

Agricultural area increases the infection risk of free ranging birds to St. Louis encephalitis and  
West Nile viruses (*Flavivirus*)

SLEV and WNV infection risk for avian hosts in agroecosystems

Ana Paula Mansilla<sup>1</sup>, Juan Manuel Grande<sup>1</sup> and Adrián Díaz<sup>2\*</sup>

<sup>1</sup>Colaboratorio de Biodiversidad, Ecología y Conservación (ColBEC), INCITAP-CONICET-  
UNLPam / FCEyN-UNLPam, Mendoza 109 (6300), Santa Rosa, LP, Argentina.

<sup>2</sup> Laboratorio de Arbovirus - Instituto de Virología “Dr. J. M. Vanella” - Facultad de Ciencias  
Médicas – Universidad Nacional de Córdoba. Ciudad Universitaria, Enfermera Gordillo Gómez  
s/n, CP X5016GCA Córdoba, Argentina.

\*Corresponding author:

[adrian.diaz@conicet.gov.ar](mailto:adrian.diaz@conicet.gov.ar)

## Abstract

Anthropogenic changes affect biological communities of host and vectors driving arbovirus activity. In general, urban and agricultural ecosystems harbor less avian and mosquito diversity than native ecosystems and are dominated by few species. Human activities have led to the emergence / re-emergence of different infectious pathogens particularly arboviruses representing a threat to both public health and biodiversity. Saint Louis encephalitis (SLEV) and West Nile viruses (WNV) are transmitted by *Culex* spp. mosquitoes as main vectors and several bird species as hosts. With the aim to study the exposure of free-ranging bird communities to SLEV and WNV in Pampean agroecosystems as well as to evaluate the environmental/ biological factors potentially associated we collected and bleed free ranging birds in 12 sites. Serum samples were analyzed by Plaque Reduction Neutralization Test (PRNT) for both viruses. Generalized Linear Mixed Models (GLMM) were performed to analyze the association between environmental / biological variables from each sampled site and avian exposition. A total of 1019 birds were sampled during 2017 - 2019. Overall, we found SLEV NTAbs in 60 out of 1019 samples (5.8%) while WNV seroprevalence was 2.1% (21/1019). SLEV and WNV seroprevalence were different among the sampled sites. Agricultural area was positively associated with the SLEV-WNV infection risk for an avian host. Forested area also had a strong association but in a negative way. Our results suggest that open agricultural area increase the infection risk of free ranging birds to SLEV and WNV while forested area diminishes the infection risk maybe through a dilution effect of vector and host communities.

**Key words:** West Nile virus; Saint Louis encephalitis virus; wildlife; birds; anthropogenic changes; agroecosystems

## 45 1 INTRODUCTION

46 Infections in animals that are transmitted to humans (zoonoses), and within them those  
47 transmitted from one vertebrate to another by an arthropod vector (vector-borne diseases), have  
48 been frequently identified among the most important emerging infectious diseases (Morens,  
49 Folkers, & Fauci, 2004). During the last decades many of these pathogens such as dengue virus  
50 (DENV), yellow fever virus (YFV), zika virus (ZIKV), chikungunya virus (CHIKV), Saint Louis  
51 encephalitis virus (SLEV) and West Nile virus (WNV) have emerged / reemerged, representing a  
52 real threat to both human health and wildlife conservation (Gould, Pettersson, Higgs, Charrel, &  
53 Lamballerie, 2017).

54 St. Louis encephalitis virus (SLEV) and West Nile virus (WNV) belong to the *Flavivirus*  
55 genus (Flaviviridae family) (ICTV, 2019). SLEV is an endemic virus in the American continent  
56 experiencing a re-emergence process in the central region of Argentina and southern area of  
57 Brazil (Diaz et al., 2018). Recently, it also re-emerged in western USA causing human  
58 encephalitis cases in Arizona and California (Venkat et al., 2015; White et al., 2016). According  
59 to ecological studies the SLEV transmission network is integrated by *Cx. quinquefasciatus*, *Cx.*  
60 *interfor* and *Cx. saltanensis* as mosquito vectors (Beranek et al., 2020) and Eared dove (*Zenaida*  
61 *auriculata*) and Picui Ground dove (*Columbina picui*) as amplifying urban host (Diaz et al.,  
62 2018). West Nile virus (WNV), originally from the Old World, appeared in the American  
63 continent in 1999, causing encephalitis outbreak in humans and massive mortality events in  
64 American crows (*Corvus brachyrhynchos*) (Hayes et al., 2005). In Argentina, the virus was  
65 isolated in Buenos Aires and Entre Ríos provinces from sick horses in 2006 (Morales et al.,  
66 2006). However, serological evidence indicates older endemic WNV activity in a large mosaic of  
67 resident avian species from central and northern Argentina since 2004 (Diaz et al., 2008). Host  
68 competence assays identified the Picui Ground dove as an amplifier host for WNV, indicating

both viruses could have similar ecological requirements for their maintenance (Diaz et al., 2011). Vector competence studies indicate that both *Cx. quinquefasciatus* and *Cx. interfor* are able to transmit WNV local viral strain (Giayetto, Beranek, Nazar, & Diaz, 2019, 2021).

Land use changes can impact on the disease dynamics by modifying the abundance, distribution, behavior, movement, immune response, vectors and hosts community composition and interactions between hosts and vectors species (Gottdenker, Streicker, Faust, & Carroll, 2014). In Argentina, the expansion of the agricultural border over the native ecosystems has generated great modifications in the landscape and in the biological communities that inhabit these regions. Specifically, the Pampas region, located in the central-eastern part of Argentina, is one of the areas that has undergone a greatest modification by human activities due to the quality of its soils. This large scale landscape modification has generated changes in the abundance of small mammals and birds (Bilenca, Codesido, Maria, & Fischer, 2009). However, some rodent and native dove species have successfully adapted to these changes so well that because of their abundances are considered agricultural pests (Bilenca and Kravetz, 1995; Codesido et al., 2015). The large populations of several columbid species (Eared doves, Picui Ground doves, Spotted wing Pigeons), could generate good ecological conditions for an increase in the activity of SLEV and WNV. In this context, the aim of this work was to study the exposure of free-ranging bird communities to SLEV and WNV and to evaluate environmental/ biological factors potentially associated with that exposure in Pampean agroecosystems.

## 93 2 MATERIAL AND METHODS

### 94 2.1 Study area and sampling sites

95 The study was carried out in the northeastern region of La Pampa province, Argentina  
96 during arbovirus activity period (February - April) of 2017, 2018 and 2019. We select twelve  
97 sampling sites within the agroecosystems of the province to perform birds captures (Fig. 1). The  
98 area is part of the former Pampean grasslands ecoregion but is currently entirely transformed into  
99 agricultural lands. The northeastern of the study area is mostly devoted to intensive soybean  
100 production under no tillage method with scarce rotation to wheat. Some farms may also produce  
101 sunflower and corn, or may have small extensions of seminatural or cultivated pastures for  
102 livestock raising; towards the center of the province, soybean is less common and cultivated and  
103 seminatural pastures dominate the landscape alternating with different crops such as wheat, corn  
104 and sunflower. In this central area there are also some small isolated patches of Caldén forest in  
105 the transition to the Espinal ecoregion (Fig. 1). Across the study area but more markedly in the  
106 northeast, there are exotic tree stands (sometimes of up to 20-30 hectares) around settlements.

107 The climate is dry sub-humid, with rainfall distributed throughout the year but with  
108 highest monthly precipitation in the summer semester (from October to March) and with  
109 precipitation increasing in a southwest-northeast gradient (Cano et al., 1980).

110

### 111 2.2 Bird collection and sera samples

112 Seven mist nets were operated during three or four days at each site during dawn and late  
113 afternoon. Collected birds were marked with an alphanumeric aluminum leg ring provided by  
114 Aves Argentinas and the species, age, sex, and regular morphometric measurements of each bird  
115 were recorded. Before being released, sampled birds were hydrated with sugar water. Blood was

collected by jugular (most species) or brachial vein (columbids), using 27 G sterile needles. Birds that weighted less than 10 grams were not bleed. Blood was collected in plastic tubes containing 0.45 mL or 0.9 mL (according to sample volume: 0.1 ml or 0.2 ml, respectively) of Minimum Essential Medium (MEM) for an approximate 1:10 serum dilution. The tubes were held at room temperature for 20–30 min for coagulation and then placed into coolers. At the laboratory, samples were centrifuged at 5,000g for 15 min for serum separation. Sera samples were stored at -20°C.

### **2.3 Viral stocks preparation**

Low-passage SLEV CbaAr-4005 and WNV E/7229/06 strains were used for serologic assays. CbaAr-4005 strain was isolated from *Culex quinquefasciatus* mosquitoes collected in Córdoba during a human encephalitis outbreak (Diaz et al., 2006). E/7229/06 WNV strain was isolated from a dead horse in Buenos Aires province, Argentina (Morales et al., 2006). Viral stocks were obtained from infected Vero cell monolayers harvested on day 7 and 5 post-inoculation for SLEV and WNV, respectively.

### **2.4 Serological assays and data interpretation**

Sera samples were analyzed by plaque-reduction neutralization test (PRNT) according to this protocol: samples were heat-inactivated for 30 minutes at 56°C for non-specific inhibitors inactivation. Sixty µl of serum was mixed with 60 µl of viral dilution containing theoretical 100 plaque forming units (PFU), estimated on the previous titration of viral stock. Serum-viral dilution mixture was incubated at 37° C for 60 minutes to allow binding of NTAb to viral particles (neutralization period). After that, 100 µl of the mixture (virus-serum) was inoculated

into a Vero cell monolayer in each well and incubated for 60 minutes at 37 °C. Finally, 0.5 ml of nutrient medium with 1% agarose was added to each well and the plate was incubated at 37° C for 5 (WNV) and 7 (SLEV) days. After the incubation period, plates were fixed for 2 hours with a 10% formaldehyde solution and stained with crystal violet. All serum sample that neutralized 80% or more of the PFU was considered positive. Positive samples for both viruses were subject to titration. Serum samples with antibody titers higher than 20 were considered positive for the tested virus.

## **2.5 Environmental and biological data**

In order to determine the influence of different environmental and biological variables on SLEV and WNV activity, we built a buffer area of 1.5 km radius around the sampling site within which we calculated the area occupied by various land cover classes. We used SPOT 6 images provided by the National Commission for Space Activities (CONAE) on which we created a shape or layer file on which polygons corresponding to the different land cover uses were digitized. We estimated the area and distances to variables relevant for arbovirus transmission within the buffer area. Free open software QGIS version 3.4.10 was used for all the procedures. Analyzed environmental variables were: agricultural area (it included crops and pasture lands), areas covered by trees (included native forest patches and exotic tree stands), distance to the nearest water body and distance to the closest urban settlement. Based on previous host competence studies (Diaz et al., 2018), dove abundance (Picui Ground dove, Eared dove and Spot-winged Pigeon abundances), cowbird abundance (Bay-winged cowbird and Shiny cowbird abundances) and House sparrow abundance were considered as biological variables. Dove, cowbird and sparrow abundance was estimated by an observational and acoustic bird count on each site. We used the fixed width transect method of 50m x 200m and we performed six

transects in each site according to a rarefaction analysis. The six transects were randomly distributed to cover as much of the site as possible and at least 200 m apart to minimize possible biases by double counting of birds (Bibby, Jones, & Marsden, 1998). All linear transects were surveyed once by a single observer. Bird surveys took place during March and April, 2018 and 2019, between 6:00 and 10:00 AM.

## 2.6 Statistical analysis

SLEV and WNV activity were estimated by detection of Neutralizing antibodies (NtAb) seroprevalence in each sampled site. The punctual seroprevalence and the 95% confidence intervals were calculated with the package “*binom*” (Dorai-Raj 2014). Association between environmental and biological variables and the SLEV-WNV infection risk for an avian host in each sampling site was investigated through Generalized Linear Mixed Models (GLMM) with Binomial error distribution and *logit* link function and considering the sampled year as a random variable in all models. Collinearity between explanatory variables was evaluated using Pearson correlation with  $r < 0.60$  as a limit. Because environmental variables "agricultural area" and "area covered by trees" were strongly correlated ( $r = -0.99$ ), we decided to remove the second one from the set of proposed models; the same happened with dove and cowbird abundance ( $r = 0.85$ ), so we decided to eliminate cowbird abundance from the analyses. Table 1 shows the proposed models with their variables, hypotheses, predictions and biological justification. Model selection was carried out using the Akaike Information Criterion (AIC) and its corrected calculation for small sample sizes (AICc) (Burnham & Anderson, 2002). The level of support for each model was evaluated using the  $\Delta AICc$  value. Competing models were those differing by  $\leq 2$   $\Delta AICc$  from the top model, and we used Akaike weights ( $w$ ) as an indication of support for each model (Burnham & Anderson, 2002). Parameter estimates were calculated using model-averaged



parameter estimates based on  $w_i$  from all candidate models. To supplement parameter-likelihood evidence of important effects, we calculated 95% confidence interval limits (CL) of parameter estimates. All statistical analysis were made using R 3.5.1 software (R Core Team 2017).

### 3 RESULTS

A total of 1019 free-ranging birds belonging to 45 species were collected and sampled for detection of SLEV and WNV NtAb. Overall, 5.8% (60/1019) and 2.1% (21/1019) seroprevalence for SLEV and WNV were detected, respectively. Twelve individuals showed NtAb titer higher than 40 for each virus, and those were considered a heterotypic infection.

Nine out of twelve sampled sites showed activity for SLEV by means of NtAb, while seven sites had seropositive birds for WNV (Fig. 2). The highest seroprevalence values for SLEV and WNV were detected in sites LP1 and LP2, while at sites LP5 and LP8, no seropositive bird was found for either virus (Fig. 1, 2).

Seventeen avian species showed SLEV NtAb while 8 species turned out to be WNV seropositive. House wren (*Troglodytes aedon*), Chalk-Browed Mockingbird (*Mimus saturninus*), Monk Parakeet (*Myiopsitta monachus*), Eared dove and House sparrow were the most exposed species for SLEV (Table 2). Interestingly, Monk Parakeet, Rufous hornero (*Furnarius rufus*) and Bay-Winged Cowbird (*Agelaioides badius*) were the most frequently infected species for WNV.

The best model explaining the variation in SLEV infection risk for an avian host included “agricultural area” as explanatory variable ( $w_i = 0.44$ ; Table 3). The SLEV infection risk for an avian host increased with agricultural area (Table 4). Odd Ratios value for this model was 1.97, which means that by each unit that increases the agricultural area, the SLEV infection risk for an avian host increases on average 1.97 times. Among the competing models there were two others,

which besides containing the variable “agricultural area” included the variable “distance to water body” and the other, the variable “dove abundance”, although there were considerable uncertainty in the models because for both variables the CL included zero (Table 3; 4). While the effect of these two variables could be considered marginal, it should be noted that the sense of these effects were negative or inverse, meaning that the SLEV infection risk for an avian host increased as the distance to water body decreased as well as the dove’s abundance.

On the other hand, the model that best explained the variation in WNV infection risk for an avian host included “distance to water body” and “agricultural area” as explanatory variables ( $w_i = 0.36$ ; Table 5), but neither of the two variables was related to WNV infection risk for an avian host, as both CL include zero (Table 6). The same happened with the others competing models that containing the variables “agricultural area” and “dove abundance” for which the CL also included zero. Therefore, although none of the variables were statistically significant, the agricultural area is again present in the three best models demonstrating its importance to explain the variation in the WNV infection risk for an avian host.

## 4 DISCUSSION

Anthropogenic disturbance produces radical changes across natural communities and thus, also affecting the infectious pathogens dynamics. Intensive agricultural activities drastically transform native vegetation into simplified monocultures such as soybean, wheat, sunflower, corn, etc. These changes, completely alter former avian and mosquito communities affecting the arbovirus transmission through host and vector abundance, host and vector communities composition, mosquito host-preference and mosquito host-seeking activity (Chuang, Hildreth, Vanroekel, & Wimberly, 2011; Gottdenker et al., 2014; Keesing et al., 2010).

Our estimations of viral activity in avian hosts (SLEV = 6%, WNV = 2%) are similar to those detected in other areas of Argentina during enzootic periods (SLEV [min= 3.9%, max= 11.95%]; WNV [min= 0.81%, max= 9.61%]) (Berrón, 2014; Flores, 2014; Diaz et al., 2016; Quaglia, 2017; Seiler, 2019) suggesting enzootic activity for both viruses. Moreover, most of seropositive avian individuals belonged to resident bird species, reinforcing the idea of endemic activity in agriculture ecosystems for both viruses. SLEV and WNV are multi-host multi-vector flaviviruses mainly vectored by many *Culex* mosquito species. In Argentina, vector competence studies demonstrate the ability of *Culex quinquefasciatus*, *Cx. saltanensis* and *Cx. interfor* to transmit SLEV (Beranek et al., 2020) and *Culex quinquefasciatus* and *Cx. interfor* to transmit WNV (Giayetto et al., 2019, 2021). *Culex* mosquito species are temperate mosquitoes with a wide geographic range encompassing neotropical and austral latitudes such as La Pampa, Neuquén and Rio Negro provinces (Patagonia Argentina). Our data confirm the expansion and adaptation of both SLEV and WNV to this temperate region of Argentina.

Most exposed avian species were different between both viruses. A total of 17 SLEV seropositive avian species were detected, being House wren, Chalk-Browed Mockingbird, House sparrow, Eared dove and Monk parakeet the most exposed (Table 2). On the other hand, only 7 bird species were seropositive for WNV and Rufous hornero, Monk parakeet, Picui Ground-dove and Bay-Winged Cowbird were the most exposed (>5%). Interesting differences show up comparing specific seroprevalences for both viruses in other ecosystems. In studies performed in Córdoba city and Mar Chiquita, Picui Ground-dove, Brown Cacholote, and Rufous hornero were the most exposed species to SLEV, while Rufous hornero appears as the most exposed species to WNV coinciding with this work and highlighting its possible role in the WNV transmission and maintenance (Diaz et al., 2016; Quaglia, 2017; Seiler, 2019). House sparrow have not been detected seropositive for SLEV in more than 200 tested sera collected in neotropical areas (Northeastern region) (Monath et al., 1985). In urbanized temperate areas (Center region –

Cordoba city) seroprevalence rates for this species were low for both viruses (3.92% and 1.96%, for SLEV and WNV respectively) (Diaz et al., 2016). Although House sparrows show low host competence index value (Diaz et al., 2018), its high abundance and great exposition to SLEV observed in this study would indicate an efficient role as amplifying host for SLEV in agricultural areas. Different mosquito species that vector both viruses with different host feeding preferences could be a possible explanation for the differences observed in the infected avian species. It's interesting as well, the low exposition to SLEV and WNV of raptors species (birds of prey) in Pampean agricultural systems. Recent studies reported that resident birds of prey in Pampean agricultural ecosystem showed almost no activity for neither tested viruses (SLEV nor WNV). In that study, seven out of 523 samples tested SLEV positive and none for WNV (considering just resident species) (Mansilla et al., 2020). These differences found in the exposition between the raptors and the passeriforms and columbiforms analyzed in this study, perhaps are related with ecological features or life history traits from birds of prey, such as perching behavior or nest heights affecting vector-host meeting.

The SLEV-WNV infection risk for an avian host was positively associated with the agricultural area, and thus, negatively affected by the cover of forested area. Highest seroprevalence values were observed at sites located north of the study area (Fig. 1, 2), the area most impacted and transformed by intensive agricultural activities. We hypothesized that agricultural area promote SLEV/WNV activity by increasing the abundance of amplifying host such as Eared doves and Picui Ground doves and *Culex quinquefasciatus* and *Cx. interfor* mosquito vectors. Although highest seroprevalence values were observed at sites located north of the study area these sites showed smaller Eared dove and Picui Ground dove abundance than sites located in the southern zone of the study area. Dove's abundance was positively associated with native forest patches located in southern studied sites. These forested patches are places for dove communal roosting and nesting sites and so becoming a suitable place for dove rearing

283 (Murton et al., 1974; Bernardos 2010; Bucher and Ranvaud, 2006). Mosquito communities in  
284 agricultural area are dominated by *Culex* mosquito species accounting for more than 90% of the  
285 total registered abundance. The most abundant groups reported were *Culex bidens/interfor* and  
286 *Culex mollis/tatoi* (Mansilla, Laurito, Gallardo, Farías, & Diaz, 2019). Likely, *Culex* mosquito  
287 vector abundance is playing a critical role driving SLEV and WNV activity in agricultural land  
288 areas increasing avian host risk exposition. Maybe, Eared dove and Picui Ground dove  
289 abundance have a secondary effect on the SLEV and WNV activity in avian host. The tested  
290 viruses are amplified and vectored by several bird and mosquito species. So, maybe alternative  
291 bird species such as House sparrow, Rufous hornero or House wren are amplifying the viruses in  
292 places where dove abundance is lower.

293         Unfortunately, there are few studies on the effect of land use on SLEV activity. In  
294 Argentina, a study about how the landscape elements affect the distribution of SLEV infections  
295 in humans revealed that the proximity to places with high vegetation cover and low density  
296 urban constructions were the best variables to explain the human infections by SLEV while the  
297 proximity to agricultural fields was not associated to SLEV human infections (Vergara Cid,  
298 Estallo, Almirón, Contigiani, & Spinsanti, 2013). However, these results cannot be compared  
299 with those obtained in this study, because the data on SLEV infections in humans come from an  
300 urban environment, where the composition of both birds and mosquitoes is different from that  
301 found in agroecosystems and therefore, the SLEV maintenance networks will also be different.

302         On the other hand, land use effect on WNV activity has been extensively studied in US  
303 (Bowden, Magori, & Drake, 2011; Bradley, Gibbs, & Altizer, 2008; Brown, Childs, Diuk-  
304 Wasser, & Fish, 2008; Chuang, Hockett, Kightlinger, & Wimberly, 2012; Ezenwa et al., 2007;  
305 Kovach & Kilpatrick, 2018; Miramontes, Lafferty, Lind, & Oberle, 2006). Studies show that  
306 mosquito vector abundance and distribution pattern is a key factor to determine viral activity and

that it is hardly affected by land use. For example, urbanization affected positively human WNV disease incidence in Northeastern US, where *Culex pipiens* and *Culex quinquefasciatus* are the main vectors in the region (Bowden et al., 2011), while on the west coast, where *Culex tarsalis* is the most efficient vector, agriculture irrigated areas such as rice field and orchards are the main land cover associated with the increase WNV activity (Kovach & Kilpatrick, 2018).

Land use (cattle, crops production, urbanization, minning) drastically affects vegetation and the composition of biological communities inhabiting the environment. Mosquito vectors and avian host communities are directly influenced by these modifications that impoverish biological communities, and which become dominated by potentially more efficient vectors and hosts. Moreover, changes in vegetation structure can also modify vectors and hosts encountering interactions influencing the overall arboviral activity. Anthropogenic activities are one of the most important factors affecting emergence of infectious diseases, particularly viral vector-borne zoonoses. Aedes-borne viruses (chikungunya, dengue and zika viruses) are positively affected by urbanization as the main breeding substrates of *Aedes aegypti* and *Aedes albopictus* vectors which become highly abundant in those anthropogenic and urban habits (Wilke et al., 2019; Zahouli et al., 2017). However, in *Culex*-borne viruses (Japanese encephalitis, West Nile, St. Louis encephalitis, Usutu viruses) it is not well established how anthropogenic changes affect viral activity. Maybe, the generalist host-feeding and host seeking behavior and wide tolerance for rearing sites by their *Culex* mosquito vectors makes difficult to determine the effect of land use on *Culex*-borne viruses activity.

The surface covered by agricultural lands is expanding globally (Bruinsma, 2003; Tilman, Balzer, Hill, & Befort, 2011). In Argentina, as in other areas of Latin America, a large portion of the agricultural expansion has been driven by large scale deforestation processes in dry forests (Graesser, Aide, Grau, & Ramankutty, 2015; Vallejos et al., 2015). Our results here,

suggest that these changes in landscape composition could favor the incidence of emergent diseases such as the SLEV and WNV. Further research on ecology and biology of these viruses will be needed to understand how crop production, monoculture areas and associated landscapes affect vector borne diseases transmission dynamics. New evidence on this topic will increase our understanding in environmental and biological interactions and improve our predictive capabilities on emergence of arboviral zoonoses.

## **ACKNOWLEDGEMENTS**

We thank Luis Alberto Mansilla, Lucas Gelid, Giovana Peralta, Diego Gallego, Aitué Farana, Mikel Larrea, Amaia Frade, Haizea Otaola, Ibai Alcelay and Milagros Mansilla for their invaluable help in the field work as well as private properties owners, who allowed us access to their lands. We thank Brenda Konigheim, Javier Aguilar, and Romina Gallardo for their technical support with cell cultures.

## **ETHICAL APPROVAL**

The bird capture was authorized by the Dirección de Recursos Naturales (La Pampa province, Argentina). Birds were handled following guidelines for the use of wild birds in research elaborated by the Ornithological Council (<https://birdnet.org/info-for-ornithologists/guidelines-to-the-use-of-wild-birds-in-research/guia/>).

Field studies did not involve endangered or protected species. All blood sampling procedures were specifically approved as part of the obtaining field permit.

353 **CONFLICTS OF INTEREST**

354 The authors declare no conflicts of interest.

355

356 **DATA AVAILABILITY STATEMENT**

357 Data is available upon request since no digital repository from our institutions are available at  
358 this moment.

359

360 **FUNDING**

361 This research received funding from Ministerio Nacional de Ciencia y Tecnología de Argentina  
362 (PICT 2016-3283, PICT 2018-1172), Consejo Nacional de Investigaciones Científicas y  
363 Técnicas (CONICET-PUE IIBYT 2017), and Secretaría de Ciencia y Tecnología - Universidad  
364 Nacional de Córdoba (Secyt CONSOLIDAR 2018). APM holds a doctoral scholarship from  
365 CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas). JMG and AD are  
366 researchers at CONICET.

367

368

369

370

371

372

373

374



375   **REFERENCES**

- 376   Beranek, M. D., Quaglia, A. I., Peralta, G. C., Flores, F. S., Stein, M., Diaz, L. A., ... Contigiani,  
377       M. S. (2020). *Culex interfor* and *Culex saltanensis* (Diptera: Culicidae) are susceptible and  
378       competent to transmit St. Louis encephalitis virus (*Flavivirus: Flaviviridae*) in central  
379       Argentina. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 114(10),  
380       725–729. <https://doi.org/10.1093/trstmh/traa058>
- 381   Bernardos, J. (2010). Informe sobre el Estado de Avance del Estudio de la sobreabundancia de la  
382       Paloma Torcaza en la Localidad de Embajador Martini. Inédito. 5p.
- 383   Berrón, C. I. (2014). *Preferencia de hospedadores aviares en especies de mosquitos Género*  
384       *Culex asociadas a la transmisión de Flavivirus (Flaviviridae) en el arco sur de la Laguna*  
385       *Mar Chiquita*. Universidad Nacional de Córdoba.
- 386   Bibby, C., Jones, M., & Marsden, S. (1998). *Expedition Field Techniques: Bird surveys*. Royal  
387       Geographical Society, London.
- 388   Bilenca, D., Codesido, M., Maria, C., & Fischer, G. (2009). Cambios en la fauna pampeana.  
389       *Ciencia Hoy*.
- 390   Bilenca, D. N., & Kravetz, F. O. (1995). Patrones de abundancia relativa en ensambles de  
391       pequeños roedores de la región pampeana. *Ecología Austral*, 5, 21–30.
- 392   Bowden, S. E., Magori, K., & Drake, J. M. (2011). Regional differences in the association  
393       between land cover and West Nile virus disease incidence in humans in the United States.  
394       *American Journal of Tropical Medicine and Hygiene*, 84(2), 234–238.  
395       <https://doi.org/10.4269/ajtmh.2011.10-0134>
- 396   Bradley, C. A., Gibbs, S. E. J., & Altizer, S. (2008). Urban land use predicts West Nile virus  
397       exposure in songbirds. *Ecological Applications*, 18(5), 1083–1092.

398 <https://doi.org/10.1890/07-0822.1>

399 Brown, H. E., Childs, J. E., Diuk-Wasser, M. A., & Fish, D. (2008). Ecological factors  
400 associated with West Nile virus transmission, northeastern United States. *Emerging*  
401 *Infectious Diseases*, 14(10), 1539–1545. <https://doi.org/10.3201/eid1410.071396>

402 Bruinsma, J. (2003). *World agriculture: Towards 2015/2030: An FAO perspective. World*  
403 *Agriculture: Towards 2015/2030: An FAO Study*. Earthscan.  
404 <https://doi.org/10.4324/9781315083858>

405 Bucher, E. H., & Ranvaud, R. D. (2006). Eared dove outbreaks in South America : patterns and  
406 characteristics. *Acta Zoologica Sinica*, 52, 564–567.

407 Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Multimodel Inference: A*  
408 *practical Information-theoretic Approach (2nd ed). Ecological Modelling* (Vol. 172).  
409 <https://doi.org/10.1016/j.ecolmodel.2003.11.004>

410 Cano, E., Casagrande, G., Conti, H., Salazar Lea Plaza, J., Peña Zubiarte, C., Maldonado Pinedo,  
411 D., ... Pittaluga, A. (1980). *Inventario integrado de los recursos naturales de la provincia*  
412 *de La Pampa. Clima, geomorfología, suelo y vegetación*. Universidad Nacional de La  
413 Pampa, Instituto Nacional de Tecnología Agropecuaria. Buenos Aires, Argentina.

414 Chuang, T. W., Hildreth, M. B., Vanroekel, D. L., & Wimberly, M. C. (2011). Weather and land  
415 cover influences on mosquito populations in Sioux Falls, South Dakota. *Journal of Medical*  
416 *Entomology*, 48(3), 669–679. <https://doi.org/10.1603/ME10246>

417 Chuang, T. W., Hockett, C. W., Kightlinger, L., & Wimberly, M. C. (2012). Landscape-level  
418 spatial patterns of West Nile virus risk in the northern Great Plains. *American Journal of*  
419 *Tropical Medicine and Hygiene*, 86(4), 724–731. [https://doi.org/10.4269/ajtmh.2012.11-](https://doi.org/10.4269/ajtmh.2012.11-0515)  
420 0515

421 Codesido, M., Zufiaurre, E., & Bilenca, D. (2015). Relationship between pest birds and  
422 landscape elements in the Pampas of Central Argentina. *Emu*, 115(1), 80–84.  
423 <https://doi.org/10.1071/MU13110>

424 Diaz, A., Coffey, L. L., Burkett-Cadena, N., & Day, J. F. (2018). Reemergence of St. Louis  
425 Encephalitis Virus in the Americas. *Emerging Infectious Diseases*, 24(12), 2150–2157.  
426 <https://doi.org/10.3201/eid2412.180372>

427 Diaz, A., Flores, F. S., Quaglia, A. I., & Contigiani, M. S. (2018). Evaluation of argentinean bird  
428 species as amplifying hosts for St. Louis encephalitis virus (*Flavivirus*, *Flaviviridae*).  
429 *American Journal of Tropical Medicine and Hygiene*, 99(1), 216–221.  
430 <https://doi.org/10.4269/ajtmh.17-0856>

431 Diaz, L. A., Flores, F. S., & Contigiani, M. S. (2011). Viremia profiles and host competence  
432 index for West Nile virus (*Flavivirus*, *Flaviviridae*) in three autochthonous birds species  
433 from Argentina. *Journal of Ornithology*, 152(1), 21–25. [https://doi.org/10.1007/s10336-](https://doi.org/10.1007/s10336-010-0538-4)  
434 [010-0538-4](https://doi.org/10.1007/s10336-010-0538-4)

435 Diaz, L. A., Komar, N., Visintin, A., Dantur Juri, M. J., Lobo Allende, R., Spinsanti, L., ...  
436 Contigiani, M. S. (2008). West Nile Virus in birds, Argentina. *Emerging Infectious*  
437 *Diseases*, 14(4), 689–690. <https://doi.org/10.1016/b978-1-4377-1986-4.00043-3>

438 Diaz, L. A., Quaglia, A. I., Konigheim, B. S., Boris, A. S., Aguilar, J. J., Komar, N., &  
439 Contigiani, M. S. (2016). Activity patterns of St. Louis encephalitis and West Nile viruses  
440 in free ranging birds during a human encephalitis outbreak in Argentina. *PLoS ONE*, 11(8).  
441 <https://doi.org/10.1371/journal.pone.0161871>

442 Diaz, L. A., Ré, V., Almirón, W. R., Farías, A., Vázquez, A., Sanchez-seco, M. P., ...  
443 Contigiani, M. (2006). Genotype III SLEV Outbreak, Argentina, 2005. *Emerging Infectious*

444 *Diseases*, 12(11), 2005–2007.

445 Dorai-Raj, S. (2014). binom: Binomial confidence intervals for several parameterizations. R  
 446 package version, 1, 1-1.

447 Ezenwa, V. O., Milheim, L. E., Coffey, M. F., Godsey, M. S., King, R. J., & Guptill, S. C.  
 448 (2007). Land cover variation and West Nile virus prevalence: Patterns, processes, and  
 449 implications for disease control. *Vector-Borne and Zoonotic Diseases*, 7(2), 173–180.  
 450 <https://doi.org/10.1089/vbz.2006.0584>

451 Flores, F. S. (2014). *Estudio de garrapatas (Acari: Ixodidae y Argasidae) como vectores*  
 452 *alternativos de los virus St. Louis Encephalitis y West Nile (Flavivirus) en el centro-norte*  
 453 *de Argentina*. Universidad Nacional de Córdoba.

454 Giayetto, O., Beranek, M. D., Nazar, F. N., & Diaz, A. (2019). Vector competence of two  
 455 Argentinean *Culex* mosquito species for West Nile virus (*Flavivirus*, Flaviviridae). In  
 456 *American Society of Tropical Medicine and Hygiene, 68th Annual Meeting*.

457 Giayetto, O., Beranek, M. D., Nazar, F. N., & Diaz, A. (2021). Dose dependence of  
 458 susceptibility and transmission for an Argentinean West Nile virus strain in local *Culex*  
 459 *pipiens quinquefasciatus* (Diptera: Culicidae). *Transactions of The Royal Society of*  
 460 *Tropical Medicine and Hygiene*, 1–4. <https://doi.org/10.1093/trstmh/traa185>

461 Gottdenker, N. L., Streicker, D. G., Faust, C. L., & Carroll, C. R. (2014). Anthropogenic Land  
 462 Use Change and Infectious Diseases: A Review of the Evidence. *EcoHealth*, 11(4), 619–  
 463 632. <https://doi.org/10.1007/s10393-014-0941-z>

464 Gould, E., Pettersson, J., Higgs, S., Charrel, R., & de Lamballerie, X. (2017). Emerging  
 465 arboviruses : Why today ? *One Health*, 4(April), 1–13.  
 466 <https://doi.org/10.1016/j.onehlt.2017.06.001>

467 Graesser, J., Aide, T. M., Grau, H. R., & Ramankutty, N. (2015). Cropland/pastureland dynamics  
468 and the slowdown of deforestation in Latin America. *Environmental Research Letters*,  
469 10(3). <https://doi.org/10.1088/1748-9326/10/3/034017>

470 Hayes, E. B., Komar, N., Nasci, R. S., Montgomery, S. P., O'Leary, D. R., & Campbell, G. L.  
471 (2005). Epidemiology and Transmission Dynamics of West Nile Virus Disease. *Emerging*  
472 *Infectious Diseases*, 11(8), 1167–1173.

473 ICTV (International Committee on Taxonomy of Viruses). Virus Taxonomy: 2019 Release.  
474 <https://talk.ictvonline.org/taxonomy/>.

475 Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., ... Ostfeld, R. S.  
476 (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases.  
477 *Nature*, 468(7324), 647–652. <https://doi.org/10.1038/nature09575>

478 Kovach, T. J., & Kilpatrick, A. M. (2018). Increased Human Incidence of West Nile Virus  
479 Disease near Rice Fields in California but Not in Southern United States. *American Journal*  
480 *of Tropical Medicine and Hygiene*, 99(1), 222–228. <https://doi.org/10.4269/ajtmh.18-0120>

481 Mansilla, A. P., Laurito, M., Gallardo, R., Fariás, A., & Diaz, A. (2019). Efecto de los  
482 agroecosistemas en la actividad del virus St. Louis encephalitis (Flavivirus) y en la  
483 comunidad de mosquitos vectores. In *XXXIX Reunión Científica Anual de la Sociedad*  
484 *Argentina de Virología*. Valle Hermoso, Córdoba: Química Viva. Retrieved from  
485 <http://www.quimicaviva.qb.fcen.uba.ar/v19n1/sav.html>

486 Mansilla, A. P., Solaro, C., Orozco-Valor, P. M., Grande, J. M., Sarasola, J. H., & Diaz, A.  
487 (2020). Exposure of raptors in central Argentina to St. Louis encephalitis and West Nile  
488 viruses. *Journal of Raptor Research*, 54(3), 279–286. [https://doi.org/10.3356/0892-1016-](https://doi.org/10.3356/0892-1016-54.3.279)  
489 54.3.279

490 Miramontes, R., Lafferty, W. E., Lind, B. K., & Oberle, M. W. (2006). Is agricultural activity  
 491 linked to the incidence of human West Nile virus? *American Journal of Preventive*  
 492 *Medicine*, 30(2), 160–163. <https://doi.org/10.1016/j.amepre.2005.10.008>

493 Monath, T. P., Sabattini, M. S., Pauli, R., Daffner, J. F., Mitchell, C. J., Bowen, G. S., & Cropp,  
 494 C. B. (1985). Arbovirus investigations in Argentina, 1977-1980. IV. Serologic surveys and  
 495 sentinel equine program. *American Journal of Tropical Medicine and Hygiene*, 34(5), 966–  
 496 975. <https://doi.org/10.4269/ajtmh.1985.34.966>

497 Morales, M. A., Barrandeguy, M., Fabbri, C., Garcia, J. B., Vissani, A., Trono, K., ... Enría, D.  
 498 (2006). West Nile virus isolation from equines in Argentina, 2006. *Emerging Infectious*  
 499 *Diseases*, 12(10), 1559–1561. <https://doi.org/10.3201/eid1210.060852>

500 Morens, D. M., Folkers, G. K., & Fauci, A. S. (2004). The challenge of emerging and re-  
 501 emerging infectious diseases. *Nature*, 430, 242–249. <https://doi.org/10.1038/nature02759>

502 Murton, R. K., Bucher, E. H., Nores, M., & Reartes, J. (1974). The Ecology of the Eared Dove  
 503 (*Zenaida auriculata*) in Argentina. *The Condor*, 76(1), 80–88.  
 504 <https://doi.org/10.2307/1365986>

505 Quaglia, A. I. (2017). *Comunidades de Mosquitos Vectores y Hospedadores Aviares y su*  
 506 *Asociación en el Mantenimiento del virus St. Louis Encephalitis (Flavivirus)*. Universidad  
 507 Nacional de Córdoba.

508 R Core Team. (2017). R: A language and environment for statistical computing. R Foundation  
 509 for Statistical Computing, Vienna. <https://www.R-project.org>.

510 Seiler, E. N. (2019). *Actividad estacional de los virus Saint Louis encephalitis y West Nile en la*  
 511 *comunidad de aves silvestres en un parche de Espinal, Córdoba*. Universidad Nacional de  
 512 Córdoba.

513 Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable  
 514 intensification of agriculture. *Proceedings of the National Academy of Sciences of the*  
 515 *United States of America*, 108(50), 20260–20264. <https://doi.org/10.1073/pnas.1116437108>

516 Vallejos, M., Volante, J. N., Mosciaro, M. J., Vale, L. M., Bustamante, M. L., & Paruelo, J. M.  
 517 (2015). Transformation dynamics of the natural cover in the Dry Chaco ecoregion: A plot  
 518 level geo-database from 1976 to 2012. *Journal of Arid Environments*, 123(26), 3–11.  
 519 <https://doi.org/10.1016/j.jaridenv.2014.11.009>

520 Venkat, H., Krow-Lucal, E., Hennessey, M., Jones, J., Adams, L., Fischer, M.,...Hills, S. (2015).  
 521 Concurrent outbreaks of St. Louis encephalitis virus and West Nile virus disease—Arizona,  
 522 2015. *MMWR Morb Mortal Wkly Rep*. 2015; 64:1349–50. DOI:  
 523 10.15585/mmwr.mm6448a5.

524 Vergara Cid, C., Estallo, E. L., Almirón, W. R., Contigiani, M. S., & Spinsanti, L. I. (2013).  
 525 Landscape determinants of Saint Louis encephalitis human infections in Córdoba city,  
 526 Argentina during 2010. *Acta Tropica*, 125(3), 303–308.  
 527 <https://doi.org/10.1016/j.actatropica.2012.12.005>

528 White, G. S., Symmes, K., Sun, P., Fang, Y., Garcia, S., Steiner, C., ... Coffey, L. L. (2016).  
 529 Reemergence of St. Louis encephalitis virus, California, 2015. *Emerging Infectious*  
 530 *Diseases*, 22(12), 2185–2188. <https://doi.org/10.3201/eid2212.160805>

531 Wilke, A. B. B., Chase, C., Vasquez, C., Carvajal, A., Medina, J., Petrie, W. D., & Beier, J. C.  
 532 (2019). Urbanization creates diverse aquatic habitats for immature mosquitoes in urban  
 533 areas. *Scientific Reports*, 9(1). <https://doi.org/10.1038/s41598-019-51787-5>

534 Zahouli, J. B. Z., Koudou, B. G., Müller, P., Malone, D., Tano, Y., & Utzinger, J. (2017).  
 535 Urbanization is a main driver for the larval ecology of Aedes mosquitoes in arbovirus-

536 endemic settings in south-eastern Côte d'Ivoire. *PLoS Neglected Tropical Diseases*, 11(7),  
537 1–23. <https://doi.org/10.1371/journal.pntd.0005751>



538 **Table 1.** Proposed models to analyze the association between environmental and biological variables and the SLEV-WNV infection risk for  
539 an avian host. Models 1 to 9 correspond to those proposed for SLEV and 10 to 18 correspond to WNV.

Model	Variables of the model	Hypotheses	Prediction	Biological justification
M1/M10	Null model	The SLEV-WNV infection risk for an avian host is randomly distributed among sites	The SLEV-WNV infection risk values for an avian host does not vary significantly between sites	The biological and environmental variables considered in this study do not explain the SLEV-WNV infection risk for an avian host
M2/M11	Distance to water body	The SLEV-WNV infection risk for an avian host is negatively associated with the distance to a water body	The SLEV-WNV infection risk for an avian host will be greater in places closer to water bodies	The water bodies are favorable habitat for the development of immature mosquitoes, especially of the genus <i>Culex</i> , for which a greater abundance of potential mosquito vectors will be generated in these sites. Also, birds use these sites for drinking, facilitating the encounter between hosts and vectors
M3/M12	Agricultural area	The SLEV-WNV infection risk for an avian host is influenced by the agricultural area	The SLEV-WNV infection risk for an avian host will be higher in places with larger areas covered by crops and pastures	Places with a homogeneous agricultural matrix will have impoverished biological communities dominated by few bird species such as <i>Z. auriculata</i> and <i>C. picui</i> with the potential to amplify viruses
M4/M13	Distance to urban site	The SLEV-WNV infection risk for an avian host is affected by the vicinity to urban areas	The SLEV-WNV infection risk for an avian host will be higher in places closer to urban areas	Peri-urban areas present better conditions for the establishment of different <i>Culex</i> mosquito species, generating a greater abundance of potential vectors
M5/M14	Dove abundance ( <i>Z. auriculata</i> + <i>C. picui</i> + <i>P. maculosa</i> )	The SLEV-WNV infection risk for an avian host is positive associated with doves abundance	The SLEV-WNV infection risk for an avian host will be higher in places with higher dove abundance	Host competence assays identified Columbiform species as the main amplifying hosts for SLEV in Argentina, so a greater abundance of these species will produce greater viral circulation in those sites
M6/M15	Sparrow abundance	The SLEV-WNV infection risk for an avian host is negative associated with the house sparrow abundance	The SLEV-WNV infection risk for an avian host will be lower in places with higher house sparrow abundance	House sparrow populations in Córdoba province were not very efficient for SLEV amplification, so higher abundance of this species would generate a viral dilution effect in the sites
M7/M16	Agricultural area + dove abundance	The SLEV-WNV infection risk for an avian host is influenced by the relationship between agricultural area and dove abundance	The SLEV-WNV infection risk for an avian host will be higher in places with larger agricultural area and higher dove abundance	Doves have a high capacity to SLEV and WNV amplification and very abundant in disturbed environments occupied by crops and pastures, providing greater viral circulation in those places
M8/M17	Distance to water body + Agricultural area	The SLEV-WNV infection risk for an avian host is associated with the presence of water bodies and the agricultural area	The SLEV-WNV infection risk for an avian host will be higher in places closer to water bodies and with larger cover of agricultural area	Places with larger agricultural area and closer to water bodies will have impoverished biological communities dominated by <i>Z. auriculata</i> and <i>C. picui</i> and high mosquitoes abundance
M9/M18	Local land use	The SLEV-WNV infection risk for an avian host is influenced by local land use	The SLEV-WNV infection risk for an avian host will be lower in places with mixed land use than in agricultural areas	Places with mixed land use will present low viral activity due to greater landscape heterogeneity and a more enriched biological community in which viral activity could be diluted

540 **Table 2.** SLEV and WNV species-specific seroprevalence in birds collected in different  
541 agroecosystems of La Pampa province.

Specie	Seroprevalence			
	SLEV	%[CI]	WNV	%[CI]
House sparrow ( <i>Passer domesticus</i> )	17/237	7.17 [4.23–11.23]	2/237	0.84 [0.10–3.01]
Rufous-collared Sparrow ( <i>Zonotrichia capensis</i> )	9/181	4.97 [2.29–9.23]	0/181	0 [0–2.02]
Rufous hornero ( <i>Furnarius rufus</i> )	6/90	6.66 [2.48–13.94]	7/90	7.77 [3.18–15.37]
Picui Ground dove ( <i>Columbina picui</i> )	5/100	5 [1.64–11.28]	4/100	4 [1.10–9.92]
Bay-Winged Cowbird ( <i>Agelaioides badius</i> )	2/63	3.17 [0.38–11]	3/63	4.76 [0.99–13.29]
Monk Parakeet ( <i>Myiopsitta monachus</i> )	2/25	8 [0.98–26.03]	2/25	8 [0.98–26.03]
Eared dove ( <i>Zenaida auriculata</i> )	2/26	7.69 [0.94–25.13]	1/26	3.84 [0.09–19.63]
Shiny Cowbird ( <i>Molothrus bonariensis</i> )	0/72	0 [0–4.99]	1/72	1.38 [0.03–7.49]
House wren ( <i>Troglodytes aedon</i> )	6/63	9.52 [3.57–19.58]	0/63	0 [0–5.68]
Double-collared Seedeater ( <i>Sporophila caerulea</i> )	1/19	5.26 [0.13–26.02]	0/19	0 [0–17.64]
Grassland Yellow-finch ( <i>Sicalis luteola</i> )	1/20	5 [0.12–24.87]	0/20	0 [0–16.84]
Chalk-Browed Mockingbird ( <i>Mimus saturninus</i> )	1/12	8.33 [0.21–38.47]	0/12	0 [0–26.46]
Tropical kingbird ( <i>Tyrannus melancholicus</i> )*	2/4	-	0/4	-

American kestrel ( <i>Falco sparverius</i> )*	2/4	-	0/4	-
Saffron Finch ( <i>Sicalis flaveola</i> )*	1/6	-	0/6	-
Pale-breasted Spinetail ( <i>Synallaxis albens</i> )*	1/1	-	0/1	-
White-winged Black-tyrant ( <i>Knipolegus aterrimus</i> )*	1/1	-	1/1	-
Hudson's Black-tyrant ( <i>Knipolegus hudsoni</i> )*	1/1	-	0/1	-

\*For seropositive species with less than ten individuals the punctual seroprevalence was not calculated.

**Table 3.** Models for SLEV infection risk for an avian host based on the generated hypotheses ranked by their Akaike information criterion (AIC) scores.

Model	Variables of the model	<i>k</i>	AICc	ΔAICc	<i>W<sub>i</sub></i>
GLMM3	Agricultural area	3	388.819	0.000	0.441
GLMM8	Distance to water body + Agricultural area	4	390.198	1.379	0.221
GLMM7	Agricultural area + dove abundance	4	390.244	1.425	0.216
GLMM5	Dove abundance	3	391.421	2.602	0.120
<i>(Z. auriculata + C. picui + P. maculosa)</i>					
GLMM2	Distance to water body	3	401.845	13.026	0.001
GLMM1	Null model	2	408.710	19.892	0.000
GLMM9	Local land use	4	409.230	20.412	0.000
GLMM6	Sparrow abundance	3	410.216	21.397	0.000
GLMM4	Distance to urban site	3	410.465	21.647	0.000

549

550

551 **Table 4.** Parameter likelihoods, estimates ( $\pm$  SE) and 95% confidence interval limits (CL) for  
 552 explanatory variables describing variation in SLEV infection risk for an avian host with  $\Delta\text{AICc} <$   
 553 2. Explanatory variables with CL excluding zero are in bold.

Explanatory variable	Parameter likelihood	Parameter estimate $\pm$ SE	CL	
			Lower	Upper
Intercept		<b>-3.79 <math>\pm</math> 1.10</b>	<b>-5.96</b>	<b>-1.61</b>
Agricultural area	<b>1.00</b>	<b>0.68 <math>\pm</math> 0.22</b>	<b>0.23</b>	<b>1.13</b>
Distance to water body	0.25	-0.18 $\pm$ 0.22	-0.63	0.27
Dove abundance	0.25	-0.87 $\pm$ 1.43	-3.68	1.94

554

555

556 **Table 5.** Models for WNV infection risk for an avian host based on the generated hypotheses  
 557 ranked by their Akaike information criterion (AIC) scores.

558

Model	Variables of the model	$k$	AICc	$\Delta\text{AICc}$	$W_i$
GLMM17	Distance to water body + Agricultural area	4	202.760	0.000	0.362
GLMM12	Agricultural area	3	202.903	0.143	0.337
GLMM16	Agricultural area + dove abundance	4	204.134	1.374	0.182
GLMM10	Null model	2	207.459	4.699	0.035
GLMM18	Local land use	4	207.916	5.156	0.027
GLMM14	Dove abundance	3	209.011	6.252	0.016
<i>(Z. auriculata + C. picui + P. maculosa)</i>					
GLMM11	Distance to water body	3	209.227	6.468	0.014
GLMM13	Distance to urban site	3	209.237	6.478	0.014

GLMM15	Sparrow abundance	3	209.367	6.607	0.013
--------	-------------------	---	---------	-------	-------

**Table 6.** Parameter likelihoods, estimates ( $\pm$  SE) and 95% confidence interval limits (CL) for explanatory variables describing variation in WNV infection risk for an avian host with  $\Delta\text{AICc} < 2$ . Explanatory variables with CL excluding zero are in bold.

Explanatory variable	Parameter likelihood	Parameter estimate $\pm$ SE	CL	
			Lower	Upper
Intercept		<b>-4.23 <math>\pm</math> 0.53</b>	<b>-5.28</b>	<b>-3.17</b>
Agricultural area	1.00	1.07 $\pm$ 0.61	-0.19	2.34
Distance to water body	0.41	0.65 $\pm$ 0.50	-0.32	1.64
Dove abundance	0.21	0.35 $\pm$ 0.36	-0.36	1.07

574

575

576 **Figure 1.** Sampling sites in the northeastern of La Pampa province into the Pampean Grasslands,  
577 Argentina.

578

579

580 **Figure 2.** Spatial distribution of SLEV and WNV neutralizing antibodies seroprevalence in free  
581 ranging birds collected in twelve sampling sites in La Pampa province.

582

583

584

585