

# Identification of animal hosts of Fort Sherman virus, a New World zoonotic orthobunyavirus

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## Abstract

An orthobunyavirus termed Fort Sherman virus (FSV) was isolated in 1985 from a febrile US soldier in Panama, yet potential animal reservoirs remained unknown. We investigated sera from 192 clinically healthy peri-domestic animals sampled in northeastern Brazil during 2014–2018 by broadly reactive RT-PCR for orthobunyavirus RNA, including 50 cattle, 57 sheep, 35 goats and 50 horses. One horse sampled in 2018 was positive (0.5%; 95% CI, 0.01–3.2) at  $6.2 \times 10^3$  viral RNA copies/mL. Genomic comparisons following virus isolation in Vero cells and deep sequencing revealed high identity of translated amino acid sequences between the new orthobunyavirus and the Panamanian FSV prototype (genes: L, 98.8%; M, 83.5%; S, 100%), suggesting these viruses are conspecific. Database comparisons revealed even higher genomic identity between the Brazilian FSV and taxonomically unassigned Argentinian mosquito- and horse-derived viruses sampled in 1965, 1982 and 2013 with only 1.1% maximum translated amino acid distances across viral genes, suggesting the Argentinian viruses were also distinct FSV strains. The Panamanian FSV strain was an M gene reassortant relative to all Southern American FSV strains, clustering phylogenetically with Cache Valley virus (CVV). Mean dN/dS ratios among FSV genes ranged from 0.03 to 0.07, compatible with strong purifying selection. FSV-specific neutralizing antibodies occurred at relatively high end-point titres in the range of 1:300 in 22.0% of horses (11 out of 50 animals), 8.0% of cattle (4/50 animals), 7.0% of sheep (4/57 animals) and 2.9% of goats (1/35 animals). High specificity of serologic testing was suggested by significantly higher overall FSV-specific compared to CVV- and Bunyamwera virus-specific end-point titres ( $p = .009$ ), corroborating a broad vertebrate host range within peri-domestic animals. Growth kinetics using mosquito-, midge- and sandfly-derived cell lines suggested *Aedes* mosquitoes as potential vectors. Our findings highlight the occurrence of FSV across a geographic range exceeding 7,000 km, surprising genomic conservation across a time span exceeding 50 years, M gene-based reassortment events, and the existence of multiple animal hosts of FSV.

## KEYWORDS

arbovirus, livestock, orthobunyavirus, PCR, reservoirs, serology, zoonosis

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## 1 | INTRODUCTION

Orthobunyaviruses are arthropod-borne viruses associated with zoonotic transmission and animal disease worldwide. Known vectors include mosquitoes, midges, bugs and ticks (Elliott, 2014). Currently, 88 species, gathered in at least 18 serogroups (Elliott, 2014), are assigned to the *Orthobunyavirus* genus in the *Peribunyaviridae* family (Abudurexiti et al., 2019). Human infection is generally asymptomatic but can cause febrile disease and severe acute neurological disease, exemplified by viruses from the Bunyamwera serogroup such as Maguari virus (MAGV) and Cachey Valley virus (CVV) (Calisher & Sever, 1995; Campbell et al., 2006; Wilson et al., 2017). Besides humans, data from serological studies point to a wide vertebrate host range of orthobunyaviruses including horses, cattle, goat, sheep, bison, caribou, grizzly bear, moose and deer (Calisher et al., 1986).

Fort Sherman virus (FSV) was isolated in 1985 from a US soldier with acute febrile disease based in Panama. The virus was antigenically related to MAGV, CVV, and the mosquito-associated Tensaw virus (TENV) and assigned to the Bunyamwera serogroup (Mangiafico, Sanchez, Figueiredo, LeDuc, & Peters, 1988). FSV viruses were previously isolated in Argentina from *Culicidae* spp. mosquitoes in the 60s (strain CbaAr426) and 80s (strain AG83-1746) and later from horses sampled in 2013 (strains SFCrEq231 SFCrEq232 and SFCrEq238), yet they were back then classified as 'Cordoba' (Groseth et al., 2017) and 'Argentina' Bunyamwera lineages (Tauro et al., 2015, 2019). The most recent ICTV taxonomy update lists FSV as a distinct *Orthobunyavirus* species (Abudurexiti et al., 2019). The animal host range or potential invertebrate vectors associated with FSV are largely unknown.

## 2 | MATERIAL AND METHODS

A total of 192 sera were collected from peri-domestic animals including 50 horses, 50 cattle, 57 sheep and 35 goats during 2014–2018 in northeastern Brazil (Table 1). Animals were sampled during veterinary surveillance activities in different farms that were 200–600 km distant from each other. Animals were handled following procedures approved by the animal ethics committee of the Federal University of Bahia with the authorization no. 55/2017.

Viral RNA was extracted using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Life Sciences). Samples were

screened for orthobunyavirus RNA using a broadly reactive and highly sensitive RT-PCR assay targeting the viral L gene (Lambert & Lanciotti, 2009). Viral RNA from serum and cell supernatant was quantified using a strain-specific real-time RT-PCR with the following primers FSHV-rtF (5'-TGTTGGTGATTGTGCATATATTGG), FSHV-rtR (5'-GGCGGACAACCATGTTAATACT) and the probe FSHV-rtP (5'-ATCTAGCCAGTAGGTTATCTGCCACGCAGC), labelled with fluorescein amidite (FAM) at the 5'-end and a dark quencher at the 3'-end. The assay was controlled by photometrically quantified in vitro-transcribed RNA controls generated from synthesized DNA fragments (IDT) containing a T7-RNA polymerase promoter region as described previously (Drexler et al., 2009). Thermocycling involved reverse transcription at 55°C for 20 min followed by 94°C for 3 min and then 45 cycles of 94°C for 15 s and 58°C for 30 s. RT-PCR was done using the OneStep SuperScript III RT-PCR kit (Thermo Fisher) with 5 µl RNA input and reaction components according to the manufacturer's instructions.

Virus was isolated from the PCR-positive horse serum diluted at 1:10 and 1:100 and inoculated onto Vero E6 cells maintained at 37°C and cultivated in DMEM. After 1 hr of incubation, the inoculum was removed and replaced by medium supplemented with 5% foetal calf serum (FCS), 1% penicillin/Streptomycin (100 U/ml) (PS, Thermo Fisher) and 1% non-essential amino acids. Infected cells were passaged three times every 7 days and controlled daily for cytopathic effect (CPE) and increases in FSV RNA via real-time RT-PCR. FSV stocks were produced by propagation in Vero E6 cells as described above. Cells and supernatant were harvested 3 days post-infection, centrifuged at 2000 x g for 10 min and titrated via plaque assay in Vero E6 cells.

For FSV growth kinetics, we compared cell lines from primate (Vero: *Cercopithecus aethiops*), mosquito (C6/36: *Aedes albopictus* and HSU: *Culex quinquefasciatus*), biting midges (KC: *Culicoides sonorensis*) and sandfly (LL5: *Lutzomyia longipalpis*) hosts. Vero cells were cultivated under conditions described above, while invertebrate cell lines were maintained at 28°C. C6/36 cells were cultivated in Leibovitz's L15 medium supplemented with 5% FCS; HSU cells were cultivated in L15 supplemented with 10% FCS and 10% tryptose phosphate (29.5 g/L). LL-5 and KC cells were cultivated in Schneider's medium supplemented with 10% FCS, 1% L-Glutamine (200 mM), 1% sodium pyruvate (100 mM) and 1% NEAA. Cells were infected at a multiplicity of infection (MOI) of 0.1, and supernatant was harvested and analysed at 0, 12, 24, 36, 48, 72, 96, 120, 144 and 168 hr post-infection (hpi).

**TABLE 1** Sampling table

Species	N	Sampling year	PCR-positive (%; 95% CI)	Seroprevalence (%) (95% CI)	Median end-point titres (interquartile range)
Horse	50	2018	1 (2.0; 0.01–11.5)	22.0 (12.6–35.4)	262.6 (95.2–422.1)
Cattle	50	2014–2016	0	8.0 (2.6–19.3)	499.7 (232.7–594.3)
Sheep	57	2016–2017	0	7.0 (2.3–17.2)	475.2 (184.1–690.8)
Goat	35	2017	0	2.9 (0.1–15.8)	340.0
Total	192	2014–2018	1 (0.5; 0.01–3.2)	10.4 (6.8–15.6)	342.7 (140.7–584.1)

Neutralizing antibodies against FSV, CVV and Bunyamwera virus (BUNV) were detected using a plaque reduction neutralization test (PRNT<sub>50</sub>) conducted in monolayers of  $8 \times 10^4$  Vero E6 cells, seeded in 24-well plates one day before the infection. Thirty plaque-forming units were incubated with serum dilutions for one hour, added onto the cell monolayer, incubated for another hour before adding an overlayer containing DMEM with 2% FCS and 0.75% carboxymethyl cellulose. After three days for BUNV and four days for FSV and CVV, the medium was removed, and cells were fixed with 6% paraformaldehyde and stained with crystal violet. For initial FSV screening, a cut-off of  $\geq 50\%$  PFU reduction at a serum dilution of 1:100 was selected. End-point titration of the positive sera was done by testing 1:50, 1:100, 1:200, 1:400, 1:800 and 1:1,600 serum dilutions. Beyond the Brazilian FSV, viruses used for PRNT were the well-characterized CVV strain 6V633 (Dunlop et al., 2018) and a BUNV prototype strain (GenBank accession number: X14383). Titres were calculated using a logistic regression function in Graphpad prism 6 (GraphPad software, www.graphpad.com).

Deep sequencing was done using MiSeq reagent v2 chemistry (Illumina), the KAPA Frag Kit and a KAPA RNA HyperPrep library (Roche Life Sciences). All MiSeq-derived sequences were confirmed by sets of strain-specific PCR assays and Sanger sequencing. Sequence assembly and MAFFT alignments were done in Geneious 9.1.8 (www.geneious.com).

Maximum likelihood (ML) phylogenies were done on translated genes using MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018) with a WAG amino acid substitution model, 1,000 bootstrap replicates, and complete deletion of all alignment positions containing gaps. Viral reassortment was assessed using the Recombination Detection Package V4 (Martin et al., 2010) and GARD (Kosakovsky Pond, Posada, Gravenor, Woelk, & Frost, 2006) using an alignment containing concatenated orthobunyavirus segments. Selection pressure analyses were performed for each FSV gene using fixed effects likelihood (FEL) and single-likelihood ancestor counting (SLAC) implemented in Datamonkey from the HYPHY package (Pond & Frost, 2005) and comparing the M1 versus M2 and M7 versus M8 models implemented in the CodeML program in the PAML package (Xu & Yang, 2013) as described previously (de Oliveira Filho et al., 2019). For selection pressure analyses, we included the FSV prototype strains (86MSP18) and the Argentinian strains found in mosquitoes in 1965 (CbaAr426) and 1982 (AG83-1746), and in horses in 2013 (SFCrEq231, SFBzEq232 and SFABCrEq238). The M gene of the reassortant FSV prototype strain was excluded in selection pressure analyses. For comparison, we used all CVV strains available (details on those viruses are provided in the figure legend). Sequences showing 100% identity were removed from the alignment. N-linked glycosylation sites were predicted with NetNGlyc 1.0 (<http://www.cbs.dtu.dk/services/NetNGlyc>) and putative transmembrane regions were predicted based on the hydropathy profile by the TMHMM 2.0 (<http://www.cbs.dtu.dk/services/TMHMM-2.0/>) as previously described (Tchouassi et al., 2019). GenBank accession numbers for the Brazilian FSV strain described here are MN379835 (L segment), MN379834 (M segment) and MN379833 (S segment).

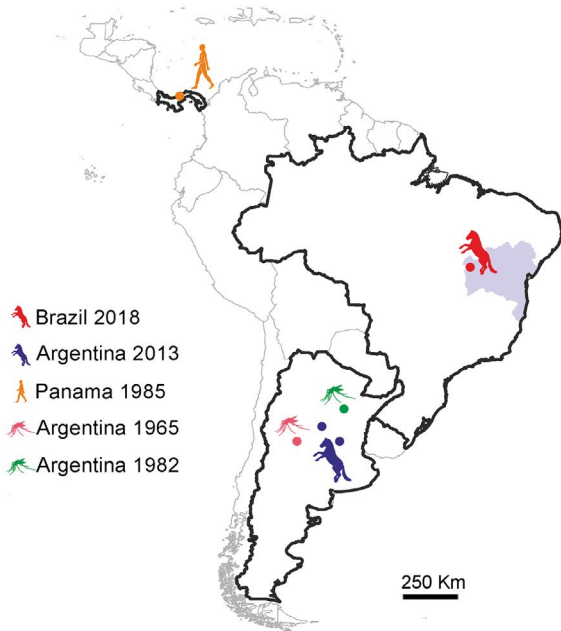
### 3 | RESULTS

The broadly reactive RT-PCR detected one viremic horse out of 192 peri-domestic animals (overall detection rate 0.5%; 95% confidence interval (CI), 0.01–3.2; sampling coordinates 12°08'54"S 44°59'33"W). In horses, the detection rate was 2.0% (95% CI, 0.01–11.5) (Table 1 and Figure 1a). The viral RNA concentration in that serum as determined by strain-specific real-time RT-PCR was low at  $6.2 \times 10^3$  RNA copies/ml. The virus was isolated from the positive serum in Vero E6 cells, showing cytopathic effects (Figure 1b) and reaching viral titres of  $4.6 \times 10^7$  PFU/ml after 7 days. Initial Sanger sequencing of the screening PCR amplicon revealed close genetic relatedness with FSV. The complete genomic sequence was obtained by deep sequencing from the cell culture supernatant.

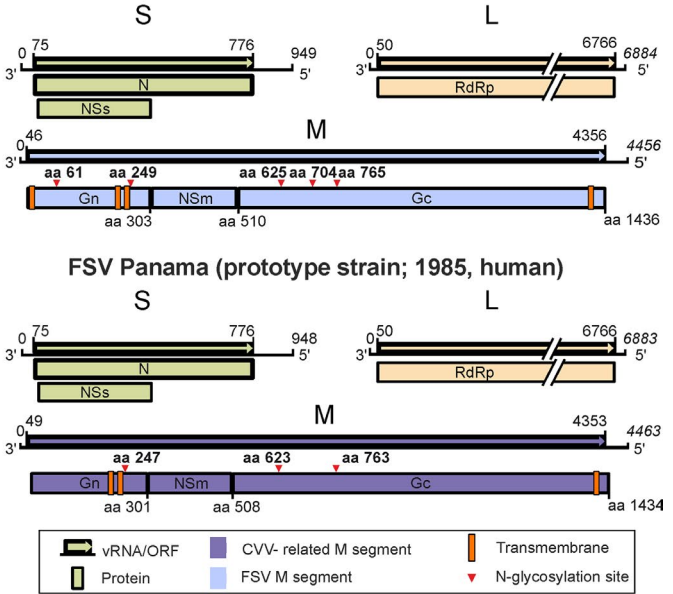
The virus exhibited the typical orthobunyavirus tripartite genome comprising an S segment with 949, the M with 4,456 and L with 6,884 nucleotides. L and S segments were similar in length with the FSV prototype strain and the Argentinian FSV strains, while the M segment showed differences in comparison with the FSV prototype regarding length, typical number and location of transmembrane and N-glycosylation domains (Figure 1c). The Brazilian FSV strain was closely related to the prototype FSV from Panama and both horse- and mosquito-derived viruses from Argentina. Translated amino acid sequence identity was very high in S (100.0%), L (98.8–99.7%) and M (99.5–98.9) segments between the Brazilian and the Argentinian FSV strains. Only the M gene of the FSV prototype strain from Panama showed lower translated amino acid sequence identity with the Brazilian strain at 83.5% (Table 2). Based on the recently updated ICTV *Orthobunyavirus* species list (Abudurexiti et al., 2019) and species demarcation criteria for the genus *Orthobunyavirus* defined as at least 96% genetic identity of the translated L segment coding region (Marklewitz et al., 2019; Reguera et al., 2016), our isolate and the Argentinian strains previously assigned to 'Cordoba' (Groseth et al., 2017) and 'Argentina' (Tauro et al., 2019) viruses are all conspecific FSV variants.

Phylogenetic analyses were performed based on translated amino acid sequences of all three genes and a data set comprising *Orthobunyavirus* ICTV reference sequences. Interestingly, ML trees displayed distinct topology in the different segments (Figure 1d). In the L and S segments, the Brazilian FSV strain clustered with the FSV prototype and the Argentinian FSV strains. In the M segment, the Brazilian FSV strain clustered with the Argentinian strains while the Panamanian FSV prototype strain clustered together with CVV, strongly suggesting reassortment events during the genealogy of the Panamanian prototype strain, as previously noted (Groseth et al., 2017; Tauro et al., 2019). Due to the remarkable genomic conservation of FSV strains across over 50 years in time and 7,000 km in space, we performed selection pressure analyses for each deduced FSV gene. Mean dN/dS ratios ranged from 0.03 to 0.07 (Figure 1e), with no site showing significant evidence for positive selection. Since the number of available FSV sequences was relatively small, we conducted the same analyses for a larger CVV data set, yielding highly similar results with mean dN/dS ratios

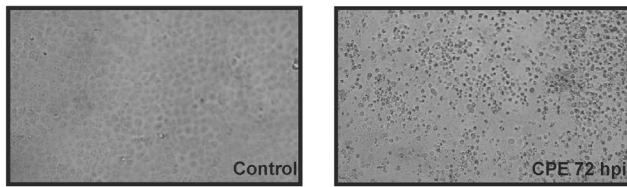
(a)



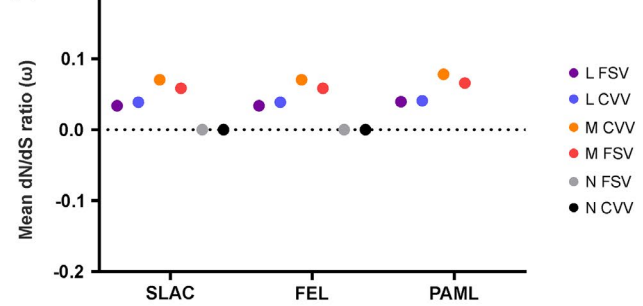
(c) FSV Brazil (this study; 2018, horse)



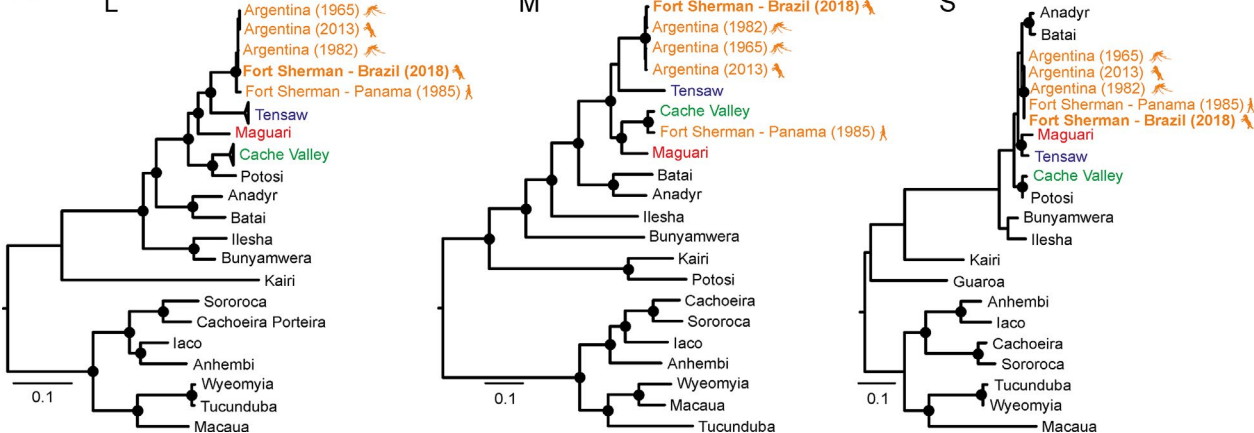
(b)



(e)



(d)



ranging from 0.04 to 0.08. This indicates that FSV and the closely related CVV are evolving under similar purifying selection, likely associated with needs to adapt to vertebrate and invertebrate hosts, as observed among other vector-borne RNA viruses (Woelk & Holmes, 2002).

Next, to assess the FSV host range, neutralizing antibodies were detected using PRNT<sub>50</sub>. Initial screening results revealed the presence of neutralizing antibodies against FSV in 22.0% of

horses (11 of 50 animals; 95% confidence interval, 12.5–35.4), in 8.0% of cattle (4 of 50 animals; 95% CI, 2.6–19.3), in 7.0% of sheep (4 of 57 animals; 95% CI, 2.3–17.2) and in 2.9% of goats (1 of 35 animals; 95% CI, 0.1–15.8) (Figure 2a, Table 1), without spatial clustering in regard to the geographic distribution of seropositive animals (Figure 2b). The seroprevalence in horses was significantly higher than in other livestock species (chi-square,  $p < .01$ ). However, end-point titres were comparably high in all



**FIGURE 1** Location, isolation and evolutionary characteristics of Fort Sherman virus from Brazil. Geographic distribution of FSV found in this study (red star), and in previous studies including the FSV prototype found in a soldier in Panama (orange circle), in mosquitos from Argentina previously assigned as Cordoba virus (purple circle) and the horse strains formerly classified as Argentina lineage in the Bunyamwera serogroup (red circle). Map built with Quantum GIS (<http://qgis.osgeo.org/wileyonlinelibrary.com>) (a). Visualization of cellular cytopathic effect and comparison between non-infected and FSV-infected Vero cells showing cytopathic effect after 72 hr (b). Comparison of the genome organization of FSV from our study with the FSV prototype strain from Panama. Sequence length of large (L), medium (M) and small (S) genomic segments and ORF positions in nucleotides (nt). RNA-dependent RNA polymerase (RdRp), glycoproteins (Gn and Gc), non-structural protein NSm, nucleocapsid (N) and non-structural protein NSs are indicated, vRNA: viral RNA (c). Maximum Likelihood phylogenetic trees based on deduced amino acid sequences of the L, M and S genes. Black circles at nodes represent support values of  $\geq 0.75$  from 1,000 bootstrap replicates (d). Mean dN/dS ( $\omega$ ) ratios on L, M and S genes of FSV and CVV using different methods indicated below. High nucleotide identity prevented selection pressure analyses in FSV and CVV S genes in PAML (e). Reference sequences: Anhembi virus (JN572062, JN572063, JN572064), Anadyr virus (KM496335, KM496336, KM496337), Batai virus (KC168048, KC168047, KC168046), Bunyamwera virus (NC\_001925, NC\_001926, NC\_001927), Cache Valley virus (KC436106, KC436107, KC436108), Cachoeira Porteira virus (JN968590, JN968591, JN968592), Fort Sherman virus (KX100132, KX100131, KX100130), Iaco virus (JN572065, JN572066, JN572067), Ilesha virus (KF234075, KF234074, KF234073), Kairi virus (KR260738, KR260739, KR260740), Maguari virus (KX100117, KX100116, KX100115), Macaua virus (JN572068, JN572069, JN572070), Potosi virus (MF066368, MF066369, MF066370), Sororoca virus (N572071, N572072, N572073), Tensaw virus (FJ943510, FJ943509, FJ943508), Tucunduba virus (KX579496, KX579497, KX579498), Wyeomyia virus (NC\_039186, NC\_039187, NC\_039188) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 2** Estimates of evolutionary divergence between Fort Sherman and closely related orthobunyaviruses

Gene	Distance between Brazilian FSV and related strains (%)*					Distance between Panamanian FSV and related strains (%)			
	FSV Panama 1985	FSV Argentina	TSV	MAGV	CVV	FSV Argentina	TSV	MAGV	CVV
L	1.2	0.3–0.5	9.3	9.5	11.6–12.0	1.0–1.1	9.3	9.8	12.1–12.2
M	16.5	0.7–1.1	16.5–17.0	14.9	15.7–16.4	16.2–16.5	19.6–20.1	13.9	2.5–3.3
S	0.0	0.0	3.4	4.3	3.9	0.0	3.4	4.3	3.9

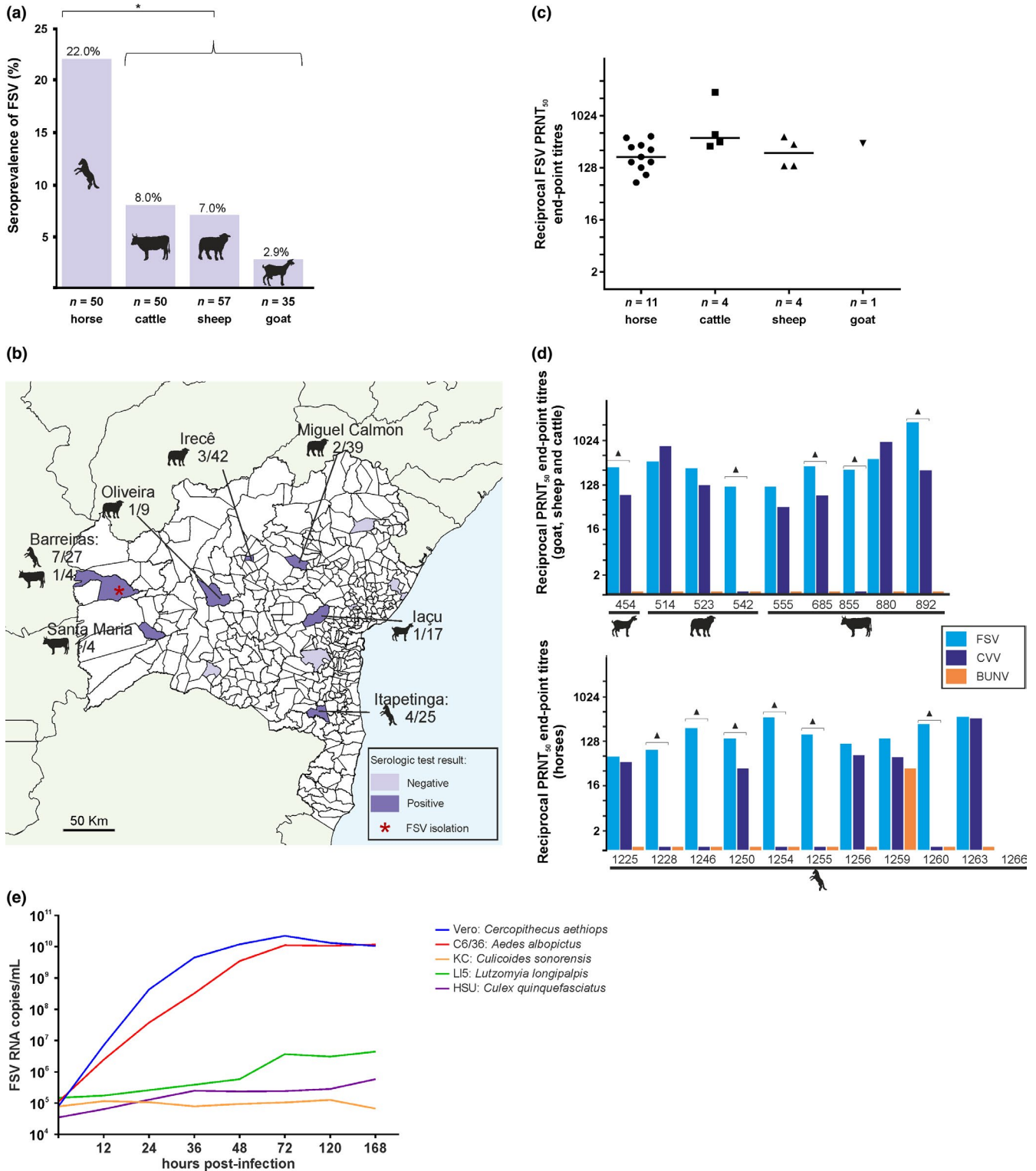
\*Amino acid pairwise sequence distance between sequences is shown (bold: smallest distance value). Viruses Abbreviations: Fort Sherman Virus (FSV), Tensaw virus (TSV), Maguari virus (MAGV) and Cache Valley virus (CVV). "FSV Argentina" viruses include two mosquito isolates from 1965 (CbaAr426) and 1982 (AG83-1746 and CbaAr426) previously classified as MAGV and the equine isolates (SFCrEq231, SFCrEq232 and SFCrEq238) from 2013 (previously classified as Bunyamwera virus strains).

seropositive animals at around 1:300 serum dilution (Kruskal–Wallis,  $p = .38$ ) (Figure 2c, Table 1). These data implied a wide vertebrate host range of FSV beyond horses, which is consistent with previous studies from Argentina that showed neutralizing antibodies against FSV in different vertebrates including humans, cattle and wild birds by PRNT<sub>50</sub> (Tauro, Almeida, & Contigiani, 2009; Tauro, Diaz, Almiron, & Contigiani, 2009; Tauro, Venezuela, Spinsanti, Konigheim, & Contigiani, 2012).

Next, we investigated the specificity of FSV neutralization by conducting PRNT<sub>50</sub> using CVV and BUNV in all sera neutralizing FSV. We used CVV because of its broad vertebrate host range and because the Panamanian FSV prototype strain was not available. Because CVV and the Panamanian FSV prototype strain are genetically closely related at 96.2%–97.3% translated amino acid identity in the M gene, M-directed antibody responses are likely indistinguishable between those two viruses. We used BUNV because that virus is genetically about equidistant from the Brazilian FSV at 65.1% and from CVV at 64.7% amino acid identity in the translated M gene. Of the 20 FSV-seropositive Brazilian animal sera, only one showed relatively weaker neutralization of the more divergent BUNV and 13 sera showed concomitant neutralization of CVV (Figure 2d). FSV end-point titres were significantly higher than CVV end-point titres in all but two of those 13 sera (Wilcoxon's signed-rank test,  $p = .009$ ). The median

difference between FSV- and CVV-specific end-point titres in those 11 sera yielding relatively higher FSV titres was 120, which exceeded CVV-specific end-point titres about fourfold or higher in all cases, irrespective of the host species (Figure 2d, Table 3). In sum, the serologic data confirmed a wide FSV animal host range and suggested that viruses closely related to the Panamanian FSV prototype strain or CVV might also be circulating in Brazil.

The two Argentinian mosquito-derived FSV strains were isolated in *Aedes albifasciatus* and *Psorophora varinervis* (Groseth et al., 2017), hinting at a broad invertebrate host range. To experimentally assess invertebrate host range, we infected different invertebrate lineages including mosquito-, sandfly-, and midge-derived cell lines with the Brazilian FSV. Beyond Vero, only the *Ae. albopictus*-derived cell line showed statistically significant ( $t$  test,  $p < .05$ ) increments of viral RNA by five orders of magnitude, and reaching titres similar to those obtained in Vero cells (Figure 2e). This suggested a narrow invertebrate host range with *Aedes* mosquitoes as main vectors. *Psorophora* sp. cell lines exist (Bello et al., 2001), but were unfortunately not available for our study. Formal vector competence and field studies will be required to confirm the FSV invertebrate host range, ideally including different Southern American *Aedes* species with differential host preferences such as the anthropophilic *Ae. aegypti*, but also other species including *Ae. albopictus*, *Ae. fluviatilis*, *Ae. scapularis* and *Ae. albifasciatus*.



**FIGURE 2** Seroprevalence, end-point titration and insect in vitro permissiveness of Fort Sherman virus. FSV seroprevalence in different animal species obtained by 50% plaque reduction at a serum dilution of 1:100. \*, statistical significance (chi-square,  $p < .01$ ) (a). Geographic distribution of the seropositive samples (b). Reciprocal PRNT<sub>50</sub> FSV-specific end-point titres. Line, median (c). Serological differentiation based on end-point titration of FSV, Cache Valley virus (CVV), Bunyamwera virus (BUNV). The symbol ▲ indicates end-point titre differences between FSV and CVV of about fourfold or higher (rounded) (d). In vitro growth kinetics of FSV in different invertebrate cell lines (e) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 3** Comparative neutralization test results of Fort Sherman virus and related orthobunyaviruses

Sample ID	PRNT <sub>50</sub> end-point titres			Species	PRNT <sub>50</sub> interpretation
	FSV	CVV	BUNV		
454	1:340	1:96	0	Goat	FSV
514	1:438	1:894	0	Sheep	Undetermined
523	1:322	1:152	0	Sheep	Undetermined
542	1:138	0	0	Sheep	FSV
555	1:137	1:56	0	Sheep	Undetermined
685	1:358	1:94	0	Cattle	FSV
855	1:302	0	0	Cattle	FSV
880	1:485	1:1,090	0	Cattle	Undetermined
892	1:2,598	1:293	0	Cattle	FSV
1,225	1:71	1:56	0	Horse	Undetermined
1,228	1:96	0	0	Horse	FSV
1,246	1:263	0	0	Horse	FSV
1,250	1:161	1:41	0	Horse	FSV
1,254	1:422	0	0	Horse	FSV
1,255	1:197	0	0	Horse	FSV
1,256	1:129	1:74	0	Horse	Undetermined
1,259	1:160	1:71	1:41	Horse	Undetermined
1,260	1:314	0	0	Horse	FSV
1,263	1:449	1:410	0	Horse	Undetermined
1,266	1:290	1:123	0	Horse	Undetermined

Abbreviations: BUNV, Bunyamwera virus; CVV, Cache valley virus; FSV, Fort Sherman virus.

## 4 | DISCUSSION

The inconsistencies observed in the assignment of FSV strains from Argentina are probably related to changes in the ICTV *Orthobunyavirus* species definition criteria. Until 2018, related viruses such as FSV, CVV and MAGV were considered different strains from a single species termed *Bunyamwera orthobunyavirus* (Maes et al., 2019). On another hand, the fact that the prototype strain is a reassortant virus relative to other FSV strains may also have led to confusion. According to the revised taxonomy, all of these strains belong to the recently recognized species *Fort Sherman orthobunyavirus* (Abudurexiti et al., 2019).

Our results show that FSV is circulating over a wide geographic range among South American horses. Interestingly, the viremic horse reported here was clinically healthy, which is different from the fatal neurologic disease reported in Argentina (Tauro et al., 2015). Different courses of FSV infection are consistent with CVV that has been isolated from both symptomatic and asymptomatic horses (Calisher et al., 1986). While our serological data suggest a broad vertebrate host range of FSV beyond horses, antibodies elicited by antigenically related Bunyamwera serogroup viruses may cross-react among each other (Hunt & Calisher, 1979; Johnson et al., 2014), and to date, no isolation or molecular detection of FSV has been reported for vertebrates other than horses. Therefore, our serological data should be interpreted with caution until further proof by direct FSV detection.

The reassortant FSV prototype strain originated from Panama and consists of FSV-related L and S segments with a CVV-related M segment. Because CVV circulates in Northern America (Calisher et al., 1986; Waddell et al., 2019), and our data show wide circulation of FSV in Southern America, it seems feasible that a reassortant virus would emerge in Central America, where the two subcontinents are connected. Since orthobunyaviruses show frequent reassortment events (Briese, Calisher, & Higgs, 2013), it will be interesting to see whether this potential geography-driven reassortment pattern can also be observed in other American orthobunyaviruses in future studies.

The orthobunyavirus M-derived glycoproteins are responsible for attachment and cellular entry and therefore major components of the viral host range (Elliott, 2014). Zoonotic potential is clearest for the reassortant FSV strain Panama, whereas it remains unknown whether the divergent Southern American FSV strains can infect humans. However, Southern American non-reassortant FSV seroprevalence in asymptomatic humans from the city of Cordoba in Argentina was 5.7% (27/472) (Tauro, Almeida, et al., 2009) and FSV seroconversion was reported in a single patient, presenting rash, fever, myalgia, arthralgia and retroocular pain that tested negative for other arboviruses such as Dengue virus, West Nile virus and Saint Louis Encephalitis virus (Tauro et al., 2012). Altogether, it seems likely that both the reassortant and the non-reassortant FSV strains should be included in

differential diagnostics of both humans and other animals with compatible symptoms.

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## ETHICAL STATEMENT

Hereby, the authors confirm that the ethical policies of the journal, as described on the author's guidelines page, were followed. All animals were sampled by trained veterinarians during veterinary surveillance activities and handled according to the procedures approved by the animal ethical committee of the Federal University of Bahia with the authorization no. 55/2017.

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