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Short Communication

First isolation of Bunyamwera virus (*Bunyaviridae* family) from horses with neurological disease and an abortion in Argentina

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ABSTRACT

Bunyamwera virus (BUNV) is the prototype virus for both the *Orthobunyavirus* genus and the *Bunyaviridae* family. Different strains of BUNV have been associated with clinical diseases in domestic animals, mainly ruminants. During 2013, in Argentina's Santa Fe Province, three new isolates of BUNV were recovered from the brain and spleen of two horses with encephalitis, and from the brain of an aborted equine fetus. This isolation of BUNV from domestic animals provided the first association of BUNV infection with disease of the central nervous system and abortion in equines in Argentina.

Orthobunyavirus is the largest genus of the *Bunyaviridae* family, with 48 species isolated worldwide (Dixon et al., 2012). The genus contains multiple human and animal pathogens distributed in 18 serogroups, including Bunyamwera, California, and Simbu serogroups (Calisher, 1996). *Bunyamwera* virus (BUNV) belongs to the first serogroup and is a mosquito-borne pathogen originally isolated from *Aedes* spp. in Africa in 1943. In the Americas, the first orthobunyavirus closely related to BUNV to be isolated was Cache Valley virus (CVV), recovered from *Culiseta inornata* in the USA in 1956 (Calisher, 1996). Currently, according to the International Committee of Taxonomy of Viruses, CVV is considered to be a strain or isolate of BUNV (Dixon et al., 2012).

BUNV can infect a wide range of mammals, and several strains cause diseases in domestic animals and are of veterinary concern. Infection can lead to abortion and produce teratogenic effects, especially in ruminants (Rodrigues Hoffmann et al., 2013), and diseases of the central nervous system (CNS) may occur in horses (Spence and Downs, 1968; Sanmartín et al., 1973).

In Argentina, two strains of BUNV have been recovered from mosquitoes, namely, (1) CbaAr-426, previously classified as CVV (Cordoba, 1964/1965), and (2) AG83-1746, previously classified as Maguari virus (MAGV) (Santa Fe, 1981) (Sabattini et al., 1998). Serological surveys have detected high prevalence of BUNV infection in humans as well as in domestic and wild life animals (Sabattini et al., 1998; Tauro et al., 2009), but only febrile diseases have been reported in humans infected by virus of the Bunyamwera serogroup (Tauro et al., 2012). We describe here the first isolation of BUNV from two horses showing clinical signs of neurological disease and from an aborted horse fetus.

In April 2013, two horses from Santa Fe Province developed neurological signs and died; the first was a 2-year-old male (Eq001), from Las Colonias Department, and the second was a 3-years old stallion (Eq002) from San Cristobal Department (Fig. 1). Clinical signs included circular displacement, disorientation, ataxia, muscular hypotonia, sensory depression, partial blindness, tongue protrusion and decubitus. Both horses died, 10 and 6 days, respectively, after the onset of clinical signs.

In July 2013, a mare that had commingled with Eq002 aborted after 8 months of pregnancy. Histopathological studies on the brains of Eq001 and Eq002 showed non suppurative encephalitis; these samples, as well as those from the aborted fetus, had previously produced negative PCR results for other pathogens frequently associated with equine encephalitis and/or abortions in horses, including rabies, equine herpesvirus 1 and 4, West Nile virus, Saint Louis encephalitis

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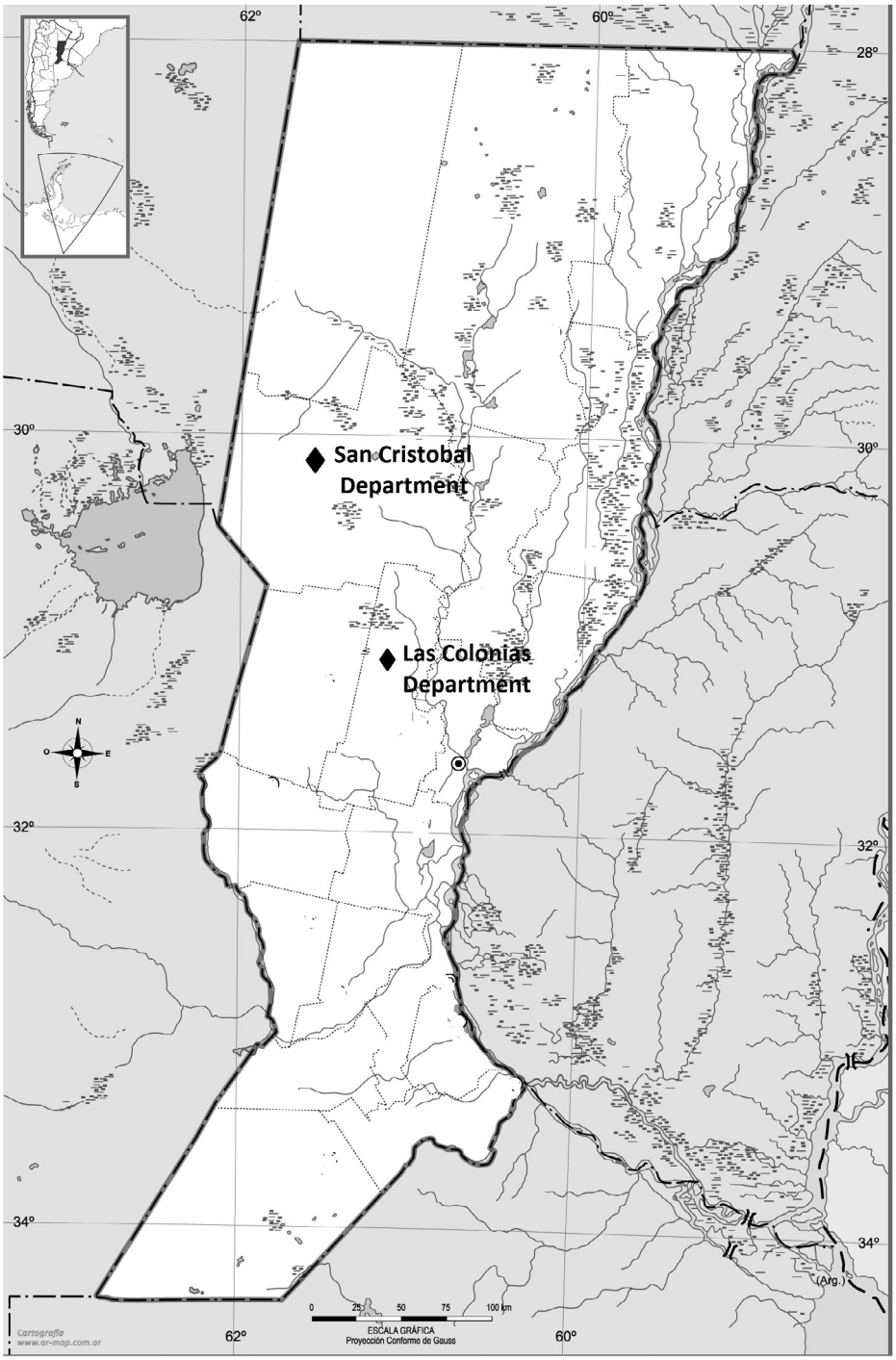

Fig. 1. Sampling locations of the BUNV investigation conducted in Santa Fe province, Argentina.

virus, Eastern equine encephalitis virus, Western equine encephalitis virus and Venezuelan encephalitis virus. Samples of brain, kidney, spleen and liver from the horses and from the brain of the fetus were screened for *Orthobunyavirus* genus by reverse transcriptase (RT)-PCR, as described by Kuno et al. (1996). The brain and kidney of Eq001, the brain and spleen of Eq002 and fetal brain all yielded positive results.

An aliquot of 0.1 mL of each tissue fragment was inoculated into a mosquito cell line C6/36 (*Ae. albopictus*) monolayer. Since the cytopathic effect could not be observed in these cells, all viral passages were analysed by RT-PCR to confirm the presence of virus RNA. Three new isolates of *Orthobunyavirus* were recovered, one from the brain of Eq001 (SFCrEq231), another from the spleen of Eq002 (SFBzEq232) and the third from the fetal brain (SFAbCrEq238). All isolates were recovered after a 5-day incubation period in the third blind passage in C6/36 cells. Titration of supernatants and cells by plaque assay in Vero cells yielded titres of 3×10^8 plaque forming units (PFUs)/mL (SFCrEq231), 9×10^6 PFUs/mL (SFBzEq232) and 11×10^6 PFUs/mL (SFAbCrEq328).

PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) and sequenced by an ABI 3130 automatic sequencer in both directions. The consensus sequences obtained with Geneious

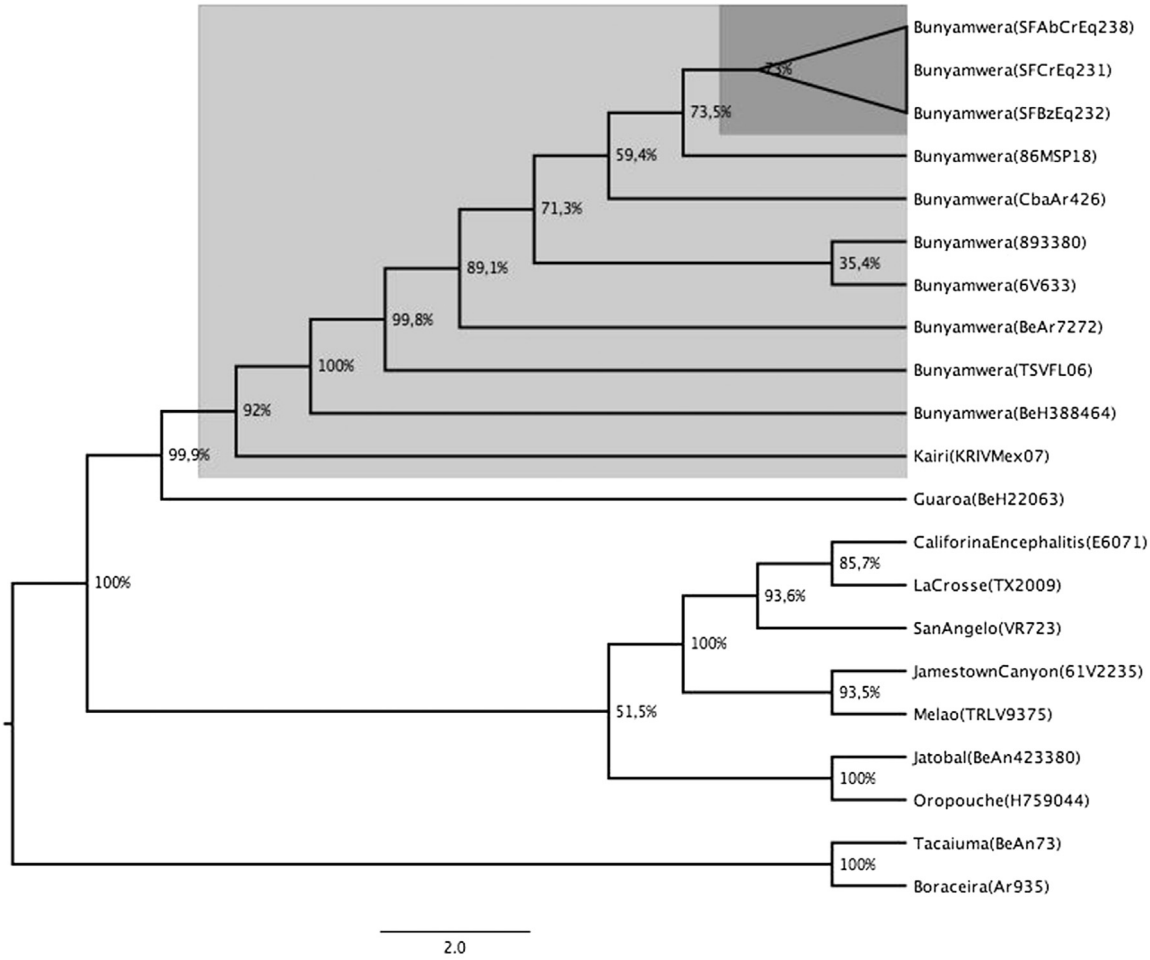

Fig. 2. Consensus tree analysing a 251 bp fragment of the S segment of *Orthobunyavirus* using the Neighbour joining method and a p-distance model bootstrapped 1000 times. Viruses of the *Orthobunyavirus* genus detected in America belonging to serogroups Anopheles A, Anopheles B, California, Simbu and Bunyamwera were included. The Bunyamwera serogroup is indicated in light grey and the three new strains detected in Argentina in dark grey. GenBank accession numbers for sequences used in the phylogenetic analysis are as follows: Bunyamwera virus (SFCrEq231): KM113024, Bunyamwera virus (SFBzEq232): KM113023, Bunyamwera virus (SFAbCrEq238): KM113025, Bunyamwera virus (86MSP18): EU564829, Bunyamwera virus (CbaAr-426): KM205207, Bunyamwera virus (BeAr7272): M28380, Bunyamwera virus (89-3380): AY729652, Bunyamwera virus (BeH388464): EU564830, Bunyamwera virus (6V633): X73465, Bunyamwera virus (TSV-FL06): FJ943507; Kairi virus (KRIV-Mex07): EU879063; Guaroa virus (BeH22063): X73466; Jatobal virus (BeAn423380): JQ675601; Oropouche virus (H759044): HQ830491; Jamestown Canyon virus (61V2235): HM007350; Melao virus (TRLV9375): U12802; San Angelo virus (VR723) U47139; California Encephalitis virus (E6071): U12800; La Crosse virus (TX2009): K00610; Tacaiuma virus (BeAn73): FJ660416; Boraceira virus (A935): FJ660418.

v.7.1.7 software were subjected to a BLASTn analysis[1] in order to identify the degree of homology with other orthobunyaviruses previously detected in America.

Multiple sequence alignments were performed with MAFFT v7. For phylogenetic analysis we used Mega version 4.0 software by means of the Neighbour joining method and a p-distance model bootstrapped 1000 times (Fig. 2). The resulting sequences grouped within the Bunyamwera serogroup and were closely related to BUNV. The three sequences showed 100% nucleotide identity and 99.6% with BUNV strain 86MSP18 (previously classified as Forth Sherman virus) that was isolated in Panama in 1985 from a febrile human (Mangiafico et al., 1998). The only strain of BUNV isolated in Argentina and available in the GenBank was the sequence corresponding to the strain CbaAr426, which showed a nucleotide identity of 97.5% with the new sequences.

We are therefore able to report the finding of three new isolates of BUNV in Argentina, two from horses that died due to a neurological disease and one from an aborted equine fetus. Even though a high seroprevalence of BUNV in cows and horses has previously been detected in Argentina, particularly in Córdoba and Santa Fe Provinces, there are no records of disease, death or abortions induced by the BUNV strains that are currently known to be circulating in the country. This is the first report associating BUNV isolated from domestic animals with neurological disease and death.

In the aborted equine fetus, known viruses that frequently produce abortions in pregnant mares were ruled out, so the isolation of BUNV from the brain of the fetus suggested that infection with SFAbCrEq238 could have been responsible for the abortion.

It is important to note that the new strains identified here had a high percentage identity (95.5%) with the Brazilian strain BeAr7272, which was originally classified as MAGV, like the strains isolated in Argentina and the strains recovered from sick horses in Guyana and Colombia. Our results highlight that BUNV can produce severe disease in animals and should be included in epidemiological surveillance systems in order to determine its likely real impact and to improve differential diagnosis.

[1] See: http://blast.ncbi.nlm.nih.gov (accessed 15.06.17).

Conflict of interest statement

None of the authors has financial or personal relation with other people or organizations that could inappropriately influence or bias the content of the paper.

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