Scientific Note

First detection of *Mansonia titillans* (Diptera: Culicidae) infected with St. Louis encephalitis virus (Flaviviridae: Flavivirus) and Bunyamwera serogroup (Peribunyaviridae: Orthobunyavirus) in Argentina

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Mansonia titillans is distributed in the central-northern provinces of Argentina (Rossi 2015). The females are very aggressive and can feed on several animals, including humans, which they prefer (Almirón 2002, Stein et al. 2013). The aggressiveness is such that they can also attack other mosquitoes, piercing the abdomen and sucking blood and other fluids in the intestine (Burton 1963). It is an abundant species in certain environments and times of the year, increasing the chances of mosquito-human contact.

In Argentina, the genus *Mansonia* has been associated with the epizootic of western equine encephalitis during 1982-1983 in Santa Fe Province (Mitchell et al. 1987) and from infected *Mansonia* spp. collected in chicken-baited traps where isolations of the Rio Negro virus (Venezuelan equine encephalitis virus subtype VI) were collected in the Chaco Province during 1977-1980 (Mitchell et al. 1985).

The zoonoses produced by St. Louis encephalitis virus (SLEV) and Bunyamwera serogroup can have multiple vectors and infect a variety of vertebrate host species. Infection with these viruses can range from asymptomatic to febrile syndrome to meningitis, encephalitis or even death; although humans are terminal hosts that occasionally become infected (Weaver and Reisen 2010). In addition, different strains of Bunyamwera orthobunyavirus can cause congenital malformations and abortions in animals, mainly in ruminants (Tauro et al. 2015b).

SLEV is transmitted in nature between mosquitoes of the genus *Culex* and avian hosts (Reisen 2003). It has been detected in 26 species of mosquitoes belonging to the genera *Aedes*, *Coquillettidia*, *Culex*, *Deinocerites*, *Haemagogus*, *Mansonia*, *Psorophora, Sabethes*, *Trichoprosopon,* and *Wyeomyia* collected in tropical and subtropical regions of America (Spence 1980, Kramer and Chandler 2001). SLEV was detected in *Ma. titillans* for the first time in Colombia (Hoyos-López et al. 2015).

In Argentina, several species of mosquitoes were found naturally infected with SLEV: *Aedes aegypti*, *Aedes albifasciatus*, *Aedes scapularis, Anopheles albitarsis*, *Culex apicinus*, *Culex interfor, Culex quinquefasciatus*, *Culex saltanensis,* and *Psorophora ferox* collected in Córdoba and Santa Fe Province (Mitchell et al. 1985, Diaz et al. 2012). Bunyamwera serogroup is one of the most important serogroups in the Orthobunyavirus genus (Maes et al. 2018). The cycle of transmission and amplification of Bunyamwera serogroup in nature occurs mainly between mosquitoes and vertebrates (Schmaljohn and Nichol 2007). In 1956, the first Orthobunyavirus in America, (Cache Valley virus) was isolated from *Culiseta inornata* (Holden and Hess 1959), currently classified as a subtype of Bunyamwera orthobunyavirus (Maes et al. 2018). Around the world, numerous strains have been recovered from different species of mosquitoes of the genera *Aedes*, *Anopheles*, *Culex*, *Culiseta,* and *Psorophora* (Schmaljohn and Nichol 2007). In Argentina, *Ae. albifasciatus, Ae. scapularis, Cx. quinquefasciatus,* and *Psorophora varinervis* collected in Córdoba and Santa Fe Province were found to be naturally infected with Bunyamwera serogroup (Bianchini et al. 1968, Mitchell et al. 1987, Tauro et al. 2015a).

The susceptibility of *Ma. titillans* to infection by SLEV and Bunyamwera serogroup is unknown in Argentina, therefore, due to the existing antecedents for this species, the objective of this study was to detect the presence of SLEV and Bunyamwera serogroup in adults of *Ma. titillans* in the city of Córdoba during 2013-2014.

The selected sites for the collection of mosquitoes were the Zoological Garden (ZO) (31°25'34.51"S; 64°10'33.45"W), Botanical Garden (BT) (31°23′13″S; 64°14′58″W), and the sewage plant Bajo Grande (BG) (31°23′38″S; 64°04′36″W). Adult capture was carried out for a consecutive year, covering the seasons (fall-spring of 2013) and (summer-fall of 2014). Four CDC-type light traps supplemented with dry ice were used, active from 18:00 to 09:00 the following day, for two consecutive nights. The collected mosquitoes were transported to the laboratory on dry ice, with identification based on morphological keys by Darsie (1985). Mosquitoes were grouped in pools of one to 50 individuals, separated by sex, physiological state of the female, and place and date of collection. The shaped pools were crushed in sterile mortars and diluted in minimal essential medium (Gibco®) supplemented with 10% fetal bovine serum (Natocor®) and 1% gentamicin (Klonal®). The homogenates were centrifuged at 11,000 rpm for 30 min and the recovered supernatant was used to extract the viral RNA by means of the commercial kit

Table 1. Pools of *Mansonia titillans* caught with CDC-type light traps supplemented with dry ice from BG site covering the seasons fall-spring of 2013 and summer-fall of 2014.

Pools	Date	N	Flavivirus	SLEV*	Orthobunyavirus**	Abundance
260	23/04/2013	27	Positive	Genotipo III	Positive	76
247	10/10/2013	2	Negative	Negative	Positive	2
287	14/02/2014	50	Negative	Negative	Bunyamwera serogroup	902
289	14/02/2014	37	Negative	Negative	Positive	902
290	14/02/2014	38	Negative	Negative	Bunyamwera serogroup	902
292	14/02/2014	50	Positive	Negative	NA	902
294	14/02/2014	47	Positive	Negative	NA	902
184	24/04/2014	38	Negative	Negative	Positive	869
185	24/04/2014	31	Negative	Negative	Positive	869
134	24/04/2014	30	Positive	Negative	Negative	869

*St. Louis encephalitis virus. **Bunyamwera orthobunyavirus and California encephalitis orthobunyavirus serogroup. NA: no data.

(Qiagen, Germany), according to manufacturer's instructions. Viral RNA detection was performed using the technique of RT-Nested PCR using generic primers for Flavivirus (Sánchez-Seco et al. 2005) and specific to SLEV (Ré et al. 2008). In Bunyamwera serogroup, RT-PCR was used based on the protocol developed by Kuno et al. (1996) using generic primers for Orthobunyavirus (Bunyamwera orthobunyavirus and California encephalitis orthobunyavirus serogroup). The PCR products were developed in 1.5% agarose gel stained with ethidium bromide. The amplified fragments obtained were purified and sequenced by the company, Macrogen – Korea. The obtained sequences were subjected to a BLASTn 2.2.19 - "Basic Local Alignment Search Tool"- (http://blast.ncbi.nlm.nih.gov) (Zhang et al. 2000) and they were compared with viral sequences available in GenBank.

Out of all individuals (N=6,491) captured during fall-spring of 2013 and summer-fall of 2014, 28.45 % belong to *Ma. titillans*, 60.14 % were species belonging to the genus *Culex* (*Cx. acharistus*, *Cx. apicinus*, *Cx. dolosus*, *Cx. interfor*, *Cx. mollis*, *Cx. quinquefasciatus,* and *Cx. saltanensis*), and 11.41% were of three species of *Aedes* (*Ae. aegypti*, *Ae. albifasciatus,* and *Ae. scapularis*). *Mansonia titillans* was collected only on site BG (N=1,849) and no specimens were captured on the BT and ZO sites. The largest catches of *Ma. titillans* occurred in 2014 (N=1,771) with only 78 individuals in the previous year. Summer was the season that contributed the largest numbers of individuals (Table 1).

Out of forty-eight pools of *Ma. titillans*, four pools were positive for Flavivirus. Only pool 260 was positive for SLEV, coinciding with genotype III; the other three pools were not identified as containing the Flavivirus. Regarding Bunyamwera serogroup, seven positive pools were found using the protocol of Kuno et al. (1996), although only pools 287 and 290 could be sequenced and had a 100% homology with Cache Valley virus (strain CbaAr-426) and length of 246-nucleotide (Sequence data submitted in GenBank). In addition, the 260 pool was coinfected with both viruses.

In Argentina, to date, there has been no history of natural infection in *Ma. titillans* for SLEV and Bunyamwera serogroup. The highest abundances of *Ma. titillans* were in the BG site during the summer, coinciding with the peak months of SLEV and Bunyamwera serogroup (unpublished observations). In our study, *Ma. titillans* was the second most abundant species in the BG site.

Phylogenetic analyses based on the full-length E gene sequences of the SLEV have shown the existence of eight viral genotypes (I-VIII) with heterogeneous distribution in the American continent. Genotype III is distributed in Argentina and Brazil (Rodrigues et al. 2010). In Córdoba there are four genotypes (II, III, V, and VII) of the eight described by Rodrigues et al. (2010). In Córdoba City, during the summer of 2005, there was an outbreak of encephalitis in humans caused by SLEV with 47 confirmed cases and nine deaths (Spinsanti et al. 2008). Two strains of SLEV of genotype III were isolated (CbaAr-4005 and CbaAr-4006) (Diaz et al. 2006). The molecular characterization determined that both variants are closely related to the strain SLEV 79V-2533, isolated 27 years ago in Santa Fe Province (Mitchell et al. 1985). In this study, the sequence of the SLEV genotype III strain detected is related to the strains CbaAr-4005 and CbaAr-4006 (Diaz et al. 2006).

The history of Bunyamwera serogroup activity throughout the Americas, whether it be due to isolation and/or serology, has very few records of the disease in humans. Nevertheless, Tauro et al. (2009) recorded the first Argentinian case of human infection by the Bunyamwera serogroup. Moreover, during 2013, in Argentina's Santa Fe Province, three new isolates of Bunyamwera serogroup groups with Cache Valley virus (strain CbaAr- 426) were recovered from the brain and spleen of two horses with encephalitis and from the brain of an aborted equine fetus (Tauro et al. 2015b). In this study with *Ma. titillans* it was possible to amplify Bunyamwera serogroup that grouped with the Cache Valley virus (strain CbaAr-426).

In the sewage plant Bajo Grande and Zoological Garden, there are wild and exotic vertebrates that could be a blood

source for *Ma. titillans*. Among mammals, we can find rodents, foxes, hares, chickens, cats, dogs, and humans (personal observation). On the other hand, the bird communities could be important reservoir hosts due to their active participation in the enzootic cycles. Birds increase their abundance between spring-summer coinciding with the presence of *Ma. titillans*.

In conclusion, *Ma. titillans* could play a role in the transmission of SLEV and Bunyamwera serogroup because it combines biological characteristics such as abundance in the environment coinciding with the months of greatest viral activity, with a food preference for humans. However, it would be necessary to perform vector competence tests to determine the participation of *Ma. titillans* in the transmission of SLEV and Bunyamwera serogroup.

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