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BRIEF REPORT

Arbovirus serosurvey (*Orthobunyavirus*, *Flavivirus*, and *Alphavirus*) in a draft horse population from Santa Fe, Argentina (2013-2016)

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Abstract

In the present study, we serosurveyed the exposure of 222 draft horses to different arboviruses in the city of Santa Fe, Argentina. Plaque reduction neutralization tests confirmed exposure to Fort Sherman virus (FSV), Saint Louis encephalitis virus (SLEV), West Nile virus (WNV), and Río Negro virus (RNV). Apparently, Western and Eastern equine encephalitis viruses did not circulate in the population tested. The confirmation of five seroconversions for WNV, FSV, and SLEV and the association between prevalence and age are indicative of recent circulation. These results highlight the importance of considering draft horses in arboviral surveillance in urban and rural areas of developing countries.

Many arthropod-borne viruses (arboviruses) can cause viral encephalitis in vertebrates. Within the genus *Flavivirus* (family *Flaviviridae*), Saint Louis encephalitis virus (SLEV) re-emerged as a human pathogen in Argentina in 2002 and caused the first epidemic in 2005, and West Nile virus (WNV) was first isolated in Argentina from horses with encephalitis in 2006, although human deaths were not reported [1, 2]. The New World alphaviruses (family *Togaviridae*) western equine encephalitis virus (WEEV), eastern equine encephalitis virus (EEEV), and the Venezuelan equine encephalitis virus (VEEV) complex can cause febrile syndromes and sometimes fatal encephalitis in equines and humans. In Argentina, the circulation of VEEV has been confirmed in the enzootic part of the transmission network, but human and equine exposure has also been reported [3]. No activity has been reported for WEEV or EEEV since the mid-1980s. However, enzootic circulation of Madariaga virus (MADV, South American EEEV) occurs [4]. Viruses belonging to the genus *Orthobunyavirus* (family *Peribunyaviridae*) have expanded their geographical range from North America to South America. In Argentina, new strains have been isolated recently from cases of encephalitis and abortion in horses [5]. Originally classified as Cache Valley virus, they are now considered different variants of Fort Sherman virus (FSV) [6].

Equines are a valuable target for detecting the circulation of arboviruses because mosquitoes exploit them for blood meals, they share rural and urban transmission scenarios with humans, they develop robust and durable immune responses to infections, and they are susceptible to disease [7]. Draft horses in particular, are widely used by informal waste pickers in urban settlements in South America, they are not included in vaccination programs, and they are often malnourished and used for hard labor. Therefore, to identify zoonotic arboviruses circulating in the city of Santa Fe, we conducted a serosurvey on draft horses that move across the city during the day and spend the night in a well-vegetated suburban area.

We examined the arbovirus exposure pattern based on 222 blood samples collected in a three-year cross-sectional

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opportunistic survey (2013 and 2014, 73 samples; 2015, 76 samples). In addition, current transmission was surveyed with 47 paired samples (2 to 6 repeated samples) and seroconversion events were recorded. We analyzed the presence of neutralizing antibodies by plaque reduction neutralization tests ($PRNT_{80}$) in Vero cell monolayers, using SLEV 78V-6507 [8], WNV E/7229/06 [2], FSV SFCrEq231 [5], Río Negro virus AG80-663 (RNV, VEEV subtype VI), WEEV 646, and EEEV Cba 55 strains [9]. Samples that neutralized at least the 80% of the inoculated plaque forming units (PFU) were considered positive, and they were titrated using twofold serial dilutions. A generalized linear model with a binomial distribution family was fitted to explore the contribution of sampling year (levels: I, 2013-2014; II, 2014-2015; III, 2015-2016) and "age class" (A, 0-5 years old [y.o.]; B, 6-10 y.o.; C, 11-15 y.o.; D, >15 y.o.) in the observed seroprevalence. *A posteriori* contrasts were performed to check the significance ($\alpha = 0.05$) of the different levels of age and year. In addition, the association between titers of neutralizing antibodies and aging was explored for each virus by ANOVA by determining the covariation between the inverse of the antibody titer (log10 transformed) and the age class. Statistical analysis was performed using the computing environment R [10].

The prevalence and the titer range of neutralizing antibodies for viruses assayed were as follows: 76.8% FSV (152/198, 10– >1280), 60% SLEV (112/188, 10–320), 27% WNV (56/203, 10–640), 7.4% RNV (16/216, 40– >1280), 0% WEEV (0/195), and 0% EEEV (0/196). The distribution of seroprevalence rates for individual viruses, as well as the annual rates and data from paired specimens are summarized in Table 1.

Twelve seroconversions were detected during the study period: five for FSV, five for WNV, and two for SLEV (Table 2). Moreover, six seroconversions occurred in animals younger than 5 years old (4/5 for FSV, 1/5 for WNV, and 1/2 for SLEV). The recent exposure of horses indicates endemic circulation of SLEV, WNV and FSV.

Table 2 Arbovirus seroconversion events detected in a draft horse population from Santa Fe, Argentina

	ID	Year of seroconversion	Titer change of neutralizing antibodies
FSV	37	2015	<10 - 10
	114	2015	<10 - 320
	172	2016	<10 - 320
	179	2015	<10 - 320
	226	2016	<10 - 320
SLEV	32	2014	<10 - 80
	172	2016	<10 - 10
	2	2015	<10 - 10
	39	2014	<10 - 320
WNV	52	2014	<10 - 40
	98	2015	<10 - 160
	109	2015	<10 - 20

FSV, Fort Sherman virus; SLEV, Saint Louis encephalitis virus; WNV, West Nile virus

Our study revealed a remarkably high prevalence of FSV, and it provides the first data about the exposure level of horses in Argentina. Importantly, FSV has been isolated from encephalitis and abortion cases in horses. Consequently, the biological and epidemiological characteristics of FSV should be investigated further to ascertain the potential health impact in horse and human populations. The seroprevalence of flaviviruses was notably higher than previously observed in farm horses from Santa Fe province (SLEV, 12.2%; WNV, 16.2%) [11]. This could be due to environmental factors that might modulate the composition of mosquito vector communities and host exposure.

Table 1 Annual infection rates and overall seroprevalence of arboviruses in an equine population from Santa Fe, Argentina

		FSV	SLEV	WNV	RNV
Positive/total by period	2013-2014 (I)	52/74	33/59	15/70	5/84
	2014-2015 (II)	52/61	43/64	20/66	7/99
	2015-2016 (III)	48/63	36/65	21/67	4/33
Total Prevalence [CI95%][a] (Positive/total)		76.8% [71.51%-83.06%] (152/198)	59.6% [53.36%-67.32%] (112/188)	27.5% [24.21%-37.02%] (56/203)	7.4% [4.29%-11.75%] (16/216)
Titer range		10 – >1280	10 – 320	10 – 640	40 – >1280
Total paired samples (total individuals)		91 (38)	85 (37)	100 (41)	110 (42)
Seroconversion events (Titer range)		5	2	5	0

FSV, Fort Sherman virus; SLEV, Saint Louis encephalitis virus; WNV, West Nile virus; RNV: Río Negro virus

[a]95% confidence interval

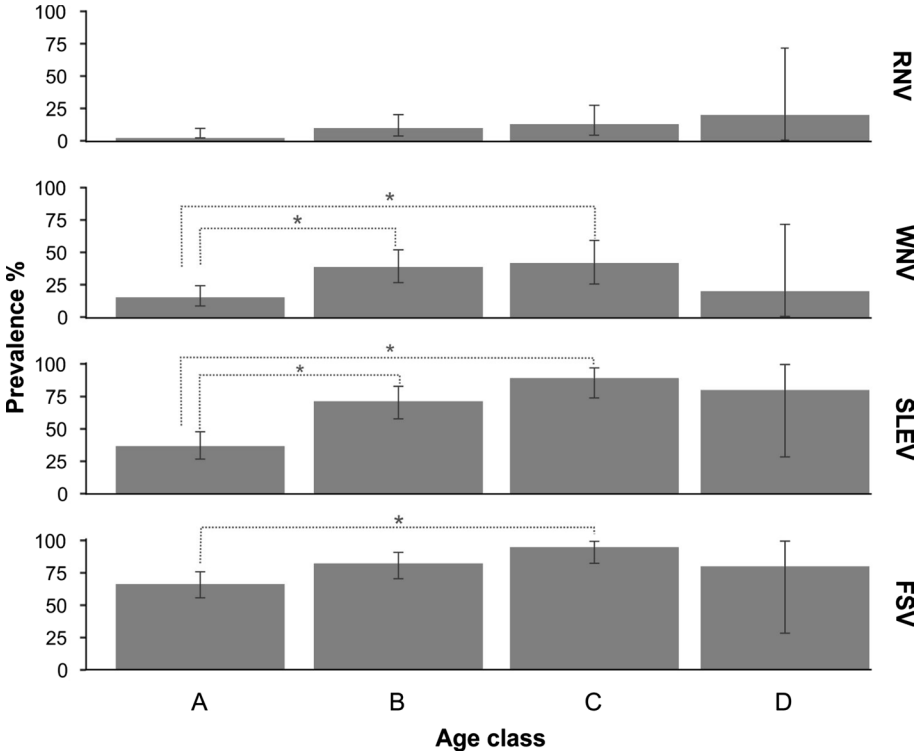

Fig. 1 Seroprevalence by age in a population of draft horses from Santa Fe, Argentina. A, 0-5 years old (y.o.); B, 6-10 y.o.; C, 11-15 y.o.; D, >15 y.o.; RNV, Rio Negro virus; WNV, West Nile virus; SLEV, Saint Louis encephalitis virus; FSV, Fort Sherman virus. A significant pairwise odds ratio ($p < 0.005$) is indicated *. Error bars represent 95% confidence intervals.

For instance, here, the horses tend to spend the night out in the open or in small crude stables without screens near a wooded nature reserve, which could increase the exposure to mosquitoes. Almost 23% of the samples exhibited heterotypic serological responses to both flavivirus. Following the criterion presented by Tauro et al. [11], we considered that sequential infections by different viruses (SLEV and WNV) can result in heterotypic responses. The samples that were positive for RNV were also analyzed for other South American members of the VEEV serocomplex, as cross-reactions are common between subtypes IF, IAB, and IV. Positive samples were not detected. Since all the samples screened for WEEV and EEEV were negative, we still lack data about the activity of these alphaviruses. However, the monitoring efforts should be sustained in order to evaluate whether "silent" sylvatic cycles are occurring and virus emergence is a potential epidemiological scenario. In this sense, the report of a fatal case of encephalitis caused by WEEV in Uruguay in 2009 and the recent detection of MADV in *Culex* mosquitoes in northeastern Argentina highlight the need to survey equine populations [4, 12]. In our study no significant differences in prevalence where observed when different time periods were compared (I, 2013-2014; II, 2014-2015; III, 2015-2016). Although there was also no significant effect of aging on antibodies titers (data not shown), when individuals were grouped into age classes, significant associations between prevalence and age were observed for WNV, SLEV, and FSV (Fig. 1), at least partially supporting the endemic circulation proposed above.

The use of draft animals in the urban environment is a frequent practice, not only in Santa Fe, but also in the rest of Argentina and many other developing countries. The recent activity and the endemicity of the analyzed viruses should encourage health authorities to promote the standardization and maintenance of larger-scale arboviral surveillance programs in Argentina and South America. In particular, draft horses are proposed as sentinels in locations where they are in common use that involves daily contact between equines and humans.

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Author contributions GA-L performed research and wrote the paper. RG and BSK performed research. AIQ analyzed data. BM and JC collected the samples. RM and MSC designed the study. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval No experimental procedures were performed on animals.

Availability of data and material The dataset analysed in the current study is available from the corresponding author on reasonable request.

References

1. Spinsanti L, Diaz LA, Glatstein N, Arselán S, Morales MA, Farías A, Fabbri C, Aguilar J, Ré V, Frías M, Almiron W, Hunsperger E, Siirin M, Travassos da Rosa A, Tesh R, Enría D, Contigiani M (2008) Human outbreak of St. Louis encephalitis detected in Argentina, 2005. J Clin Virol 42:27–33. https://doi.org/10.1016/j.jcv.2007.11.022
2. Morales ME, Barrandeguy M, Fabbri C, Garcia J, Vissani A, Trono K, Gutierrez G, Pigretti S, Menchaca H, Garrido N, Taylor N, Fernandez F, Levis S, Enría D (2006) West Nile virus isolation from equines in Argentina. Emerg Infect Dis 12:1559–1561. https://doi.org/10.3201/eid1210.060852
3. Pisano MB, Ré V, Contigiani M, Ré V (2016) Venezuelan equine encephalitis. In: Liu D (ed) Molecular detection of animal viral pathogens, 1st edn. CRC Press, Boca Raton, pp 269–275
4. Stechina OS, Oria GI, Torres C, Diaz LA, Contigiani M, Stein M (2019) First detection of Madariaga virus in mosquitoes collected in a wild environment of Northeastern Argentina. Am J Trop Med Hyg 101:916–918. https://doi.org/10.4269/ajtmh.19-0475
5. Tauro LB, Rivarola ME, Lucca E, Mariño B, Mazzini R, Ferreira Cardoso J, Barrandeguy ME, Teixeira Nunes MR, Contigiani MS (2015) First isolation of Bunyamwera virus (*Bunyaviridae* family) from horses with neurological disease and abortion in Argentina. Vet J 206:111–114. https://doi.org/10.1016/j.tvjl.2015.06.013
6. De Oliveira Filho EF, Carneiro IO, Ribas JRL, Fischer C, Marklewitz M, Junglen S, Netto EM, Franke CR, Drexler JF (2020) Identification of animal hosts of Fort Sherman virus, a New World zoonotic orthobunyavirus. Transbound Emerg Dis 00:1–9. https://doi.org/10.1111/tbed.13499
7. Vanhomwegen J, Beck C, Desprès P, Figuerola A, García R, Lecollinet S, López-Roig M, Manuguerra J-C, Serra-Cobo J (2017) Circulation of zoonotic arboviruses in equine populations of Mallorca Island (Spain). Vector Borne Zoonotic Dis 17:340–346. https://doi.org/10.1089/vbz.2016.2042
8. Sabattini MS, Avilés G, Monath TP (1998) Historical, epidemiological and ecological aspects of arbovirus in Argentina: Flaviviridae, Bunyaviridae and Rhabdoviridae. In: Travassos da Rosa APA, Vaconcelos PFC, Travassos da Rosa JFS (eds) An overview of arbovirology in Brazil and neighboring countries, 1st edn. Belém: Instituto Evandro Chagas, Belém, pp 113–134
9. Mitchell CJ, Monath TP, Sabattini MS, Cropp CB, Daffner JF, Calisher CH, Jakob WL, Christensen HA (1985) Arbovirus Investigations in Argentina, 1977–1980. II. Arthropod collections and virus isolations from Argentine mosquitoes. Am J Trop Med Hyg 34:945–955
10. R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
11. Tauro LB, Marino B, Díaz LA, Lucca E, Gallozo D, Spinsanti L, Contigiani M (2012) Serological detection of St. Louis encephalitis virus and West Nile virus in equines from Santa Fe Argentina. Mem Inst Oswaldo Cruz 107:553–556. https://doi.org/10.1590/S0074-02762012000400019
12. Delfraro A, Burgueño A, Morel N, González G, García A, Morelli J, Pérez W, Chiparelli H, Arbiza J (2011) Fatal Human Case of Western Equine Encephalitis, Uruguay. Emerg Infect Dis 17:952–954. https://doi.org/10.3201/eid1705.101068