil is caused mainly by Vesiculovirus Alagoas (VSAV), a member of the Vesiculovirus genus from the Rhabdoviridae family. The VSV is an arbovirus, enveloped, bullet-shaped containing a non-segmented, RNA negative sense genome. The VSAV is epitheliotropic, provokes proliferative skin and mucosal lesions as blisters- like vesicles, hypersalivation, erosion and ulcers around the mouth, tongue, naso-oral mucosa, on the udder, on the teats, and on the coronary bands of the feet. Several municipalities from the Maranhão (MA) State suffered with the VSAV outbreaks that occurred from 2016 until 2024. Those outbreaks started in municipalities within the biome of Cerrado and expanded to municipalities belonging to Amazon biomes inside of the MA state. The laboratorial diagnosis and molecular investigation of the field VSV lineage is a paramount tool in strategies to control the virus as well, it is important to consider several ecological factors that might infer the virus' natural evolution.*

Materials and Methods*: Surveying data of the VSAV outbreaks from 2014 up to 2023 were obtained from the official laboratory records files. In 2024, twelve VSAV outbreaks that occurred in ten municipalities between January and February were described and plotted in a map. The VSV clinical finds and symptoms of the cattle, equids and suids from the first three outbreaks occurred in the municipalities of Ribamar Fiquene, Davinópolis and João Lisboa has reported by the AGED-MA. In 2025, the AGED-MA reported four VSAV suspicious cases being one equine and three bovine occurrences in three distinct municipalities. Serum samples, epithelium from desquamations of the recent lesions from each suspicious of VSAV infectious animals from the 12 VSV outbreaks notified in 2024, were collected. The virus isolation, antibody neutralization and RTqPCR, were performed. The genomic analysis is based on partial phosphoprotein gene sequences.*

Results: *The VSV outbreaks in the MA State from 2014 to 2024were plotted in a map allowed to observe the VSV outbreaks were first detected in the municipalities from the Cerrado biome, northwest region, and moved towards the Amazon biome, in the north region within the MA state, concomitantly, with the livestock expansion. Clinically, oral, and interdigital space lesions in cattle, lesions on the udder and teats of mare and foal lips were observed. The virus isolation results of the VSAV have been fully confirmed by the RTqPCR performed. Likewise, the VN test of the sera samples from bovine and equine, the highest antibodies titres against the VSAV reach at least to 2,7and >3,0, respectively. The phylogenetic tree based on a partial sequence of the Phosphoprotein gene (gene P) has been built successfully. Since it is allowed to notice the VSAV variants' proximity.*

Conclusion: *The existence of a cladistic grouping in the Amazon and Cerrado biomes within the MA state area suggests an evolutionary path of this virus which should be associated with that environment and all susceptible fauna or vectors.*

Key Word: *vesicular stomatitis virus, clinic finds, laboratory diagnosis, phylogeny, Cerrado biome, Amazon biome.*

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I. Introduction

Vesicular stomatitis (VS) is one of the most relevant viral enzootic diseases of the livestock in the tropical and subtropical regions of the Americas[1]. The VS virus (VSV) is a member of the Vesiculovirus genus and the prototype of the family the Rhabdoviridae family[2]. The VSV is an arbovirus, enveloped, bullet-shaped containing a non-segmented, RNA negative sense genome, with around 11kb in size[3]. From the 3' to the 5' terminal sense, the proteins gene transcripts are arranged as nucleoprotein (N), the phosphoprotein (P), the matrix (M), the surface glycoprotein (G), and the RNA-dependent RNA polymerase (L)[2] .The genus Vesiculovirus shelters four species denominated Vesiculovirus Indiana –VI (formerly IND-1), Vesiculovirus Cocal –VC (formerly IND-2), Vesiculovirus Alagoas – VSAV (formerly IND-3) and Vesiculovirus New Jersey – VNJ. The VSAV has occurred seasonally, particularly, in rainy periods in tropical climate regions, suggesting that the virus has been remaining stable in these ecological niches. Moreover, prior VSV phylogenetic studies have suggested that VSAV is endemic in several regions from Brazil with the formation of two genotype groups evolving independently[2,4,5,6]. This would make the disease spread predictable, however the complexity VSAV transmission mechanisms which can involve different hosts and several possible arthropod vectors turn the prediction of the VSAV transmission and the disease control a continuous challenging[8].The VSAV is an epitheliotropic virus causing severe vesicular disease in cattle, sheep, goats, pigs, horses, and wildlife hosts. Clinically, the VSAV symptoms in cattle and pigs are not differentiated from the foot and mouth disease (FMD) and other vesicular diseases thus, the differential laboratory examination is essential[6]. Besides, the molecular investigation of the field VSAV could be a paramount tool in strategies to control the virus.

The Maranhão State (MA) is a federal state from the Brazilian Northeast region is constituted by the following three mainly biomes: Amazon (Dense evergreen terra firme amazon rainforest biome); Cerrado (Seasonal Tropical Savanna Biome); and the Caatinga (Semi-arid Seasonal Tropical Savanna Biome), occupying a territorial area around 35%, 64%, and 1%, respectively[9]. In the last 30 years, the highest incidences of drought and rainfall events were recorded in the south and the north of the MA, respectively[11].Likewise, in this period, the VSAV outbreaks had been increased[6]. Besides global climate changes, in 2024, the MA state had suffered with the "El Niño and La Niña phenomenon" occurrence, which could have seriously interfered with insect population growth dynamics. Since the VSAV can be transmitted biologically or mechanically by insects, myriads of mosquitoes and flies could be involved in the VSAV rise[8]. As one can infer, several factors have a potential to increase the VSAV outbreaks. Due to, in this work, we focused on a thoughtful analysis about the virus outbreaks, animals affected, and the virus lineage. The VSAV outbreaks and cases that occurred in the MA between 2016 and 2024 were scrutinized. In addition to differential laboratory tests for other vesicular diseases, the virus isolation, antibodies survey against the virus and the phylogeny analysis of virus lineages were performed. Only for the January to April 2024, 12 outbreaks took place in ten municipalities, which the first three ones have occurred in the municipalities Ribamar Ficane, Davinópolis and João Lisboa gave us the opportunity to screen the VSAV clinical finds of the cattle, equids and suids. This study showed that in both the Cerrado and the Amazon biome regions from the MA State, the VSAV outbreaks and the livestock expansion production has increased following the same pace.

II. Material And Methods

Surveying data of the VSV outbreaks from 2014 up to 2024. Data of occurrence of VS outbreaks were obtained from the Laboratório de Diagnóstico de Doenças a virus – LDDV, from Federal Agricultural Defense Laboratory, of the Ministry of Agriculture and Livestock (LDDV/LFDA/MG-MAPA), official laboratory records files. The samples had been investigated in serology, virology, and molecular methods Twelve VSV outbreaks that occurred in ten municipalities in 2024, between January and February were described and plotted in map. The VSV clinical finds and symptoms of the cattle, equids and suids from the first three outbreaks of 2024 occurred in the municipalities of Ribamar Fiquene, Davinópolis and João Lisboa has reported by the AGED-MA. All the samples that arrived in LDDV were submitted to differential diagnosis followed the WOAH Standard protocol: antibodies against non-structural proteins of the Foot and Mouth disease Virus (FMDV) in the ELISA assay; the epithelium samples from bovine tested for FMDV, Orthopox and Parapoxvirus. As well, the swine tissue samples were tested for FMDV, Senecavirus valley, Vesicular Exanthema of Swine Virus (VESV); Swine Vesicular Disease (SVD) by virus isolation and RTqPCR.

Sampling. Serum samples and tissues. Briefly: epithelium from desquamations of the recent lesions from each suspicious of VSAV infectious animals from the 12 VSV outbreaks notified in 2024, were drawn into a plastic flask containing Minimum Essential Medium (MEM). After identified, the samples were sent to the LDDV/LFDA/MG-MAPA, in Pedro Leopoldo, Minas Gerais State.

Methodologies. Upon arrival at the laboratory, the VSAV serum neutralization assays, virus isolation and real time quantitative PCR (RTqPCR) for fast diagnosis, were performed. The VSAV positive samples chosen depending on the value of the Threshold cycle (Ct), preferentially, below 30, were submitted to the conventional retro-transcription PCR (RT-PCR) for sequencing. The vesicular disease differential diagnose were performed as WOAH recommendation.

Virus Neutralization (VN) and Virus Isolation (VI). The humoral antibody against the VSAV by the viral neutralization method, was performed. The antibody has been titrated face a standard VSAV at concentration previously determined. The cut-off has been determined to be 1.50 $TCID_{50}$. The serum will be considered positive or reagent when the titre is equal or higher the cut-off value. The ELISA (Enzymatic linked immunosorbent assay) method has been applied to the detection antibody against non-structural proteins of FMDV. The VSV isolation and the virus neutralization assays was performed with BHK-21 [C-13] cells (Baby hamster kidney – ATCC CCL-10) and Vero cell line (ATCC-CCL-81) in accordance with the WOAH/OIE (2023).

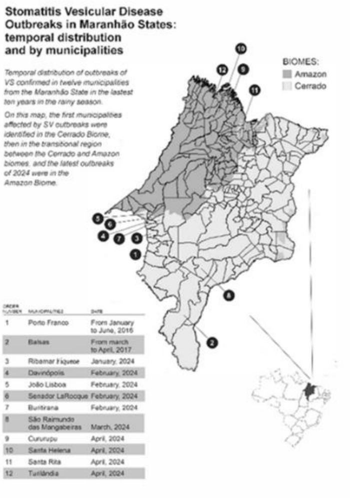
The RTqPCR. Initially, the presence of the VSAV genomic fragment from suspicious samples had faster analyzed applying the RTqPCR[12]. Briefly: primers and probes have been designed using the Primer3 Plus program based on the alignment of nucleotide sequences of the glycoprotein gene in the RT-qPCR and of the phosphoprotein for RT-PCR. The RT-qPCR was performed using the Quantifast RT-PCR Kit (Qiagen, Germany) and the reaction occurred in a single step. The reactions were assayed in the Bio-Rad CFX 96 – C1000 thermocycler.

RT- PCR. The VSAV's RNA was extracted from the second passage in the Baby Hamster Kidney cell line (BHK). The Trizol method (Invitrogen, Carlsbad, CA) was used to extract total RNA from the samples according to the manufacturer's instructions. The cDNA synthesis has been carried out by the reverse transcription with 200U M-MLV; 10µM random primers, 2.5nM dNTPs, 0.1M DTT, 20U RNAse-OUT (Invitrogen, Waltham, MA, USA) and RNase-free water. The reaction was incubated at 37ºC for 1 h, followed by enzyme inactivation at 70ºC for 15 min. The cDNA samples were stored at -20ºC until processed.

The partial Phosphoprotein gene sequencing. The RT-PCR product from the positive samples, has been evaluated on a 1% agarose gel stained with ethidium bromide. The 722 base pairs bands were cut out and purified using the Quick-spin Total Fragment DNA purification Kit (Intron Biotechnology). The amplicons were sent for Sanger sequencing on the ABI 3500 analyzer (Thermo, USA). Both forward and reverse sequences generated were aligned using the BioEdit software. The sequences were submitted to BLAST software for identification. The MEGA 6.02 program was used to reconstruct the phylogenetic tree using the Kimura 2-parameter model with gamma distribution and evolutionary inference from Maximum Likelihood with 1000 bootstrap replicates. The phylogenetic relationship among consensus sequences obtained from Ribamar Fiquene, Davinópolis and João Lisboa and 37 sequences from Brazilian Northeast, Southeast regions have been inferred allowing the building of the phylogenetic tree (Figure 5).

III. Result

Differential diagnosis: bovine and swine serum samples were negative for antibodies to non-structural proteins of the FMDV in the ELISA assay. And the epithelium samples from bovine showed negative results for FMDV, Orthopox and Parapoxvirus. Swine tissue samples were negative for FMDV, Senecavirus A.; Swine Vesicular Disease (SVD). The municipalities where VSAV outbreaks have occurred; their geolocation within the Cerrado and Amazon biomes from MA state have been plotted. (Figure 1).



Temporal distribution of outbreaks of VS confirmed in twelve municipalities from the Maranhão State in the lastest ten years in the rainy season.

On this map, the first municipalities affected by SV outbreaks were identified in the Cerrado Biome, then in the transitional region between the Cerrado and Amazon biomes, and the latest outbreaks of 2024 were in the Amazon Biome.

ORDER NUMBER	MUNICIPALITIES	DATE
1	Porto Franco	From January to June, 2016
2	Balsas	From march to April, 2017
3	Ribamar Fiquene	January, 2024
4	Davinópolis	February, 2024
5	João Lisboa	February, 2024
6	Senador LaRocque	February, 2024
7	Buritirana	February, 2024
8	São Raimundo das Mangabeiras	March, 2024
9	Cururupu	April, 2024
10	Santa Helena	April, 2024
11	Santa Rita	April, 2024
12	Turilândia	April, 2024

Figure 1. The distribution of VS outbreaks from 2014 to 2024 in Cerrado, Transitional, and Amazon Biomes within the Maranhão State, Brazil. The 12 municipalities were numbered from the first to last outbreak, have pointed out in the map.

The epidemiological survey from 2016 to 2017 demonstrated the presence of viral infection in cattle and horses in Porto Franco and Balsas municipalities from the Cerrado biome from MA State. In 2016, nine VSV outbreaks have arisen in three farms from January until June in Porto Franco municipality. VSAV was detected in cattle, equine and swine herds. The virus neutralization highest antibody titre of three sequential of bovine and equine sampling was 3.2 and 3.7 respectively[9]. In 2017, two VSAV outbreaks in the same farm have taken place in Balsas municipality detected only in cattle herds (Table 1).

Table 1. The Vesicular Stomatitis Alagoas virus (VSAV) outbreaks occurred in the Maranhão State before 2024. Laboratory Analysis data (LFDA-Sample registration files.

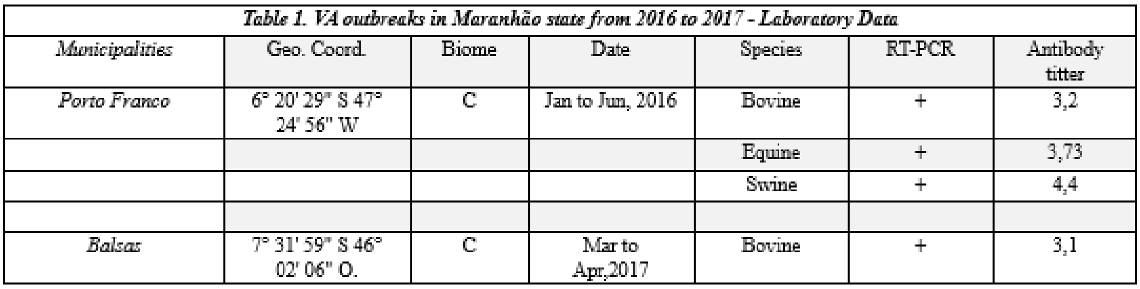


Table 1. VA outbreaks in Maranhão state from 2016 to 2017 - Laboratory Data

Municipalities	Geo. Coord.	Biome	Date	Species	RT-PCR	Antibody titter
Porto Franco	6° 20' 29" S 47° 24' 56" W	C	Jan to Jun, 2016	Bovine	+	3,2
				Equine	+	3,73
				Swine	+	4,4
Balsas	7° 31' 59" S 46° 02' 06" O.	C	Mar to Apr,2017	Bovine	+	3,1

Furthermore, it is important to report that no VSAV outbreaks were recorded in the period 2018 to 2023. However, the number of the VSAV from 2024 was 12 which were distributed in 10 municipalities.

The first epidemiologic survey of the AGED-MA has begun in Ribamar Fiquene (Farm 1). Despite of one of the sick cattle showed apathetic behavior or even hypersalivation. The VSAV main lesions found in an adult Nellore bull were oral erosions on the skin and mucosa and interdigital injuries (Figure 2 A).

The second investigation (Farm 2), in Davinópolis municipality, an athlete equine (runner), from "Vaquejada", a type of rustic race involving bovine and equine which two knights persecute one bovine until it brings down to the ground, has presented rupture VSAV oral lesions which may progress to an ulcerative lesion (Figure 2.B). Surveying all the equine competitors, ten more equines were found in similar condition were investigated.

The third surveying (Farm 3) took place in the municipality of João Lisboa. The investigation has found out infected equines that have participated in the "Vaquejada", being four horses and two mules with healed lesions on the lips, tongue and several lesions on her udder and teats due to the foal's suckling, some lesions still had not healed effectively (Figure 2C). In addition, six cows, over 36 months old, had lesions on the gums and tongue, in an advanced state of healing. However, the pig did not show any clinical signs of vesicular disease.

Figure 2. A

Figure 2. B

Figure 2. C

Figure 2. In the photo A, one can see the VSAV lesions seven days pos-infection on the bovine foot from Farm 1 (Municipality Ribamar Fiquene, MA). In the photo B is shown the ruptured VSAV vesicular lesions on the gum of an athlete equine (runner) from Farm 2 (Municipality Davinópolis, MA). In the photo C is shown a nursing mare has presented several vesicular lesions on her udder and teats from the Farm 3 (Municipality João Lisboa, MA). All photos were courtesy of the AGED-MA.

The Ribamar Fiquene and Davinópolis municipalities, where the VSAV outbreaks were identified early, belong to Cerrado biome (C). While the third municipality João Lisboa is in a territorial strip intermediate between Amazon and Cerrado (A/C).

The following nine VSAV outbreaks have occurred in A/C and in the Amazon Biome (A). Only one VSAV outbreak recurrence was recorded in bovine herds in Cururupu (A) (Table 2).

Table 2. VA outbreaks in Maranhão state from Jan to Apr 2024 -Laboratory Data

Municipalities	Geo. Coord.	Biome	Date	Species	RT-qPCR	VI	Antibody titter
Ribamar Fiquene	5° 55' 48" S 47° 23' 02" W	C	Jan 29th	Bovine	+	+	-
Davinópolis	5° 33′ 28″ S, 47° 25′ 33″ W	C	Feb 02nd	Equine	+	+	≥3.0
João Lisboa	5° 26' 47" S 47° 24' 11" W	A/C	Feb 09th	Bovine Equine	+	+	B=2.4 E=2.7
Senador La Rocque	5° 26' 51" S 47° 17' 35" W	A/C	Feb 24th	Equine	+	+	≥2.7
Buritirana	5° 35′ 55″ S 47° 0′ 59″ W	A/C	Feb 24th	Equine			≥2.7
São Raimundo das Mangabeiras	7° 1' 20" S 45° 28' 53" W	C	Mar 06th	Bovine	+	+	≥2.7
Cururupu	1° 49' 44" S 44° 51' 48" W	A	Apr 06th Apr 13th Apr 23rd	Bovine Bovine Bovine	+ + +	+ + +	- - -
Santa Helena	2° 13′ 44″ S 45° 17′ 48″ W	A	Apr 11th	Equine	+	+	≥2.7
Santa Rita	3º08'49" S 44º19'11"W	A 95% C 5%	Apr 23rd	Bovine Equine	+ +	+ +	≥2.7 ≥2.7
Turilândia	2° 11' 57" S 45°20' 35"W	A	Apr 23rd	Bovine	+	+	≥2.7

Table 2. The Vesicular Stomatitis Alagoas virus (VSAV) last outbreaks had occurred in the Maranhão State from January to April 2024. Laboratory Analysis data (LFDA Sample registration files.

In the first three VSAV outbreaks investigated, for the bovine and equine hydration were common a collective drinking fountain mainly for horses and for both bovine and equine the dams and small watercourses. A large infestation of the horsefly, a Diptera from the Tabanidae family, was observed disturbing the animals.

The VSAV has been isolated after two passages with a 48-hour interval in cell line from all samples of bovine and equine epithelium collected during outbreaks reported in early 2024 from the Ribamar Fiquene, Davinópolis, João Lisboa municipalities and other municipalities where outbreaks have occurred.

These results of the VSAV have been fully confirmed by the RTqPCR performed. Likewise, the VN test of the sera samples from bovine and equine, the highest antibodies titres against the VSAV reach at least to 2,7 and >3,0, respectively. The previously determined positive samples have attained a threshold cycle (Ct) value of 17, 27; 16,64; 26, 35 from Ribamar Fiquene, Davinópolis and João Lisboa samples, respectively. Since these values reflect the amplification and genetic expression efficiency, these samples have been considered suitable for sequencing.

The phylogenetic tree based on a partial sequence of the Phosphoprotein gene (gene P) has been built successfully (Figure 5). Since it is allowed to notice the VSAV variants' proximity. On the superior branches of the tree, samples from Ribamar Fiquene and Davinópolis outbreaks came together on the same branch. The sample from the VSAV outbreak from João Lisboa has been situated in the branch below. However, all of them were located closed with samples from 2013/2014 and 2016 of Porto Franco, MA.

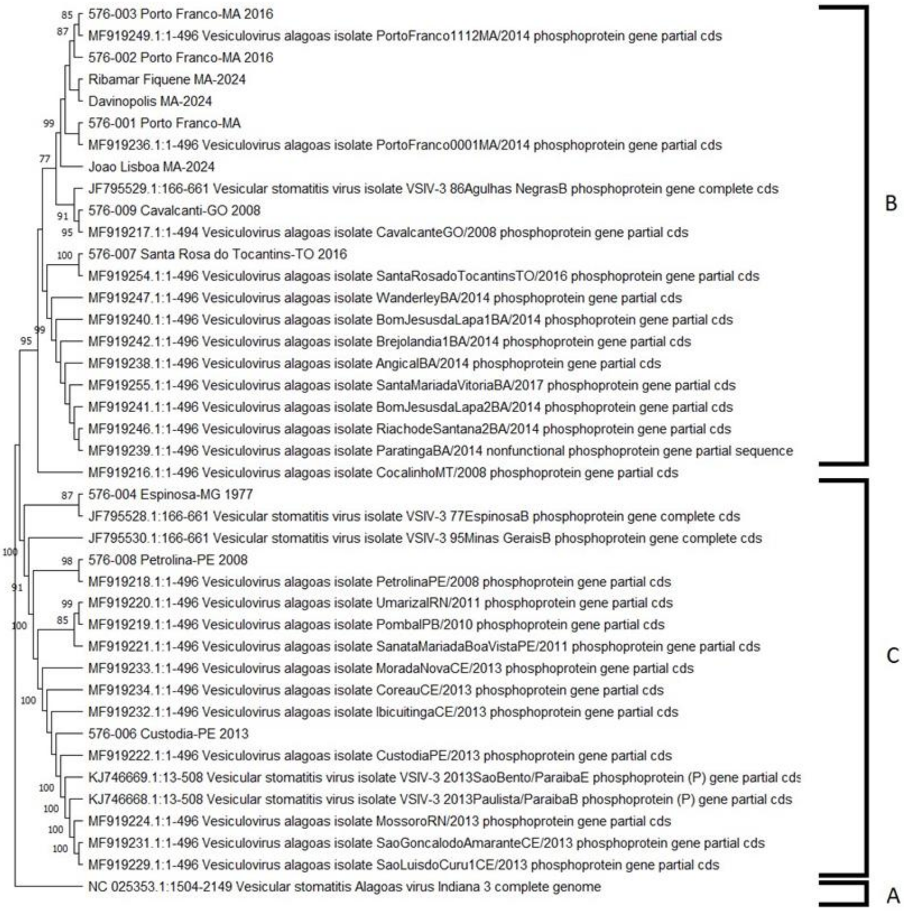


Figure 5. Maximum likelihood phylogenetic analysis of VSAV virus based on the partial sequence of the phosphoprotein gene from samples collected during the VSAV outbreaks occurred Ribamar Fiquene, Davinópolis and João Lisboa municipalities. The genetic relatedness among viruses in lineages from the VSAV outbreaks since 2013; 2014, 2016 to 2024 have been analyzed.

IV. Discussion

The controlling of vesicular diseases is essential as livestock farming seeks to improve its quality and competitiveness in the national and international market. Emphasizing that MA livestock production has been consolidating since 2021 the cattle herd grew by 2.9%, becoming the 11th largest in the country and the 2nd largest in the Northeast in 2021, with 8.6 million head. In 2022, this number reached 9.428,128 bovine, 984.666 swine and 257.423 equine[13]. Furthermore, MA's livestock heritage was strengthened by obtaining the qualification of a herd free of foot-and-mouth disease without vaccination with international recognition from the World Organization for Animal Health (WOAH). However, this increase in livestock production has been generating growing concern due to its direct relationship with the increase in deforestation and fires. It is well known that the burning of native vegetation for pasture production has contributed to worsening climate conditions. In 2023, the MA state had the most deforestation of the Cerrado biome in the country, putting pressure on indigenous lands[14]. Concern environmental situation is the occurrence of VSAV in domestic herds farms at least cattle and horses, from Cururupu, Santa Helena, Santa Rita and Turilândia municipalities within the Amazon biome of MA State (Table 2). The livestock introduction probably comes along with other human interference such as deforestation and fire that may aggravate the effects of the El Niño phenomenon and worsen the perception of global warming. Moreover, at the end of 2023 the El Niño phenomenon began to weaken but its effects can last for several months. However, some of the main typical summer weather systems, such as the Intertropical Convergence Zone (ITCZ), will contribute to the occurrence of heavy rain, accompanied by gusts of wind in the Northeast, as has occurred in the summer season of 2024 in MA state which increased the rain accumulations above the historical average[15]. Particularly, in MA, the average annual temperature had been registered above 26ºC and annual rainfall had varied from 700 mm on average in the central region to more than 2200 mm in the northern region[15].

The MA state territory is mostly constituted by the Cerrado (64%) and the Amazon (35%) biomes It is worth mentioning, both biomes are composed of many water resources such as rivers sustaining a high degree of "humidity", forests, and woods with temperature considered elevated[9]. A major implication is that it makes up a suitable scenario which favors the increasing of several insect populations[16]. Consequently, the risk of arbovirus sprawling, as one could suggest from 2014 to 2023, since the Porto Franco and Balsas municipalities (Table 1) are under the same environmental condition in MA State[6,17].

However, in the following years from 2018 to 2023, no outbreak has been detected in that region suggesting that the decreasing of VSAV outbreaks could be associated both with the presence of neutralizing antibodies and the change of season which acts as a reducing agent to arthropod population[19,20]. It is significant to add that in the Caatinga, the third and smallest biome of MA State, which occupies 1% of the landscape mosaic in MA, considered the driest biome, no VSV outbreaks were identified.

Unfortunately, the VSAV outbreaks have still occurred. After seven years, in 2024 from January to May 12 VSAV outbreaks distributed in ten municipalities were confirmed in the Cerrado and Amazon biomes from the MA State, representing an increase of 20% of municipalities affected in relation of between 2016 and 2017, mentioned above. It suggests an increase in the virus spread. However, in these regions despite the oscillatory climate in the beginning summer season, February, presented significant episodes of rainfall throughout the state, and March was the rainiest month of the year. Thus, it lasted until May, and was seen as a quarterly precipitation anomaly in Brazil[21]. Simultaneously, the VSAV outbreaks notifications began in January 2024. Conjointly with laboratory analysis, the VSV has been scrutinized thoroughly.

The Ribamar Fiquene municipality, in Jan 26, 2024 (rainy season, with a large infestation of hematophagous insects), one VSAV outbreak in cattle herds occurred. Right away, on Jan 31, 2024, in Davinópolis, equines from a "vaquejada" race were identified. Similarly, the third notification of the VSAV outbreak that occurred on February 7, 2024, has taken place again among the equine runner's crawl together from the "vaquejada" race in the municipality of João Lisboa. Since cattle and horses interact closely during "vaquejada", this race may favor virus dissemination among the animals. As one might expect, the VSAV in bovine herds was also detected. It was realized that the João Lisboa city is in a transitory biome between Amazon and Cerrado. Suggesting a migration from the south to the northwest of the state, that is, from the Cerrado to the Amazon biome (Figure 1). As can be seen in table 2, the VSAV outbreaks has affected equine herds farms from the transitory biome municipalities at the end of February, as Senador La Rocque, and Buritirana municipalities. In March 2024, a VSV outbreak in cattle in the municipality of São Raimundo das Mangabeiras. In the MA State's Amazon biome, the VSAV outbreaks have affected cattle and equine herds. As seen in Table 2, one VSAV outbreak has occurred in Santa Helena municipality, representative of the micro-region of maranhense lowlands (flooded region). Then, four VSAV outbreaks were diagnosed in cattle herds in Turilandia, and three in Cururupu municipalities. In Santa Rita, specifically, in the transition area between the Cerrado and the Amazon since its territory is composed of 95% of the Amazon and 5% of the Cerrado biomes, one (01) VSAV outbreak in equine herds (Table 2 and Figure 1). The serologic studies from the VSAV outbreaks that have taken place in Brazil have demonstrated that this virus is endemic in several Brazilian regions with high frequency in the north, northeast, southeast and central-west regions[22].

To deepen understanding of the VSAV occurrence in the MA state, the partial Phosphoprotein gene phylogenetic analysis of the virus samples from cattle or horses during the VSAV outbreaks occurred in 2013; 2016 and 2024 have been indicated that VSAV remained predominant throughout the decade in MA state, this data agrees of the VSAV occurrence thoroughly Northeast regions[23]. In spite of the VSAV genome have shown coherence among the virus samples collected from MA, the observed divergence from the virus sampling from other country regions, suggesting the climatic conditions, biomes and the geolocation may infer in the VSAV genetic profile and generate a distinguish VSAV local environmental endemic in the MA State's Cerrado and Amazon biomes[5;7]. The phylogenetic relationship similarity among VSAV samples from the outbreaks of 2013, 2016 and 2024 has been allowed to form a distinguished closed clade (Figure 5). The Ribamar Fiquene, Davinópolis and João Lisboa VSAV outbreaks occurred in the summer season, in January and February 2024. Although, in the Ribamar Fiquene and Davinópolis, samples have been collected from distinct species bovine and equine, respectively, they have been placed on the same branch on the top of the tree. While João Lisboa where the VSAV outbreaks affected bovine and equines, samples have been located a little below, on the other branch of the tree. The João Lisboa municipality is in the transition region of the biomes. Since, all the samples were displayed on the top of the phylogenetic tree close to the Porto Franco 2013/14 and 2016 branches we can infer that the VSAV lineages isolated in 2024 belong to the group B[5]. The virus lineages that circulating during these last ten years might accruing little mutations associated with several ecological factors[8].

Therefore, the impact of mutations in the VSAV evolution could be affected by effective population size, initial genetic variability in populations, and environmental heterogeneity shape. As already known, RNA genome virus as the Rhabdovirus has the RNA-dependent polymerase of RNA with low fidelity affecting the virus replication because lacking the proofreading machinery to correct misincorporated nucleotides is responsible for high mutation rates[7]. However, it is becoming evident that the ecological diversity displayed by

Rhabdovirus is also apparent in the complexity of their genome organization and the proteins encoded[24]. However, more studies must be done to understand the consequence of the mutations for VSAV evolution.

The mechanisms of the geographic spread during endemic and epizootic cycles variables may involve different factors including a broad range of vertebrate hosts, including several native animal specimens, multiple routes of transmission, and an extensive diversity of suspected arthropods species acting as both mechanical and biological vector, as well as environmental factors, including temperature and seasonality[8]. In addition, the virus may disseminate among the animals in the herd by the close contact with wearing infected skin lesions and/or fomites and winds[1].

The VSAV control measures internationally recommended is based in the righteous sanitation and quarantine practices on affected farms since there is no specific treatment or cure for vesicular stomatitis. It means the rapid actions to investigate in the local area where the infection is running; move apart the animals with lesions from healthy animals until at least 14 days after the onset of lesions and, also to collect samples and send to laboratory diagnosis confirmation. The control of native arthropods is a knotty challenge since they belong to the local environment. In turn, the elimination or reduction of insect breeding areas, including the manure management measures also, insect prevention on animals and around facilities are recommended. Last, but also important, avoid human exposure to this disease.

V. Conclusion

As expected, the VSAV outbreaks either in bovine or equine herds have occurred in summer during the rainy season and ceased in the dry season.

The VSAV outbreaks had run from the northwest region towards the north region or from the Cerrado biome region to Amazon biome within MA State.

The existence of the VSAV cladistic grouping in both biomes may suggest that an evolutionary path of this virus is associated with that environment and all susceptible fauna or vectors living there.

New studies should be accomplished to evaluate the continuous occurrence of this clade in similar environmental conditions as well as the emergence of new ones.

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