Ciência Rural, Santa Maria, v.53:2, e20210713, 2023
http://doi.org/10.1590/0103-8478cr20210713
ISSNe 1678-4596
MICROBIOLOGY

Seroprevalence of arboviruses in *Nasua nasua* (Mammalia, Carnivora, Procyonidae) of synanthropic habitats in the Iguaçu National Park, Brazilian Atlantic Forest

Jéssica Cecília Pinheiro Rodrigues[1] Isabela de Souza Parry[2] Thais de Cássia Mouzinho Lopes[3] Éder Barros dos Santos[1] Lívia Carício Martins[1] Adivaldo Henrique da Fonseca[4] Paulo Cesar Magalhães-Matos[5*]

[1]Departamento de Arbovirologia e Febres Hemorrágicas, Instituto Evandro Chagas (SAARB-IEC), Ananindeua, PA, Brasil.
[2]Laboratório de Zoonoses e Saúde Pública, Programa de Pós-graduação em Saúde Animal na Amazônia (PPGSAAM), Instituto de Medicina Veterinária, Universidade Federal do Pará (UFPA), Castanhal, PA, Brasil.
[3]Setor de Diagnóstico por Imagem do Hospital Veterinário Prof. Mário Dias Teixeira, Universidade Federal Rural da Amazônia (UFRA), Belém, PA, Brasil.
[4]Laboratório de Doenças Parasitárias, Departamento de Epidemiologia e Saúde Pública, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, RJ, Brasil.
[5]Curso de Medicina Veterinária, Instituto Federal de Educação, Ciência e Tecnologia do Amapá (IFAP), 68997-000, Porto Grande, AP, Brasil. E-mail: paulo.matos@ifap.edu.br. *Corresponding author.

ABSTRACT: *Arboviruses are agents transmitted by arthropods and represent a considerable threat to public health worldwide, causing diseases in humans and animals. A serological investigation was carried out to detect total antibodies against different types of arboviruses in free-living coatis (Nasua nasua) from the Iguaçu National Park (INP) through the Hemagglutination Inhibition test. Serum samples were tested using antigens from 33 arboviruses belonging to the genera Alphavirus, Flavivirus, Orthobunyavirus, and Phlebovirus. The data showed that 23.6% (17/72) of coatis were seropositive for at least one of the tested antigens, including monotypic and heterotypic reactions. Seropositivity was detected for Alphavirus (5.9%, 1/17; WEEV), Flavivirus (64.7%, 11/17; YFV, ILHV, SLEV, BSQV, ROCV, WNV, DENV-1, DENV-2, DENV-3, DENV-4, and NJLV), Phlebovirus (88.2%, 15/17; ICOV and BUJV) and Orthobunyavirus (5.9%, 1/17; ORIV). The presence of antibodies to these viruses in coatis from INP indicated an apparent silent circulation of arbovirus, implying N. nasua to be a possible amplifying host of these arboviruses in the studied area. The data reported also serve as a warning about the possible risk of establishing an arbovirus transmission cycle involving vector arthropods and coatis, or even other wild animals, consequently, including humans in this transmission chain.*
Key words: *arboviruses, coati, hemagglutination inhibition, serology, zoonoses.*

Soroprevalência de arbovirus em *Nasua nasua* (Mammalia, Carnivora, Procyonidae) de hábitos sinantrópicos no Parque Nacional do Iguaçu, Mata Atlântica brasileira

RESUMO: *Os arbovírus são agentes transmitidos por artrópodes que representam considerável ameaça à saúde pública em todo o mundo, causando doenças em humanos e animais. Neste trabalho foi realizada investigação sorológica para detecção de anticorpos totais contra diferentes tipos de arbovírus em quatis (Nasua nasua) de vida livre do Parque Nacional do Iguaçu (PNI) através do teste de Inibição da Hemaglutinação. Amostras de soro foram testadas utilizando-se antígenos de 33 arbovírus pertencentes aos gêneros Alphavirus, Flavivirus, Orthobunyavirus e Phlebovirus. As análises evidenciaram que 23,6% (17/72) dos quatis apresentaram soropositividade para pelo menos um dos antígenos testados, incluindo reações monotípicas e heterotípicas. Foi detectada soropositividade para Alphavirus (5,9%, 1/17; WEEV), Flavivirus (64,7%, 11/17; YFV, ILHV, SLEV, BSQV, ROCV, VNO, DEN1, DEN2, DEN3, DEN4, NJLV), Phlebovirus (88,2%, 15/17; ICOV, BUJV) e Orthobunyavirus (5,9%, 1/17; ORIV). A presença de anticorpos para esses vírus em quatis do PNI indica uma aparente transmissão silenciosa de arbovírus, incluindo N. nasua como um possível amplificador destes arbovírus na área estudada. Os dados encontrados servem de alerta quanto ao possível risco de estabelecimento de um ciclo de transmissão de arbovírus envolvendo insetos vetores e quatis, ou ainda, outros animais silvestres, consequentemente, podendo incluir o homem nessa cadeia de transmissão.*
Palavras-chave: *arboviroses, inibição da hemaglutinação, quati, sorologia, zoonoses.*

INTRODUCTION

The Iguaçu National Park (INP) is one of the main centers of ecotourism convergence in Brazil and is part of the official list of Natural Heritage of Humanity by the United Nations Educational, Scientific and Cultural Organization (UNESCO) since 1986 (GUIMARÃES et al., 2003). The INP has an abundant

Received 10.01.21 Approved 02.20.22 Returned by the author 04.30.22
CR-2021-0713.R1
Editor: Rudi Weiblen

faunal diversity of mammals, such as jaguars, tapirs, and coatis (FERNANDES & GARCIA, 2011).

Coatis (*Nasua nasua*) are omnivorous animals that have a rich diet characterized mainly by insects and wild fruits (GOMPPER & DECKER, 1998). Populations of coatis are quite tolerant of anthropogenic disorders, which has facilitated the synanthropic process, and thus, the risk of transmission of zoonotic pathogens, such as those causing rabies, arboviruses, and leishmaniasis (ALVES-COSTA & ETEROVICK, 2007; GUIMARÃES et al., 2012).

Arboviruses are agents transmitted by arthropods and represent a considerable threat to public health worldwide (WEAVER & REISEN, 2010), causing diseases in humans and animals (ICTV, 2020). In infected humans, arbovirus can range from undifferentiated, moderate, or severe febrile illnesses, skin rashes, and arthralgia to neurological and hemorrhagic syndromes (CLETON et al., 2012). Therefore, this study investigated the seroprevalence of arbovirus in coatis of synanthropic habits in the INP, Paraná State, Brazil.

MATERIALS AND METHODS

Study animals and sample collection

Serum samples were collected between 2014 and 2015 from 72 coatis, males and females, of synanthropic habits in the INP, Paraná State, Southern Brazil, using three sampling locations (Figure 1). Coatis were attracted to peanut baits, captured with a hand net, and anesthetized with a combination of tiletamine-zolazepan (Zoletil 50, Virbac®, Carros/France, 7 mg/kg), and xylazine hydrochloride 2% (Xilazin, Syntec®, São Paulo/Brazil, 2 mg/kg), administered intramuscularly. After recovery, all animals were reintroduced into their original habitat. Blood samples obtained through the jugular vein were centrifuged (2000 rpm, 5 minutes) to obtain serum samples, which were identified and stored at -70 ºC until further analysis.

Hemagglutination inhibition test

The serum samples were subjected to the hemagglutination inhibition (HI) test according to CLARKE & CASALS (1958), with adaptation to microplate by Shope (1963). The study was carried out at the Section of Arbovirology and Hemorrhagic Fevers of the Evandro Chagas Institute (SAARB/IEC), municipality of Ananindeua, Pará State, Brazil.

In the screening step, the samples were tested against an antigen panel composed of 33 different types of arbovirus that antigens were prepared by sucrose-acetone and sorovirus extraction methods (CASALS & BROWN, 1954). Then, 25 µL of the treated serum (diluted 1:20) and 25 µL of antigens from the 33 types of arbovirus were added: *Alphavirus* [Eastern Equine Encephalitis (EEEV), western equine encephalitis (WEEV), Mayaro (MAYV), Mucambo (MUCV), Aura (AURAV), and Pixuna virus (PXV)], *Flavivirus* [yellow fever (YFV), Ilheus (ILHV), Saint Louis encephalitis (SLEV), Rocio (ROCV), Bussuquara (BSQV), Cacipacoré (CPCV), West Nile (WNV), Dengue 1 (DENV-1), Dengue 2 (DENV-2), Dengue 3 (DENV-3), Dengue 4 (DENV-4), Zika (ZIKAV), and Naranjal-like virus (NJLV)], *Orthobunyavirus* [Maguari (MAGV), Tacaiuma (TCMV), Caraparu (CARV), Oropouche (OROV), Catu (CATUV), Utinga (UTV), Itaqui (ITV), Apeu (APEUV), Murucutu (MURV), Oriboca (ORIV), and Marituba virus (MTBV)], and *Phlebovirus* [Icoaraci (ICOV), Bujaru (BUJV), and Urucuri virus (URUV)], diluted in the proper proportion in order to obtain four hemagglutinating units in each well of the microplate and incubated at 4 ºC for 12h. Then, 50 µL of the HI test developer system, consisting of goose red blood cells in a solution diluted in dextrose, gelatin, and veronal (DGV), in a proportion of 1:5 ratio at an appropriate pH (6.0-7.0, according to the virus), was added to each well. The microplate was shaken and incubated for 30 min at the appropriate temperature (15-37 ºC, according to the virus).

Samples that showed antibody titers during the screening step were tested in the titration step. Positive serum (25 µL) was added and diluted using 0.4 % bovine albumin serum at pH 9.0. Next, 25 µL of the antigen was added and incubated for 12 h at 4 ºC. The result was considered positive when the erythrocyte sedimentation and the titer were greater than or equal to 1:20.

RESULTS AND DISCUSSION

Of the 72 samples analyzed, 17 (23.6%) were positive for at least one of the tested antigens, including monotypic (when the test result is positive for only one of the tested antigens in the same viral genus) and heterotypic (when the result is positive for more than one antigen of the same viral genus) reactions (RODRIGUES et al. 2010). Seropositivity was detected for *Alphavirus* (5.9%, 1/17; WEEV), *Flavivirus* (64.7%, 11/17; YFV, ILHV, SLEV, BSQV, ROCV, WNV, DENV-1, DENV-2, DENV-3, DENV-4, and NJLV), *Phlebovirus* (88.2%, 15/17; ICOV, BUJV), and *Orthobunyavirus* (5.9%, 1/17; ORIV) (Table 1), with titers ranging from 1:20 to 1:80.

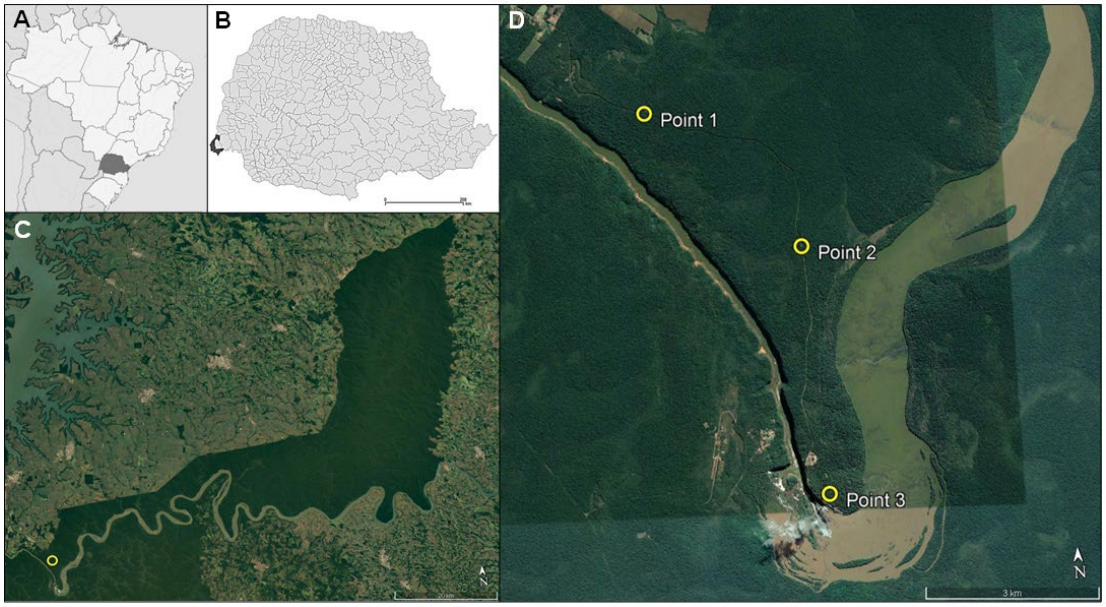

Figure 1 - Geographic location of the study area. A) Brazil, with emphasis on the Paraná State; B) The municipality of Foz do Iguaçu (west region) is highlighted in the State; C) Iguaçu National Park area, highlighting the tourist area (point); D) Sampling locations in the tourist area. Source: A, B: present study; C, D: Google Earth™ software (georeferenced).

Seven monotypic reactions were identified, and the frequency of these reactions was 71.4 % (5/7) for BUJV, 14.3 % (1/7) for DENV-4, and 14.3 % (1/7) for WEEV. Most of the individually detected antibodies belonged to the genus *Flavivirus*. Antibodies against the genus *Alphavirus* were detected in only one sample. Similarly, antibodies against *Orthobunyavirus* were detected in only one sample but with simultaneous reactions for the genera *Flavivirus* and *Phlebovirus*.

Of the seropositive animals, 58.82% (10/17) presented a heterotypic reaction for *Flavivirus*, *Phlebovirus*, and/or *Orthobunyavirus*. *Phlebovirus* was the most prevalent genus, occurring in 88.23% of seropositive animals (15/17), and *Flavivirus* exhibited the highest cross-reactivity, due to the high sensitivity of the HI test, it is common for there to be a high rate of cross-reactions between viruses belonging to the same genus, as they are similar to each other (VASCONCELOS, 2003).

This study is the first to specifically investigate the role of coatis as possible hosts of arbovirus and shows the distribution and diversity of the arboviruses who belong to the genera *Alphavirus*, *Flavivirus*, *Phlebovirus*, and *Orthobunyavirus*, in the INP. In Brazil, remnants of the Atlantic Forest and the Amazon rainforest are known to be the largest reservoirs of arbovirus (SILVA & ANGERAMI, 2008), and the serological findings of the present study suggested the same trend in the INP.

The Foz de Iguaçu region brings together the factors considered ideal for the proliferation of vectors: it has a large circulation of people because it is a tourist city, and it provides a variety of food options (animals and humans), which facilitates pathogen transmission, thereby making it a city prone to the emergence or reemergence of diseases (GÓIS, 2017). According to the municipality's Epidemiological Bulletin (BRASIL, 2018), the case records for Chikungunya and Zika started in 2015. In this study, antibodies were detected for the *Alphavirus* and *Flavivirus* genera (the genera to which the Chikungunya and Zika viruses belong to), suggesting that coatis may play a role as host of arbovirus.

Although, mosquitoes have low host specificity, feeding on several wild species (GÓIS, 2017), references confirming the importance of coatis as a food source for mosquitoes are scarce. However, these animals are known hosts of other ectoparasites, such as ticks and lice, which even

Table 1 - Individual serological result for Arbovirus in coatis according to sex, age, and type of antibodies.

Identification	Gender	Age	Antibodies detected†
06	Female	Adult	BUJV
13	Male	Adult	ILHV, SLEV, DENV-1, DENV-2, DENV-3, DENV-4, NJLV, ICOV, BUJV
18	Male	Young	BUJV
29	Female	Young	SLEV, DENV-2, DENV-3, NJLV, ICOV, BUJV
30	Female	Adult	BUJV
35	Female	Adult	WEEV
38	Female	Adult	DENV-4
42	Male	Young	BUJV
52	Female	Cub	ILHV, SLEV, BSQV, DENV-1, DENV-2, DENV-3, DENV-4, NJLV, ICOV, BUJV
55	Male	Adult	ILHV, SLEV, BSQV, DENV-1, DENV-2, DENV-3, DENV-4, NJLV, ICOV, BUJV, ORIV
63	Female	Young	ILHV, SLEV, DENV-2, DENV-3, NJLV, BUJV
64	Female	Adult	ILHV, SLEV, DENV-1, DENV-2, DENV-3, NJLV, BUJV
65	Female	Young	YFV, ILHV, SLEV, BSQV, DENV-1, DENV-2, DENV-3, DENV-4, NJLV, BUJV
66	Female	Young	SLEV, DENV-1, DENV-2, DENV-3, DENV-4, NJLV, BUJV
67	Female	Young	ILHV, SLEV, DENV-2, DENV-3, NJLV, BUJV
69	Female	Adult	BUJV
70	Male	Adult	YFV, ILHV, SLEV, ROCV, WNV, DENV-1, BUJV

†West equine encephalitis (WEEV), yellow fever (YFV), Ilheus (ILHV), Saint Louis (SLEV), Rocio (ROCV), Bussuquara (BSQV), West Nile virus (WNV), Dengue 1 (DENV-1), Dengue 2 (DENV-2), Dengue 3 (DENV-3), Dengue 4 (DENV-4), Naranjal-like (NJLV), Oriboca (ORIV), Icoaraci (ICOV), Bujaru (BUJV), and Urucuri (URUV).

infested the same animals evaluated in this study (MAGALHÃES-MATOS et al., 2017). Research shows that almost 10% of tick species can be vectors of viruses, including arboviruses (KAZIMÍROVÁ et al., 2017), which serves as a warning when we observe that 99% of the animals in this work were infested by ticks (MAGALHÃES-MATOS et al., 2017). Although, none of the tick species reported on coatis is currently recognized as an arbovirus vector, further studies are needed to clarify whether ticks or other arthropods of INP participate in cycles of arbovirus transmission to coatis and other animals.

The highest frequency of antibodies per individual was for *Phlebovirus*, followed by *Flavivirus*, *Orthobunyavirus*, and *Alphavirus*, corroborating a serological study done by CATENACCI et al. (2018) with arboviruses in primates and sloths in the Atlantic Forest, which described 33.1% seroprevalence mainly for *Flavivirus*, followed by *Phlebovirus*, *Orthobunyavirus*, and *Alphavirus*.

It was possible to detect in this study a frequency of 64.7% of animals with antibodies to the genus *Flavivirus*, corroborating PEREIRA et al. (2001) who carried out a study in the state of São Paulo and managed to detect monotypic reactions to the Ilheus virus (belonging to the *Flavivirus* genus) through the HI test in serum samples from birds (*Columbina talpacoti*, *Geopelia cuneata*, *Molothrus bonariensis* and *Sicalis flaveola*), marmosets (*Callithrix jacchus* and *Callithrix penicillata*) and including coatis. Ilheus virus represents a major public health problem in all Brazilian regions due to its wide circulation. It has Aedes and Psorophora mosquitoes as vectors and migratory wild birds as amplification hosts, with human being an accidental host (REIS & NUNES-NETO, 2021).

Conversely, monotypic reactions were observed for *Alphavirus* (WEEV), *Flavivirus* (DENV-4), and *Phlebovirus* (BUJV), which suggested the circulation of these or other viruses related to them in the studied population. Paraná State, among other states in Brazil and countries in South America, is part of the migratory route for wild birds, which may constitute natural reservoirs of viruses that cause encephalomyelitis (FERNÁNDEZ et al., 2000). Wild birds and horses from a region of the Brazilian Pantanal were seroreactive for EEEV, WEEV, Venezuelan equine encephalitis virus (VEEV), ILHV, ROCV, SLEV, and TCMV (IVERSSON et al., 1993), which is similar to the observation of the current study. The INP has a great diversity of wild birds

(FERNANDES & GARCIA, 2011), which, among other free-living species in the park, may be potential transmitters of these arboviruses to coatis, and further studies are needed to clarify this role.

The dengue virus has four serotypes (1, 2, 3, and 4). In wild transmission, primates are the main hosts, while humans are accidental hosts. There is no reported cross-immunity, so infection by one of the serotypes only confers immunity to that serotype (CHEN & WILSON, 2010; KORSMAN et al., 2012; TAUIL, 2001). BATISTA (2019) reported cases of dengue in Foz do Iguaçu, noting that in 2010, 2011, 2013, and 2015, the highest numbers of cases of the disease were experienced. Among the monotypic reactions observed in this study, antibodies against DENV-4 were observed in 8.3% of the samples.

The genus *Phlebovirus* has ten species (ICTV, 2020), which cause a variety of syndromes ranging from a mild febrile illness to infections of the central nervous system (BRAITO et al., 1998; DEPAQUIT et al., 2010; PAPA et al., 2011). In this study, the *Phlebovirus* species that showed the most monotypic reaction in coatis was the Bujaru virus, suggesting that the INP may be a new area of circulation for this virus. Bujaru virus is an arbovirus whose information is still scarce. It was isolated in Amazonas and Pará (Brazilian Amazon) in rodents Proechimys guyannensis, being serologically detected also in other rodents and marsupials. Arthropod vector and its occurrence in humans are still poorly understood (CDC, 2021).

The monotypic reactions observed suggested the occurrence of infections by at least three of the investigated arbovirus or other antigenically related ones. The Bujaru, western equine encephalitis, and dengue 4 viruses were the only ones that showed a monotypic reaction. In addition to wild cycle viruses, arbovirus with predominantly urban cycles have been detected, such as DENV, which are responsible for outbreaks and epidemics in Brazil (LEÃO et al., 2013; BRASIL, 2018).

CONCLUSION

The presence of viral antibodies in coatis from INP indicate diversity, distribution and an apparent silent circulation of arbovirus of the genera *Flavivirus*, *Alphavirus*, *Orthobunyavirus*, and *Phlebovirus*, suggesting *N. nasua* to be a possible amplifying host of these arbovirus in the studied area. The data reported also serve as a warning about the possible risk of establishing an arbovirus transmission cycle involving arthropods vectors and coatis, or even other wild animals, and consequently, may include man as an accidental host in this transmission chain.

ACKNOWLEDGEMENTS

We would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil, process Nº 140968/2014-8) and the Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ/Brazil, process Nº E-26/221145/2016) for financial support.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The Ethics Committee for the Use of Animals of the Universidade Federal Rural do Rio de Janeiro approved this study under protocol number 058/2014. Animal capture and field collection were authorized by the Biodiversity Authorization and Information System (SISBio) under protocol number 43614-3.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

ALVES-COSTA, C. P.; ETEROVICK, P. C. Seed dispersal services by coatis (*Nasua nasua*, Procyonidae) and their redundancy with other frugivores in southeastern Brazil. **Acta Oecologica**, v.32, n.1, p.77–92, 2007. Available from: <http://dx.doi.org/10.1016/j.actao.2007.03.001>. Accessed: Nov. 10, 2019. doi: 10.1016/j.actao.2007.03.001.

BATISTA, G. Mining of climatological data and urban variables associated to the dengue incidence: Study of case in Iguassu Falls, Paraná. In: CISBETI 2019 - International Congress of Health, Well-Being, Technology and Innovation, 2019, Foz do Iguaçu, PR. **BMC Proceedings**, 13 (Suppl 5), 5. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6651897/>. Accessed: Mar. 10, 2020. doi: 10.1186/s12919-019-0166-9.

BRAITO, A. et al. Toscana virus infections of the central nervous system in children: a report of 14 cases. **Journal of Pediatrics**, v.132, n.1, p.144–148, 1998. Available from: <https://doi.org/10.1016/S0022-3476(98)70500-1>. Accessed: Nov. 1, 2019. doi: 10.1016/S0022-3476(98)70500-1.

BRASIL. **Situação da Dengue, Chikungunya e Zika Vírus no Paraná – 2017/2018**. Brasília: Secretaria de Vigilância em Saúde, Ministério da Saúde, 2018. 14p. Informe técnico, 17.

CATENACCI, L. S et al. Surveillance of Arboviruses in Primates and Sloths in the Atlantic Forest, Bahia, Brazil. **EcoHealth**, v.15, p.777–791, 2018. Available from: <https://link.springer.com/article/10.1007/s10393-018-1361-2#citeas>. Accessed: Dec. 10, 2019. doi: 10.1007/s10393-018-1361-2.

CASALS, J.; BROWN, M. Hemagglutination with arthropod-borne viruses. **Journal of Experimental Medicine**, v.99, p.429-449, 1954. Available from: <010.1084/jem.99.5.429>. Accessed: Jan. 5, 2022.

CDC (CENTERS FOR DISEASE CONTROL AND PREVENTION). 2021. **Arbovirus Catalog**. Virus Name: Bujaru. Available from: <https://wwwn.cdc.gov/arbocat/VirusDetails.aspx?ID=78&SID=7>. Accessed: Jan. 15, 2022.

CHEN, L. H.; WILSON, M. E. Dengue and chikungunya infections in travelers. **Current Opinion in Infectious Diseases**, v.23, n.5, p.438–444, 2010. Available from: <https://journals.lww.com/co-infectiousdiseases/Abstract/2010/10000/Dengue_and_chikungunya_infections_in_travelers.8.aspx>. Accessed: Oct. 13, 2019. doi: 10.1097/QCO.0b013e32833c1d16.

CLARKE, D. H.; CASALS, J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. **American Journal of Tropical Medicine and Hygiene**, v.7, n.5, p.561–573, 1958. Available from: <https://www.ajtmh.org/view/journals/tpmd/7/5/article-p561.xml>. Accessed: Nov. 20, 2019. doi: 10.4269/ajtmh.1958.7.561.

CLETON, N. et al. Come fly with me: review of clinically important arboviruses for global travelers. **Journal of Clinical Virology**, v.55, n.3, p.191–203, 2012. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1386653212002570?via%3Dihub>. Accessed: Nov. 09, 2019. doi: 10.1016/j.jcv.2012.07.004.

DEPAQUIT, J. et al. Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review. **Eurosurveillance**, v.15, n.10, p.1-8. Available from: <https://www.eurosurveillance.org/content/10.2807/ese.15.10.19507-en>. Accessed: Oct. 28, 2019.

FERNANDES, C. R.; GARCIA, H. **Parque Nacional do Iguaçu: Patrimônio natural da humanidade**. 1 ed. São Paulo: Ipsis Gráfica e Editora, 2011. 252p.

FERNÁNDEZ, Z. et al. Identificação do vírus causador de encefalomielite eqüina, Paraná, Brasil. **Revista de Saúde Pública**, v.34, p.232–235, 2000. Available from: <https://doi.org/10.1590/S0034-89102000000300004>. Accessed: Oct. 10, 2019.

GÓIS, F. R. **Investigação de arbovírus (gênero Flavivirus) de interesse à saúde pública em mosquitos (Aedes aegypti e Aedes albopictus) em Foz do Iguaçu, Paraná**. 2017. 81f. Dissertation (Master in Pharmaceutical Sciences) – Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Paraná.

GOMPPER, M. E.; DECKER, D. M. *Nasua nasua*. **Mammalian Species**, n.580, p.1–9, 1998. Available from: <https://academic.oup.com/mspecies/article/doi/10.2307/3504444/2600752>. Accessed: Nov. 10, 2019.

GUIMARÃES, A. É. et al. Ecologia de mosquitos (Diptera, *Culicidae*) em áreas do Parque Nacional do Iguaçu, Brasil: 1 Distribuição por habitat. **Cadernos de Saúde Pública**, v.19, n.4, p.1107–1116, 2003. Available from: <https://doi.org/10.1590/S0102-311X2003000400032>. Accessed: Oct. 28, 2019.

GUIMARÃES, F. D. R. et al. Estudo de patógenos de potencial zoonótico em procionídeos. **Revista de patologia tropical**, v.41, p.253–269, 2012. Available from: <https://doi.org/10.5216/rpt.v41i3.20747>. Accessed: Aug. 12, 2019.

ICTV. **International Committee on Taxonomy of Viruses**. (website). Available from: <https://talk.ictvonline.org/>. Accessed: Dec. 12, 2020.

IVERSSON, L. B. et al. Circulation of eastern equine encephalitis, western equine encephalitis, Ilheus, Maguari and Tacaiuma viruses in equines of the Brazilian Pantanal, South America. **Revista do Instituto de Medicina Tropical de São Paulo**, v.35, n.4, p.355–359, 1993. Available from: <https://doi.org/10.1590/S0036-46651993000400009>. Accessed: Aug. 24, 2021.

KAZIMÍROVÁ, M. et al. Tick-Borne Viruses and Biological Processes at the Tick-Host-Virus Interface. **Frontiers in Cellular and Infection Microbiology**, v.7, n.339, 2017. Available from: <10.3389/fcimb.2017.00339>. Accessed: Jan. 15, 2022.

KORSMAN, S. N. et al. **Virology: An Illustrated Colour Text**. 1 ed. London: Churchill Livingstone Elsevier. 2012. 148p.

LEÃO, R. N. Q. D. et al. **Medicina tropical e infectologia na Amazônia**. 1 ed. Belém: Samauma, 2013. 848p.

MAGALHÃES-MATOS, P. C. et al. Ticks (Acari: *Ixodidae*) and lice (Phthiraptera: Trichodectidae) infesting free-living coatis (*Nasua nasua* Linnaeus, 1766) with sylvatic and synanthropic habits in the Atlantic rainforest of Southern Brazil. **Systematic & Applied Acarology**, v.22, n.6, p.779–784, 2017. Available from: <http://doi.org/10.11158/saa.22.6.3>. Accessed: Jan. 16, 2022.

PAPA, A. et al. A novel phlebovirus in Albanian sandflies. **Clinical Microbiology and Infection**, v.17, n.4, p.585–587, 2011. Avaible from: <https://doi.org/10.1111/j.1469-0691.2010.03371.x>. Accessed: Aug. 24, 2021.

PEREIRA, L. E. et al. Arbovírus Ilheus em aves silvestres (*Sporophila caerulescens* e *Molothrus bonariensis*). **Revista de Saúde Pública**, v.35, p.119–123, 2001. Available from: <https://doi.org/10.1590/S0034-89102001000200003>. Accessed: Jun. 14, 2020.

REIS, L. A. M.; NUNES-NETO, J.P. Ilheus Virus: revisão sobre um arbovirus emergente. **Brazilian Journal of Development**, v.7, n.4, p.37675-37695, 2021. Available from: <https://doi.org/10.34117/bjdv7n4-300>. Accessed: Jan. 16, 2022.

RODRIGUES, S. G. et al. Epidemiology of *Saint Louis encephalitis virus* in the Brazilian Amazon region and in the State of Mato Grosso do Sul, Brazil: elevated prevalence of antibodies in horses. **Revista Pan-Amazônica de Saude**, v.1, n.1, p.81-86, 2010. Available from: <http://dx.doi.org/10.5123/S2176-62232010000100012>. Accessed: Jan. 5, 2022.

SHOPE, R. E. The use of a micro hemagglutination inhibition test to follow antibody response after arthropod-borne virus infection in a community of forest animals. **Anais de**

Microbiologia, v.11, p.167–169, 1963. Available from: <https://patua.iec.gov.br/handle/iec/3436>. Accessed: Jan. 03, 2020.

SILVA, L. J. D.; ANGERAMI, R. N. **Viroses emergentes no Brasil**. 1 ed. Rio de Janeiro: Editora Fiocruz, 2008. 134p.

TAUIL, P. L. Urbanização e ecologia do dengue. **Cadernos de Saúde Pública**, v.17, p.S99–S102, 2001. Available from: <https://doi.org/10.1590/S0102-311X2001000700018>. Accessed: Oct. 10, 2019.

VASCONCELOS, P. F. Yellow fever. **Revista da Sociedade Brasileira de Medicina Tropical**, v.36, n.2, p.275-293, 2003. Available from: <https://doi.org/10.1590/S0037-86822003000200012>. Accessed: Jan. 5, 2022.

WEAVER, S. C.; REISEN, W. K. Present and future arboviral threats. **Antiviral Research**, v.85, n.2, p.328–345, 2010. Available from: <https://doi.org/10.1016/j.antiviral.2009.10.008>. Accessed: Aug. 24, 2021. doi: 10.1016/j.antiviral.2009.10.008.