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Selecionados Prêmio HGP

HGP.1 a HGP.6

20/9/2024

HGP.1

RESISTANCE IN RICE GENOTYPES AGAINST RICE STRIPE NECROSIS VIRUS AND ITS VECTOR: DEVELOPMENT OF A SEVERITY SCALE AND RT-QPCR ASSAY FOR SCREENING

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Rice stripe necrosis virus (RSNV) causes rice crinkling disease and is transmitted by the protozoan *Polymyxa graminis*. Although genetic resistance has been researched, no resistant commercial cultivars are currently available. *Oryza glaberrima* has shown potential resistance, but it is unclear whether this resistance is effective against the virus, the vector, or both, and whether it can be transferred to Oryza sativa cultivars. Visual observation of symptom expression is the only method for selecting disease-resistant genotypes. However, the lack of a severity scale for RSNV makes this process challenging, and relying solely on visual assessments can lead to introduce subjectivity. Our aim was to develop a severity scale for RSNV and an absolute quantification test for the virus and the vector, as well as to explore degrees of genetic resistance in contrasting rice materials. Experiments were conducted using *O. glaberrima* and three *O. sativa* cultivars to achieve absolute quantification. Inoculation occurred naturally using soil from an area with a history of the disease. Visual symptoms were recorded, and disease intensity was evaluated. Subsequently, total nucleic acid extraction was performed on the samples, and viral and vector loads were quantified through RT-qPCR and qPCR, respectively. The *O. glaberrima* showed a lower viral and vector load than the other cultivars. SCS123 Pérola is originated from hybridization between *O. glaberrima* and *O. sativa*, and did not differ from *O. glaberrima*. The weekly recorded symptoms, together with the field reports, allowed for the development of a severity scale for the disease that showed a significant correlation between virus load.

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HGP.2

Reemergence of Oropouche virus between 2023 and 2024 in Brazil: a virological study

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Oropouche virus (OROV) is a neglected arbovirus endemic to Latin America and the Caribbean, where it has caused outbreaks of Oropouche fever since the 1950s. In this study, we investigate virological factors contributing to the reemergence of Oropouche fever in Brazil between 2023 and 2024. For this, we combined OROV epidemiological, genomic, molecular, and serological data from Brazil from 1 January 2015 to 29 June 2024, along with *in vitro* and *in vivo* characterization. We found that autochthonous OROV infections in 2024 were detected in previously non-endemic areas, with cases reported in 19 of 27 federal units, which 83.2% (6,895 of 8,284) occurred in Northern Brazil. We demonstrate that the 2023-2024 epidemic was caused by a novel OROV reassortant that replicated approximately 100-fold higher titers in mammalian cells compared to the prototype OROV strain BeAn 19991. The 2023-2024 OROV reassortant displayed plaques earlier than the prototype, produced 1.7 times more plaques, and plaque sizes were 2.5 larger compared to the prototype. Furthermore, serum collected in 2016 from previously OROV-infected individuals showed at least a 32-fold reduction in neutralizing capacity against the reassortment strain compared to the prototype. In conclusion, we demonstrated that the increased incidence of Oropouche fever in Brazil may be related to a higher replication efficiency of a new reassortant virus that also evades previous immunity, contributing to a better understanding of the OROV reemergence in 2023-2024.

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HGP.3

RAB27A GTPase AND ITS EFFECTOR MYOSIN VA ARE HOST FACTORS REQUIRED FOR EFFICIENT OROPOUCHE VIRUS CELL EGRESS.

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Oropouche virus (OROV) is an arbovirus that belong to the *Peribunyaviridae* family, a large group of RNA viruses. OROV causes a debilitating illness common in the Amazon region of South America, but that has recently spread to all five regions of Brazil. Despite this, little is known about the cell biology of OROV in host cells, especially assembly and egress processes. We have discovered that the small GTPase Rab27a mediates intracellular transport of OROV induced compartments and viral release from infected cells. We found colocalization between Rab27a and OROV during late phases of the infection cycle and depletion of Rab27a using RNAi or overexpression of dominant negative/positive mutants produce a change in the intracellular distribution of the virus, which remain retained in the perinuclear region, and compromise the release of infectious particles. Moreover, we show that Rab27a interacts with OROV glycoproteins and associates with released viral particles. Consistently, depleting Rab27a's downstream effector, Myosin Va, also interferes with the transport of OROV compartments to the cell periphery and infectious viral particle production. Interestingly, the morphology of OROV compartments show strong alterations in Myosin Va depleted cells. Finally, inhibiting actin polymerization also hinders OROV compartments targeting to the cell periphery and infectious viral particle egress. Taken together these data indicate that OROV use the Rab27a – Myosin Va – Actin filaments system for intracellular trafficking and egress from host cells.

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HGP.4

Evaluating Neutralizing Antibody Responses Post-Immunization with a COVID-19 Bivalent Vaccine: Implications for Future Vaccine Design

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The emergence of SARS-CoV-2 variants has led to the development of bivalent COVID-19 vaccines, incorporating antigens from both the wild-type virus and the Omicron strain. This study aimed to evaluate the impact of bivalent vaccination on the neutralizing antibody (NAb) response. We enrolled 93 volunteers who had received either three or four doses of monovalent vaccines targeting the original virus (n=61) or a bivalent booster shot (n=32). Serum samples from these volunteers underwent neutralization assays using wild-type SARS-CoV-2 and Omicron subvariants. Additionally, immunoinformatics was employed to quantify and localize highly conserved NAb epitopes. The primary finding was that neutralization titers in samples from individuals vaccinated with the bivalent vaccine were higher for the original virus compared to their capacity to neutralize the Omicron variant and its subvariants. NAbs recognizing epitopes mostly conserved in the wild-type SARS-CoV-2 were significantly boosted, whereas those recognizing epitopes predominantly present in the Omicron variant and its subvariants were merely primed. These results suggest that future vaccine formulations should target circulating viruses rather than those that are no longer prevalent.

Keywords: SARS-CoV-2, Omicron, epitope, bivalent vaccine, boost.
Financial support: FINEP, MCTI, CNPq, CAPES

HGP.5

MOLECULAR SCREENING OF SELECTED VIRUSES IN VULNERABLE CARNIVORANS IN MATO GROSSO DO SUL, BRAZIL

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Wild carnivorans are vulnerable to pathogens typically found in domestic species, and increasing interactions between the two groups heighten the risk of disease transmission, posing significant threats to the conservation. This study aimed to assess the presence of several viruses, including bocaparvoviruses, parvoviruses, hepadnaviruses, coronaviruses, paramyxoviruses, canine distemper virus (CDV), poxviruses, feline herpesvirus, feline calicivirus, feline immunodeficiency virus, feline leukemia virus (FeLV), and gammaherpesviruses in wild carnivorans. A total of 30 biological samples from animals in the Canidae, Felidae, Mephitidae, Mustelidae, and Procyonidae families were analyzed, all of which had been killed by vehicles in Mato Grosso do Sul, Brazil. The study detected canine parvovirus (CPV-2) DNA in the spleens of a bush dog (Speothos venaticus), a jaguarundi (Puma yagouaroundi), and a jaguar (Panthera onca). Additionally, FeLV proviral DNA was identified in the spleen of an ocelot (Leopardus pardalis), and CDV RNA was found in the liver of a jaguarundi. Phylogenetic analysis of the partial CPV-2 VP2 gene sequence and the FeLV U3 (LTR) gag region revealed a 100% identity with strains from domestic dogs and cats, respectively. The proximity of wild and domestic animals facilitates pathogen transmission, especially among closely related species like Canidae and Felidae. The discovery of important and potentially fatal viruses, such as CPV-2, FeLV, and CDV in four endangered and under-researched wild species highlights the importance of understanding the pathogens circulating within these vulnerable populations to better guide actions to cotain or mitigate their dissemination among wild carnivorans.

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HGP.6

MORTALITY IN SEA LIONS IS ASSOCIATED WITH THE INTRODUCTION OF THE H5N1 CLADE 2.3.4.4B VIRUS IN BRAZIL, OCTOBER 2023: WHOLE GENOME SEQUENCING AND PHYLOGENETIC ANALYSIS

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The highly pathogenic avian influenza (HPAI) H5N1 virus Clade 2.3.4.4b was spread throughout Europe, Asia, Africa, and North America into wild bird populations in late 2021 and, shortly after, in South America and Antartida. The virus has also been detected in numerous mammal species (wild and livestock), including humans. In October 2023, a high mortality of South American sea lions (*Otaria flavescens*) was reported in Santa Catarina. Sixteen samples (lung and intestine) from eight sea lions were collected for molecular tests. Four animals were positive for the RT-qPCR-specific test of avian influenza viruses. Complete genome sequences from two samples (lungs) were obtained. Viruses, named OF-358R/23 and OF-359R/23, clustered with South American H5N1 HPAI viruses clade 2.3.4.4b, genotype B3.2, within sea lion sequences identified in Chile and Peru. The hemagglutinin cleavage site had the HPAI motif PLREKRRKR/GLF, matching recent South American clade 2.3.4.4b H5N1 viruses. Amino acid substitutions, known to increase virulence, transmissibility, and replication in mammals, were identified in the PB2(Q591K and D701N), PA (R57Q), and NS (V226T) genes. Bayesian phylogenetic analysis indicated these substitutions arose between September 2022 and February 2023. Enhanced surveillance and monitoring are essential to understand transmission among marine mammals and prevent potential human spillover.

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