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Short Communication

Molecular survey of flaviviruses and orthobunyaviruses in *Amblyomma* spp. ticks collected in Minas Gerais, Brazil

Investigação molecular de flavivírus e orthobunyaviruses em carrapatos do gênero *Amblyomma* spp. coletados em Minas Gerais, Brasil

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Abstract

Due to anthropic environmental changes, vector-borne diseases are emerging worldwide. Ticks are known vectors of several pathogens of concern among humans and animals. In recent decades, several examples of tick-borne emerging viral diseases have been reported (Crimean Congo hemorrhagic fever virus, Powassan virus, encephalitis virus, heartland virus, severe fever with thrombocytopenia syndrome virus). Unfortunately, few studies addressing the presence of viruses in wild ticks have been carried out in South America. With the aim of detecting flaviviruses and orthobunyaviruses in ticks, we carried out molecular detection in wild ticks collected in the state of Minas Gerais, Brazil. No *Flavivirus*-positive ticks were detected; however, we detected activity of *Orthobunyavirus* in 8 *Amblyomma* tick specimens. One of those individuals was positive for *Bunyamwera orthobunyavirus,* which represents the first report of this virus among ticks in South America. Further studies related to the ecology of zoonotic diseases are needed to increase knowledge of this topic, including attempts at viral isolation, full genome sequencing and biological characterization. In this way, we will obtain a better picture of the real risk of ticks as a vector for viral diseases for humans and animals on our continent, where no tick-borne viral disease is known to occur.

Keywords: *Bunyamwera orthobunyavirus*, ticks, *Orthobunyavirus*, *Amblyomma* spp.

Resumo

Alterações ambientais causadas pelo homem têm levado à emergência de doenças transmitidas por vetores no mundo. Carrapatos são vetores conhecidos de vários patógenos de importância médica e veterinária, tendo sido reportado nas últimas décadas um grande número de enfermidades virais emergentes transmitidas por eles (vírus da Febre Hemorrágica da Crimeia-Congo, vírus Powassan, vírus da Encefalite, vírus Heartland e vírus da Síndrome da Febre Trombocitopênica Severa). Infelizmente, poucos estudos envolvendo a pesquisa de vírus em carrapatos foram conduzidos na América do Sul até o momento, e nas últimas décadas um elevado número de enfermidades virais emergentes transmitidas por estes artrópodes foi relatado. Com o objetivo de investigar a presença de flavivírus e orthobunyavírus em carrapatos, foi conduzida uma análise molecular em espécimes coletados no estado de Minas Gerais, Brasil. Em nenhum carrapato foi detectada a presença de *Flavivirus*, no entanto, em 8 espécimes do gênero *Amblyomma*, foi detectada a presença de *Orthobunyavirus*, dos quais um espécime foi positivo para *Bunyamwera orthobunyavirus*. Novos estudos relacionados à ecologia de doenças zoonóticas, incluindo tentativas de isolamento viral, sequenciamento completo do genoma e caracterização biológica, são necessários. Desta forma, será possível ter uma base sobre os riscos da transmissão de vírus patogênicos por carrapatos em nosso continente, uma vez que até agora isso é desconhecido.

Palavras-chave: *Bunyamwera orthobunyavirus*, carrapatos, *Orthobunyavirus*, *Amblyomma* spp.

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Introduction

Vector-borne viruses are the most important infectious diseases to have emerged in the last three decades, affecting human populations, wildlife and animal production. Chikungunya, Dengue, Japanese encephalitis, Rift Valley Fever, St. Louis encephalitis, yellow fever, West Nile and Zika are emerging mosquito-borne viruses of concern in our region and worldwide (HUANG et al., 2019). Along with mosquitoes, ticks are major vectors of pathogens and can transmit viruses, bacteria and parasites. Among the viruses transmitted by ticks, members of the *Flaviviridae* and *Peribunyaviridae* families play a major role (BARTÍKOVÁ et al., 2017). Within the genus *Flavivirus* (family *Flaviviridae*), the Tick-borne encephalitis virus serogroup (TBEV) (European, Far-eastern and Siberian subtypes) represents a public health issue in countries such as Russia, Hungary, Romania and Bulgaria, among other eastern European countries (RUZEK et al., 2019). Powassan virus (POWV) (*Flavivirus*), another encephalitogenic flavivirus that is endemic in the northeast area of the US, is transmitted by *Ixodes cookei* Packard, *Ixodes marxi* Banks, and *Ixodes scapularis* Say (KEMENESI & BÁNYAI, 2018). Kyasanur Forest disease virus (KFDV) is an endemic hemorrhagic flavivirus in India, where it is vectored by *Haemaphysalis spinigera* Neumann (MANSFIELD et al., 2017). Crimean-Congo hemorrhagic fever orthonairovirus (CCHFV, *Orthonairovirus*, *Peribunyaviridae*) is transmitted by *Hyalomma* spp. ticks and is the causative agent of hemorrhagic fever cases in the Crimean and Congo regions, although notable outbreaks have recently been reported in the Mediterranean area (MANSFIELD et al., 2017). Currently, there is no evidence of tick-borne viruses affecting humans or animals in South America, although there have been a few records of different viruses infecting ticks. In a recent review presented by Nuttall (2014), it was indicated that only two tick-borne viruses have been reported in South America: Matucare virus (*Reoviridae: Orbivirus*) in *Ornithodoros kohlsi* Guglielmone & Keirans (reported as *Ornithodoros boliviensis*) in Bolivia and Huachovirus (*Reoviridae: Orbivirus*) in *Ornithodoros amblus* Chamberlin in Peru. More recently, Figueiredo et al. (2017) reported Cacipacoré virus (*Flaviviridae: Flavivirus*) in *Amblyomma sculptum* Berlese (reported as *Amblyomma cajennense*) in São Paulo state, Brazil, and Maruyama et al. (2014) and Pascoal et al. (2019) reported Mogiana tick virus (*Flaviviridae: Jingmenvirus*) in *Rhipicephalus microplus* (Canestrini) ticks from different regions of Brazil. This scarcity of records makes South America the continent with the fewest tick-borne viruses in the world (NUTTALL, 2014). Hence, the aim of this study was to search for flaviviruses and orthobunyaviruses in ticks collected in Brazil.

Materials and Methods

Sampling sites and tick collection

Ticks were collected between 2007 and 2013 at three sites in the state of Minas Gerais, southeastern Brazil: Serra da Canastra National Park (20°18′S, 46°32′W) - ticks collected from vegetation; Grande Sertão Veredas National Park (15°18', 45°49') - ticks collected from vegetation; and the Guarda Mor municipality (17°42'S, 47°04'W) – ticks collected from a road-killed capybara. Details on the collection of these ticks in each locality have been published elsewhere (COSTA et al., 2017; SZABÓ et al., 2018; BARBIERI et al., 2019). Although all three areas were within the Cerrado biome, the two National Park areas were highly preserved, whereas the area where the road-killed capybara was found was dominated by an agricultural landscape.

Molecular detection and phylogenetic analyses

All ticks were brought to the laboratory alive, where they were kept frozen at -80°C until RNA extraction. Individual ticks were tested for the presence of orthobunyaviruses and flaviviruses. Viral RNA was extracted using the QIAamp Viral RNA Minikit (Qiagen, Germany) according to the manufacturer's instructions. The RT conditions were as follows: an initial step of 10 min at 65 °C for denaturation, then 60 min at 40°C for reverse transcription; and 10 min at 65 °C for denaturation. Reverse-transcription polymerase chain reaction (RT-PCR) was performed for *Orthobunyavirus* (Bunyamwera and California serogroups) detection, amplifying a fragment of 251 bp of the S segment using the primers described by Kuno et al. (1996). For the detection of flavivirus, we performed a generic RT-PCR assay developed by Sánchez-Seco et al. (2005) that amplifies an NS5 protein fragment of 143 nucleotides in length. Each PCR was performed including a positive control: BeAr-8226 Kairi virus strain (*Orthobunyavirus*) and CbaAr-4005 St. Louis encephalitis virus strain (*Flavivirus*), respectively. PCR products of the expected size were purified using the QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA, U.S.A.) and then sequenced by the Sanger method by an external contractor (Macrogen, Korea). The consensus sequence obtained was subjected to a BLASTn 2.2.19 search (Basic Local Alignment Search Tool) (ZHANG et al., 2000). For the phylogenetic analyses an alignment containing *Orthobunyavirus* genus sequences detected in America belonging to the Anopheles A, Anopheles B, Simbú, California and Bunyamwera serogroups were included (see Figure 1 legend for GenBank accession numbers). A maximum likelihood tree was constructed using Kimura 2-parameter model and branch supported was estimated with bootstrap (1000 times). The nucleotide substitution model was estimated by used of ModelTest (POSADA & CRANDALL, 1998).

Results and Discussion

Between 2007 and 2013, a total of 98 ticks of the genus *Amblyomma* were collected in the three above mentioned areas of the state of Minas Gerais: 40 *A. sculptum*, 6 *Amblyomma dubitatum* Neumann and 4 *Amblyomma brasiliense* Aragão collected on vegetation at Serra da Canastra National Park; 12 *A. sculptum* and 6 *Amblyomma parvum* Aragão collected on vegetation at Grande Sertão Veredas National Park; and 30 *A. dubitatum* collected on a road-killed capybara (*Hydrochoerus hydrochaeris*) in the Guarda Mor municipality. All ticks analyzed by RT-PCR were negative for *Flavivirus*. Overall, 5 ticks (4 *A. dubitatum*, 1 *A. sculptum*) were positive for *Orthobunyavirus* (Table 1).

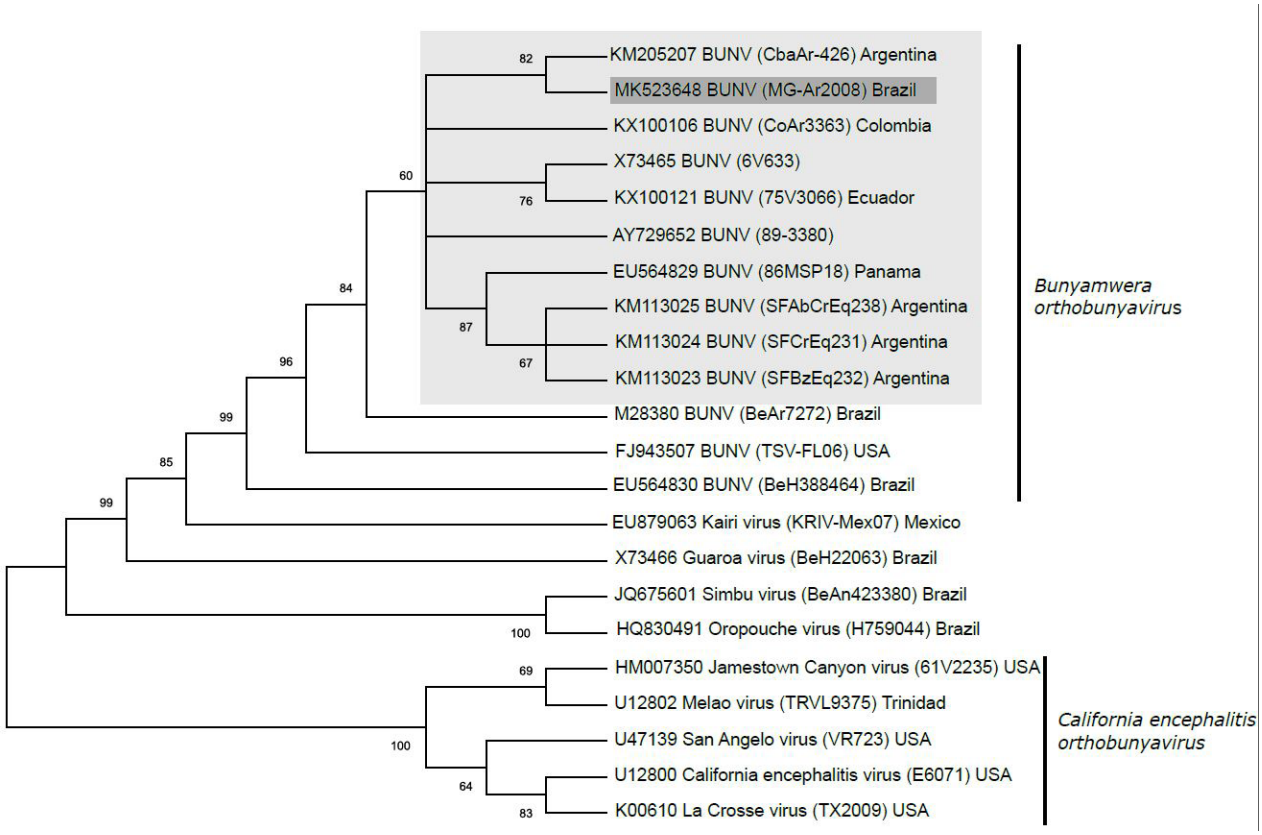

Figure 1. Consensus tree obtained from the analysis of a 251 bp fragment of the S segment of *Orthobunyavirus* using the maximum likelihood method and a Kimura 2-parameter model bootstrapped 1000 times. Viruses of the *Orthobunyavirus* genus detected in America belonging to the Anopheles A, Anopheles B, Simbú, California and Bunyamwera serogroups were included. The GenBank accession numbers for the sequences used in the phylogenetic analysis are as follows: Bunyamwera virus (CoAr 3363): KX100106, Bunyamwera virus (MGAr-2008): MK523648, Bunyamwera virus (75V3066): KX100121, 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.

Table 1. Ticks positive for *Orthobunyavirus* collected in Minas Gerais, Brazil.

ID	Tick species	Sex	Collection site	Collection date	RT-PCR and sequencing results	
					Flavivirus	***Orthobunyavirus***
213	*A. dubitatum*	male	Grande Sertão Veredas, Guarda Mor - MG	August 2013	Negative	Unidentified
225	*A. dubitatum*	female	Grande Sertão Veredas, Guarda Mor - MG	August 2013	Negative	Unidentified
235	*A. dubitatum*	male	Grande Sertão Veredas, Guarda Mor - MG	August 2013	Negative	Unidentified
238	*A. dubitatum*	male	Grande Sertão Veredas, Guarda Mor - MG	August 2013	Negative	Unidentified
20	*A. sculptum*	female	Serra da Canastra National Park- MG	November 2008	Negative	BUNV **(Genbank Acc. Nbr.: MK523648)**

BUNV: *Bunyamwera orthobunyavirus*.

Most of the amplified fragments exhibited a weak band in agarose gels, and only one sample was able to be sequenced (sample #20, GenBank accession number MK523648). According to the BLASTn analysis, the sequence showed 98% nucleotide identity with *Bunyamwera orthobunyavirus* (BUNV), Laguna Larga strain CbaAr-426 (KM205207.1) isolated in Córdoba, Argentina, in 1964 from an *Aedes albifasciatus* mosquito (SABATTINI et al., 1998) (Figure 1). The sequence shared high nucleotide identity (95%-98%) with other BUNV strains isolated in Colombia, Mexico, Panamá and USA (Figure 1). Orthobunyaviruses are highly associated with mosquitoes as vectors; however, a previous study detected a high infection rate of BUNV (prototype virus

of the *Orthobunyavirus* genus) in ticks collected from several mammals in an Old-World region (LWANDE et al., 2013). In that study, BUNV strains were isolated from *Amblyomma lepidum* Dönit, *Amblyomma gemma* Dönit, *Hyalomma* spp., *Hyalomma truncatum* Koch and *Rhipicephalus pulchellus* (Gerstäcker) ticks collected from cattle, sheep, goats, giraffe and warthog in Kenya (LWANDE et al., 2013). *Bunyamwera orthobunyavirus* exhibits a wide distribution on the American continent, from the USA to the central region of Argentina. In Brazil, the former Maguari virus is the only member of the *Bunyamwera orthobunyavirus* species with known activity. BUNV is considered the etiological agent of acute febrile syndrome, central nervous system (CNS) pathologies (encephalitis and meningitis) and congenital malformations in humans (ELLIOTT & SCHMALJOHN, 2013). Several strains cause diseases in domestic animals and are of veterinary concern. Infection can produce teratogenic effects, especially in ruminants (RODRIGUES HOFFMANN et al., 2013), and diseases of the CNS and abortions in horses (TAURO et al., 2015).

Tick-borne viral zoonoses have emerged/reemerged in the last decade (MANSFIELD et al., 2017). Recently, CCHFV has emerged in Spain and Turkey, largely because of the dispersion of its vector *Hyalomma* spp. ticks and as consequence of environmental changes, such as deforestation for livestock production (HAWMAN & FELDMANN, 2018). Human cases of POWV reported in the Great Lake regions and along the northeast coast of the USA have increased in recent years (KEMENESI & BÁNYAI, 2018). The geographic distribution of KFDV in India is expanding, with viral activity being reported in new areas such as the provinces of Karnataka, Kerala, Tamil Nadu and Maharashtra (MANSFIELD et al., 2017). New human-pathogenic phleboviruses, such as heartland virus (*Orthophlebovirus*) and SFTSV (*Orthophlebovirus*), were discovered in the last decade (MCMULLAN et al., 2012; YU et al., 2011). Improvements in diagnostic techniques and better training of medical teams can explain this pattern. However, anthropic changes such as deforestation and expansion of urbanized areas are increasing the risk of human exposure to vectors and, thus, the likelihood of increasing emergence of viral zoonoses (MANSFIELD et al., 2017).

There are ≈200 tick species described in Latin America (GUGLIELMONE et al., 2003; NAVA et al., 2017), including 73 species specifically in Brazil (GIANIZELLA et al., 2018; MUÑOZ-LEAL et al., 2018). Several species have been reported as vectors of bacteria, especially those of the genera *Rickettsia* and *Borrelia* (PADDOCK et al., 2016). Ticks can also be vectors of several arboviruses, although this has yet to be demonstrated in South America.

We are aware of the limitations of our data reported herein; nevertheless, our findings represent confirmation of tick-borne viruses circulating in South America, particularly in Brazil. Studies on tick-borne viruses are lacking in South American countries, and we therefore have no evidence of the role of ticks as vectors for viral infections of human and veterinary concern. We encourage the scientific community interested in the ecology of zoonotic diseases to increase studies on this topic, including attempts at viral isolation, full genome sequencing and biological characterization. In this way, we will obtain a better picture of the real risk of ticks as a vector for viral diseases in humans and animals.

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