



OPEN

Serological survey reveals enzootic circulation of St. Louis encephalitis and West Nile viruses in semiarid Monte ecosystem of Argentina

Kevin A. Rucci^{1,2}, Diego L. Arias-Builes³, Andrés M. Visintin^{2,4,5} & Adrián Díaz^{1,2}✉

St. Louis encephalitis virus (SLEV) and West Nile virus (WNV) are arboviruses transmitted by *Culex* mosquitoes and amplified in avian hosts. The present study aimed to investigate the presence and seasonal circulation of SLEV and WNV in La Rioja province, within the semiarid ecoregion of the Monte, Argentina. Over a two-year period, avian sera were collected and tested for neutralizing antibodies against SLEV and WNV. Our results reveal the enzootic activity of both viruses in this challenging environment. SLEV seroprevalence was 4.5% (35/778), with higher activity in spring (2016) and autumn (2017). WNV seroprevalence was 3.5% (27/778), peaking during the summer 2016–2017. Greater seroprevalence for SLEV in 2016 was detected for the Lark-like Brushrunner (*Coryphistera alaudina*) and the Short-billed Canastero (*Asthenes baeri*) and in 2017 for the Black-crested Finch (*Lophospingus pusillus*) and Lark-like Brushrunner, whereas for WNV greater seroprevalence in 2016 was detected for the Picui Ground Dove (*Columbina picui*) and in 2017 for the Lark-like Brushrunner and Band-tailed Seedeater (*Catamenia analis*). Additionally, five avian individuals experienced seroconversion during the sampling period, namely the Lark-like Brushrunner and White-fronted Woodpecker (*Melanerpes cactorum*) for SLEV, and the Lark-like Brushrunner, Greater Wagtail Tyrant (*Stigmatura budytoides*) and Many-colored Chaco Finch (*Saltatricula multicolor*) for WNV. The study highlights the persistence and circulation of these viruses in a semiarid ecosystem, raising questions about overwintering mechanisms and transmission dynamics. This research contributes to understanding arbovirus ecology in diverse environments. Further investigations are needed to assess the specific mechanisms facilitating virus persistence in the Monte ecoregion.

St. Louis encephalitis (SLEV) and West Nile (WNV) viruses (*Flaviviridae*, *Flavivirus*) are two neurotropic arboviruses belonging to the Japanese encephalitis virus serocomplex which cause clinical manifestations, ranging from an asymptomatic or undifferentiated febrile condition to a febrile illness and encephalitis, rarely evolving to a more complex neuroinvasive condition¹. Both viruses are maintained in nature by enzootic transmission networks between several species of *Culex* mosquitoes and different avian species, mainly Passeriformes and Columbiformes, as vectors and amplifying hosts, respectively^{2,3}.

St. Louis encephalitis virus was first detected in St. Louis, Missouri, US in the summer of 1933, during an epidemic with more than 1000 cases within areas of the city adjacent to storm drains and sewage channels that generated high densities of *Culex* mosquitoes. Since its discovery, several outbreaks have occurred, the last major outbreak occurring in Florida during the summer of 1990¹. Up to date, SLEV has been detected throughout the Americas, from Canada to Argentina. However, outbreaks in South America have been rare or sporadic². Principal amplifying hosts in North America include house sparrows, house finches, blue jays, pigeons, and mourning doves¹. In Argentina, SLEV reemerged in 2002 and produced the first encephalitis outbreak in Córdoba city in

¹Laboratorio de Arbovirus, Instituto de Virología “Dr. J. M. Vanella”, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina. ²Ministerio de Ciencia, Tecnología e Innovación, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires, Argentina. ³Departamento de Ciencias Básicas y Tecnológicas, Universidad Nacional de Chilecito, Chilecito, La Rioja, Argentina. ⁴Centro de Investigación e Innovación Tecnológica (CENIIT), Instituto de Biología de la Conservación y Paleobiología (IBICOPA), Universidad Nacional de La Rioja, La Rioja, Argentina. ⁵Centro de Investigaciones Entomológicas de Córdoba, Instituto de Investigaciones Biológicas y Tecnológicas (IIByT), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina. ✉email: adrian.diaz@fcm.unc.edu.ar

2005, where 47 human cases were detected⁴. Later, a small new outbreak occurred in 2010 in the same city, as well as sporadic cases in several provinces in the northeast and center of Argentina^{5,6}. In Argentina, SLEV has been isolated from humans (Buenos Aires province), *Culex* mosquitoes, and wild rodents (Córdoba), but never from birds. However, neutralizing antibodies have been detected in several families of wild birds (Furnariidae, Columbidae, Tyrannidae, Fringillidae, Icteridae, Ardeidae, and Cotingidae)⁷.

West Nile virus was first isolated in the West Nile district in Uganda in 1937, and subsequently also detected in other areas of Africa, Europe, South Asia, Australia, and America. In America, it was first detected in New York city in 1999, during an encephalomyelitis outbreak in humans. In South America, specific antibodies against WNV have been detected in birds and equines in the North (Colombia and Venezuela)^{8–10}. Principal WNV hosts include house sparrows and robins¹¹. In Argentina, WNV was first detected in 2006 in horses of Buenos Aires province, however, several studies indicate that it would have been introduced in late 2004, based on the detection of neutralizing antibodies in resident birds¹².

The ability of both viruses to be transmitted and amplified by various species of *Culex* mosquitoes and avian hosts enables them to successfully colonize and establish themselves in diverse and novel biomes. For instance, following its introduction to the US, WNV effectively adapted and thrived across a broad spectrum of ecosystems, ranging from arid deserts (California, Arizona, New Mexico) and shrublands to pastures (Central Great Plains) and forests (northeast coast)³. A comparable expansive geographical distribution is observed for SLEV¹. In Argentina, the majority of arbovirus studies have primarily focused on subtropical and temperate regions (such as Buenos Aires, Córdoba, Chaco, Tucumán, and Salta), which are anticipated to exhibit favorable conditions for arbovirus transmission and maintenance^{7,12–15}. Recently, Batallán et al.¹⁶ have reported the circulation of both viruses by means of detection of neutralizing antibodies in wild birds in the Monte biogeographic region. This finding was unexpected since the Monte ecosystem is a semiarid region with limited water availability. We questioned if this detected circulation was because of an established enzootic transmission focus or represented sporadic circulation. Hence, we aimed to further expand the knowledge of SLEV and WNV circulation in this semiarid area in La Rioja province, employing a serological survey. To assess this, we focused on two aspects, studying the seasonal circulation of these viruses in wild bird communities, and investigating whether birds undergo seroconversion. This allowed us to determine if there exists an enzootic activity and establishment of these viruses in this challenging environment¹⁶.

Materials and methods

Study site

The study was conducted at “Camping del Club Legislativo La Rioja”, located in the vicinity of La Rioja city within La Rioja province, situated in the Argentine northwest region (29° 26 23 S; 66° 54 29 W; 620 masl.) (Fig. 1).

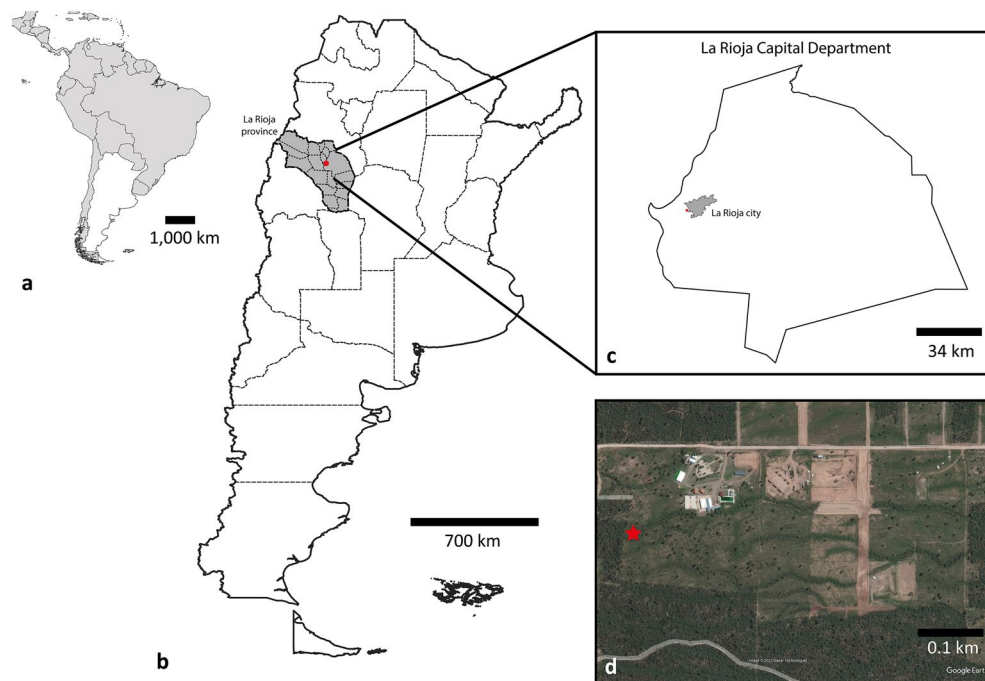


Figure 1. Sample site in the northwest region of Argentina. (a) Overview map of South America highlighting in grey the location of the Argentina Republic within the continent. (b) Argentine Republic, indicating in grey La Rioja province and as a red spot the sample site, “Camping del Club Legislativo La Rioja”. (c) La Rioja Capital department, with La Rioja city shaded in grey and the sample site as a red spot. (d) Landscape from the “Camping del Club Legislativo La Rioja”, extracted from Google Earth. Red star indicates sample site.

Arana et al.¹⁷ described that a part of La Rioja belongs to the biogeographic region of Monte province, specifically to the Septentrional district. This area is characterized by an average annual precipitation of 200 to 400 mm, accumulated mainly during the warm season (October to March)^{17,18}. Dominant vegetation is of the xerophytic type, which has developed a set of morphological, anatomical, and physiological adaptations to efficiently use the water and tolerate the temperatures of the region. Zonal vegetation (= climatic) is composed of a steppe of shrubs that do not exceed 3 m in height, characterized by scarcity of pasture and absence of trees. The most important shrub-steppe is the *jarillal*, formed by densely arranged shrubs of genus *Larrea* (*jarilla*), always characterized by abundant Zygophyllaceae and often featuring trees of the genus *Neltuma* (= *Prosopis*). The climate of this ecoregion does not allow the development of large forest masses and those present are edaphic communities forming riparian forests along rivers of permanent flow, which are dominated by *Neltuma nigra* (= *P. nigra*), *N. alpataco* (= *P. alpataco*), *N. argentina* (= *P. argentina*) (Fabaceae), and *Bulnesia retama* (Zygophyllaceae)^{17,19}.

Bird capture and sample collection

Bird collection was carried out using six mist nets (9 m length × 2.5 m height, 36 mm mesh) operated during sunrise (about 05:00 to 11:00 h depending on the season). Field sampling was performed during 4 days in each season (spring, summer, autumn, and winter) for 2 consecutive years (2016 and 2017). An alphanumeric aluminum band was placed on the tarsus to identify individuals in case of recapture, following the Centro Nacional de Anillado de Aves (National Center of Bird Banding) guidelines (<https://www.csnat.unt.edu.ar/investigacion/institutos/cenaa>). Bird species were identified by using a specialized field guide for bird species from Argentina and Uruguay²⁰. The species and body mass of each bird were recorded. Prior to release, sampled birds were hydrated with sugar water. Blood was collected by jugular (for most species) or brachial (for columbids) venipuncture, using 27 G sterile needles. Birds weighing less than 10 g were not bled. Blood was collected in plastic tubes containing 0.45 mL or 0.9 mL (depending on the required sample volume: 100 µL or 200 µL, respectively) of phosphate buffered saline (PBS) for an approximate 1:10 serum dilution. The tubes were held at room temperature for 20–30 min to allow clotting, after which they were transferred to coolers. In the laboratory, samples were centrifuged at 5000g for 15 min for serum separation. Serum samples were stored at – 20 °C.

Serological assays and data interpretation

Detection of neutralizing antibodies against SLEV and WNV was performed through Plaque-Reduction Neutralization Test (PRNT) in Vero Cl76 cell line (ATCC CRL-587). The screening test serum samples were incubated with 100 PFUs of each virus. Autochthonous low-passage SLEV CbaAr-4005 and WNV E/7229/06 strains were used. The SLEV CbaAr-4005 strain was isolated from *Culex quinquefasciatus* mosquitoes collected in Córdoba province²¹, whereas the WNV E/7229/06 strain was isolated from a dead horse in Buenos Aires province, Argentina²². Positive control corresponded to a known serum with neutralizing antibodies for each virus, while negative control was represented by a serum without neutralizing antibodies. Samples were analyzed with an initial dilution of 1:20. All serum samples that neutralized 80% or more of the inoculated plaque-forming units at dilutions ≥ 1:20 were considered positive. Samples positive for both viruses were subjected to titration. Seven serial two-fold dilutions of serum were prepared, resulting in final dilutions of 1:20, 1:40, 1:80, 1:160 and 1:320. Endpoint titers were assigned as the reciprocal of the greatest dilution in which > 80% neutralization of the challenge virus was observed. Seroconversion was defined as a bird that was seronegative when first captured and became seropositive at recapture. Seroreversion was defined as a seropositive bird whose antibodies decreased below the cut-off value of 20 at recapture²³. Serological data were interpreted following the same criteria explained by Diaz et al.⁷.

Statistical analysis

Seroprevalence with its 95% confidence interval was calculated for both SLEV and WNV using the *binom* library and the Pearson-Klopper method in R²⁴. Proportions were considered for each season, year, and species (with sample size > 10 individuals). The effects of season, year, and avian species were assessed using Generalized Linear Models with binomial error distribution and logit link function. We used the presence and absence of neutralizing antibodies for both viruses, SLEV and WNV, as binary response variables. Every single explanatory variable was modeled as a main effect and in combination as additive effects. To select those models that explained the greatest variability of the response variable, we employed the second-order Akaike information criterion (AICc) for small samples. The support for each model was evaluated using the Δ AICc, defined as the difference in AICc values between the model with the minimum AICc and the AICc of the model under consideration. We selected the best model as the one with a Δ AICc < 2. Additionally, Akaike weights (ω_i) and evidence ratio (ER), which is the ratio between the weight of the best model and the second best, were calculated as additional levels of support for the best models²⁵. Model selection was carried out using the *AICcmodavg* library in R²⁶. All analyses were conducted in R Studio statistical software, v.4.2.1²⁷.

Ethical approval

The capture, manipulation, blood collection, and transport of samples were approved by Fauna Silvestre and the Secretaría de Ambiente of La Rioja province (resolution S.A. 0215/13). The study was performed in accordance with relevant guidelines and regulations for the use of birds in research developed by the Ornithological Council (<https://www.aaalac.org/pub/?id=E9019213-EE55-98AB-F68E-EF2B10C31360>) and in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. All methods and procedures complied with the ARRIVE guidelines for reporting animal research.

Results

Seasonal circulation of SLEV and WNV

A total of 778 sera belonging to 51 avian species from 17 families were subjected to PRNT for detection of neutralizing antibodies against SLEV and WNV. The most frequently captured birds in each season were the tanagers (family Thraupidae), which represented 30.4% and 50% in winter and spring 2016, respectively, and 54.9% and 67.1% in autumn and winter 2017, respectively. The Columbidae (15.7%) and Furnariidae (13.2%) families were most prevalent during the summer of 2016–2017. Conversely, the family Emberizidae (buntings) showed high representation in winter 2016 (28.3%) and autumn 2017 (23.7%).

The best model, selected by the ΔAICc criterion, for SLEV seroprevalence, included the variables season and year ($\omega_i = 0.85$, ER = 13.51) (Table 1). Overall, SLEV seroprevalence accounted for 4.5% (35/778), being higher in 2016 (5.68%, 17/299) than in 2017 (3.76%, 18/479). Regarding seasonal variation, two peaks of seroprevalence were observed: one in spring 2016 (5.26%, 9/171) and another in autumn 2017 (6.22%, 16/257). Positivity for winter 2016 (4.35%, 4/92) and summer 2016–2017 (4.13%, 5/121) were similar, with a marked decline towards winter 2017 (0.73%, 1/137) (Table 2). The most seropositive bird species during 2016 were the Lark-like Brushrunner (*Coryphistera alaudina*) (14.29%, 2/14) and the Short-billed Canastero (*Asthenes baeri*) (7.14%, 1/14), both resident (i.e., non-migratory) birds. During the following year, the most seroprevalent birds were the resident Black-crested Finch (*Lophospingus pusillus*) (8.33%, 1/12) and the Lark-like Brushrunner (7.69%, 1/13) (Table 3, Fig. 2).

For WNV, the best model included only the year variable ($\omega_i = 0.81$, ER = 6.58). Overall, 3.5% (27/778) of the total were positive for this virus, with higher seroprevalence in 2017 (4.8%, 23/479) than in 2016 (1.34%, 4/299). Although season was not selected as an explanatory variable, it could be observed a seasonal pattern in WNV seroprevalence. The lowest seroprevalences were recorded in winter (1.09%, 1/92) and spring (1.17%, 2/171) 2016, rising to a peak in summer 2016–17 (6.61%, 8/121) and decreasing but maintaining high levels towards autumn (3.9%, 10/257) and winter (4.38%, 6/137) 2017 (Table 2). The most frequently positive bird species in 2016 was

Model	K	AICc	ΔAICc	ω_i
SLEV				
Season + year	6	272	0.00	0.85
Season	5	277	5.21	0.06
Null	1	278	5.78	0.05
Year	2	278	6.42	0.03
Season + species	56	354	82.01	0.00
Species	51	358	86.43	0.00
Species + year	52	359	87.39	0.00
WNV				
Year	2	203	0.00	0.81
Null	1	207	3.77	0.12
Season	5	209	5.66	0.05
Season + year	6	210	7.18	0.02
Species + year	52	273	70.43	0.00
Species	51	279	75.82	0.00
Season + species	56	282	78.98	0.00

Table 1. Results of model selection of logistic regression models for St. Louis encephalitis (SLE) and West Nile (WN) viruses seroprevalence during 2016–2017 in La Rioja, Argentina. The selected model was the one with the least ΔAICc . K degrees of freedom, ω_i Akaike weight.

Season	SLEV		WNV	
	Pos/test	%[IC95]	Pos/test	%[IC95]
Winter (2016)	4/92	4.35 [1.20–10.76]	1/92	1.09 [0.03–5.90]
Spring (2016)	9/171	5.26 [2.43–9.75]	2/171	1.17 [0.14–4.16]
Summer (2016–2017)	5/121	4.13 [1.35–9.38]	8/121	6.61 [2.90–12.61]
Autumn (2017)	16/257	6.22 [3.60–9.91]	10/257	3.90 [1.88–7.03]
Winter (2017)	1/137	0.73 [0.018–4.00]	6/137	4.38 [1.62–9.29]
Total	35/778	4.50 [3.15–6.20]	27/778	3.47 [2.30–5.00]

Table 2. SLEV and WNV neutralizing antibody prevalence among all bird species combined, by season, La Rioja, Argentina.

Family	Species	Migratory status	2016				2017			
			SLEV		WNV		SLEV		WNV	
			Pos/test	%[IC95]	Pos/test	%[IC95]	Pos/test	%[IC95]	Pos/test	%[IC95]
Icteridae	Grayish Baywing (<i>Agelaioides badius</i>)	Resident	0/13	0 [0–24.71]	0/13	0 [0–24.71]	–	–	–	–
Furnariidae	Short-billed Canastero (<i>Asthenes baeri</i>)	Resident	1/14	7.14 [0.18–33.87]	0/14	0 [0–23.16]	–	–	–	–
Thraupidae	Band-tailed Seedeater (<i>Catamenia analis</i>)	Resident	4/63	6.35 [1.76–15.47]	0/63	0 [0–5.69]	1/14	7.14 [0.18–33.87]	1/14	7.14 [0.18–33.87]
Columbidae	Picui Ground Dove (<i>Columbina picui</i>)	Resident	0/14	0 [0–23.16]	1/14	7.14 [0.18–33.87]	0/34	0 [0–10.28]	0/34	0 [0–10.28]
Furnariidae	Lark-like Brushrunner (<i>Coryphistera alaudina</i>)	Resident	2/14	14.29 [1.78–42.81]	0/14	0 [0–23.16]	1/13	7.69 [0.19–36.03]	2/13	15.38 [1.92–45.45]
Thraupidae	Diuca Finch (<i>Diuca diuca</i>)	Resident	–	–	–	–	1/38	2.63 [0.07–13.81]	2/38	5.26 [0.64–17.75]
Thraupidae	Black-crested Finch (<i>Lophospingus pusillus</i>)	Resident	–	–	–	–	1/12	8.33 [0.21–38.48]	0/12	0 [0–26.46]
Thraupidae	Cinnamon Warbling Finch (<i>Poospiza ornata</i>)	Resident	–	–	–	–	8/119	6.72 [2.95–12.82]	3/119	2.52 [0.52–7.19]
Thraupidae	Many-colored Chaco Finch (<i>Saltatricula multicolor</i>)	Resident	3/45	6.67 [1.40–18.27]	0/45	0 [0–7.87]	2/45	4.44 [0.54–15.15]	1/45	2.22 [0.06–11.77]
Passerellidae	Rufous-collared Sparrow (<i>Zonotrichia capensis</i>)	Resident	3/44	6.82 [1.43–18.66]	1/44	2.27 [0.06–12.02]	3/87	3.45 [0.72–9.75]	3/87	3.45 [0.72–9.75]
TOTAL	–	–	13/207	6.28 [3.39–10.5]	2/207	0.97 [0.12–3.45]	17/362	4.7 [2.76–7.41]	12/362	3.31 [1.72–5.72]

Table 3. SLEV and WNV seroprevalence among bird species, by year, La Rioja, Argentina. Only species with sample size > 10 are shown. Seropositive species with sample size < 10. 2016 = *Cranioleuca pyrrhophia* (1/2), *Melanerpes cactorum* (1/7), *Pseudoseisura lophotes* (1/5), *Stigmatura budytoides* (1/9). 2017 = no positive species. Seronegative species with sample size < 10. 2016 = *Agriornis Micropterus* (0/1), *Ammodramus humeralis* (0/2), *Anairetes flavirostris* (0/1), *Cyclarhis gujanensis* (0/1), *Diuca diuca* (0/4), *Elaenia parvirostris* (0/4), *Falco sparverius* (0/4), *Furnarius rufus* (0/1), *Knipolegus aterrimus* (0/1), *Leptasthenura platensis* (0/1), *Leptotila verreauxi* (0/1), *Lophospingus pusillus* (0/2), *Molothrus bonariensis* (0/2), *Molothrus rufoaxillaris* (0/4), *Myiarchus tyrannulus* (0/3), *Passer domesticus* (0/1), *Pitangus sulphuratus* (0/1), *Poospiza melanoleuca* (0/6), *Poospiza ornata* (0/2), *Poospiza torquata* (0/1), *Saltator aurantirostris* (0/2), *Synallaxis albescens* (0/7), *Troglodytes aedon* (0/8), *Turdus amaurochalinus* (0/3), *Turdus chiguanco* (0/1), *Tyrannus melancholicus* (0/1), *Veniliornis mixtus* (0/2), *Vireo olivaceus* (0/1), *Zenaida auriculata* (0/2). 2017 = *Agelaioides badius* (0/9), *Anairetes flavirostris* (0/1), *Asthenes baeri* (0/9), *Athene cunicularia* (0/5), *Coccyzus melacoryphus* (0/1), *Colaptes melanochloros* (0/1), *Coryphospingus cucullatus* (0/2), *Elaenia parvirostris* (0/3), *Embernagra platensis* (0/2), *Furnarius rufus* (0/2), *Melanerpes cactorum* (0/2), *Mimus patagonicus* (0/2), *Myiarchus tyrannulus* (0/2), *Myioborus bruniceps* (0/1), *Nothura maculosa* (0/2), *Patagioenas maculosa* (0/5), *Poospiza melanoleuca* (0/2), *Pseudoseisura lophotes* (0/1), *Saltator aurantirostris* (0/4), *Spizapteryx circumcincta* (0/1), *Stigmatura budytoides* (0/4), *Troglodytes aedon* (0/4), *Turdus amaurochalinus* (0/2), *Turdus chiguanco* (0/4), *Veniliornis mixtus* (0/2), *Vireo olivaceus* (0/1), *Xolmis irupero* (0/1), *Zenaida auriculata* (0/3).

the Picui Ground Dove (*Columbina picui*) (7.14%, 1/14), and in 2017 was the Lark-like Brushrunner (15.38%, 2/13) and the Band-tailed Seedeater (*Catamenia analis*) (7.14%, 1/14), all resident avian species (Table 3, Fig. 2).

Seroconversion survey

From all captured birds, 36 were recaptured, being 34 of these seronegative for both SLEV and WNV at the first sampling event. Among these, 14% (5/34) underwent seroconversion during the sampling period. For SLEV, birds that seroconverted were the Lark-like Brushrunner (from June to October 2016) and the White-fronted Woodpecker (*Melanerpes cactorum*) (from September to November 2016). In the case of WNV, the birds that seroconverted included the Lark-like Brushrunner (from November 2016 to January 2017), the Greater Wagtail-Tyrant (*Stigmatura budytoides*) (from September to December 2016), and the Many-colored Chaco Finch (*Saltatricula multicolor*) (from April to May 2017). Additionally, two birds seroreversed, namely the Many-colored Chaco Finch (from December 2016 to December 2017) for SLEV and the Rufous Hornero (*Furnarius rufus*) (from April to June 2017) for WNV. Lastly, two birds were positive during their first capture and recapture event, the many-colored Chaco Finch (from December 2016 to March 2017) for SLEV and the Short-billed Canastero (from January to February 2017) for WNV.

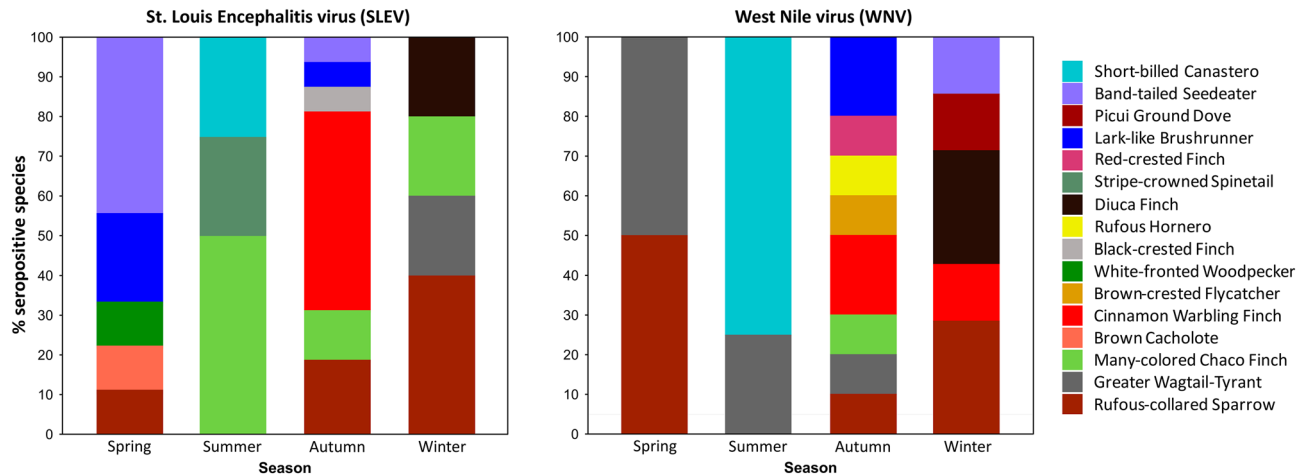


Figure 2. Seasonal species composition seroprevalence of St. Louis encephalitis virus (SLEV) (left) and West Nile virus (WNV) (right) for the period 2016–2017 in La Rioja, Argentina.

Discussion

St. Louis encephalitis and West Nile viruses are multi-host/multi-vector flaviviruses, endowing them with the capacity to be amplified and transmitted by a variety of avian and *Culex* mosquito species²⁸. This ecological adaptability has facilitated their wide geographic distribution and colonization of numerous ecoregions. In Argentina, both viruses have been identified across diverse ecological zones, encompassing subtropical forests, thorned forests, shrublands, pastures, agricultural areas, and urbanized regions^{7,12,15,29,30}. Intriguingly, Batallán et al.¹⁶ detected the presence of both viruses in semiarid ecosystems within the Monte region (La Rioja province), as evidenced by the identification of neutralizing antibodies in wild birds. Their study reported a lower seroprevalence for both viruses compared to our findings. Specifically, Batallán et al.¹⁶ recorded an overall seroprevalence of 2.4% for SLEV and 0.8% for WNV, in contrast to our results of 4.5% and 3.5%, respectively. Notably, the Cinnamon Warbling Finch (*Poospiza ornata*) exhibited higher seropositivity to SLEV, whereas the White-tipped Dove (*Leptotila verreauxi*) and Grayish Baywing (*Agelaioides badius*) displayed greater seroprevalence to WNV. Nevertheless, the seroprevalence of these viruses was lower compared to other regions in Argentina^{7,12,30}. This reduced avian host seropositivity rate may stem from the relatively low mosquito population abundance in the semiarid Monte ecosystem. The mosquito community within this ecosystem is characterized by various *Culex* mosquito species, among which the most abundant are *Cx. apicinus*, *Cx. bidens*, *Cx. coronator*, *Cx. dolosus*, *Cx. interfor*, *Cx. maxi*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. saltanensis* and *Cx. tramazayguesi*^{31–33}. These species, in turn, can act as vectors for SLEV and WNV^{34,35}. Mosquito populations in the Monte ecoregion show remarkable stationary population dynamics with population peaks primarily occurring at the beginning of the warm season (October–December), and at the end of the same season (March–April), corresponding to periods of increased rainfall.

One of the most noteworthy attributes of vector-borne diseases is their distinct seasonality, intricately linked with the biology of their vectors. Consequently, the pathogens they carry must employ mechanisms to persist through adverse periods, adapting to the seasonal dynamics of their vectors (overwintering)³⁶. Conceptually, three distinct overwintering mechanisms could come into play: (i) sustained yet diminished transmission, linked to the survival and residual biting activity of adult vectors, (ii) persistence within vector/hosts, and (iii) survival within the resistant stages of the vector³⁶. Sustained transmission might occur in regions where vectors continue to bite hosts throughout the year. In these areas, the elongated interval between blood meals due to winter temperatures, coupled with an extended extrinsic incubation period (latency period), leads to infrequent clinical cases that may easily go unnoticed (as observed in the persistence of the *Culex*/West Nile virus system in southern California)³⁷. It has been observed that Argentine populations of *Cx. quinquefasciatus* do not enter diapause during unfavorable periods (winter), remaining physiologically active, which allows for low but steady vectorial transmission³⁸. The persistence of the virus within an avian host could result from a prolonged presence in tissues, subsequently leading to resurgence (as observed in West Nile virus infections in house sparrows)³⁹. In the *Culex*/Western equine encephalitis virus system, a reemergence of viremia was experimentally observed in snakes following hibernation⁴⁰. Furthermore, a pathogen can persist by establishing itself within the overwintering stages of the vector through vertical transmission, either in eggs or in newly emerged adults. This phenomenon is exemplified by the *Culex*/St. Louis encephalitis virus system and the *Aedes triseriatus*/La Crosse virus system, where persistence is maintained respectively in the eggs and the recently emerged larval/adult mosquitoes^{41,42}. Persistence in unfavorable environments could also be explained by the repeated annual introduction of the virus through the dispersal by migratory birds¹². SLEV and WNV are flaviviruses amplified by birds that could be dispersed by this type of host. It would be interesting to study the phylodynamics of these viruses in the Monte ecosystem to obtain evidence about the potential annual introduction or local establishment of viral strains. The consistent presence of antibodies against SLE and WN viruses in different resident bird species over a two-year sampling period, along with the observed seroconversions in resident birds (Lark-like

Brushrunner, White-fronted Woodpecker, Greater Wagtail Tyrant, Many-colored Chaco Finch) at different times of the year, confirms the enzootic activity and establishment of these viruses within this challenging environment.

Our findings unequivocally affirm the enzootic establishment and activity of SLE and WN viruses within an ecosystem traditionally deemed unfavorable for arbovirus transmission, as exemplified by the Monte ecoregion. Consequently, future research endeavors aimed at pinpointing the specific overwintering mechanisms driving in this ecosystem are imperative.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Received: 6 September 2023; Accepted: 27 February 2024

Published online: 29 February 2024

References

1. Reisen, W. K. Epidemiology of St. Louis encephalitis virus. In *The Flaviviruses—Detection, Diagnosis and Vaccine Development* (ed. Reisen, W. K.) 139–183 (Elsevier, 2003). [https://doi.org/10.1016/S0065-3527\(03\)61004-3](https://doi.org/10.1016/S0065-3527(03)61004-3).
2. Diaz, A., Coffey, L. L., Burkett-Cadena, N. & Day, J. F. Reemergence of St. Louis encephalitis virus in the Americas. *Emerg. Infect. Dis.* **24**, 2150–2157 (2018).
3. Kramer, L. D., Ciota, A. T. & Kilpatrick, A. M. Introduction, spread, and establishment of West Nile virus in the Americas. *J. Med. Entomol.* **56**, 1448–1455 (2019).
4. Spinsanti, L. I. *et al.* Human outbreak of St. Louis encephalitis detected in Argentina, 2005. *J. Clin. Virol.* **42**, 27–33 (2008).
5. Seijo, A. *et al.* Outbreak of St. Luis encephalitis in the metropolitan Buenos Aires area. *Medicina (Buenos Aires)* **71**, 211–217 (2011).
6. Fabbri, C. M. *et al.* Brote de encefalitis de San Luis en la provincia de San Juan, Argentina, 2011. *Rev. Argent. Microbiol.* **1**, 89–89 (2011).
7. Diaz, L. A. *et al.* Activity patterns of St. Louis encephalitis and West Nile viruses in free ranging birds during a human encephalitis outbreak in Argentina. *PLoS One* **11**, e0161871 (2016).
8. Mattar, S. *et al.* West Nile virus antibodies in Colombian Horses. *Emerg. Infect. Dis.* **11**, 1497–1498 (2005).
9. Bosch, I. *et al.* West Nile virus, Venezuela. *Emerg. Infect. Dis.* **13**, 651–653 (2007).
10. Hoyos-López, R., Suaza-Vasco, J., Rúa-Urbe, G., Uribe, S. & Gallego-Gómez, J. C. Molecular detection of flaviviruses and alphaviruses in mosquitoes (Diptera: Culicidae) from coastal ecosystems in the Colombian Caribbean. *Mem. Inst. Oswaldo Cruz* **111**, 625–634 (2016).
11. Komar, N. West Nile virus: Epidemiology and ecology in North America. In *The Flaviviruses—Detection, Diagnosis and Vaccine Development* (ed. Komar, N.) 185–234 (Elsevier, 2003). [https://doi.org/10.1016/S0065-3527\(03\)61005-5](https://doi.org/10.1016/S0065-3527(03)61005-5).
12. Diaz, L. A. *et al.* West Nile virus in birds, Argentina. *Emerg. Infect. Dis.* **14**, 689–691 (2008).
13. Pauli, R. *et al.* Arbovirus investigations in Argentina, 1977–1980. *Am. J. Trop. Med. Hyg.* **34**, 966–975 (1985).
14. Mitchell, C. J. *et al.* Arbovirus isolations from mosquitoes collected during and after the 1982–1983 epizootic of western equine encephalitis in Argentina. *Am. J. Trop. Med. Hyg.* **36**, 107–113 (1987).
15. Tauro, L. *et al.* Serological detection of St. Louis encephalitis virus and West Nile virus in equines from Santa Fe, Argentina. *Mem. Inst. Oswaldo Cruz* **107**, 553–556 (2012).
16. Batallán, G. P. *et al.* Autochthonous circulation of Saint Louis encephalitis and West Nile viruses in the province of La Rioja, Argentina. *Rev. Argent. Microbiol.* **53**, 154–161 (2021).
17. Arana, M. D. *et al.* *Esquema Biogeográfico de la República Argentina* (Opera Lilloana, 2021).
18. Burkart, R., Bárbaro, N. O., Sánchez, R. O. & Gómez, D. O. *Eco-Regiones de la Argentina* (Administración de Parques Nacionales, 1999).
19. Morello, J. *et al.* *Ecorregiones y complejos Ecosistémicos de Argentina* (Orientación Gráfica Editora, 2012).
20. Narosky, T. & Yzurrieta, D. *Guía de Identificación de Aves de Argentina y Uruguay* (Vazquez Mazzini Editores, 2010).
21. Diaz, L. *et al.* Genotype III Saint Louis encephalitis virus outbreak, Argentina, 2005. *Emerg. Infect. Dis.* **12**, 1752–1754 (2006).
22. Morales, M. *et al.* West Nile virus isolation from equines in Argentina, 2006. *Emerg. Infect. Dis.* **12**, 1559–1561 (2006).
23. Figuerola, J., Soriguer, R., Rojo, G., Tejedor, C. G. & Jimenez-Clavero, M. A. Seroconversion in wild birds and local circulation of West Nile Virus, Spain. *Emerg. Infect. Dis.* **13**, 1915 (2007).
24. Dorai-Raj, S. binom: Binomial confidence intervals for several parameterizations. *R package version 1.1–1.1* (2014).
25. Burnham, K. P. & Anderson, D. R. *Model Selection and Multimodel Inference* (Springer New York, 2004). <https://doi.org/10.1007/b97636>.
26. Mazerolle, M. J. Model selection and multimodel inference based on (Q)AIC(c). *R package version 2.3–1* (2020).
27. R Core Team. R: A language and environment for statistical computing (2022).
28. Diaz, L. A. *et al.* Transmission of endemic St Louis encephalitis virus strains by local *Culex quinquefasciatus* populations in Cordoba, Argentina. *Trans. R. Soc. Trop. Med. Hyg.* **107**, 332–334 (2013).
29. Albrieu-Llinás, G. *et al.* Arbovirus serosurvey (Orthobunyavirus, Flavivirus, and Alphavirus) in a draft horse population from Santa Fe, Argentina (2013–2016). *Arch. Virol.* **166**, 881–884 (2021).
30. Mansilla, A. P., Grande, J. M. & Diaz, A. Effect of agroecosystems on seroprevalence of St. Louis encephalitis and West Nile Viruses in Birds, La Pampa, Argentina, 2017–2019. *Emerg. Infect. Dis.* **28**, 1393–1402 (2022).
31. Visintin, A. M. *et al.* Two new mosquito species and six new provincial records in Argentina. *J. Am. Mosq. Control Assoc.* **26**, 91–94 (2010).
32. Visintin, A. M., Batallán, G. P., Beranek, M., Laurito, M. & Almirón, W. R. Diversidad de Culicidae (Diptera) de las ciudades de La Rioja y Chilecito, provincia de La Rioja, Argentina. In *X Jornadas Regionales sobre Mosquitos* (2016).
33. Rossi, G. C. Annotated checklist, distribution, and taxonomic bibliography of the mosquitoes (Insecta: Diptera: Culicidae) of Argentina. *Check List* **11**, 1712 (2016).
34. Beranek, M. D. *et al.* *Culex* interfor and *Culex saltanensis* (Diptera: Culicidae) are susceptible and competent to transmit St. Louis encephalitis virus (Flavivirus: Flaviviridae) in central Argentina. *Trans. R. Soc. Trop. Med. Hyg.* **114**, 725–729 (2020).
35. Giayetto, O., Beranek, M. D., Nazari, F. N. & Diaz, A. Dose dependence of susceptibility and transmission for an Argentinean West Nile virus strain in local *Culex pipiens quinquefasciatus* (Diptera: Culicidae). *Trans. R. Soc. Trop. Med. Hyg.* **115**, 1066–1069 (2021).
36. Charron, M. V. P., Balenghien, T., Seegers, H., Langlais, M. & Ezanno, P. How much can diptera-borne viruses persist over unfavourable seasons?. *PLoS One* **8**, e74213 (2013).
37. Reisen, W. K. *et al.* Overwintering of West Nile virus in Southern California. *J. Med. Entomol.* **43**, 344–355 (2006).
38. Branda, M. F., Laurito, M., Visintin, A. M. & Almirón, W. R. Gonoactivity of *Culex* (*Culex*) (Diptera: Culicidae) mosquitoes during winter in temperate Argentina. *J. Med. Entomol.* **58**, 1454–1458 (2021).
39. Nemeth, N. *et al.* Persistent West Nile virus infection in the house sparrow (*Passer domesticus*). *Arch. Virol.* **154**, 783–789 (2009).

40. Thomas, L. A. & Eklund, C. M. Overwintering of western equine encephalomyelitis virus in garter snakes experimentally infected by *Culex tarsalis*. *Exp. Biol. Med.* **109**, 421–424 (1962).
41. Miller, B. R., DeFoliart, G. R. & Yuill, T. M. Vertical transmission of La Crosse Virus (California Encephalitis Group): Transovarial and filial infection rates in *Aedes Triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* **14**, 437–440 (1977).
42. Flores, F. S., Diaz, L. A., Batallán, G. P., Almirón, W. R. & Contigiani, M. S. vertical transmission of St. Louis encephalitis virus in *Culex quinquefasciatus* (Diptera: Culicidae) in Córdoba, Argentina. *Vector-Borne Zoonotic Dis.* **10**, 999–1002 (2010).

Acknowledgements

The study was funded by the projects 0052/2016 from the Consejo de Investigación Científicas y Tecnológicas (CICyT), Universidad Nacional de La Rioja, and PICT 2016-3283 and 2018-1172 from the Fondo para la Investigación Científica y Tecnológica (FONCYT). We thank Hernán Brizuela for permission and support to carry on sampling activities in the “Camping del Club Legislativo La Rioja”.

Author contributions

A.M.V. and A.D. conceptualized the research idea and designed the study; D.L.A.-B., A.M.V., and A.D. collected the samples and performed the experiments; K.A.R. and A.D. analyzed and interpreted the data; K.A.R., D.L.A.-B., A.M.V. and A.D. wrote and prepared the manuscript for publication and revision. A.M.V. and A.D. administrated the funds.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to A.D.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2024