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Selecionados Prêmio HGP

HGP.1 a HGP.6

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HGP.1

CHARACTERIZATION OF CUCURBIT APHID-BORNE YELLOWS VIRUS (CABYV) FROM PASSION FRUIT IN BRAZIL: EVIDENCE OF A COMPLEX OF SPECIES WITHIN CABYV ISOLATES

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Passion fruit (PF, genus *Passiflora*, family *Passifloraceae*) is susceptible to several viruses. In 2015, an outbreak of virus-like symptoms was observed in several PF - producing regions in Bahia state, Brazil. Using high-throughput sequencing technology, bioinformatics, RT-PCR, and Sanger sequencing, we identified the cucurbit aphid-borne yellows virus (CABYV, *Polerovirus*, *Solemoviridae*) and determined the full genome sequence of a variety of isolates (CABYV-PF) from different localities in Bahia. CABYV was also identified in co-infection with cowpea aphid-borne mosaic virus (CABMV, *Potyvirus*, *Potyviridae*) in PF, green manure, and spontaneous plants. Based on previous reports, the green manure and spontaneous plants *Macroptilium* spp., *Stylosanthes* spp., *Sida* spp., and *Bignonia* spp. are new hosts for CABYV, as well as *Sida* spp. and *Bignonia* spp. of CABMV. Phylogenetic analysis and pairwise identity of CABYV-PF isolates and CABYV sequences available in GenBank unveiled a complex of different species within CABYV. Based on this evidence, we proposed reclassifying all CABYV isolates from different countries/hosts and CABYV-PF in at least ten distinct species into the genus *Polerovirus*. According to the novel binominal nomenclature adopted for viruses' species, we tentatively named these isolates "*Polerovirus curcubitaeprimum*" to "*Polerovirus curcubitaenonum*", and "*Polerovirus melo*". CABYV-PF is more closely related to French and Spanish isolates, and were classified and named as "*Polerovirus curcubitaeprimum*".
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HGP.2

The virome of the invasive Asian bush mosquito *Aedes japonicus* **in Europe**

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The Asian bush mosquito *Aedes japonicus* is rapidly invading North America and Europe. Due to its potential to transmit multiple arboviruses it is important to understand the biology of this vector mosquito. Mosquitos also carry insect specific viruses that are receiving increasing attention due to their effects on host physiology and arbovirus transmission. We characterized the virome of *Ae. japonicus* populations in the Netherlands and France. Applying a small RNA-based metagenomic approach, we uncovered a distinct group of viruses present in both locations. These included one known virus, *Ae. japonicus narnavirus1* (AejapNV1), and three new virus species named *Ae. japonicus* totivirus1 (AejapTV1), *Ae. japonicus* anphevirus1 (AejapAV1) and *Ae. japonicus* bunyavirus 1 (AejapBV1). We also discovered sequences presumably derived from two additional novel viruses: *Ae. japonicus* bunyavirus2 (AejapBV2) and *Ae. japonicus* rhabdovirus1 (AejapRV1). All six viruses induced strong RNA interference responses, including the production of 21nt siRNAs, a signature of active replication in the host. Notably, AejapBV1 and AejapBV2 belong to different viral families; however, no RdRP sequence has been found for AejapBV2. Intriguingly, our small RNA-based approach identified an ~1Kb long ambigrammatic sequence associated with AejapNV1 as a secondary segment but showed no similarity to any sequence in public databases. We confirmed the presence of AejapNV1 primary and secondary segments, AejapTV1, AejapAV1, and AejapBV1 by RT-PCR in wild-caught mosquitoes. Using a small RNA-based sequence-independent metagenomic strategy, we uncovered a conserved and prevalent virome among *Ae. japonicus* mosquito populations and characterized a *bona fide* example of the viral metagenomic dark matter.

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HGP.3

DIVERSITY OF SURFACE FIBRIL PATTERNS IN MIMIVIRUS ISOLATES

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Among the most intriguing structural features in the known virosphere are mimivirus surface fibrils, proteinaceous filaments approximately 150 nm long, covering the mimivirus capsid surface. Fibrils are important to promote particle adhesion to host cells, triggering phagocytosis and cell infection. Although mimiviruses are abundant viral entities in a plethora of worldwide biomes, there is no comparative analysis on fibrils organization and abundance among distinct mimiviruses isolates. Here, by combining a set of methods and analyses, including transmission and scanning electron microscopy, image processing, genomic sequencing and viral prospection, we obtained evidence of at least three main patterns of surface fibrils that can be found in mimiviruses: (i) isolates containing particles with abundant fibrils, distributed homogeneously on the capsid surface; (ii) isolates with particles almost fibrilless; and (iii) isolates with particles containing fibrils in abundance, but organized as clumps, as observed in Megavirus caiporensis. A total of 15 mimivirus isolates (SisGen - A702EB8) were analyzed by microscopy, and their DNA polymerase subunit B genes were sequenced for phylogenetic analysis. We observed a unique match between evolutionarily-related viruses and their fibril profiles. To demonstrate that fibrils appearance is not due to microscopy artifacts, isolates were analyzed concomitantly in pairs or trios, reinforcing the aforementioned diversity on fibrils organization. At last, biological assays suggested that patterns of fibrils can influence viral entry in host cells. Taken together, our data contribute to the knowledge of mimivirus fibril organization and abundance, as well as raising questions on the evolution of those intriguing structures.

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HGP.4

SARS-CoV-2 uses CD4 to infect T helper lymphocytes

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The agent responsible for the global outbreak of coronavirus disease 2019 (COVID-19) is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus mainly targets the lungs, potentially leading to various immune-related complications, including lymphocytopenia and cytokine storm, which are significant predictors of disease severity and mortality. The precise mechanism through which SARS-CoV-2 causes immune system dysfunction remains unclear. In our study, we provide evidence that SARS-CoV-2 infects human $CD4^+$ T helper cells, while sparing $CD8^+$ T cells, and can be found in the blood and bronchoalveolar lavage T helper cells of severe COVID-19 patients. Our research indicates that the spike glycoprotein (S) of SARS-CoV-2 directly binds to the CD4 molecule, facilitating the virus entry into T helper cells. Consequently, this interaction impairs the function of $CD4^+$ T cells and may lead to cell death. Moreover, SARS-CoV-2 infected T helper cells express elevated levels of IL-10, which has been associated to viral persistence and disease severity. The findings suggest that CD4-mediated infection of T helper cells by SARS-CoV-2 may contribute to an unsatisfactory immune response in COVID-19 patients. Furthermore, this study offers potential insights for future research to comprehending how viruses mediate infection of T cells. Financial Support: FAPESP, CNPq, INCT-NIM, FINEP, FAEPEX and CAPES.

HGP.5

TONSILS ARE MAJOR SITES OF PERSISTENCE SARS-COV-2 IN CHILDREN

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In this study, we show that SARS-CoV-2 can infect palatine tonsils and adenoids in children without symptoms, without history of recent upper airway infection. We studied 48 children undergoing tonsillectomy due to snoring/OSA or recurrent tonsillitis between October 2020 and September 2021. Patients were aged 3 to 11 years [5.9 ± 2.2], 62.5% were boys and 50% had no associated diseases. Briefly, nasal cytobrush, nasal wash and tonsillar tissue fragments obtained at surgery were tested by RT-qPCR, we detected the presence of SARS-CoV-2 in at least one specimen tested in 27% of patients, and viral loads ranged widely from 186 to 7114 genome copies per µg of total RNA. Immunohistochemistry revealed the presence of viral nucleoprotein in epithelial surface and in lymphoid cells in both extrafollicular and follicular regions, in adenoids and palatine tonsils. Also, immunohistochemistry for the SARS-CoV-2 non-structural protein NSP-16 indicated the presence of viral replication in 53.8% of the positive tissues. Flow cytometry showed that $CD20^{+}$ B lymphocytes were the most infected phenotypes by SARS-CoV-2 NP, followed by CD4+, CD123, CD8+ and CD14+. Additionally, immunofluorescence indicated that SARS-CoV-2-infected tonsillar tissues had increased expression of ACE2 and TMPRSS2. NGS sequencing demonstrated the presence of different SARS CoV-2 variants in tonsils from different tissues. SARS-CoV-2 antigen detection was not restricted to tonsils, but was also detected in nasal cells from the olfactory region. In conclusion, palatine tonsils and adenoids are sites of prolonged viral components by SARS-CoV-2 in children, even without COVID-19 symptoms.
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HGP.6

EVOLUTION OF RABIES VIRUS ISOLATES : SEARCH FOR VIRULENCE GENE SIGNATURES

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Rabies is a fatal encephalitis caused by the negative-sense single-stranded RNA virus *Lyssavirus rabies* (RABV). Its genome encodes five proteins that accomplish a variety of functions; therefore, nonsynonymous substitutions can result in major consequences. RNA viruses could exist as viral quasispecies, with a consensus sequence surrounded by a mutant spectrum, which would be selected under environmental changes resulting in improved fitness and virulence. Here, we investigated possible virulence gene signatures in RABV isolates and their phenotypical consequences. Six naturally occurring RABV isolates from Brazil were submitted to 10 serial passages in neuronal and non-neuronal cell systems, and the evolution of the nucleoprotein (N) and glycoprotein (G) genes was analysed. In the neuronal system, the N gene was highly conserved for all isolates. Conversely, nonsynonymous mutations in the G gene were detected and mapped to loop regions, domain linkers, and the antigenic site III. Molecular dynamics simulations showed that some mutants presented more conformational flexibility, probably enhancing endosome fusion during RABV penetration in the host cells. Molecular docking indicated a possible secondary receptor after mutation. *In vitro* and *in vivo* assays revealed a better replicative fitness and improvement in neurotropism for the mutants. Only one RABV isolate could maintain replication cycles in the non-neuronal system, which could reflect its evolutionary history. In summary, we identified three amino acid residues in RABV G which could be associated with the virulence mechanisms as well as neurotropism in wild type isolates.