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Poster session I

Individual and comparative analysis of RNAseq data from Caco-2 cells infected with classical and non-classical human astroviruses

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Human classical astroviruses (HAstV-1–HastV-8) are infectious agents that typically infect the gastrointestinal tract producing gastroenteritis in children and older adults. However, in 2009, a new astrovirus was discovered through next-generation sequencing called VA1 (HAstV-VA1). Astrovirus VA1 has been found in immunocompromised patients with encephalitis, which is why it has been described as a neurotropic strain. The adaptation of this astrovirus strain to grow in cell culture was recently described, especially in Caco-2 cells, a cell line derived from the human intestinal epithelium that allows the replication of VA1, as well as classical astroviruses. Differences between Yuc8 (HAstV-8) and VA1 have been observed in terms of receptor binding specificity, monoclonal antibody neutralization, the kinetics of one-step replication, and the proteases responsible for processing the capsid protein precursor. Several studies have reported the single-cell transcriptomics of VA1-infected enteroids. However, no reports on classical astrovirus transcriptomics have been published. In this work, we decided to study the comparative gene expression profiles of Caco-2 cells infected with either a classical gastroenteric astrovirus (Yuc8) and the neurotropic astrovirus strain VA1. Given the biological differences exhibited by these viruses, we decided to undertake a temporal study spanning intervals of 0, 6, 12, 18, 24, and 36 hours using uninfected Caco-2 cells and cells independently infected with both viruses. The infection kinetics was done in triplicate. Subsequent to this, poly(A)+ RNA was isolated from each sample and subjected to next-generation sequencing, to generate RNAseq data. We obtained 20 million reads per sample. This study provides a platform to advance our knowledge of both astroviruses and their effects on the expression of cellular genes.

Keywords: Astrovirus; Transcriptomics; RNAseq.

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Tetraspanin CD81 blocking decreases dengue virus infection

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The tetraspanin CD81 has been reported to play an essential role as viral receptor, in the fusion of endosomal and viral membranes, genome replication, the release of viral particles, and the formation of extracellular vesicles with infectious components. However, the role of CD81 during dengue virus infection is little known. In this study, we analyzed the participation of CD81 in the infection of dengue virus serotype 2 incubating antibodies anti-CD81 in Huh-7 cells before interaction with exosomes or viral particles. Our results showed a significant reduction in the expression levels of viral proteins and the percentage of infected cells compared to infected cells without antibodies. These results suggest that CD81 is involved in the initial steps of dengue virus replicative cycle.

Keywords: Tetraspanin; Exosome; Replicative cycle; Viral entry.

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Participation of the Kinesin Light Chain 1 protein in the secretion of the dengue virus NS1 protein

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The discovery of DENV NS1 secretion in mosquito cells suggests its potential role in the vector's pathogenesis. Determine the mechanisms governing its secretion into the extracellular milieu would provide valuable insights into virus-host interaction. A non-biased protein-protein interaction assay conducted in our laboratory suggested an interaction between KLC1 (kinesin light chain 1) and DENV-2 NS1 in mosquito cells. KLC1 is a component of the kinesin motor complex involved in anterograde transport along microtubules of numerous cellular elements like the cell membrane periphery. Previous research has demonstrated the involvement of some kinesin members in viral infections. Several studies indicate that the secretion of certain viral effectors occurs via unconventional secretion pathways. Therefore, we hypothesize that the kinesin motor complex, by participating in these pathways, could facilitate the secretion of NS1. The present study aims to characterize the KLC1-NS1 interaction in mosquito cells. Through proximity ligation and co-immunoprecipitation essays, we confirmed the KLC1-NS1 interaction in DENV-2-infected C6/36 mosquito cells. Treatment of DENV-2 infected C6/36 cells with the microtubule polymerization inhibitor colchicine resulted in a significant reduction in NS1 secretion. These findings suggest the involvement of tubulin cytoskeleton and its accessory proteins in the transport of NS1 in mosquito cells.

Keywords: Dengue; Secretion; Mosquito; Kinesin; NS1.

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The role of the ubiquitination in the replication of the neurotropic astrovirus VA1

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Human astroviruses (HAstV) have been associated with gastroenteritis in children. However, fatal encephalitis caused by the HAstV VA1. The Ubiquitin - Proteosome System has been shown to be required for the replication cycle of HAstV8 and VA1. It was reported that HAstV8 requires the proteasome activity during the early stages of the virus replication cycle and requires to produce the virus infectious progeny. It has been reported that the production of VA1 progeny decreases when the proteasome activity is inhibited, but the role of ubiquitination on the replication of this virus is unknown. In this work, we investigated the importance of ubiquitination in the VA1 replication cycle, using the ubiquitination inhibitor Pyr41. We have found that the production of viral progeny and the synthesis of viral protein were reduced in the presence of Pyr-41 in a dose-dependent manner suggesting that ubiquitination is necessary for the replication. In addition, the infectivity and the progeny production were lower when Pyr-41 was added during the adsorption of the virus to the cells. These results suggest that ubiquitination plays an important role in the early stages of VA1 infection. Even though the importance of the ubiquitination for VA1 replication was shown, there are still many questions to be explored.

Keywords: Astrovirus; Ubiquitination; Viral replication; Proteosome.

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Vol. 11 | Special Supplement

Extracellular vesicles promote Rotavirus infection in low-susceptible cells

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Rotaviruses (RV) are the leading cause of acute dehydrating gastroenteritis in children under 5 years of age worldwide. Apart from gastroenteritis, rotavirus infection has also been infrequently associated with non-gastrointestinal manifestations such as hepatitis, pneumonia or neurological complications. In this work, we tested the existence of a receptor-independent extra-intestinal transmission pathway mediated by extracellular vesicles (EV). The EV-RV association has been reported *in vitro* and *in vivo*, observing that these vesicles, without the independent ability to replicate, could promote active infection. We selected cell lines from distinct organs (kidney, ovary, CNS) that show low susceptibility to RV infection, but are permissive when virus particles are introduced into the cytoplasm. The EV were purified from rotavirus-infected cells and, were added to the cell lines of interest. The purified EV-RV complexes were found to promote infection in low and non-susceptible cell lines. Furthermore, the purified EV-RV complexes retained some level of infectivity even after treatment with a strong neutralizing antibody prior to incubation with the cells, suggesting that the viral particles are not accessible to the action of the neutralizing antibody. Our data provide evidence for the existence of an alternative pathway by which rotaviruses could enter cells in a receptor-independent mechanism.

Keywords: Rotavirus; Extracellular vesicles; Extraintestinal infection; Microvesicles.

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Vol. 11 | Special Supplement

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Pharmacological inhibition of Lipid Droplets biogenesis and its impact in the replication of Respiratory Syncytial Virus

The viruses can recruit cellular factors for their infection cycle to be successful. In this sense, lipid droplets (LD), which are cellular structures that store lipids and can function as an energy reservoir, play an essential role during viral infections. These structures may be involved in viral particles' entry, assembly, replication, and exit during infections. We characterized the effects of biogenesis and pharmacological blockade of LD and its impact on replicating the Respiratory Syncytial Virus (RSV) in a pulmonary epithelial cell line (A549). As a result, we observed that in RSV-infected cells, there is a decrease in LD and a change in the spatial distribution pattern. The LD biogenesis caused an increase in the number of RSV-infected cells. The pharmacological inhibition of LD caused a reduction in the expression of viral M2-1 protein and significant downregulation of the RSV viral F protein gene. Finally, we found a decrease in PFU in cells treated with LD inhibitor compared to control cells. The results show that RSV infection in A549 cells can promote a reduction of lipid droplets, and pharmacological inhibition of lipid droplets has a negative effect on viral replication and the production of infective viral particles.

Keywords: Lipid Droplets; DGTA; Triacylglycerol; Metabolism; Pathogen; Infection.

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Subcellular localization of RNA-dependent RNA polymerase nsP1b in infected CaCo2 cells and its interaction with the 5' and 3' Untranslated Region of Human Astrovirus 8

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Human astroviruses (HAstV) cause gastroenteritis and neurologic disorders. These envelopeless viruses have +ssRNA genomes with three overlapping open reading frames (ORF): ORF1a, ORF1b, and ORF2, flanked by 80-100 nucleotide Untranslated Regions (UTRs). VPg, is a viral protein, covalently linked to the 5′ end of the genome, helps in viral infection. ORF1b encodes nsP1b, an RNA-dependent RNA polymerase (RdRp) involved in the synthesis of genomic RNA. The viral replication complex (VRC) forms within the intracellular membrane during astrovirus replication. To understand HAstV's role in viral replication and know what other factors are involved, we determine if whether nsP1b is a VRC component and whether it interacts with 5′ and 3′ UTRs of HAstV-8. In this study, we found that recombinant nsP1b interacts with the 5′ and 3′ UTRs using electrophoretic mobility shift and north western blot assays. By confocal microscopy, at 8 hours post infection we found nsP1b near the perinuclear region, where VPg and the cellular protein Calnexin are located, suggesting that these cellular factors are part of the complexes involved in viral replication. We are currently investigating on more detail the possible association of nsP1b with other viral proteins as from CaCo2 cells in the replication of this virus.

Keywords: Astrovirus; RNA; Replication; Cellular factors.

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Study of 3T3-L1 cell permissiveness to Adenovirus type 36 infection

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Human adenovirus (HAdV-D36) was the first adenovirus that was shown to cause obesity in animal models and has been shown to exert an adipogenic affect in cell culture. In humans, HAdV-D36 has been correlated with obesity in several studies, but whether the infection can be considered an etiologic factor of obesity has not been determined and many outstanding questions remain of the effect of HAdV-D36 infection on the metabolism and differentiation of adipocytes. The 3T3-L1 murine cells have served as an adipocyte model to study HAdV-D36 infection. These cells are susceptible but not permissive to virus replication before entering adipocyte differentiation, and we have recently shown that HAdV-D36 productive infection of 3T3-L1 cells that are committed to adipocyte differentiation increases the expression of adipogenic key markers (C/EBPβ C/EBPβ PPARγ) and accumulation of intracellular lipids, nevertheless, as differentiation of adipocytes continues to a mature state the expression of viral genes and progeny production decreases, suggesting that the cells may be only permissive to infection at the state of adipocyte commitment. Therefore, in this work the HAdV-D36 permissiveness of 3T3-L1 cells in an adipocyte mature state was evaluated through the quantification of intracellular lipids by Oil-Red O (ORO) staining, as well as of viral production through plaque assays. The results shown that HAdV-D36 infection of 3T3-L1 cells in an adipocyte mature state leads to an exponential increase in intracellular lipids, but highly reduced viral replication.

Keywords: Human adenovirus 36; Obesity; Adipocyte commitment; Adenovirus replication; Permissive replication.

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Incidence of Lyssavirus in vampire bat: The importance of the life cycle

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Desmodus rotundus (common vampire bat) is the primary vector of rabies. The dynamics of its populations involve recurrent migrations, colonization of caves near livestock, and a reproductive behavior where males maintain a harem that they defend against other young males seeking reproductive events. The aim of this study was to identify the differential incidence of Lyssavirus among demographic categories of the vector. Reverse transcription technique was used on samples from all individuals collected from four shelters in Puebla. The results indicate that shelters closer to livestock have a higher incidence of the virus. Reproductive adults, particularly females, have a higher frequency of infection than other categories, but only in populations where the structure indicated reproductive events and turnover due to maternity, not in peripheral shelters. These data are important for delineating vector control measures necessary to prevent livestock losses and occasional cases of rabies in humans.

Keywords: Prevalence; Reverse transcription; Population dynamics; Vector; Rabies virus.

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Apoptosis regulation in the midgut and salivary gland of mosquitoes *Aedes aegypty* infected with dengue virus

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Dengue virus (DENV) is transmitted by female Aedes aegypti and Aedes albopictus mosquitoes, causing 390 million new infections each year, mainly in tropical and subtropical regions of the world. Mosquitoes acquire DENV during their hematophagous feeding on an infected person, the virus spreads in the epithelial cells of the midgut, subsequently crosses the basal lamina into the hemocoel and disseminates in the hemolymph infecting other organs, including the salivary glands. Where it replicates before being transmitted to another person in a subsequent blood meal. In the insect-virus interaction, apoptosis functions as an antiviral response, however, apoptosis is not observed in mosquitoes infected with DENV, indicating its modulation. This study aims to analyze the gene expression of apoptosis modulators of the extrinsic pathway and the intrinsic pathway in the Aedes aegypti mosquito infected with DENV-2 at various times post-infection. Preliminary results suggest that there is a modulation of the expression of several genes. A significant overexpression of AeDrice was observed during 4, 7 and 14dpi, suggesting a fundamental role in the regulation of apoptosis; however, its modulation is not clear since it is an effector caspase. While AeDeBcl, an adapter caspase of the intrinsic pathway, shows repression at 4dpi and 14dpi. On the other hand, the expression of the AeIAP1 gene (negative regulator of the intrinsic pathway) increases at 4 and 7dpi but decreases significantly at 14dpi. Therefore, the downregulation of apoptosis during infection of Aedes aegypti mosquitoes with DENV-2 is upregulated and shows significant differences when feeding on uninfected blood.

Keywords: Apoptosis; *Aedes aegypti*; Dengue virus; Caspases; Cell death; Mosquitoes.

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Studying the role of the viral protein NSP3 in mRNA translation and stability

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Rotavirus NSP3 protein is involved in the regulation of translation of cellular and viral proteins, however, the precise mechanism by which this protein exerts its functions is still undefined. NSP3 binds specifically to the 3´ GACC consensus sequence (present in all mRNAs) and also binds to the translation initiation factor eIF4G. It has been proposed that these interactions promote the circularization of viral mRNAs favoring their translation. However, we have found that the absence of NSP3 during the infection does not affect the translation of viral proteins. To learn about the possible effects of NSP3 on the translation efficiency and stability of viral and cellular mRNAs, we are evaluating the expression of reporter mRNAs with cellular or viral 5´and 3´UTRs in the presence or absence of NSP3. So far, our results indicate that there are no significant differences in the translation efficiency or stability of the reporter mRNAs analyzed, suggesting that NSP3 might not be necessary for viral mRNA translation and stability, the role of NSP3 on cellular reporters is still under investigation.

Keywords: Rotavirus; NSP3; UTRs (untranslated regions).

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Moringa oleifera cMoL lectin has antiviral activity against Hepatitis C Virus genotype 2a

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INTRODUCTION: The envelope glycoproteins of Hepatitis C Virus (HCV) are heavily glycosylated, with carbohydrates comprising one-third of the molecular weight of E1E2 heterodimer. Plant or microbial lectins exhibit antiviral activity against viruses with glycosylated envelope proteins. *Moringa oleifera* seeds yield lectins that have various biological activities, including nematicidal, bactericidal, insecticidal, anticarcinogenic effects. However, research linking *Moringa oleifera* lectins to their potential antiviral activity against HCV remains unexplored in existing studies. OBJECTIVE: Identify antiviral activity of the cMoL lectin purified from *Moringa oleifera* seeds against HCV genotype 2a in Huh7.5.1 infected cells. METHODS: Huh7.5.1 cells were cultured in DMEM medium supplemented with 10 % FBS. Cell culture medium was replaced by different concentrations of cMoL lectin (0.1, 0.03, 0.01, 0.003, 0.001, 0.0003, 0.0001, and 0.00003 µg / µL) in combination with HCV genotype 2a (1 x 105 focus forming units / mL). The cells were incubated at 37 °C for two days and subsequently, the Renilla Luciferase assay was performed. *Galanthus nivalis* lectin and Fluoxazolevir were used as positive controls. RESULTS: cMoL generated 90.8 % inhibition of HCV RLuc (genotype 2a) at the 0.1 µg / µl concentration in Huh7.5.1 cells. CONCLUSION: The results indicate that cMoL lectin exhibits antiviral activity against HCV genotype 2a. Future studies are needed to understand the underlying mechanism through which cMoL lectin mediates this antiviral effect. Funding Conahcyt scholarship numbers: 965468 462092 and 785514, to J.S.-M, M.C.-V and MA-C-G respectively. PIN 2021-II, CUCS, Universidad de Guadalajara.

Keywords: Hepatitis C Virus; Moringa oleifera; cMoL lectin.

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Feline calicivirus LC viroporin interacts with cellular proteins during viral replication

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The *Feline calicivirus* (FCV) leader of the capsid (LC) protein is a viroporin required for an efficient viral replication. It is responsible for the induction of the cytopathic effect during infection and for the mitochondrial apoptosis when expressed in CRFK cells. The LC viroporin sequence contains two conserved regions: a polycisteine sequence implicated in the formation of disulfide bonds and a polyproline region (PVVPP) at the carboxyl terminus with an unknown function. Polyproline regions are implicated in the interaction with SH3 domain containing proteins. To determine if the LC protein interacts with cellular proteins, immunoprecipitation (IP) and mass spectrometry (MS) assays were performed. We found 135 LC-interacting cellular proteins; among them, the Fucosyltransferase 8 (FUT8), an enzyme that catalyzes the addition of fucose in alpha 1-6 linkage to the first GlcNAc residue and has an SH3 domain, characterized by having a beta barrel fold consisting on six β-strands arranged as two tightly packed anti-parallel β sheets. We analyzed the *in silico* interaction of both proteins and we will study the relevance of this interaction in infected cells.

Keywords: *Feline calicivirus*; Fucosyltransferase 8; LC viroporin, SH3 domain.

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Understanding the highly dynamic phagome of a superbug

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Bacteriophages can affect bacterial community dynamics depending on whether the phages cause lytic or lysogenic infections. Although we have an idea of the effect of lytic phages on bacterial communities influencing human health, the impact of lysogenic phages is much less understood. *Acinetobacter baumannii* is an important nosocomial bacterium of high importance for human health. Recent studies, some from our group, have shown that *A. baumannii* isolates can have several prophages. Importantly, the presence of antibiotic resistance and virulence genes within the prophage sequences suggests that they could be potential mediators of horizontal transfers. This study aims to better understand the genomic diversity of the prophages and how they might affect *A. baumannii*. Key to our approach is to consider the spatial and temporal dimensions of both the bacterium and its phages. In analyzing 1 547 previously reported and genotyped *A. baumannii* genomes we found 3 980 prophage signals, with an average of three prophages per isolate. To understand the diversity of the prophages, we created a network considering the average nucleotide identity across them. Many of the clusters in the network have just one prophage and most clusters tend to correspond to individual Sequence Types (ST) of *A. baumannii*. Our results suggest a high diversity of prophages, which show a narrow host range (same ST). In future analyses, we will characterize the level of polylysogeny and the potential factors determining it.

Keywords: Bacteriophages; Prophages; *Acinetobacter baumannii*; Genomic diversity.

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The nucleolar protein fibrillarin modulates its expression and distribution during Respiratory Sincital Virus infection

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In this work, we focus on the respiratory syncytial virus matrix protein. However,the primary function of the matrix protein is to serve as a site for viral assembly and budding at the plasma membrane. There is evidence that the matrix protein of Nipah and Hendra viruses can be transported to the nucleolus and interact with nucleolar proteins, such as fibrillarin (FBL). The interaction between FBL and matrix causes FBL to be inhibited, silencing the rRNA biogenesis. And the latter allows for an increase in the production of viral particles. In this work, we focus on evaluating the nucleolar distribution and expression of FBL protein during the infection of RSV. Our results obtained in immunofluorescence did not identify a colocalization between the matrix protein and the FBL protein. However, different distributions and proportions of this protein were observed; and performing an analysis of medium-intensity fluorescence determined a more significant signal of FBL in conditions of infection. This data was corroborated by a western blot identifying a greater expression of this nuclear protein in cells infected with RSV. In conclusion, RSV infection doesn't exist to colocalize between FBL and matrix protein: however, it identified that RSV promotes an increase in the expression of the FBL protein. Therefore, the next step is to analyze the implication that this increase in FBL could have during RSV infection. during respiratory syncytial virus infection.

Keywords: RSV; Matrix protein; Nucleolus; FBL; rRNA.

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Vol. 11 | Special Supplement

Molecular detection of *Orthoflavivirus* in wild birds from Yucatan, Mexico

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Orthoflaviviruses annually infect over 400 million people worldwide. These arboviruses are transmitted by mosquitoes of the *Aedes* and *Culex* genera, and some bird species are recognized as reservoirs, mostly of the Passeriformes order. Given Yucatan's bird richness, it is necessary to emphasize the need to monitor *Orthoflavivirus* circulation in this taxon from the region. The main aim of this study is to identify *Orthoflavivirus* presence in wild birds inhabiting a gradient of different land use (Conserved, Diversified, Rural and Urban) in Yucatan, Mexico. The captured birds were classified by species and a cloacal swab was collected. Samples of the species considered as reservoirs or possible reservoirs of *Orthoflavivirus* were selected and analyzed using a nested RT-PCR for the amplification of a fragment of the NS5 gene and positive results were confirmed by Sanger sequencing. A total of 423 birds were sampled which 218 samples are from reservoirs and possible reservoirs, being the Rural habitat with the greatest abundance of these (88 of 233 birds). So far, 134 samples have been analyzed; with two positive results obtained, but continuous monitoring of bird communities is necessary to assess the relationship between land-use changes and the *Orthoflavivirus* cycle in Yucatan.

Keywords: Orthoflaviviruses; Zoonoses; Wildlife; Vectors; Transmission dynamics.

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Vol. 11 | Special Supplement

Changes in distribution and number of mitochondria associated with chikungunya virus infection

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Viruses depend on cell machinery to replicate, which strategically modulates the metabolism and physiology of host cells, changing cell architecture and functions. In this sense, it has been shown that alphavirus induces changes in the membrane, alters glycolysis rates, and even affects autophagy/apoptosis. The genus *Alfavirus* belongs to the family *Togaviridae*, including Venezuelan equine encephalitis (VEEV) and Chikungunya virus (CHIKV). The impact of CHIKV on mitochondria structure has not been determined, so this project aimed to evaluate the effects of CHIKV on mitochondria. For this purpose, the immunofluorescence technique evaluated changes in morphology, distribution, and number of mitochondria at 12, 14, 16, and 18 hpi. The data obtained suggest perinuclear clustering, a decrease in the number of mitochondria, and modification of mitochondrial morphology depending on the time of infection. Subsequently, the Macintosh MitoProt program was used to determine whether structural proteins or peptides (capsid, E1, E2, E3, and 6K) had a mitochondrial target, with the capsid protein having the highest score. We hypothesized CHIKV capsid protein is related to altered mitochondrial function, which would contribute to mitochondrial damage.

Keywords: Mitochondria; Chikungunya; Virus.

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Vol. 11 | Special Supplement

Two short low complexity regions (LCRs) are hallmark sequences of the Delta SARS-CoV-2 variant spike protein

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Low complexity regions (LCRs) are protein sequences formed by a set of compositionally biased residues. LCRs are extremely abundant in cellular proteins and have also been reported in viruses, where they may partake in evasion of the host immune system. Analyses of 28 231 SARS-CoV-2 whole proteomes revealed the presence of four extremely conserved LCRs in the spike protein of several SARS-CoV-2 variants. With the exception of Iota, where it is absent, the Spike LCR-1 is present in other variants of concern and interest. The Spike LCR-2 is highly prevalent (79.87 %) in Iota. Two distinctive LCRs are present in the Delta spike protein. The Delta Spike LCR-3 is present in 99.19 % of the analyzed sequences, and the Delta Spike LCR-4 in 98.3 % of the same set of proteins. These two LCRs are located in the furin cleavage site and HR1 domain, respectively, and may be considered hallmark traits of the Delta variant. The presence of the medically-important point mutations P681R and D950N in these LCRs, combined with the ubiquity of these regions in the highly contagious Delta variant opens the possibility that they may play a role in its rapid spread.

Keywords: Low complexity regions; SARS-CoV-2; Spike protein; Delta variant.

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Vol. 11 | Special Supplement

Permissivity of BeWo cells differentiated to syncytiotrophoblasts to human respiratory syncytial virus infection

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The Human Respiratory Syncytial Virus (RSV) is one of the leading agents causing respiratory tract infections in children. Transmission of RSV is mainly respiratory. However, vertical transmission from mother to fetus during pregnancy is poorly understood. Until now, *in-vitro* studies using primary cultures of trophoblasts and cell lines such as BeWo have shown to be permissive to infection by this virus. Also, infection with murine models is able of vertical transmission. Reports in humans have suggested vertical transmission of this virus, which could interfere with fetal programming, thus reducing fetal developmental pathways and modifying postnatal metabolism, making it susceptible to respiratory diseases. For this reason, it is crucial to understand the viral mechanisms during vertical transmission to develop possible treatments for infection by this virus. In this sense, RSV can infect human placental cytotrophoblast (BeWo) cell lines, but the permissiveness of syncytiotrophoblasts (first placental barrier) is unknown. In this work, we developed the differentiation of the BeWo cell line to syncytiotrophoblasts *in-vitro*, under cAMP treatment. The syncytiotrophoblasts derived from the BeWo line, whose differentiation was evidenced by the expression of SYNCYTIN2 and SIK1 at the mRNA and protein level, as well as the formation of syncytia revealed by Immunofluorescence, also we demonstrated using Immunofluorescence and Western Blot assays, that they are permissive to RSV infection. Additionally, in syncytiotrophoblast, an increase in the expression of the viral receptor for RSV (nucleolin) was demonstrated, which could help us to understand this cell model as a target of RSV infection and vertical transmission.

Keywords: VSR; Trophoblast; Transmission; Pregnancy.

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Vol. 11 | Special Supplement

Molecular characterization and phylogenetic analysis of HIV-1 Tat and Rev proteins from antiretroviral therapy-naïve Mexican infected individuals

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To modulate viral replication in early infection, HIV directs the synthesis of Tat and Rev regulatory proteins at the transcriptional and post-transcriptional level respectively. We provided a detailed molecular analysis of 29 Tat and Rev protein sequences derived from PBMCs obtained from 22 antiretroviral therapy-naïve Mexican HIV-1 infected individuals. Of the 29 sequences, seven were sampled from the same patient at different times, spanning between 1 and 3 years between samples, to evaluate genetic evolution. The 3D-protein molecular structure, evolutionary relationships among populations and phylogeny were evaluated. Tajima's-D test was used to assess genetic diversity. Tat and Rev showed a low diversity (Tajima's D value: Rev = 0.811, Tat = 0.758) and reduced genetic distance (Kimura 2-parameters: Rev = 0.109, Tat = 0.067). Low intraindividual evolutionary divergence was found in samples obtained from the same patient at different times (Poisson-correction model: Rev = 0.14, Tat = 0.13) with respect to interindividual divergence (Rev = 0.174, Tat = 0.170). Q54R mutation which decrease Tat-TAR binding affinity was found in 2/29 sequences. RGD motif which is associated with Tat-mediated LTR-promoter activity was replaced by QGD in a sample of non-progressor to AIDS. High polymorphism in Rev oligomerization domain was observed and stop codons were identified in turn region and C-term domain. The 3D protein analysis showed a conserved structure of Tat and Rev proteins. Tajima's D test showed a population expansion on both proteins indicating a recent selective pressure attributable to the immune system or transcriptase reverse error prone. Broadening our knowledge of the genetic diversity of HIV-1 regulatory genes is critical to develop novel therapeutic anti-HIV-1 strategies.

Keywords: HIV-1; Tat; Rev; Phylogenetic analysis; Protein structure.

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Vol. 11 | Special Supplement

Participation Of The Endoplasmic Membrane Complex 1 (EMC1) In The Localization Of The Dengue Virus Nonstructural Protein 1 (NS1) To The Viral Replication Complex

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Dengue virus (DENV) has a monocistronic genome, which encodes 3 structural and 7 non-structural proteins. Nonstructural protein 1 (NS1) is proteolytically matured, generating monomers in the lumen of the endoplasmic reticulum (ER) that rapidly dimerize. The NS1 dimer participates in the formation of replication complexes (RC); but, in addition, it leaves the ER to be secreted, as a hexamer, to the extracellular space. The mechanisms by which NS1 localizes to the RCs and its exit from the ER are uncertain. Protein-protein interaction studies from our laboratory indicate the possible interaction of NS1 protein with the Sec61 translocation complex. Other investigations shows the possible interaction with other translocation chaperone proteins, such as EMC, which is a complex of ER membrane resident proteins involved in the correct folding, maturation and insertion of proteins from the lumen. In this work we identified the proximity and interaction of NS1 with EMC1 and Sec61 by immunofluorescence, proximity ligation assays and immunoprecipitation in vertebrate cells infected or expressing recombinant NS1. In addition, we performed assays to elucidate the function of the EMC1 protein in the infection process by silencing the expression of this cellular protein. These results suggest that NS1 is translocated out of the ER lumen using Sec61 and EMC1.

Keywords: Dengue virus; Flaviviruses; EMC1; Sec61; Translocation.

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Vol. 11 | Special Supplement

Zika virus infection affects the integrity of α- and β-karyopherins

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Zika virus is an arbovirus belonging to the Flaviviridae family; its genome is a single-stranded RNA of positive polarity that encodes a polyprotein which is processed into three structural and seven non-structural proteins. Although its replication is cytoplasmic, there is evidence of the presence of some of its proteins in the nucleus and studies performed in cultured cells infected with ZIKV show the degradation of some of the proteins of the nuclear pore complex, the main structure involved in the control of nucleus-cytoplasm transport. This degradation has effects on the distribution of messenger RNA and cellular proteins important for both the activation of the immune response and the maintenance of cellular homeostasis. Active transport between the nucleus and cytoplasm in addition to NPC requires transport proteins or karyopherins (KPNA). Recently, overexpression of KPNA6 has been reported in ZIKV-infected cells, suggesting its involvement in the viral replicative cycle. Likewise, it was demonstrated that during infection the expression levels of other transport proteins such as KPNA2 are affected. In this work, we studied the integrity and subcellular localization of α- and β-karyopherin family nuclear transporter proteins during ZIKV infection in Huh-7 and U-87 cells. Our results indicate that ZIKV infection affects KPNA1 and KPNA2 finding changes in their expression and localization, mainly in U-87 cells; as well as KPNB1 and TNPO1 whose expression is decreased in Huh-7 cells. This suggests possible interactions between viral and cellular components that could promote viral persistence in the host and/or changes in the immune response.

Keywords: Zika; virus; Karyoherins; Integrity; Localization.

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Vol. 11 | Special Supplement

Sars-CoV-2 Nucleocapsid Protein and Lipofuscin Accumulation in Tissues of a Young Adult with Fatal COVID-19

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The pathogenesis of COVID-19 extends beyond the respiratory system. Initially identified as a respiratory illness, extensive autopsy evidence using immunochemistry, electron microscopy, and molecular techniques revealed the presence of SARS-CoV-2 in multiple organs. In this study, we report the detection of SARS-CoV-2 N protein in different tissues of a young adult with fatal COVID-19. For indirect immunofluorescence (IIF) assays, paraffin sections were stained with anti-SARS-CoV nucleocapsid (N) antibody, anti-CD31/PECAM-1 and analyzed by confocal microscopy. The SARS-CoV-2 N protein was abundantly detected in multiple skeletal muscle fibers distributed throughout the sarcoplasm in a distinctive punctuated pattern with many CD31+ infiltrating cells. The N protein was detected in the sarcoplasm of the cardiac tissue, but the distribution was concentrated in heterogeneous clusters. The cardiac muscle tissue showed minimal structural changes. Interestingly, an extensive lipofuscin accumulation was abundantly detected in skeletal and cardiac muscle fibers. Similarly, the kidney was positive for N protein and lipofuscin around the tunica externa of the blood vessel. The extensive presence of the SARS-CoV-2 N protein in the kidney, cardiac, and skeletal myocytes, as well as lipofuscin accumulation, provides further evidence of the potential role of SARS-CoV-2 in cellular senescence in the tissues of a young adult.

Keywords: COVID-19; Pathology; Nucleocapsid; Lipofuscin; Heart.

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Vol. 11 | Special Supplement

Phenotypic and genotypic analysis of influenza A H1N1pdm09 virus isolates from the 2016-17 and 2018-19 seasons

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Introduction: The influenza virus is the cause of pandemics and in the case of H1N1pdm09 it has been associated with acute severe respiratory disease. According to this, the present work focused on phenotypic and genomic characterization of influenza A H1N1pdm09 isolates from the 2016-17, 2018-19 winter seasons. Methodology: Influenza H1N1pdm09 virus was isolated in MDCK cells, from nasopharyngeal swabs of hospitalized patients. Growth kinetics of virus in cells and whole genome sequencing were performed. Subsequently, Mann Whitney tests were performed to determine significant differences in replication kinetics. Results: Eight influenza A H1N1pdm09 viruses were isolated from the 2016-17 and 2018-19 winter seasons, observing significant differences in their replicative capacity. This was associated with mutations present in the isolated viruses, mainly in HA (N162H, S190V), NS1 (Insertion Ile113) and polymerases. Also we found that In comparison with viruses carrying less mutations, viruses carrying substitutions through the viral genome replicate less efficiently. Conclusions: Significant differences were observed in the growth kinetics, An increase in mutations in the viral genome was related to less replication in vitro. The phenotypic and genomic characterization of the influenza virus is necessary to know the mutations that could be associated with better viral fitness and higher pathogenicity.

Keywords: Growth kinetics; Influenza virus; h1n1; Viral genome.

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Vol. 11 | Special Supplement

Study of the secretion of the Leader of the Capsid (LC) viroporin from CrFK cells infected with Feline calicivirus

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The Leader of the Capsid (LC) protein is unique among the members of the *Vesivirus* genus, in the *Caliciviridae* family, and it is responsible for the cytopathic effect produced by Feline calicivirus (FCV) infection. When the LC protein is expressed in a virus-free context, induces apoptosis through the intrinsic pathway; moreover, our workgroup has found that it has several viroporin characteristics, such as its ability to form oligomers, and when expressed in bacteria is intrinsically toxic must probably through the induction of osmotic stress. During FCV infection, LC protein is present in the cytoplasm and the plasma membrane. In this work, we demonstrated that LC protein is also present in the supernatants of the infected cells and is associated with extracellular vesicles, suggesting that it is secreted during infection. The results obtained add to previous work that indicates that LC viroporin could act as an important virulence factor for the maintenance and spread of the FCV infection in the host.

Keywords: FCV; Viroporin; Secretion; Infection; LC.

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The NS1 protein of DENV causes an alteration in the midgut epithelium of the *Aedes aegypti* mosquito

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Dengue virus (DENV) is the disease agent that causes dengue fever. This virus is transmitted by the bite of infected *Aedes aegypti* and *Ae. albopictus* female mosquitoes. It is an RNA-enveloped virus composed of structural and nonstructural proteins involved in the virus replicative cycle. Dengue virus nonstructural protein 1 (NS1) is the only nonstructural protein continuously secreted by infected cells. In humans, NS1 has been associated with the pathogenesis of the disease, as it can alter endothelial homeostasis through several mechanisms. When the mosquito feeds, it ingests the virus and the NS1 protein. NS1 has the capacity of decreases the antiviral response in vectors; however, considering the ability to alter the architecture in vertebrate endothelial, this could occur in the vector's intestine. To evaluate the intestinal permeability, a colorimetric assay was carried out. The structural changes of this tissue were also analyzed by conventional histology methods, and the ultrastructural modifications were analyzed by transmission electron microscopy (TEM). In addition, the localization of some proteins involved in cell-cell junctions was evaluated by immunofluorescence assays. Finally, RT-PCR experiments were used to verify the dissemination of the virus in secondary tissues within the mosquito. Therefore, we suggest that the NS1 protein participates in the dissemination of the Dengue virus in the mosquito through the modification of the mosquito's intestinal epithelium.

Keywords: Mosquitoes; Midgut; Dengue virus; NS1; Dissemination.

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Antiviral activity of *Pentalinon andrieuxii* against *Cowpea Chlorotic Mottle Virus* (CCMV)

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Plant viruses cause great losses in commercial crops around the world and represent a serious threat to food security. Currently, there are limited controls for these types of plant infections and, consequently, the search for new antivirals of natural origin represents an attractive solution to this problem. *Pentalinon andrieuxii* (Apocynaceae), is a plant distributed in the Yucatan peninsula where is used in traditional Mayan medicine to cure lesions derived from cutaneous leishmaniasis. Even though there are no reports of antiviral activity for this plant, preliminary results showed that 10 µg/ml solutions of semi purified fractions from the methanolic extract of *P. andrieuxii* reduced the symptoms of infection by CCMV (22 % of the leaf area showing mild leaf scorch) when injected to leaf petioles of *Nicotiana benthamiana*. These results suggest that *P. andrieuxii* has the potential as a novel source of new antiviral agents.

Keywords: CCMV; Antiviral; Plant virus; *Pentalinon andrieuxii*.

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Silencing of Filamin in C6/36 cells to analyze its participation in Dengue virus infection

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Dengue disease is an endemic problem in Mexico with cases of mortality every year. Dengue virus (DENV) replicates in epithelial cells of midgut and salivary glands of its insect vectors *Aedes aegypti and Aedes albopictus*. Additionally, there is a group of proteins in the cytoskeleton, some that engage the replicative cycle of DENV and others that limit it. Actin filaments facilitate the viral entry, replication and exit thanks to a connection with the viral protein E of DENV. On the other hand, filamin, a protein associated to actin and antiviral Toll pathway, limits viral infection. When this protein is down regulated, there is an increased in viral infection; however, this effect has never been tested in vector cells during DENV infection. In this work, we identified the sequences of filamin A and B of *Aedes albopictus* mosquitoes and designed two siRNAs using the i-score designer software. A siRNA against GFP was used as a negative control. Transfecting these two siRNAs simultaneously with Lipofectamine 3000TM for 72 hours, 50 % of silencing was archived in *Aedes albopictus* C6/36 cells. These conditions will be used to evaluate the effect of filamin in DENV infection in the future.

Keywords: Filamin; Dengue virus; Antiviral response; Silencing; Toll pathway.

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Vol. 11 | Special Supplement

Modulation of RhoA GTPase in CD1 mouse microglia and astrocytes during Zika virus (ZIKV) infection

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The ZIKV belongs to the Flaviviridae family, transmitted by arthropod vectors. Currently, there is no treatment for ZIKV infection, making it a public health problem in tropical areas. ZIKV has neurotropism, causing Guillain-Barré syndrome in adults and Congenital Zika Syndrome in neonates, affecting multiple signaling pathways in the brain. In this sense, other viruses of the same family, such as Japanese encephalitis virus, have reported modulation of the RhoA GTPase in brain-derived cells, involved in the signaling of the immune response and the remodeling of the cytoskeleton of glial cells and neurons. Furthermore, the importance of RhoA in the proinflammatory and anti-inflammatory profile of microglia cells in neurodegenerative diseases has been described. Therefore, in this study, we assessed RhoA activity during infections caused by ZIKV in primary cultures of astrocytes and microglia from CD1 mice. Our results demonstrate a positive regulation of RhoA and actin cytoskeleton rearrangement during ZIKV infection. Likewise, drugs capable of inhibiting the RhoA pathway cause a decrease in viral replication, suggesting that the RhoA pathway may be a therapeutic strategy during ZIKV neuroinflammation.

Keywords: Neuroinflammation; ZIKV; RhoA; Drugs; Brain.

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High Prevalence of Syphilis and Syphilis/HIV Coinfection among Men Who Have Sex with Men Who Attend Meeting Places in Mexico

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Men who have sex with men (MSM) are disproportionately affected by syphilis, HIV, and syphilis/HIV coinfection. ART prevents HIV transmission but does not impede syphilis transmission. Information about syphilis/HIV coinfection is scarce. We aimed to determine the prevalence of syphilis/HIV coinfection in a national sample of MSM who attend meeting places in Mexico to 1) evaluate factors associated with syphilis and 2) compare the prevalence of syphilis between the current survey and Dirección General de Epidemiología (DGE) data. We performed a laboratory diagnosis to determine syphilis and HIV among MSM. National/regional prevalence of syphilis was calculated. HIV and coinfection prevalence were determined only for the survey. Descriptive, bivariate, and multivariate analyses were performed. National prevalence of syphilis, HIV, and coinfection were 15.2 %, 10.2 %, and 5.7 %, respectively. Mexico City had the highest prevalence (39.4 %). The Center region, minimal "goods", "inhalant drugs", "HIV infection", "sexual intercourse", only with men, "rewarded sex", and "early sexual debut" were risk factors for syphilis. Regional prevalence of syphilis was higher in the survey (2013) and DGE data from 2019 than in the DGE data from 2013. Mexico needs to assess elements around not only syphilis and HIV infections but also syphilis/HIV coinfection, and preventive measures focusing on MSM are needed.

Keywords: HIV; Meeting places; Men who have sex with men; Syphilis; Syphilis/HIV coinfection.

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Variability in Susceptibility to Type I Interferon Response and Subgenomic RNA Accumulation Between Clinical Isolates of Dengue and Zika Virus From Oaxaca Mexico Correlate With Replication Efficiency in Human Cells and Disease Severity

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Dengue and Zika viruses cocirculate annually in endemic areas of Mexico, causing outbreaks of different magnitude and severity every year, suggesting a continuous selection of Flavivirus variants with variable phenotypes of transmissibility and virulence. To evaluate if Flavivirus variants with different phenotypes cocirculate during outbreaks, we isolated dengue and Zika viruses from blood samples of febrile patients from Oaxaca City during the 2016 and 2019 epidemic years. We compared their replication kinetics in human cells, susceptibility to type I interferon antiviral response, and the accumulation of subgenomic RNA on infected cells. We observed correlations between type I interferon susceptibility and subgenomic RNA accumulation, with high hematocrit percentage and thrombocytopenia. Our results suggest that Flaviviruses that cocirculate in Oaxaca, Mexico, have variable sensitivity to the antiviral activity of type I interferons, and this phenotypic trait correlates with the severity of the disease.

Keywords: Dengue; Zika; Interferons; Isolates; sfRNA.

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Vol. 11 | Special Supplement

Evaluation of flaviviral subgenomic RNA as a virulence factor of Flavivirus virulence factor of Flaviviruses isolated in endemic endemic areas of Mexico

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Dengue and Zika viruses cocirculate annually in endemic areas of Mexico, causing outbreaks of different magnitude and severity every year, suggesting a continuous selection of Flavivirus variants with variable phenotypes of transmissibility and virulence. We compared the accumulation of subgenomic RNA on infected cells between Flavivirus isolates from Oaxaca from 2016 and 2019. We observed correlations between subgenomic RNA accumulation, with high hematocrit percentage and thrombocytopenia. Our results suggest that Flaviviruses that cocirculate in Oaxaca, Mexico, have variable accumulation of sfRNA, and this phenotypic trait correlates with the severity of the disease.

Keywords: Dengue; Zika; Isolates; sfRNA.

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Vol. 11 | Special Supplement

Immune characterization of the megakaryocytic lineage during Dengue virus infection

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Dengue virus (DENV) infection is a global health problem, and its incidence is increasing. Activation and decrease in the number of platelets is a clinical feature of severe dengue. Moreover, platelet precursors are known to be the main target of DENV infection in bone marrow. The objective of this project is to understand the differences in the permissiveness of DENV and its relationship with the mechanisms of the antiviral response in platelet precursors, for which we infected the erythroid-megakaryocyte precursor (cell line K-562) and the megakaryoblast (cell line MEG-01) with DENV serotype 1 (DENV1). Infection in the cells was confirmed by immunofluorescence using a primary antibody directed to DENV protein E, as well as by flow cytometry, obtaining higher percentages of viral antigen in K-562 cells (19.3 + -2.6 %) than in MEG-01 cells (14.9 + -2.4 %). When performing a 7-day infection kinetic and evaluating the viral titer by plaque formation assay, it was observed that K-562 cells are more permissive to DENV1 than MEG-01 cells since higher values were obtained (K-562: 6.49 ± 0.61 (Log10) PFU/mL; MEG-01: 3.87 ± 0.4(Log10) PFU/mL), also when performing a MTT cell viability assay, no reduction in viability was observed in both cell lines during infection.

Keywords: Megakaryocytic; Innate immunity; Dengue; K-562; MEG-01.

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Vol. 11 | Special Supplement

Association of BoLA-DRB3.2 binding pockets amino acid motifs with outcome of persistent lymphocytosis in Bovine Leukemia Virus infected cows

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Bovine leukemia virus (BLV) promotes a persistent lymphocytosis (PL) acknowledged for susceptibility. PL has been used for detection of BoLA-DRB3.2 alleles linked with susceptibility or resistance resting on amino acid (aa) motifs within five binding pockets (P1, 4, 6, 7, and 9) located in the peptide-binding cleft of MHC-II. To typify BoLA-DRB3.2 alleles in BLV infected cows, PCR- sequence-based typing (SBT) was utilized to relate pocket aa motifs positioned in the pockets with resistance or susceptibility based on PL occurrence. Odds ratios (OR) were used to categorized traits, revealing that the main associations were within P4 at TL/77-78, L/78, K /71, EK/70-71, and E/74 for resistance whereas those related to susceptibility were placed at TV/77-78, V/78, RR/70-71, K/71, and Y /78. Also, resistance motifs were identified in P9 (D/57), P6 (Y/30) and P1 (V/86). Furthermore, the P4 electrostatic charge determined via calculation of solvent accessible surface areas (SASA) showed positive charge in the BLV+LP+ cows while in the BLV+LP- and uninfected animals exhibited negative charge. Our findings suggest that both, aa motif location and electrostatic protein charge contribute to the binding cleft conformation, affecting the siting and attachment of peptide which in turn influences resistance o susceptibility to disease.

Keywords: Bovine leukemia virus; Persistent lymphocytosis; BoLA-DRB3.2 alleles; Pockets; Electrostatic charge.

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Vol. 11 | Special Supplement

Generation of a monoclonal antibody against the RBD of the Spike protein from SARS CoV-2-

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The COVID-19 is a disease caused by the SARS CoV-2; virus responsible for millions of deaths worldwide 1. SARS-CoV-2 is an enveloped, positive, and single-stranded RNA virus. Its genome encodes four structural proteins: membrane (M), envelope (E), spike (S), and nucleocapsid (N). The spike glycoprotein (S) protein is comprised of S1 & S2 subunits. These spike protein subunits enable viral attachment by binding to the host cell *via* ACE-2 (angiotensin converting enzyme-2) receptor (RBD). The Spike protein induce an immune a protective immune response and it has been used as a vaccine target, and for diagnosis, furthermore; an anti-Spike monoclonal antibody is a power tool to evaluate many aspects of virus molecular biology and many aspects of pathogenicity of the SARS CoV-2. In the present work we design express and purify the RBD protein of SARS CoV-2. in mammalian platform, to immunize BALB/c mice for the generation of monoclonal antibodies. After all steps for get monoclonal antibodies. This antibody was able to recognize the viral protein trough different approaches such as ELISA, WB and in immunofluorescence assays using cells Vero infected with the SARS CoV-2 virus. This antibody will provide us with an essential tool for studies on the pathogenicity of the SARS CoV-2.

Keywords: Antibody; Monoclonal; SARS CoV-2; Spike, RBD.

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Changes In The Cytokine Profile Of HIV/HCV Coinfected Patients Treated With Direct-Acting Antivirals

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We evaluate the behavior of cytokines in HIV/HCV coinfected patients treated with direct acting antivirals (DAAs). Through a prospective study in patients > 18 years old HIV/HCV coinfected carriers treated with DAAS for 12 weeks. A baseline sample (prior to treatment) and 12 weeks post-treatment were taken. For the statistical analysis: descriptive (median and interquartile ranges for quantitative variables, frequencies, and percentages for qualitative variables). The comparison of cytokines was done using the Wilcoxon test. 27 patients were included vih years (SD 10.56), 96.3 % (26) were men, the most frequent genotype of HVC was 1A with 55.6 % (15), the DAAS received was: 14 (51.9 %) Sofosbuvir/Ledipasvir and 13 (48.1 %) Sofosbuvir/Velpatasvir, 100 % achieved sustained viral response. A cytokine profile (pg/ml) was analyzed obtaining the following significant results: sCD163: 48.8 (27.1—68) vs 32.1 (19.3—57.6) [P = 0.001], IL-12: 98.31 (78—165.9) vs 85 (55—162.7) [P = 0.039], RANTES: 716 (248.3—1151.5) vs 853.6 (389.6—3425.5) [P = 0.046], IL-15: 147.3 (43.1—560.5) vs 253.3 (74.1—627.97) [P = 0.035], IP-10: 89.3 (57.8—222.46) vs 26 (16.3—43.9) [P = 0.000], MIG: 131.4 (73.1—321.7) vs 99.6 (52.8—130.9) [P = 0.030]. With these results we can conclude the treatment with DAAS decreases the inflammatory and profibrotic response in HIV/HCV coinfected patients with sustained viral response.

Keywords: HIV1; HVC2; DAAs3; Citokyne4; Profibrotic5.

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Discovery and optimization of neutralizing SARS-CoV-2 antibodies using ALTHEA Gold Plus Libraries™

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We recently reported the isolation and characterization of anti-SARS-CoV-2 antibodies from a phage display library built with the VH repertoire of a convalescent COVID-19 patient, paired with four naïve synthetic VL libraries. One of the antibodies, called IgG-A7, neutralized Wuhan, Delta (B.1.617.2) and Omicron (B.1.1.529) strains in authentic neutralization tests (PRNT). It also protected 100 % transgenic mice expressing the human ACE-2 from SARS-CoV-2 infection. Here, the four synthetic VL libraries were combined with the semi-synthetic VH repertoire of ALTHEA Gold Libraries™ to generate a set of fully naïve, general-purpose, libraries called ALTHEA Gold Plus Libraries™. After three rounds of panning with SARS-CoV-2 RBD wildtype as selector, 630 clones were tested for binding to RBD, yielding 125 positive and specific scFvs, with 24 being unique clones. Three out of 24 specific clones with affinity in the low nanomolar range and sub-optimal *in vitro* neutralization in PRNT, were affinity optimized via a method called "Rapid Affinity Maturation". The final molecules reached sub-nanomolar neutralization potency, slightly superior to IgG-A7, while improved the developability profile over the parental molecules. These results demonstrate that general-purpose libraries are a valuable source of potent neutralizing antibodies and it could expedite isolation of antibodies for rapidly evolving viruses.

Keywords: COVID-19; Phage display; Therapeutic antibodies; Affinity maturation; Semi-synthetic libraries.

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Vol. 11 | Special Supplement

Titer of IgM, IgA and IgG antibodies in serum and saliva of personnel occupationally exposed to SARS-CoV-2

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The presence of anti-SARS-CoV-2 antibodies in serum indicates protection against the virus; however, although circulating antibodies control infection within the body, secretory IgA could provide better protection by acting as a primary barrier in containing the infection and therefore dispersing the virus. Our objective was to evaluate the seroconversion and titers of IgG, IgA and IgM against SARS-CoV-2 in Personnel Occupationally Exposed to SARS-CoV-2 (POE) in the Manuel Gea Gonzalez General Hospital. Using the ELISA technique, the antibody titers (IgG, IgA and IgM) against the synthetic peptides Spike (RBD, Arg319-Phe541) and Nucleocapsid (NCP, Met1-Ala419) were determined in the serum of three study groups: A) infected and vaccinated POE, b) uninfected and vaccinated POE and C) uninfected and unvaccinated POE. In addition, IgA was also detected in the saliva of group C. The percentage and titers of IgM, IgA and IgG versus NCP and RBD were similar in groups A and B (above 35 %), but not in group C, in the that IgG and IgA showed values below 10 % and no seroconversion of IgM versus RBD was observed. Regarding the detection of IgA in saliva, 35 % positivity was observed with RBD antigen and 25 % with NCP, higher percentages than in serum. Although RT-qPCR did not detect SARS-CoV-2 infection in Group C, antibodies against SARS-CoV-2 were detected; this could indicate that secretory IgA was excellent protection.

Keywords: Seroconversion; Saliva samples; IgA; IgG; IgM.

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Vol. 11 | Special Supplement

P7F8, a promising human antibody against SARS-CoV-2 variants of concern

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The vaccination against COVID-19 represented a great advance in the reduction of infections and has provided patients with the disease with an improved immune response, although the prevailing public health crisis continues to cast doubt on the efficacy of current treatments due to the high rate of mutations that the virus possesses, which has led to the search for other therapeutic options such as monoclonal antibodies. In this work, we aimed to select and characterize a group of human monoclonal antibodies against SARS-CoV-2, from convalescent and/or vaccinated individuals, which are able to inhibit the interaction of protein S with the ACE-2 receptor. Using the Epstein-Barr virus memory B-cell immortalization method, we selected whole blood samples from confirmed and convalescent volunteers with antibodies with recognition of the RBD domain of the S1 protein of SARS-CoV-2. From venous blood PBMCs and by MACS and FACS selection, the number of RBD-specific antibody-producing cells was quantified and immortalized. Once the lymphoblastoid cell lines were developed, one of the best specific antibody-producing clones was determined by recognition assays and characterized. The antibody obtained is able to recognize RBD from different VOC of SARS-CoV-2 with an affinity of nM scale, and presents a reported motif with cross-reactivity to RBD and other *Sarbecoviruses* giving it a broad neutralization.

Keywords: SARS-CoV-2; COVID-19; Antibody; RBD; Sarbecoviruses.

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Effect of the absence of the AalSep2 septin protein on Dengue virus replication in C6/36 cells derived from *Aedes albopictus* mosquito

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Introduction: Septins form a family of GTP binding proteins, are components of the cytoskeleton interacting with actin filaments, microtubules, intermediate filaments, and cell membrane. Septins participate in cytokinesis, motility, chromosome segregation, vesicular trafficking, and in innate immunity against intracellular pathogenic bacteria. In *Ae. aegypti* and *Ae. albopictus* mosquito derived cell lines Aag2 and C6/36 during the DENV replication cycle, septin2 interacts with NS3 and NS5 viral proteins. Objective: Generate septin2 knockout C6/36 cells and analyze its effect on DENV replication. Methods: A guide RNA against aalsep2 gene was designed and cloned in the CRISPR/Cas expression system vector pSpCas9(BB)-2A-GFP (PX458). The plasmid was transfected into C6/36 cells, transfectants were puromycin selected cloned, expression of AalSep2 analyzed by western blot and DENV titer measured. Results: Transfectant clones did not expressed AalSep2 protein, presented changes in morphology and during DENV infection there was a significant viral titer drop. Discussion: C6/36 AalSep2 KO cells changes in morphology, supports its cytoskeletal role. In C6/36 AalSep2-KO cells DENV4 infection rendered a diminished viral progeny. At present, this system is used to investigate the specific function of septin 2 during viral infection. A hypothesis is that a cell and virus proteins are interacting to complete viral development.

Keywords: Aedes; DENV; Dengue virus; Knockout; Guanosine triphosphate.

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Human metapneumovirus infection modifies stem cell function, immunomodulatory and migratory capacity

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Human metapneumovirus (hMPV) is a major cause of acute respiratory infection worldwide, hMPV is a negative-sense single stranded RNA virus, it is a virus with a high prevalence, however our knowledge of the viral impact in some cell population remains limited. We focus on placenta stem mesenchymal cells (hMSC), cells that we prove elsewhere to be susceptible to infection by hMPV. during infection the morphology of the cells changes, however the formation of syncytia (common cytopathic effect of hMPV infection) is much lower compared to cell lines susceptible to infection. By immunofluorescence we observed the distribution of virus within cells, noting different patterns between cell lines (VERO, A549) and mesenchymal stem cells. We evaluated on a population of macrophages whether the infected cells were able to induce morphological changes and changes in the growth factor secretory profile, and we compared the results with those obtained with uninfected cells. The ability of stem cells to modulate components of the immune system by paracrine action is modified during infection, being able to activate a population of monocytes by the supernatant of infected mesenchymal stem cells. Finally, we evaluated the migratory capacity of the infected cells by evaluated related secretory molecules, we also observed a change in cytoskeletal proteins distribution (actin, tubulin and annexin).

Keywords: Metapneumovirus; Stem cells; Mesenchymal viral infection; Macrophage; Infection; Immunomodulatory.

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Effect of adenovirus infection on the intracellular distribution and activity of the cellular components of innate immunity IRF3 and IFI16

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The cellular response to viral infections involves innate antiviral mechanisms, including the production of interferons (IFNs) that inhibit viral replication. However, viruses have evolved mechanisms that inhibit the antiviral response of the cell. A common strategy among viruses in which this process has been studied involves the relocalization of cellular antiviral defense proteins to sites where the viral genome is replicated and expressed, known as viral replication compartments (RC). During adenovirus infection one of the primary antiviral responses is triggered by recognition of viral double-stranded DNA in the cytoplasm, and activation of the cGAS-STING pathway, leading to the phosphorylation and nuclear localization of the interferon response factor 3 (IRF3) and interferon gamma-induced cellular protein 16 (IFI16). IRF3 is a transcription factor that plays a crucial role in the activation of the innate immunity and inflammation in response of viral infection; IFI16 was the first Pattern Recognition Receptor (PRR) identified that detects cytosolic DNA and activates antiviral gene expression via STING triggering IFN I and III production. In this study we hypothesized that in adenovirus-infected cells IRF3 and IFI16 may be inhibited through their recruitment to RC. Therefore, we evaluated the effect of adenovirus infection on the intracellular distribution and activity of IRF3 and IFI16.

Keywords: Viruses; Innate immunity; IRF3; IFI16.

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Effect of extracellular vesicles from Dengue virus-infected endothelial cells on human monocytes

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The main cause of death in Severe Dengue patients is the hypovolemic shock Syndrome, that occurs because of vascular damage. Endothelial vascular cells (EVC) have great importance as they are the main components of vascular endothelial barrier and can modulate of other cells through different effector molecules like cytokines or extracellular vesicles (EVs) as microparticles or exosomes. Dengue virus (DENV) is capable of directly infect human endothelial vascular cells (EVC), activating them and generates different changes in the endothelium such as the expression of leukocyte adhesion proteins, procoagulant and pro inflammatory molecules and the increase in the secretion of extracellular vesicles. In this work we propose that endothelium infection by DENV favor the generation of EVs with the capacity to induce changes in the monocyte phenotype, which has been associated with greater adhesion to endothelium, and the secretion of proinflammatory, pro adherent and procoagulant molecules which may alter the endothelial barrier with an increased vascular permeability, present during Dengue Shock Syndrome cases.

Keywords: Severe dengue; Vascular barrier; Extracellular vesicles; Monocytes; Vascular permeability.

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Vol. 11 | Special Supplement

Long-lasting Chikungunya virus Neutralizing Antibodies in Convalescent patients´ sera from an endemic region of Mexico

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Chikungunya fever (CF) is caused by CHIK virus and is transmitted by an arthropod vector. Currently, the climatic conditions spread the migration of the vector, increasing the possibilities of new outbreaks around the world. We evaluate the persistence of antibodies against CHIKV in a population of Guerrero by indirect ELISA, Western Blot (WB), and plaque reduction neutralization test (PRNT), nine years after the 2014 outbreak. To considerate positive specimens that recognize E2-CHIKV protein in ELISA, the ratio between positive and negative samples absorbance is ≥ 2, using a serum from one patient obtained during CHIKV outbreak of 2014 as a positive control. From thirty-four sera, the antibody titer was determined using a three-fold serial dilution, starting with 1:300. The samples were classified in four groups: 1) 16.6 % showed very high titer (≥10 001); 2) 8.3 % high titer (10 000—5 001); 3) 41.6 % intermediate titer (5 000—1 001); and 4) 33.3 % low titer (1 000—300). One sixth of these, showed neutralizing activity against the isolate Chikungunya virus 033. The protein E2 (37.5 kDa) was identify in almost all the samples by WB. These results indicate that long-lasting humoral immunity against CHIKV (due to the presence of long-live plasma cells) is reactivated by continuous exposure to the virus.

Keywords: CHIKV; E2; Antibodies; Indirect ELISA; PRNT.

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Effects of Gamma Radiation on the Expression of Interferon Type I Stimulated Genes in Antigen Presenting Cells

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Antigen Presenting Cells (APCs) play a crucial role in the antiviral response by producing Type I Interferons (IFN-I). While ionizing radiation's immunomodulatory role is known, its effects and applications in APCs are still being studied. We evaluated the impact of 1.5, 2, 12.5, and 30 Gy on IFN-I stimulated genes (ISGs) expression in monocyte-derived dendritic cells using RT-qPCR. Overexpression of Interferon Regulatory Factor 3 occurred at 2 ($P = 0.028$) and 12.5 Gy ($P = 0.046$), and Tripartite Motif Containing 21 at 1.5 and 2 Gy ($P = 0.037$ for both). A tendency towards increased alpha interferon expression was observed at 2 and 12.5 Gy ($P = 0.054$ for both). The ISG expression alterations prompt further study of a possible promotion of the antiviral state in other APCs.

Keywords: Radiobiology; Radio-immunomodulation; ISGs; Antigen presenting cells; Antiviral.

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Non-Structural protein 5 of DENV serotype 2 localizes to the nucleolus and interacts with nucleolar protein B23

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The non-structural protein 5 (NS5) is a multifunctional molecule in all flaviviruses. In some flaviviruses, such as DENV serotype 2, this protein is located mainly in nucleus of infected cells, so far, the function of the nuclear form of NS5 has been exhaustively evaluated. Many interactome predictor has been performed discovering that NS5 protein interacts with many cellular proteins however the NS5 interaction with cellular proteins is not yet totally elucidated. In order to provide more information on the role of NS5 protein in the nucleus. We found by IF assays, co-localization of NS5 with nucleolar protein B23. To confirm interaction, we immunoprecipitated nuclear protein extracts of infected cells, with an antibody against B23. The exact mechanism through which NS5 and B23 interact remains unknown to this study, we found that NS5 mainly localized in the nucleus of infected cells and specifically appears to target the nucleolus, further investigation on mechanisms and functionality are required to get to know the advantages that DENV-2 gets with this interaction.

Keywords: DENV; NS5; B23; Interaction; Co-localization.

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Vol. 11 | Special Supplement

Beyond a cytokine storm: relevance of the action between tumor necrosis factor-alpha (TNF) and interferon-gamma (IFN-γ) in COVID-19

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In severe COVID-19, a rapid deterioration at the pulmonary level may lead to organ dysfunction and even death. Initially, this was attributed to the cytokine storm. However, it has been identified in a murine model that more than the overall action of a broad panel of cytokines could be the synergism between TNF and IFN-γ, which triggers a process of inflammatory cell death. Here, we evaluated the impact of the TNF/IFN-γ axis in COVID-19 patients. A cohort of 138 vaccine naïve COVID-19 patients were classified into four groups (G) according to the plasma level of TNF and IFN-γ (High [H] or Normal-Low [N-L]), as follows: G1: TNFH/IFN-βH; G2: TNFH/IFN-βN-L; G3: TNFN-L/IFN-βH; and G4: TNFN-L/IFN-βN-L. Apoptosis, cell death, inflammation, and senescence-related proteins were evaluated at transcriptional, soluble levels, or both. According to the patient group, we identify a specific profile of apoptosis and inflammatory-involved proteins. The p21/CDKN1A, a protein impacting the cell cycle and senescence, was increased in G2 and G3. G1 showed a higher level of TNFR1, MLKL, RIPK1, NLRP3, Caspase 1, and HMGB-1. Interestingly, a high percentage of the Gp 1 patients (81 %) had severe COVID-19, and 44 % died. Overall results suggest that an increased level of both cytokines simultaneously promotes the convergence of cell death pathways as pyroptosis, apoptosis, and necroptosis, which may converge in a cell death process called PANoptosis. This could be associated with the severity of the disease and death of patients. Additionally, some events of senescence and cell cycle deregulation could participate.

Keywords: COVID-19; SARS-CoV-2; Co-signaling; IFN-γ TNF.

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Vol. 11 | Special Supplement

In silico analysis of differentially expressed transcripts in premalignant lesions and cervical cancer

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Worldwide, cervical cancer (CC) is the second cause of women's death. Human papillomavirus (HPV) infection is the major risk agent for this cancer type, being HPV-16 and -18 the prevalent genotypes. Women with HPV-positive premalignant lesions (HSIL) and cancer shown high levels of serum cytokines. The aim of this work was to analyze in silico, the transcript profiles from HPV-16 cervical cancer and premalignant lesions from the GEO2R database. By using the Transcriptome Analysis Console tools to compare the transcript profiles of CC vs control, CC vs HSIL and HSIL vs control samples; thus, 17,857, 4,756 y 7,204 differentially expressed transcripts, were observed. Interestingly, the tumor necrosis factor (TNF-α) signaling and therein the NF-κB pathways were up-regulated in CC. TNF-α. is one of the main pro-inflammatory cytokines of the immune system, with multiple pleiotropic functions. Analysis of the transcript and protein levels of TNF-α in cervical cancer tumor derived cell lines revealed that protein and transcript levels were expressed differentially. Therefore, we initiate a more detailed analysis of mechanisms of expression and secretion of TNF-α in these cells at the different post-transcriptional and post-traductional levels and in this way to have a better understanding of its possible implications in tumorigenesis.

Keywords: Cervical cancer, HPV, TNF-α, Transcript in silico.

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Bispecific antibodies against the main SARS-CoV-2 variants of concern: an effective strategy

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COVID-19 caused by SARS-CoV-2, emerged as a global pandemic, highlighting the RNA virus's rapid mutation rate, in special affecting the Spike (S) protein, which is crucial in the binding with the host cell receptor. As a result, variants of concern (VOCs) appeared with the ability to escape from the immune response. In contrast, several monoclonal antibodies were used as treatment against COVID-19, but they failed through the time, because they lost the binding capacity. We developed bispecific antibodies from a group of human monoclonal antibodies able to recognize different RBD epitopes using the Epstein-Barr virus immortalization human B cells method. The variable regions of the heavy and light chains of these antibodies were amplified, sequenced, and the germlines were analyzed. The constructions of heterodimers VHS57G9-(G4S)4linker-VLS57G9-K6/K9linker-VHP7F8-(G4S)4linker-VLP7F8, and VHS56G9-(G4S)4linker-VLS56G9-K6/K9linker-VHG6E8-(G4S)4linker-VLG6E8 were cloned in the pFUSEss-CHIg-hG1. Each dimer was expressed in Expi293 cells. These were purified by affinity chromatography with protein A. We evaluated the binding affinity and neutralization capacity compared with the monomers used as controls. These bispecific antibodies against SARS-CoV-2 increased the constant affinity and neutralizing properties. Also, their capacity against VOCs enhanced broadly.

Keywords: SARS-CoV-2; Variants of concern; Bispecific; Tandem; B cells.

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Vol. 11 | Special Supplement

Characterization of Flavivirus-induced platelet microparticles

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The role of platelets in hemostasis has been extensively documented, but various aspects of their involvement in the immune response are still under investigation. Platelets can regulate the immune system by releasing cytokines and platelet microparticles (MPs). MPs are small extracellular vesicles ranging from 100—1000 nm in size, derived from cell membranes. They play a significant role in intercellular communication and transport, carrying various molecules related to both hemostasis and the immune system. Their content is heterogeneous, depending on the stimulus triggering their release. Under pathological conditions, MPs have been reported to contain cytokines, glycoproteins (CD42b), costimulation molecules (CD40L), adhesion molecules (CD62P), and transcription factors (NF-κB). The release of MPs increases in various diseases, including cancer, cardiovascular diseases, and viral infections. During DENV infection, MPs are known to transport proinflammatory cytokines. There have also been reports of severe thrombocytopenia associated with ZIKV infections. We evaluated MPs released from platelets stimulated by DENV and ZIKV. Particle tracking analysis was used to analyze concentration and size, which revealed no difference in size but a significant increase in concentration upon stimulation. Flow cytometric analysis identified immunoregulatory molecules in the MPs, with both DENV and ZIKV-stimulated MPs containing CD40L and pNF-κB. The results showed that flavivirus stimulation led to a substantial increase ($P < 0.05$) in MP concentration. Additionally, DENV-stimulated MPs showed an increase ($P < 0.05$) in size, while the expression of the CD62p and CD42b molecules decreased ($P < 0.05$) compared to other groups. The MPs released in response to flavivirus stimulation contained immunomodulatory molecules CD40L and p-NF-κB.

Keywords: Platelets; Microparticles; MPs; DENV; ZIKV.

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Identification of replicated genes in *de novo* DNA synthesis derived from immunological priming in the *Aedes aegypti – Dengue virus* model

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The term "immune priming" applied to insects refers to innate immune memory induced by an initial exposure to a sublethal dose of a pathogenic agent or its derivative, which elevates its immune response, and protects it against a secondary exposure. The molecular events involved in the generation of immunological memory of mosquitoes are yet to be fully elucidated. However, there is a relationship between the increase of DNA in cells involved in the immune response and immune priming. This research project aims to identify groups of genes that enrich the cell signaling pathways when the immune priming is induced. We identified about 200 genes replicated in *de novo* DNA synthesis, derived from immune priming, in midgut cells of *Ae. aegypti* challenged with inactive DENV-2. The obtained data suggest the process of immune priming-induced endoreplication replicates important genes in the anti-DENV immune response.

Keywords: Immune priming; *Aedes aegypti*; Dengue virus; Endoreplication; Immune response.

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Evaluation of the neutralizing effect of anti-Chikungunya antibodies against Dengue virus

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The neutralizing capacity of anti-CHIKV (chikungunya virus) antibodies against dengue virus (DENV) is currently unknown. In this study, the identification and titration of anti-DENV2 and anti-CHIKV antibodies was carried out in sera from patients from Veracruz during 2013 and 2015. In addition, anti-CHIKV antibodies were obtained in BALB/c mice and evaluated for their neutralizing capacity against DENV. An increase of 100 AU (arbitrary units) of anti-CHIKV antibodies was observed in the group of mice that were inoculated with DENV with respect to the control group ($P < 0.0001$); an increase of 250 AU was also observed in the group of mice that were initially inoculated with CHIKV and subsequently with DENV with respect to the control group ($P < 0.0001$). In the neutralization assay against dengue the group of mice that were inoculated only with CHIKV showed 50 % reduction of PFUs (Plaque forming units), in comparison the group of mice that were inoculated initially with dengue and subsequently with CHIKV had 65 % reduction of PFUs. The serum of patients positive for anti-CHIKV IgG and negative for anti-DENV2 IgG had a neutralizing antibody titer against DENV2 of 2 $\log_2$, in contrast those positive for anti-CHIKV IgG and anti-DENV showed a higher titer (7.5 $\log_2$, $P < 0.0001$).

Keywords: Neutralizing antibodies; Dengue; Chikungunya; DENV; CHIKV.

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Vol. 11 | Special Supplement

Human microvascular endothelial cells (HMEC-1) activation by Dengue virus infected human monocytes (THP-1) microparticles

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Dengue fever is acute febrile illness, widely distributed to tropical and subtropical zones around the world, that is caused by any of 4 serotypes of Dengue virus (DENV) which is transmitted by female mosquitos of the genera Aedes. DENV has 3 structural proteins and 7 nonstructural proteins necessaries for its replication. Mononuclear phagocyte system cells (Monocyte, macrophage, and dendritic cells) are DENV's principal target cells, when infected they release proinflammatory cytokines and extracellular vesicles (exosomes and microparticles) or VEs that have a lipid bilayer and can carry viral products (viral proteins and viral genome) and mature virions that can favor endothelial cells activation therefore expressing procoagulant, proinflammatory and pro adherent molecules. In the present work we evaluate the activation of human vascular endothelium by DENV-infected monocyte's extracellular vesicles that can favor the increase in vascular permeability (assessed by permeability transwell assay) that characterizes Dengue Shock Syndrome.

Keywords: Dengue Virus; DENV; Monocyte; Endothelium; Extracellular Vesicles.

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Isolation and characterization of single domain antibodies against SARS-CoV-2 RBD protein

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We describe here the construction of a single domain antibody (sdAb) library consisting of grafting natural human HCDR3 fragments obtained from 200 healthy human donors into a synthetic framework region designed with the human germline IGHV3-23. After three rounds of panning against the hACE-2 receptor binding domain (RBD) of SARS-CoV-2, 90 clones were randomly picked and screened for binding to RBD and BSA. A total of 35 clones specifically bound RBD, with 9 clones showing unique sequences. Out of these unique clones, four blocked RBD:hACE2 interaction.. A Clone, termed sdC3, was expressed in *E. coli* and purified by Protein A, exhibiting the expected molecular mass of ≈18 kDa. Purified sdC3 had an affinity for RBD comparable to a potent neutralizing scFv and blocked the RBD:ACE2 interaction. Further, heat-treated sdC3 at 80 °C for 10 minutes retained its binding affinity, indicating high thermal stability, an advantageous property typical of sdAbs. Thus, the platform we developed for rapid isolation of fully human sdAbs set the stage for future discovery of diverse molecules with application not only in rapidly evolving viral diseases such as SARS-CoV-2 but also in other applications that require highly stable molecules.

Keywords: ACE2; sdAbs; RBD; SARS-Cov-2, Library single domain.

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The SARS-CoV-2 E protein interacts with distinctive PDZ proteins in immune cells

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PDZ proteins are central in the assembly of multiprotein complexes that regulate cell polarity. Viral pathogens express proteins harboring PDZ-binding motifs (PBM) as a strategy to facilitate their dissemination. SARS-CoVs express the envelope protein (E) that contains a conserved PBM. SARS-CoV-1 E interaction with PDZ proteins like PALS1 in epithelia promotes disruption of cell junction, contributing to viral pathogenicity. SARS-CoV-2 infects epithelium, but also innate immunity cells like monocytes, macrophages and dendritic cells. Identification of targets of the SARS-CoV-2 E protein (2E) in immune cells might offer valuable clues to understand viral immunopathogenicity and to highlight the function of PDZ proteins in immune fitness. The ORF encoding 2E was cloned in the pEZYeGFP vector, which allowed to express 2E fused to a GFP-tag. GFP tagged proteins were expressed in the THP-1 monocytic cell line, and immunoprecipitated using the GFP-trap system. Associated proteins were analyzed by LC-MS. The interactome of 2E in THP-1 cells provided 372 proteins that fall into different functional groups; eight of these harbor PDZ domains. We found novel interactions of 2E protein with distinctive PDZ proteins whose function has been related with polarity and immune response. This support that viral targeting of PDZ proteins alters immune response

Keywords: Viral-host relationship; PDZ proteins; Viral hijacking; Envelope protein; SARS-CoV-2.

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Vol. 11 | Special Supplement

High Prevalence of Syphilis and Syphilis/HIV Coinfection among Men Who Have Sex with Men Who Attend Meeting Places in Mexico

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Men who have sex with men (MSM) are disproportionately affected by syphilis, HIV, and syphilis/HIV coinfection. ART prevents HIV transmission but does not impede syphilis transmission. Information about syphilis/HIV coinfection is scarce. We aimed to determine the prevalence of syphilis/HIV coinfection in a national sample of MSM who attend meeting places in Mexico to 1) evaluate factors associated with syphilis and 2) compare the prevalence of syphilis between the current survey and Dirección General de Epidemiología (DGE) data. We performed a laboratory diagnosis to determine syphilis and HIV among MSM. National/regional prevalence of syphilis was calculated. HIV and coinfection prevalence were determined only for the survey. Descriptive, bivariate, and multivariate analyses were performed. National prevalence of syphilis, HIV, and coinfection were 15.2 %, 10.2 %, and 5.7 %, respectively. Mexico City had the highest prevalence (39.4 %). The Center region, minimal "goods", "inhalant drugs", "HIV infection", "sexual intercourse" only with men, "rewarded sex", and "early sexual debut" were risk factors for syphilis. Regional prevalence of syphilis was higher in the survey (2013) and DGE data from 2019 than in the DGE data from 2013. Mexico needs to assess elements around not only syphilis and HIV infections but also syphilis/HIV coinfection, and preventive measures focusing on MSM are needed.

Keywords: HIV; Meeting places; Men who have sex with men; Syphilis; Syphilis/HIV coinfection.

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Rabies virus G5 immunogenic epitope as surface marker of HIV-1-infected cells: a strategy to eradicate HIV-1

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The development of an HIV-1 cure remains a huge scientific challenge due to its high genetic/antigenic variability that favors the evasion of the host's immune system and formation of the viral reservoir. We developed a molecular model to detect exclusively HIV-1 infected cells by regulating the expression of an epitope of rabies virus glycoprotein (RABVG5) through a viral infection-dependent mechanism mediated by HIV-1 Tat and Rev proteins. RABVG5 is a small linear epitope tested as a potential rabies DNA vaccine candidate, inducing high titers and sustained production of neutralizing antibodies. We designed pLG5I containing HIV-1LTR promoter, INS, and RRE sequences coupled to G5-TagHis, which was transfected to HEK293 cells in the presence and absence of HIV-1 Tat and Rev encoded by pCV1 and pNL4-3 plasmids. G5+cells were quantified by flow cytometry and fluorescence microscopy. G5 expression in whole HEK293 lysates transfected with pLG5I in the presence of Tat and Rev was verified by WB. Serum antibodies against RABVG5 were generated in Balb/c mice and used to detect the epitope on HEK293 cell surface. RABVG5 expression was controlled by HIV-1 Tat and Rev and allowed the detection of HIV-infected cells, which incorporated RABVG5 into their cell membrane associated with GPI and polyhistidine Tag. Expression of RABVG5 did not confer any non-specific viral tropism and did not affect cell viability. The regulated expression of RABVG5 system may be a reliable and accurate strategy for labeling HIV-infected cells and offers the possibility to eradicate HIV-1 by avoiding viral escape.

Keywords: HIV-1; Rabies virus; Viral regulation; Tat; Rev.

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Epidemiological and economic burden of Dengue in Mexico: Data analysis from 2010 to 2020

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Dengue is the most prevalent arboviral disease worldwide. Knowing that Mexico is an endemic country for dengue, this study analyzed the epidemiological and economic burden of dengue in the country, as well as the comorbidities and conditions associated with clinical outcomes through national healthcare databases analysis and expert Delphi panel implementation. From 2010 to 2020, the SINAVE/DGE notified 1 620 872 probable cases of dengue, 336 991 laboratory-confirmed cases, 226 554 outpatient cases, 110 437 hospitalized cases, and 1 385 deaths. The age group most affected by dengue infection and its severity was the population under 30 years of age. The case fatality rate increased from 0.8 in 2010 to 2.6 in 2020, and the comorbidities associated with hospitalization and death risk were peptic ulcer, liver cirrhosis, diabetes, kidney disease, and hypertension. The medical care costs for dengue patients were estimated at US $ 23 713 589 (pre-outbreak period) in 2018, US $ 111 851 376 (outbreak period) in 2019, and US $ 39 780 809 in 2020 (post-outbreak period). Our data shows that the incidence of dengue in Mexico has increased in recent years, the disease burden is concentrated in children and young adults (< 30 years old) and the average cost of dengue care was US $ 724.30 per patient.

Keywords: Dengue; Burden; Risk factors; Mexico.

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Vol. 11 | Special Supplement

A retrospective analysis of SARS-CoV-2 infections detected at a dedicated COVID-19 diagnostic laboratory of IPN: Observations of the pandemic behavior from 2020 through 2022

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The first SARS-CoV-2 infection reported in Mexico City occurred on February 27th, 2020. Fifty-one days later, our laboratory was authorized by the InDRE to offer the RT-PCR test as a standard gold to determine the viral load through the amplification of the E gene. Herein we present the local behavior during the four waves in Mexico City, that included Ct values frequencies, percentage of positivity rate as a function of the age and sex from 22 775 nasopharyngeal samples. Additionally, some virus lineages were identified by NGS. The averaged positivity percentage was 19.84 %. However, during the pre-vaccination period this percentage was higher (24.6 %). Higher viral loads (lowest Ct values) were observed some weeks before the infection peaks of the third and fourth waves. In 2020, the B.1.1 and B.1.1.222 lineages exhibited a higher prevalence. In 2021, during the second wave the predominant lineage was B.1.1.519. In the third wave, the AY.20 and AY.26 lineages. In early 2022, the predominant lineages were BA.1 and BA.1.1 during the fourth wave. Additionally, we analyze the correlations between age groups and the prevalence of the virus by gender. These results contributed to understand the dynamics of SARS-CoV-2 infections at a local extent.

Keywords: Cycle threshold value; Waves of infection.

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Evaluation Of Two Multi-Epitope Chimeric Constructs Of The Rabbit Hemorrhagic Disease Virus VP60 Protein

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Rabbit hemorrhagic disease type 2 (RHD2) is a highly contagious viral disease affecting rabbits and hares. In Mexico, the first outbreak of this disease was recorded in 2020. Current control methods are based on vaccination with attenuated virus. Currently, the development of vaccines based on immunogenic peptides is proposed. Therefore, the main capsid protein VP60 has been studied for its immunogenic character and its role in the target cell invasion process. In this work, two multi-epitope chimeric constructs created from predicted B epitopes of the VP60 protein were developed. Both constructs were modified with a poly-histidine tag, with the difference that one of them contains the signal peptide of the periplasmic thiamine-binding protein (thiB). Two synthetic genes were cloned into a commercial vector and used to transform bacterial cells. Protein detection was performed by western-blot using cell lysates and an anti-histidine antibody. Finally, the antigenicity of the proteins was evaluated by western-blot using sera from rabbits naturally infected with RHDV2. In conclusion, it was demonstrated that the presence of a signal peptide favors obtaining the recombinant protein in the soluble fraction in bacterial cultures. Likewise, both proteins obtained in this work contain epitopes that are present in a natural immune response against the virus causing the disease.

Keywords: VP60; Recombinant protein; Bacteria; RHDV2.

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Design of a RT-LAMP colorimetric test for the identification of influenza A virus

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The influenza virus is an emerging virus with the ability to cause epidemics and pandemics. Serological and molecular assays are used for diagnosis, however, in health emergencies, the availability of test, reagents, and equipment is limited. The goal of the study was to design a loop-mediated isothermal amplification assay to detect influenza A viruses. First, 7364 complete influenza A sequences (H1N1, H3N2), year range report (2000–2021), human host, and geographic region America were obtained using the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) platform. By cluster analysis, 73 groups were obtained, and the consensus sequence of 1034 nucleotides in a conserved region of the matrix (M) gene was used to obtain the LAMP external (F3 and B3) and internal (FIP and BIP) primers in the primer explorer program. The primer set was chosen based on its physicochemical characteristics and percentage of identity. We decided to increase the percentage of identity using degenerated primers in two nucleotides of the four primers, and the percentage of identity increased to 81 %. We designed a set of degenerated primers for the RT-LAMP assay to detect influenza A viruses. The standardization of the LAMP assay could be a potential tool, particularly in
resource-limited settings.

Keywords: RT-LAMP, Flu, Isothermal amplification.

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Vol. 11 | Special Supplement

Characterization of respiratory viruses in goats, sheep and cattle cohabiting in an intensive production unit in Mexico City

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The most important viral agents in the respiratory complex in ruminants are respiratory syncytial virus (RSV), parainfluenza virus type 3 (PI3V) and bovine viral diarrhea virus (BVD) and, been reported to have a worldwide distribution in these three species. The objective of this study was to identify the genome and perform phylogenetic analysis of respiratory viruses that are transmitted among ruminants that cohabit in an intensive production unit in Mexico City. 10 goats, 10 sheep and 10 cattle living together in an intensive production unit in Mexico City were used. Three samplings were carried out, the first was in winter, the second in summer and the third in winter. The polymerase chain reaction test with and without retro-transcription was performed to amplify a fragment of the genome of these three viral agents, the test product was purified and sent for sequencing to perform genetic characterization and phylogenetic inferences. The results confirmed that the co-habitation between different species predisposes to the transmission of these three viral agents and that there are genetic modifications in them that facilitate their adaptation to different hosts, in addition it is confirmed that in the case of viruses with respiratory tropism the season winter is a major factor and with respect to BDV, the time of year has no implication on its presentation. These results allow us to establish the bases of the genetic characteristics of the viral agents that circulate in the ruminant production units in Mexico to allow us to establish preventive and biosecurity measures in the production units to prevent productive and economic decreases to the producers

Keywords: Respiratory; Complex; Ruminant; Virus.

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Vol. 11 | Special Supplement

Characterization of small ruminant lentivirus and analysis of factors related to presence in goat and sheep production units in México

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According to the current classification small ruminant lentivirus (SRLV) includes the lentivirus species that infect goats and that were previously known as caprine arthritis-encephalitis virus (CAEV) and the Maedi/Visna virus (MVV) infects sheep. Based on phylogenetic analyses of the gag and env gene, it has been observed that this virus has five genogroups assigned with letters A to E. In genotype A, 18 subtypes (A1 to A18) and B, four subtypes (B1 to B4) have been included. Genotype E is divided into subtype E1 and E2, they have been identified exclusively in goats located in Italy. In Mexico genotypes A and B circulate naturally in sheep and goats. Currently, the factors related to the national situation that favor the presence of the virus in the production units are not known, so the objective of this study is to identify the presence of SRLV in different types of production units and determine which are the factors that favor their presence in sheep and goats of different types of production units. Thirteen sheep with goat coexistence production units (mixed) were selected, 4 units of goat production and 19 of sheep were selected. We make a serological diagnosis and work with an end point polymerase chain reaction (PCRpf) test for molecular diagnosis. In the positive samples we identification genogroup A and B with a real-time PCR test. The results demonstrate in the mixed production both genotypes circulate, in the sheep production circulate genotype A and in goat production B. The association between the signs and the frequency of infection, a significant effect of the positivity rate with respiratory signs in sheep and with mastitis and arthritis in goat. With these results we demonstrate that it is extremely important to implement biosafety strategies as part of the preventive medicine programs in the sheep and goat production in Mexico.

Keywords: Small ruminant lentivirus; Goat; Sheep; México.

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Synthetic peptide of rotavirus VP6 induces a Th cell-dependent protection in mice immunized intranasally

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Studies in mice have shown that an intranasal (i.n.) immunization, in the presence of adjuvant, with the peptide 289-302 of the internal RV protein VP6 ($VP6_{289-302}$), which represents a Th cell epitope, can induce a protective immune response against a RV infection in the small intestine, that depends on Th1-type cells. Although this protection depends on Th cells; it is unclear how the peptide-specific Th cells induced in the nasal epithelium exercise their protective function against infection. This study aimed to determine the migration pattern of the peptide-specific memory Th cells induced by the i.n. immunization. BABL/c mice were immunized i.n. two times with $VP_{6289-302}$ in the presence of cholera toxin (CT) as adjuvant, as control only CT was used. After the last immunization, cells from the mesenteric lymph nodes (MLN) (lymphoid tissue associated to the intestine) of one group of mice were stimulated *in vitro* with the peptide for 24 h, and the frequency of peptide-specific memory Th cells expressing CD69 by flow cytometry. In another group of mice, the peptide was inoculated in the foot pad and the type IV hypersensitivity evaluated every 24 h for 5 days. It was found that after the i.n. immunization, peptide-specific memory CD69+ Th cells were present in the MLN and that the peptide induced a specific type IV hypersensitivity response, showing the vaccination induces both peripheral and intestinal memory peptide-specific Th cells. However, the intestinal Th cells are more likely to be responsible of the protection against the infection.

Keywords: Rotavirus1; Th cell 2; intranasal 3; VP6 4.

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Vol. 11 | Special Supplement

Poster session II

Prevalence, genetic diversity and phylogenetic analysis of PRRSV in Mexico

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Porcine Reproductive and Respiratory Syndrome (PRRS) is a highly significant disease due to the economic losses it causes globally. PRRS affects pigs of all ages, primarily causing reproductive failure in sows and respiratory disease in piglets. The etiological agent, PRRS virus (PRRSV), belongs to the order *Nidovirales*, family *Arteriviridae*, genus *Porartevirus*, with a positive-sense single-stranded RNA genome of 15 kb. The glycoprotein GP5 is encoded in the ORF5 gene, whose sequence is commonly used to establish phylogenetic relationships and classify PRRSV isolates. During routine monitoring of various swine production units in Mexico, a total of 5,840 samples, mainly serum/whole blood, oral fluids, and lung tissue, were analyzed for PRRSV diagnosis using real-time RT-PCR, resulting in an average positivity of 18.51 %. Positive samples were selected for Sanger sequencing of the ORF5 gene. The obtained sequences were aligned, and a maximum likelihood phylogenetic tree was constructed. The circulating PRRSV strains in Mexico were found to belong to lineages 1 (57 %) and 5 (43 %). The predominant RFLP pattern within lineage 1 was 1-7-4 type, while in lineage 5, 2-5-2 type. These findings shed light on the genetic diversity of PRRSV circulating in Mexico and provide valuable insights for the surveillance and control of the disease.

Keywords: PRRSV; Phylogeny; Swine; Sequencing; Epidemiology.

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Discovery and optimization of neutralizing SARS-CoV-2 antibodies using ALTHEA Gold Plus Libraries™

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We recently reported the isolation and characterization of anti-SARS-CoV-2 antibodies from a phage display library built with the VH repertoire of a convalescent COVID-19 patient, paired with four naïve synthetic VL libraries. One of the antibodies, called IgG-A7, neutralized Wuhan, Delta (B.1.617.2) and Omicron (B.1.1.529) strains in authentic neutralization tests (PRNT). It also protected 100 % transgenic mice expressing the human ACE-2 from SARS-CoV-2 infection. Here, the four synthetic VL libraries were combined with the semi-synthetic VH repertoire of ALTHEA Gold Libraries™ to generate a set of fully naïve, general-purpose, libraries called ALTHEA Gold Plus Libraries™. After three rounds of panning with SARS-CoV-2 RBD wildtype as selector, 630 clones were tested for binding to RBD, yielding 125 positive and specific scFvs, with 24 being unique clones. Three out of 24 specific clones with affinity in the low nanomolar range and sub-optimal in vitro neutralization in PRNT, were affinity optimized via a method called "Rapid Affinity Maturation". The final molecules reached sub-nanomolar neutralization potency, slightly superior to IgG-A7, while improved the developability profile over the parental molecules. These results demonstrate that general-purpose libraries are a valuable source of potent neutralizing antibodies and it could expedite isolation of antibodies for rapidly evolving viruses.

Keywords: COVID-19; Phage display; Therapeutic antibodies; Affinity maturation; Semi-synthetic libraries.

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Vol. 11 | Special Supplement

Spike-SARS-CoV-2 pseudo-virus antibody micro-neutralization assay in a Hek 293T/ACE2 cell line

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Emerging viruses such as pandemic SARS-CoV-2 require management in BSL-3 facilities. Pseudo-virus are non-replicative recombinant particles expressing specific viral glycoproteins on their surface and a reporter gene for infection quantification and can be manipulated in BSL-2 facilities. We established a SARS-CoV-2 Spike-expressing pseudo-virus system for the evaluation of the neutralizing capacity of human serum, antibodies, and other blocking agents. Pseudo-viruses were produced by transfection of the Hek293T cell line with a set of 5 plasmids: pHDM-Spike, pHAGE-Luc-IRES-ZsGreen, pHDM-gag-pol HIV, pHDM-tat1b, pRC-CMV-rev1b. Transfection was confirmed using fluorescence microscopy. Supernatants containing pseudoviral particles were harvested at 24, 48, and 72 hours. A $TCID_{50}$ assay in Hek-293T/hACE2 cells was used to determine pseudo-virus infective titer, measuring transduction as the level of luminescence intensity at 255nm. SARS-CoV-2 Spike pseudo-virus 48h supernatant showed the best value of RLU's (above $1x10^5$) with a $10^{2.5}$ $TCID_{50}$/ml. For the micro-neutralization assay, $10^{2.5}$ $TCID_{50}$/ml of SARS-CoV-2 Spike pseudo-virus 48h supernatant was neutralized with 10^{-2}, 10^{-3}, 10^{-4} serial dilutions of polyclonal αSpike human serum of SARS-CoV-2 vaccinated individuals. Neutralizing human monoclonal IgG1 anti-S1(RBD) of SARS-Co-V-2 was used as the control of neutralization. Vesicular stomatitis virus-G protein pseudo-virus (VSV-G) 48h supernatant was included to demonstrate specificity for the SARS-CoV-2 spike protein neutralizing capacity of human serum. Human serum neutralized SARS-CoV-2 Spike pseudo-virus showed a neutralization percentage above 60 % at a 10-4 dilution, while VSV-G pseudo-virus dropped near 0 % at 10^{-4} dilution, therefore proving specific neutralization for the SARS-CoV-2 spike. Our results show that SARS-CoV-2 pseudo-virus particles can be used to perform quantitative neutralization assays in BSL2 facilities.

Keywords: SARS-CoV-2; Micro-neutralization assay; Pseudo-virus; Hek-293T/hACE2.

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Vol. 11 | Special Supplement

Viral Load Analysis Of Human Herpesviruses 4, 5, 6, and 7 In Gastric Biopsies From Pediatric Patients With Chronic And Recurrent Abdominal Pain

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Chronic/recurrent abdominal pain (C/RAP) is persistent pain lasting at least 3 months continuously or discontinuously. This problem affects 34 % of the world's population under 18 years. C/RAP is a common cause of hospital care, especially in children who do not resolve their symptoms. In these patients, *Helicobacter pylori* (HP) infection is usually explored as one of the causes. However, other species of the genus *Helicobacter spp* (NHPH) infecting the stomach of animals have been described as emerging bacteria with clinical significance and impact on gastric disease in humans. Evidence suggests that there is cooperation between HP and Epstein-Barr virus (EBV), as only individuals who are co-infected by both pathogens develop severe inflammation, and active lesions from childhood, with an increased risk of progressing to gastric cancer in adults. Whether EBV or other herpesviruses are also linked to NHPH's in the development of gastric symptomatic lesions is unknown. Body and antrum gastric biopsies from 105 patients with C/RAP were analyzed with the aim of understanding the association between individual infection or coinfection with HP, NHPH and herpesvirus in the development and clinical presentation of chronic and recurrent abdominal pain in pediatric patients.

Keywords: Herpesvirus; *Helicobacter pylori*; Non-Helicobacter pylory Helicobacter; Inflammation.

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Vol. 11 | Special Supplement

VHH8 As a Prognostic Marker of Mortality in a Cohort of Patients with Kaposi's Sarcoma And HIV

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Our objective was to evaluate the prognosis of the viral load (VL) of HHV8 in patients with HIV (PLHIV) and Kaposi Sarcoma (KS). In a cohort of PLHIV > 18 with KS in 4 different stages, treated with chemotherapy and molecular diagnosis for HVH8. Statistical analysis was for descriptive (frequencies and proportions or medians and interquartile ranges, as appropriate). The groups were compared with $\chi 2$ (qualitative variables) and Mann-Whitney U (quantitative variables). A Kaplan-Meier curve was used for survival analysis. Significance level $P < 0.05$. We include 82 PLHIV/SK and we select 46.7 % (56) who were positive for VHH8. Mortality was 27.7 %. Mortality (dead vs. alive) was compared according to clinical condition (stage I: 1; II: 1 vs. 5; III: 1 vs. 1; IV: 13 vs. 34) [$P = 0.742$]; the units for VL was copies/mL and for subgroup of lymphocytes was cel/µL. HIV VL: 114955 (1223- 347432) vs. 69677 (1745-313339) [$P = 0.153$]; CD4: 9.5 (9.5-42.5) vs 55 (11-151.5) [$P = 0.006$], CD4/CD8: 0.05 (0.02-0.09) 0.10 (0.045 -0.18) [$P = 0.001$]. Three groups were formed according to the VL of VHH8: > 19 to 3400, 3400.01 to 12000 and > 12000, showing differences in the survival curves [$P = 0.010$]. The VL of HVH8 in peripheral blood was significantly associated with the probability of mortality as a factor independent of the clinical stage of KS in PLHIV.

Keywords: HIV; VHH8; Kaposi Sarcoma; Mortality.

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Efficacy, Pharmacokinetics, and Toxicity Profiles of a Broad Anti-SARS-CoV-2 Neutralizing Antibody

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We recently reported the isolation and characterization of an anti-SARS-CoV-2 antibody, called IgG-A7, that protects transgenic mice expressing the human angiotensin-converting enzyme 2 (hACE-2) from an infection with SARS-CoV-2 Wuhan. We show here that IgG-A7 protected 100 % of the transgenic mice infected with Delta (B.1.617.2) and Omicron (B.1.1.529) at doses of 0.5 and 5 mg/kg, respectively. In addition, we studied the pharmacokinetic (PK) profile and toxicology (Tox) of IgG-A7 in CD-1 mice at single doses of 100 and 200 mg/kg. The PK parameters at these high doses were proportional to the doses, with serum half-life of ~ 10.5 days. IgG- A7 was well tolerated with no signs of toxicity in urine and blood samples, nor in histopathology analyses. Tissue cross-reactivity (TCR) with a panel of mouse and human tissues showed no evidence of IgG-A7 interaction with the tissues of these species, supporting the PK/Tox results and suggesting that, while IgG-A7 has a broad efficacy profile, it is not toxic in humans. Thus, the information generated in the CD-1 mice as a PK/Tox model complemented with the mouse and human TCR, could be of relevance as an alternative to Non-Human Primates (NHPs) in rapidly emerging viral diseases and/or quickly evolving viruses such as SARS-CoV-2

Keywords: COVID-19; Therapeutic antibody; SARS-CoV-2 Delta; SARS-CoV-2 Omicron; Toxicology.

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Vol. 11 | Special Supplement

Genomic surveillance of SARS-CoV-2 in Mexico from 2020-2022

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The objective of this work was to analyze the metadata of the complete genome sequences of SARS-CoV-2 from samples collected in Mexico from 2020-2022. The metadata was retrieved from GISAID with the inclusion criteria of samples collected in Mexico between 2020-2022 and was manually curated for homogenization. In total, 81 983 entries were obtained; the analyses indicate that 24 % of the sequences were deposited by the Mexican Consortium for Genomic Surveillance (CoViGen-Mex). In total, during 2020, 4.5 % of the data were obtained, 54.6 % in 2021, and 40.9 % in 2022. Regarding the distribution by sex, most of the metadata come from samples of female patients (49.8 %), the rest male (44.7 %), and not declared (5.5 %). At the same time, the predominant variants during this period were the delta (31.1 %) and the omicron (39.5 %). The predominant age ranges in the metadata were from 21 to 40 (37.9 %) and 41 to 60 (29.3 %). The results indicate that only 1.1 % of the confirmed cases concluded in the sequencing of the sample, so in Mexico, more collaborative efforts and investment in infrastructure are necessary to increase genomic surveillance at the national level.

Keywords: COVID-19, SARS-CoV-2, Genomic surveillance, Metadata.

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Vol. 11 | Special Supplement

Subtyping of influenza viruses in confirmed cases during 2022 in Guadalajara, Jalisco

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The annual incidence of influenza reaches around 1 000 million cases in the world. In Mexico, during 2022, 7 148 confirmed cases were reported. Descriptive study. We included 414 cases of influenza confirmed by PCR, in people over 18 years of age who attended the Diagnostic Laboratory of Emerging and Reemerging Diseases (LaDEER) of University of Guadalajara during 2022. Influenza virus subtyping was performed using the BlueFinder 22 kit (Genes2Life®). The virus subtype with the highest circulation was AH3N2 with 93.7 % of the total cases, followed by type A not subtypeable with 6.1 %, and type B Victoria with 0.2 %. The epidemiological week with the highest number of observed cases was week 1 with 86 cases (20.8 %), followed by week 46 with 65 cases (15.7 %) and week 45 with 54 cases (13 %). The age range with the highest number of cases was 20 to 24 years, with 96 % of the cases due to AH3N2; followed by the group from 25 to 29 years with 97.9 % of cases due to AH3N2. No differences were observed in the distribution of typified cases between sexes.

Keywords: Influenza; Subtypes; Epidemiology.

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Vol. 11 | Special Supplement

Design and development of an end-point RT-PCR for the detection of Hepatitis A Virus in strawberry

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WHO has reported up to 14 million cases and almost 300 000 deaths linked to Hepatitis A Virus (HAV) due to the consumption of uncooked food given by the stability of the virus in organic matter. As an example, there is a recent outbreak that occurred this past June in the United States linked to a batch of organic strawberries from Baja California. The objective of this work is to design and develop an endpoint RT-PCR test for the detection of Hepatitis A Virus in strawberry. For the in silico design the software Claustal Omega was used to align 14 different sequences pertaining genotypes I and II (A and B subgenotypes), 5'NCR region was found as the most conserved zone so was selected for primers design, GADPH gene was also included as a technique control. The Oligo Explorer 1.2 software and the Blast tool were used for primers design and in silico specificity validation respectively. Primers were tested in the experimental stage with strawberry and HAV positive samples. So far, the GADPH PCR product was cloned into pJET 1.2/blunt plasmid transforming Escherichia coli Top 10; HAV PCR product was obtained using its primers and both sequences will bind each other in order to build a protection plasmid to carry out the standarization of the test.

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Identification of Human Cytomegalovirus in placenta, brain, and liver of stillbirth cases

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One stillbirth occurs every 16 seconds worldwide. Despite medical advances, infection contributes to 10–25 % of stillbirths in high-income countries. In Mexico, during 2008–2019, national databases recorded 198 076 stillbirths and nineteen cases were attributable to viruses, being human cytomegalovirus (HCMV) identified in nine cases. Previously, our team found an HCMV prevalence of 60.65 % in placenta samples of pregnancy women; so, we decided to identify the presence of HCMV in stillbirth. A retrospective, cross sectional, descriptive study was performed. Twenty-four cases of stillbirth of the National Institute of Perinatology were selected. DNA was extracted from placenta, brain and liver using a commercial kit. To detect the DNA HCMV, a nested PCR was performed to amplify 219 base pairs of the UL123 gene. *GAPDH* gene was used as integrity control of DNA. DNA HCMV was detected in 14/23 (61 %) of the cases studied. The necropsy files of positive cases reported clinico-pathological findings of congenital HCMV infections as corioamnionitis, villitis, hydrops fetalis not due to immune disease. This study highlights the importance of implement detection of viral infection as causative of stillbirth. We recommend studying a higher number of cases and identify the HCMV genotypes to analyze the correlation between the genotypes and clinico-pathological findings.

Keywords: Stillbirth, Viral infection, Diagnosis, Placenta, HCMV.

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Antiviral effect of different metformin and phenformin analogues on Dengue virus infection

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Dengue is the disease caused by dengue virus (DENV), and is associated with significant morbidity, mortality and economic cost worldwide. Despite its relevance, there is currently no specific, safe and effective pharmacological treatment against this disease. Our group was able to demonstrate the anti-DENV effect of metformin in both in vitro and in vivo models. On the other hand, the Pharmaceutical Chemistry Laboratory of the Faculty of Pharmacy of the Autonomous University of the State of Morelos developed new structural and functional analogues of metformin and phenformin. Considering the similarity of the analogues with metformin, it is possible that their therapeutic properties are similar and with it, the antiviral activity on DENV infection. Therefore, in the present work we analyzed the effect of various metformin and phenformin analogues on DENV-2 infection in Huh-7 cells. The results demonstrate that metformin and phenformin analogues are effective antiviral agents for inhibiting DENV infection in vitro and could be good candidates for the treatment of dengue. Among them, the analog EGL-1 and EGL-2 were found to have the most promising characteristics. Thus, the latter two compounds could be an alternative to metformin, the only biguanide currently available.

Keywords: Antiviral effect; Metformin and phenformin analogs; DENV; Therapeutic potential; Dengue.

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G-quadruplex DNA structures in oncogenic high risk human papillomaviruses 16, 18 and 58

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G-quadruplexes (G4s) are non-canonical structures of DNA or RNA formed in guanine-rich regions and play crucial roles in several processes related to the central dogma of molecular biology. G4s have been identified in some medically important viruses, such as SARS-COV-2 or HIV, making them potential molecular targets for diagnostics and antiviral development. However, not much is known about DNA viruses, as the Human Papillomavirus (HPV), the etiological agent of several types of cancers worldwide. The present study aims to identify G4 structures in the genomes of High-Risk (HR) HPV types 16, 18, and 58 using *in silico* methods. Initially, we selected bioinformatics predictors based on established criteria and identified Putative Quadruplex Sequences (PQS) in the genomes of these three HR-HPVs, as well as in their circulating variants. Finally, we modeled a conserved atypical G4 structure in 3D. Our results indicated more than 100 SFG4 in each genotype, with a higher abundance in L2, E1 and L1 genes. Furthermore, we identified two conserved PQS in the L1 region, which encodes the main structural protein, across all three HPVs, showing > 80 % identity. These same motifs were also found in clinical samples (n = 992) and could therefore serve as potential molecular targets.

Keywords: DNA; Cancer; G-quadruplexes; Genes; HPV.

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Evidencing G-quadruplexes in the HPV16 genome using the quantitative PCR stop assay

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G-quadruplexes (G4s) play a crucial role in viral life cycles, regulating essential processes such as replication, transcription, and translation. Recently, G4 structures have garnered attention from the scientific community due to their potential applications as aptamers, cancer treatments, or antiviral targets. In our laboratory, we have identified putative G-quadruplex sequences in regions of the Human Papillomavirus 16 (HPV16) genome that require experimental confirmation. In this study, we standardized a real-time quantitative PCR stop assay (qPCR stop) to determine the degree of amplification inhibition caused by G4s in the HPV16 genome. Specific primers were designed to detect G4s in E1, E2, E6, L1, L2 genes, and the promoter region. To validate the technique, we used plasmid DNA, containing a DNA-G4 region reported for HPV52 (5O4D) as a reference. Additionally, we determined optimal KCl concentrations for G4 formation, as well as for the ligands PhenDC3 and Pyridostatin. Our results demonstrated that qPCR stop has the capability to distinguish between regions with and without G4s, validating its utility as a reliable tool for detecting and investigating these structures, which may have potential clinical applications in other HPV genotypes. Also, a list of potential G4s is provided for future biophysical and functional studies.

Keywords: DNA; G-quadruplexes; Papillomavirus; qPCR; Ligands; Virus.

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Vol. 11 | Special Supplement

Presence of SARS-CoV-2 at the bus station in Culiacán, Sinaloa, México

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This work aimed to determine the presence of circulating SARS-CoV-2 in the bus station of Culiacan, Sinaloa, Mexico. From February to April 2022, 45 wastewater samples were taken from different sources within the bus station facilities. 1 000 ml of water was taken from each site, and then concentrated by precipitation with 20 % PEG6000/2.5M NaCl. From the eluted, the viral RNA extraction was carried out with the QIAamp Viral RNA Mini Kit, and the detection was performed with the CDC 2019-Novel Coronavirus kit. From the total samples (n = 45), SARS-CoV-2 was detected in 8.8 % (n = 4) of wastewater from different station areas. During this period, the Culiacan bus station received approximately 670 000 passengers, of which the results suggest that at some point within the facilities, the presence of at least one passenger with an active case of the disease was found, placing the most concurred sites of each city such as station of buses and airports as a potential site of contamination and spread of the virus. The results confirm that bus stations are a strategic point of epidemiological surveillance.

Keywords: COVID-19; SARS-CoV-2; Genomic surveillance; Bus station.

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A clinical case of a heifer with mucousal disease, a variant of bovine viral diarrhea

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The mucosal disease is a form of bovine viral diarrhea and occurs in animals under 24 months of age, depending on the period of gestation and the characteristics of the viral strain, which is why it can induce embryonic or fetal death, abortion, mummification, congenital malformations, perinatal mortality and protective immune response or tolerance. The objective was to carry out the molecular diagnosis, derived from the clinical sinology suggestive of Bovine Viral Diarrhea, from nasal and vaginal swabs of Angus breed heifers, extensively produced in the State of Jalisco. A PCR was established that amplified a 214 bp segment of the UTR of the virus. The bands obtained were sent for sequencing. Likewise, viral isolations were carried out in the MBDK cell line. Of the 20 animals, one 18-month-old heifer was positive by PCR. Viral isolation was achieved in both swabs, this case coincided with an individual who had manifested clinical signs suggestive of the disease. A phylogenetic tree was made that corresponded to cytopathic biotype 1. It is concluded that it was a persistently infected animal based on clinical sinology and molecular diagnosis.

Keywords: VBD; Persistent animals; Variants VBD.

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SARS-CoV-2 contamination in surfaces from public hospital in Culiacán, Sinaloa, México

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This work aimed to determine potential sites of contamination and transmission of SARS-CoV-2 during the COVID-19 pandemic in a public hospital in Culiacan, Sinaloa, México. The sampling was carried out from February to April 2022 of surfaces in the COVID areas and the Intensive Care Unit (ICU) of one public hospital in Culiacan, Sinaloa, Mexico. Samples were taken using a sterile sponge moistened with phosphate buffer. RNA extraction was performed with the Viral RNA mini kit (Qiagen), and the detection was performed with the CDC 2019-Novel Coronavirus kit. During the surveillance period in the public hospital, 11 samples were taken from surfaces. SARS-CoV-2 was found in 63.6 % of the samples. SARS-CoV-2 was absent from the ICUs; however, it was recovered in the COVID area of the public hospital (n = 7). The COVID area was the main point of contamination during the pandemic. On the other hand, it is notable that the virus was not detected outside this area, which can be associated with the adequate hospital containment measures adopted during the health emergency.

Keywords: COVID-19; SARS-CoV-2; Hospital; Surfaces; RT-PCR.

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In vitro study of H7N3 avian Influenza A viruses with truncated NS1 protein at aminoacids 99 and 126

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In Mexico, the presence of highly pathogenic avian influenza AH7N3 affects the poultry industry since 2012, causing economic losses and being a subtype of concern due to its pandemic potential. Influenza viruses can block the immune response of the host by inhibiting interferons β/βby effects of NS1 protein. Many studies show that truncations in NS1 protein can be the key to obtaining live attenuated vaccine candidates. Using reverse genetics and based on the H7N3 influenza full genome of a Mexican strain, recombinant viruses possessing a full-length NS1 or a truncated NS1 protein at the carboxyl-terminal at 99 or 126 amino acids were generated. All rescued viruses replicated to HA titers of 1:160 in MDCK cells. In growth kinetics assays, truncated viruses showed attenuation compared with full-length NS1 viruses in MDCK cells. The measurement of interferon mRNA production by infection assay in chicken embryo primary cell cultures, showed that truncated viruses were able to reduce IFN inhibition up to 5-fold compared to wild-type viruses. This is the first report of the rescue of recombinant viruses from full genome H7N3 subtype and the generation of two H7N3 viruses with truncated NS1 protein as potential candidates of live attenuated vaccines.

Keywords: Influenza H7N3; Recombinant NS1; Truncated proteins; Virus rescue; Live attenuated vaccines.

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Design and production of a chimeric antigen against Zika virus

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Zika virus (ZIKV) is a flavivirus belonging to the family *Flaviviridae*. According to the World Health Organization, ZIKV is considered a serious public health problem worldwide because of its association with microcephaly and Guillain-Barré syndrome. There are currently no approved vaccines or specific medical treatments, making both aspects relevant targets for biomedical research. In our study we designed and produced in *Escherichia coli* a multiepitope vaccine candidate against ZIKV capable of inducing cellular and humoral responses. A chimeric protein was designed based on a series of ZIKV antigenic sequences and a bacterial carrier. Modeling, prediction, and analysis of the recombinant protein structure was performed using Phyre2 and ChimeraX servers. The antigen was produced in *Escherichia coli* and purified from inclusion bodies. Twenty-six sequences were obtained and analyzed on the Phyre2 and ChimeraX servers. The selected chimera had a hydrophobicity index of - 4.635. The protein was efficiently induced at a lactose concentration of 1.5 g/L, and the SDS-PAGE analysis shows that most of the protein remains in the insoluble fraction. However, the protein was successfully solubilized, refolded and purified. The next step is evaluation in mice to determine the immunogenic response.

Keywords: ZIKV; *Escherichia coli*; Multiepitope; Vaccine; Purification.

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In silico analysis of SARS-CoV-2 Delta and Omicron variants 3a protein mutations

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SARS-CoV-2 is the *Betacoronavirus* that caused the COVID-19 pandemic. SARS-CoV-2 genome (30 kb) encodes 16 nonstructural proteins, 4 structural proteins, and 11 accessory proteins. Within the accessory proteins of SARS-CoV-2, 3a is important for immune modulation and apoptosis, and its mutations functional effect is still poorly understood. In this work, we sequenced 50 SARS-CoV-2 positive samples obtained in a period between 2020 and 2022. and found 14 different lineages, being AY.20 (Delta) and BA.5.1 (Omicron) the most frequent. We also found additional mutations to those that define the different lineages or variants, highlighting the L106F/S165F and P267L mutations found in ORF3a. We evaluate the effect of the L106F/S165F and P267L mutations on the structure of the SARS-CoV-2 3a protein by *in silico* modelling using AlphaFold2 and analyse the structural disorder using IUPred2A. Our results show that L106F/S165F and P267L mutations on 3a did not alter its 3D structure but reduce the structural disorder at the position of residues 165 and 267. The results suggest that the L106F/S165F and P267L mutations do not modify the structure of 3a but can modify its molecular dynamics; however, functional studies are necessary.

Keywords: SARS-CoV-2, Mutations, Variants, ORF3a accessory protein.

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Vol. 11 | Special Supplement

Bioinformatic analysis of imidazopyridine-hydrazones as a possible alternative for the treatment of Influenza a virus

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The Influenza virus has been of relevance in public health history, with great socioeconomic impact. Despite having the drugs Oseltamivir and Zanamivir for its treatment, it is not enough for its control, due to the limited access to vaccination and the constant evolutionary process of the virus, so it is of great importance to develop new therapeutic alternatives for its treatment. The present work focuses the study of molecular coupling of 2-Aryl-3-((2-(pyridin-2-yl)hydrazone) methyl)imidazo[1,2-a]pyridines and 3-R-2-((2-(pyridin-2-al)hydrazone)methyl) imidazo[1,2-a]pyridines which according to the site of binding to the protein domain of the compounds under study and the reference drug is established that the imidazo[1,2-a]pyridine derivatives have the same mode of action as the reference drug (Oseltamivir) showing greater affinity. Although the series of molecules extrapolated to the coordinates of the Osetamivir binding site will not be able to completely mimic the mechanism of action of the reference drug as a neuraminidase blocker, they will possibly follow a similar signaling route, and therefore a mechanism of similar action. The neuraminidase inhibition constant obtained for imidazopyridine derivatives was better (45—993 μM), compared to the reference drug Oseltamivir (1 730 μM). This concludes that by possessing a better inhibition constant and an established recognition topology, in addition to a mechanism of action to follow, this series of molecules are potentially functional and very promising as antiviral molecules.

Keywords: Organic chemistry; Medicinal chemistry; Bioinformatic chemistry; Imidazopyridines; Antiviral compounds.

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Fibrinogen-to-platelet ratio as a predictor of COVID-19 outcomes in non-diabetic patients

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The fibrinogen-platelet ratio (FPR) is used to identify patients likely to develop thrombotic complications. COVID-19 mortality is associated with venous thromboembolism. The objective of this study was to evaluate the prognostic value of FPR as a coagulative index by examining survival in non-diabetics patients with COVID-19. This is a retrospective single-center observational study conducted at a tertiary care hospital to determine the prognostic role of FPR. Clinical predictors were identified using Cox proportional hazard regression models to calculate the hazard ratio (HR) with a corresponding 95 % confidence interval (CI). Receiver Operator Characteristic (ROC) curve and Kaplan-Meier curve were calculated. Non-survival group had higher FPR ($P < 0.0001$) levels in comparison with the survival group. Cox analysis showed that FPR (HR = 3.525, 95 % CI 1.191-10.431; $P = 0.022$) was an independent high-risk factor associated with COVID-19 mortality in non-diabetic patients. The area under the curve (AUC) of FPR was 0.715 ($P = 0.001$) with 72 % sensitivity and 62.22 % specificity. Patients admitted to the hospital with a lower FPR [< 34.2] had significantly longer overall survival compared to a higher FPR [> 34.2] ($P = 0.002$). In this study was suggested that FPR > 34.2 on admission could effectively predict COVID-19 mortality in non-diabetic patients.

Keywords: COVID-19; SARS-CoV-2; Biomarker; Fibrinogen-to-platelet ratio (FPR).

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Investigation of cases of swine Influenza virus in the state of Jalisco

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A convenience-directed study was conducted in swine farms. Nasal swab samples and oral fluids (n = 172) were collected from pigs in 13 farms with a clinical history of respiratory illness associated with swine influenza virus. Five individual serum samples were collected from the production line (3–21 weeks of age). 4 positive farms were identified in nasal swab samples (5/33.15 %), two farms in oral fluid samples (2/7.28 %) by real-time RT-PCR. Sequencing allowed the identification of two main subtypes, H3N1 and H3N2. In all cases, the sequences obtained from HA correspond to subtypes of porcine origin, identified in North America or the USA; for NA, four sequences obtained are of porcine origin and two from viruses identified as human influenza; the samples correspond to Mexican strains or from USA. In relation to the detection of antibodies, positivity was identified in all the analyzed farms, however, there were negative individuals or individuals with low titers. The highest average titers for the H1N1 subtype were identified between weeks 12 and 18. In the case of the H3N2 subtype, the highest mean titer was at week 6 and 9.

Keywords: Influenzavirus; Epidemiology; Surveillance; Diagnostic; Pigs.

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Vol. 11 | Special Supplement

Implementation of an ELISA based on the use of a fused recombinant protein (p24-p12) for the detection of antibodies against the Bovine Leukemia Virus

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Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis, a disease with worldwide distribution. Some studies in Mexico show a wide spread of the infection in dairy herds and BLV genotypes 1 and 3 have been identified, out of the 12 recognized so far. The aim of this study was to standardize an ELISA based on a recombinant protein expressed in *E. coli*. Which included capsid/nucleocapsid (p24-p12) of the BLV *gag* gene of genotype 1. Seven hundred bovine plasmas from different herds from 9 states of the country were previously categorized as seropositive/seronegative using a commercial ELISA that detects antibodies against gp51. The results showed a sensitivity of 82.4 % and a specificity of 63.1 % with respect to the commercial ELISA. These results could be due to the fact antibodies against gp51 are the first to appear indicative of an early infection, another possibility would be related to the genotype/antigen used, which is not the one prevalent in the region studied, or that the surface protein of BLV is an immunodominant antigen. Discordant results should be re-evaluated with another technique to determine if the categorization of the animals with the commercial test is adequate.

Keywords: Bovine leukemia virus; Capsid/nucleocapsid recombinant protein; Serology; Diagnosis.

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Vol. 11 | Special Supplement

Development and validation of an assay for the quantification of herpes viruses and viral bovine diarrhea viruses

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Herpes virus (HSV-1) and bovine viral diarrhea virus (BVDV) are viruses used as test models in the evaluation of viral safety of biologics. Virus quantification using the plaque assay is the gold standard for determining the amount of infective virus present in a sample. This work reports the development and validation of plaque assays to quantify the amount of HSV-1 and BVDV viral particles present in biological samples. The assays developed will demonstrate compliance with the quality attributes: linearity, precision, accuracy and quantification interval, which allows their use in the evaluation of the viral safety of biological products.

Keywords: HSV-1; BVDV; Validation; Linearity; Precision; Accuracy; Quantification Interval.

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Vol. 11 | Special Supplement

Optimization of neutralization assay against CHIKV

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Chikungunya virus (CHIKV) is a virus transmitted by Aedes mosquitoes, producing a disease that causes fever and joint pain. Quantification of neutralizing antibodies against Chikungunya virus can be crucial to evaluate possible treatments, vaccines and to understand the immune response. The process of optimizing the neutralization assay usually involves several steps; from selecting the appropriate viral and cellular strains, as well as the assay incubation conditions and time to observe the neutralizing effect of the antibodies on the cell monolayer, measuring the reproducibility and specificity between the positive and negative controls used in the assay repeats, determining the functional range and sensitivity of the assay. This work reports the optimization of the neutralization assay against CHIKV

Keywords: CHIKV; Quantification; Optimizing; Neutralization.

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Development of a reporter cell line to detect rabies virus infection

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The rapid diagnosis of rabies is essential for implementing prophylactic measures in exposed humans and animals. We developed a sensitive reporter cell line to detect rabies virus (RABV) infection. We designed a reverse genetics system emulating RABV genome coupled to an HIV-1 gene expression mechanism mediated by HIV-1 Tat and Rev regulatory proteins to sense and enhance green fluorescent protein (GFP) expression. *gfp* was first integrated into HEK293 genome and was controlled by HIV-1 LTR promoter (HEK293/LTR-GFP cells). To induce GFP expression, HEK293/LTR-GFP cells were transfected with RABV minigenome encoding *tat* and *rev* reverse genes. HEK293/LTR-GFP cells were then infected with RABV Pasteur strain and GFP+ cells were quantified by flow cytometry and fluorescence microscopy. The rabies minigenome coupled to HIV gene-expression mechanism efficiently detected RABV infection in the HEK293 reporter cell line starting at 24 h becoming frankly positive at 72 h. RABV replication mechanism recognized the minigenome encoding *tat* and *rev* genes, and HIV regulatory proteins strongly activated GFP expression. This strategy does not require the use of specific antibodies to detect rabies infection. Reverse genetics system and HEK293/LTR-GFP cell line have high sensitivity for RABV and might be used for rabies diagnosis and research. The reporter cell line needs to be validated testing clinical samples from other animal species. This novel reporter cell line is a reliable tool, easy to handle, economical, and with the potential to speed rabies diagnosis, which could be useful especially in developing countries.

Keywords: Rabies virus; Reverse genetics; HIV-1; Tat; Rev.

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Molecular analysis and modeling of Porcine Parvovirus 5 NS1 protein”

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Recently, emerging porcine parvovirus species (PPVs) have been identified. These species have designated as PPV2 to PPV8. At present, PPV5 and PPV8 remained unclassified by ICTV. The criteria for PPV taxonomy utilize the amino acid (aa) sequence of NS1 which is a multifunctional protein essential for viral replication. In this scenario, a PPV member is ascribed to the same genre and species if it shares 35–45 % and ≥ 85 % identity of NS1, respectively. To perform a NS1 molecular analysis, three PPV5+ DNA samples were used to achieve the complete ORF1 lineal nucleotide sequence via amplification of three overlapping PCR products. At editing, a NS1 lineal sequence of 1750 nucleotides comprising 96.9 % of ORF1 was obtained from each DNA sample. The aa matrices showed that the Mexican sequences have a 96.7–99.8 % identity with previously reported PPV5 sequences, and high similarity with PPV4 (76.0–87.3 %) and PPV6 (54.9–55.4 %) sequences. The analysis of aa sequences pointed at five conserved motifs and arginine fingers which are portrayed for superfamily 3 (SF3) helicases. The current work findings suggest that PPV5 might belong to the *Copiparvovirus* genre due to its proximity to PPV4 and PPV6 and that NS1. Most likely, NS1 is a SF3 helicase

Keywords: Porcine parvovirus; PPV5; NS1; Helicases; Copiparvovirus.

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Phylogenetic analysis of Chikungunya viruses isolated in America

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Chikungunya virus (CHIKV) is a re-emerging arbovirus that causes an inflammatory musculoskeletal disease in humans, characterized by fever, polyarthralgia, myalgia, rash, and headache. In this study, an analysis was performed using the partial sequences of the genes that encode the E1 and E2 proteins, obtained from samples of Mexican patients, complemented with sequences from the entire American continent. A phylogenetic tree scaled by collection date and a haplotype network were constructed to infer these sequences' evolutionary and dispersal relationships. The results show that some of the sequences from Mexican patients are related to sequences from Nicaragua, as expected, but others are more closely related to sequences from Ecuador. The fact that the Mexican sequences do not form a monophyletic group shows several introductions to our country. At the same time, the longer branches indicate that a diversification process has occurred within Mexico. These findings contribute to understanding the evolutionary dynamics of CHIKV in different populations and provide valuable information for the future design of control and prevention strategies and surveillance of the virus.

Keywords: Chikungunya; Phylogenetics; Haplotype network; Viral sequencing; Arbovirus.

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Inhibition of SARS-CoV-2 infection and reduction of cell death by polyene

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As infections from emerging or reemerging virus became a serious threat to humans in the last years, drug repurposing, is an a promising, fast and cost-effective alternative that can overcome traditional de novo drug discovery and development challenges. Given that therapeutic options for antiviral treatment of SARS–CoV–2 remain limited, through this strategy we sought to find molecules with antiviral activity. In this regard, antifungals such as itraconazole, anidulafungin, and micafungin were reported to have antiviral activity against SARS–CoV–2. Polyene antibiotics are potent antifungal agents currently used in human therapy. Among these is Amphotericin B (AmB), and its newly created less derivative A21. In addition, lipid-based formulations have been developed to deliver polyenes, such as liposomes and these alternatives are also available for AmB and A21. Therefore, it was decided to see if these compounds have antiviral activity against SARS–CoV–2 through a time-of-drug-addition strategy. We were able to observe an 83 % reduction of viral particles determine by plaque assay, when AmB is added after infection at a concentration of 10 µM. No significant reduction of the viral particles was detected with the other compounds, however, a reduction in cell death due to SARS–CoV–2 infection was observed, but only when viruses are treated before infection. The latter could be explained if other signaling pathways related to viral entry and cell death are being stimulated. Exploring these alternative pathways could provide valuable information for the development of future antiviral molecules.

Keywords: Polyene, antiviral, SARS-CoV-2, Liposomes.

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Surveillance of SARS-CoV-2 in healthcare settings

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SARS-CoV-2 is released from patients or asymptomatic carriers mostly thru saliva droplets deposited on surfaces or objects, and if not properly disinfected, the virus could be transferred to others. In the present study, surfaces and air were sampled from hospital intensive care unit, pediatric intensive care unit, and COVID-19 care unit to identify the presence of viral RNA of SARS-CoV-2 and Influenza. Results showed that SARS-CoV-2 was present in 10 % of surface samples, particularly from the COVID-19 care unit. After next-generation sequencing, we were able to identify two of the variants of concern, Delta, and Omicron. SARS-CoV-2 and Influenza were absent from the air samples collected. The presence of SARS-CoV-2 reveals the importance of constant microbiological monitoring to prevent co-infections, the occurrence of nosocomial infections during hospitalization, and to improve of public health strategies.

Keywords: SARS-CoV-2; COVID-19; Indoor air; Surfaces, Sequencing; Hospital.

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Biological evaluation of reverse transcriptase inhibition and antiviral activity against HIV-1 of Cyclopentanpyridinone analogues

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The HIV-1 reverse transcriptase (RT) enzyme is responsible for the transcription of viral RNA to produce a double-stranded DNA copy, which is subsequently integrated into the genome of target cells. The pyridin-2(1*H*)-one ring is a central structure for the development of new non-nucleoside reverse transcriptase inhibitors, because kinetic studies and mutation analysis indicate that these derivatives bind at the allosteric site of RT, showing activity against wild-type and mutant strains of HIV. Pyridinone-derived type quantitative structure-activity relationship (QSAR) models and virtual screening were developed and used to design new analogs with activity against RT-HIV-1. Twenty derivatives were synthesized and tested for cytotoxicity, inhibition against RT by biochemical assay, and activity against HIV-1 using a CD4+ T indicator cell line combined with flow cytometry. The results illustrate the utility of computer-aided drug design in the search for new small compounds against HIV-1. Biological tests allow analysis of structural determinants of antiviral effect to guide enhancement of antiviral effect.

Keywords: HIV; reverse transcriptase; pyridinone.

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Effect of human papilloma virus oncoproteins E6 and E7 on redox state and its association with radiotherapy response

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Persistent high-risk human papillomavirus (HR-HPV) infection is an important etiologic factor for the progression of invasive anogenital and oropharyngeal cancers. The main objective was to elucidate the impact of HR-HPV E6 and E7 oncoproteins on redox regulation in response to radiotherapy. Compared to the control group, a decrease in the ID_{50} of cells transfected with HPV16 E6 and E7 oncogenes was observed. The levels of some antioxidant proteins were evaluated. Total Nrf2 levels were not affected by the expression of E6 and E7 oncoproteins or by radiation treatment. In contrast, phosphorylated Nrf2 levels were increased in C33A-E6 and C33A-E7 cells before radiation, and significantly decreased after the radiotherapy. The Nrf2 negative regulator, KEAP1, showed the opposite effect after radiation as these levels increased. Additionally, SOD2 levels decreased only after radiation. Catalase protein levels were decreased in C33A-E6 cells before and after radiation, and these levels were decreased in C33A-E7 cells only after radiation. Finally, the study analyzed the levels of the marker of DNA damage, H2AX, both total and phosphorylated, and found that the levels of this total protein were elevated only in irradiated C33A-E6 cells. Phosphorylated γH2AX was decreased in C33A-E6 and C33A-E7 cells before irradiation, but increased in C33A-E6 cells after radiation. HPV-16 oncoproteins E6 and E7 induce radiosensitivity and differentially modulate redox regulation mechanisms before and after treatment with ionizing radiation.

Keywords: HPV; Oncoproteins; Radiotherapy; Redox; Antioxidants.

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Vol. 11 | Special Supplement

Community-Based Educational Approaches for Pandemic Control and Mitigation: The Teabo, Yucatan Case Study

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During the pandemic generated by the SARS-CoV-2 virus, the Mexican government implemented containment-mitigation strategies to reduce and prevent contagion, however, community participation in health issues has generated adverse reactions from the official sector, which approach indigenous communities with an imposing and handout mentality. The study aimed to develop a research-action-education work to control COVID-19 in an indigenous community of Yucatán. For this purpose, semi-structured interviews and focused observation in the municipality of Teabo, Yucatan were performed from June to December 2020. With the collected information, the located approach strategies were developed using various resources, spaces, and media for dissemination. In general, good management of sanitary measures among the population was found, nevertheless, the presence of discrimination and stigma was recorded. The community educational strategies developed for the containment-mitigation of COVID-19 in Teabo were of three types: (1) for the population in general; (2) for the young population; and (3) social networks. Action-research projects with a collaborative approach are fundamental for health to be a right in rural areas.

Keywords: SARS-CoV-2, Community, Mitigation, Indigenous, Health.

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Vol. 11 | Special Supplement

The use of AG129 mice for the evaluation of FDA-approved drugs with broad antiviral spectrum as a potential anti-flavivirus treatment

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The Dengue (DENV) and Zika (ZIKV) viruses have been increasing in the last decade in the Americas. For lack of vaccines, FDA-approved drugs have been proposed in recent years to combat these viruses, most notably lipid-lowering drugs, which have demonstrated a wide in vitro antiviral spectrum. However, despite being safe, in vivo assays remain critical to reduce the risk of adverse effects during their use as antivirals. Therefore, the present study focuses on the advantages and disadvantages of using AG129 transgenic mice, triple knock-out for interferon alpha (INF-α), beta (INF-β) and gamma receptor (INFr-γ), as an in vivo model for the evaluation of candidate lipid-lowering drugs for treatment against flaviviruses. Immunodeficient AG129 mice showed the advantage of exhibiting clinical signs of ZIKV and DENV disease. However, we observed differences between females and males, during infection and lipid-lowering drug treatments, that should be considered. MET increased survival time and decreased disease severity of DENV-infected mice. Interestingly, MET did not counteract ZIKV disease *in vivo*; on the contrary, exacerbated severe signs were observed in females that require further study.

Keywords: DENV; ZIKV; Lipid-lowering drugs; AG129-mice.

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Vol. 11 | Special Supplement

Detection of Respiratory viruses in residual clinical samples from patients with a clinical diagnosis of SARS-CoV-2, one year after the pandemic

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Acute Respiratory Infections (ARI) are the main cause of morbidity and mortality worldwide and a persistent public health problem. The identification of other ARI-causing viruses in addition to SARS-CoV-2 is important. The objective of this study was to determine the infection by other respiratory viruses in residual RNAs of patients with a clinical diagnosis of COVID-19 and negative to RT-PCR in patients of the medical service of the Autonomous University of Yucatán. From March to December 2021, 221 RNAs were selected, 77 of 221 RNAs (34.84 %) were positive for other respiratory viruses; 30 (38.96 %) were positive for influenza virus (VI), 43 (55.84 %) for parainfluenza virus (PIV), 7 (9.09 %) for rhinovirus (RV), 6 (7.79 %) for human metapneumovirus (hMPV) and 2 (2.60 %) to human coronavirus 229E (HCoV-229E). It is worth noting the presence of 12 coinfections, of which the VI-PIV coinfection was the most frequent with seven, followed by PIV-CoV-22E with two and finally the VI-MPVh, PIV-RV, and PIV-MPVH coinfections with once each. The months of March, April and May were the months with the highest number of positive cases for other viruses, months prior to the start of the second wave of coronavirus infections

Keywords: viruses, pandemic, respiratory infections, co-infections, epidemiology.

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Single infection and dual co-infection by respiratory viruses in Mexican patients with acute respiratory illness negative for SARS-CoV-2

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Objective: Determine the frequency of single infections, co-infections and viral load of respiratory syncytial virus (RSV), metapneumovirus (MPV), rhinovirus (RV) and parainfluenza (PIV) 3 in Mexican patients with acute respiratory illness (ARIs) negative for SARS-CoV-2. **Methods:** 300 patients adults with ARIs were included. The nasopharyngeal exudate was taken from each individual, from which RT-qPCR were performed for SARS-CoV-2. Samples negative for SARS-CoV-2 were processed for molecular detection by RT-qPCR for Influenza A H1N1, differential diagnosis and viral load of RSV, MPV, RV, and PIV-3. **Results:** 36 % (108 / 300) of the cases of ARIs were positive for RSV, MPV, RV and PIV-3 infection, and of these, 91.67 % had a single virus infection and 8.33 % had dual co-infections. RSV was the respiratory virus with the highest prevalence in the population with ARIs negative for SARS-CoV-2 and the most common dual co-infection were RV+RSV. On the other hand, RV infections had the highest viral loads compared to the other viruses detected. **Conclusions:** RSV was the most prevalent respiratory virus in a population of Southern Mexico with ARIs negative for SARS-CoV-2 during the COVID-19 pandemic. The most frequent co-infection was RV+RSV and RV positive patients have the highest viral loads.

Keywords: Respiratory virus; Single infection; Co-infections; Viral load; Acute respiratory illness.

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Vol. 11 | Special Supplement

In Vitro and *In Vivo* Characterization of a Broadly Neutralizing Anti-SARS-CoV-2 Antibody Isolated from a Semi-Immune Phage Display Library

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Neutralizing antibodies targeting the receptor-binding domain (RBD) of SARS-CoV-2 are among the most promising strategies to prevent and/or treat COVID-19. However, as SARS-CoV-2 has evolved into new variants, most of the neutralizing antibodies authorized by the US FDA and/or EMA to treat COVID-19 have shown reduced efficacy or have failed to neutralize the variants of concern (VOCs), particularly B.1.1.529 (Omicron). Previously, we reported the discovery and characterization of antibodies with high affinity for SARS-CoV-2 RBD Wuhan (WT), B.1.617.2 (Delta), and B.1.1.529 (Omicron) strains. One of the antibodies, called IgG-A7, also blocked the interaction of human angiotensin-converting enzyme 2 (hACE2) with the RBDs of the three strains, suggesting it may be a broadly SARS-CoV-2 neutralizing antibody. Herein, we show that IgG-A7 efficiently neutralizes all three SARS-CoV-2 strains in plaque reduction neutralization tests (PRNTs). In addition, we demonstrate that IgG-A7 fully protects K18-hACE2 transgenic mice infected with SARS-CoV-2 WT. Our findings indicate that IgG-A7 could be a suitable candidate for developing antibody-based drugs to treat and/or prevent SARS-CoV-2 VOCs infection.

Keywords: COVID-19; Broadly neutralizing antibody; Non-clinical efficacy; Delta variant; Omicron variant.

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Sequencing of Influenza A virus isolates from the Monterrey metropolitan area

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Influenza A potentially pandemic respiratory disease, requires understanding its evolution for effective health strategies. The study aimed to analyze influenza A strains during the 2022-2023 winter in Monterrey Metropolitan Area, identifying clustering patterns in clades reported by GISAID (Global Initiative on Sharing All Influenza Data). Fourteen Influenza A samples were collected at University Hospital UANL. Viral genetic material was isolated and amplified by RT-PCR. Then, we sequenced with Oxford Nanopore MinION equipment and the SQK-LSK109 kit. Basecalling was performed using Dorado basecaller, followed by assembly via minimap2, medaka, and samtools. Finally, we conducted a bioinformatic analysis utilizing the Nextstrain seasonal-flu pipeline. Fourteen complete sequences of influenza A virus were obtained, thirteen sequences of the H3N2 subtype and one of H1N1. The H1N1 HA (Hemagglutinin) and NA (Neuraminidase) sequence was classified into clade 6B.1A.5a.2a.1 and C.5.1, respectualy, both from North America. On the other hand, the sequences belonging to H3N2 HA were grouped with isolates from North and southamerica as well as Europe (HA Clade: 3C.2a1b.2a.2b, subclades: G.1.1,G.1.3 & G.2). In conclusion, this work suggest that Influenza A isolates from Metropolitan Area of Monterrey are similar to circulating viruses reported in the America and European countries.

Keywords: Influenza A virus; Sequencing Oxford Nanopore; Phylogeny; Pandemic respiratory disease; Influenza-like illness

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Vol. 11 | Special Supplement

Isolation of Flavivirus from biological samples from Oaxaca, Mexico

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Mexico is an endemic country for DENV and ZIKV infection due to the large presence of their vector. Currently, there is evidence suggesting the existence of variability in the transmissibility of Flaviviruses, which would translate into an increase in total cases between epidemic outbreaks. However, epidemiological surveillance of DENV and ZIKV is limited to genetic characterization from patient samples and captured mosquitoes. In the present project we performed the isolation of DENV and ZIKV from infected mosquitoes in cell culture with the cell lines C6/36 (Aedes albopictus) and Vero (Chlorocebus), comparing the percentage of success by titration of plaque-forming units. Flaviviruses isolated from infected mosquitoes will be an invaluable tool for the study of phenotypic variations associated with transmissibility.

Keywords: Isolation, Flavivirus, Mosquitoes.

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Metagenomic analysis of plant viruses in tropical fresh and wastewater

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Plant pathogenic viruses represent a threat to agriculture. Previous studies have shown that some plant viruses can be transmitted by contact with contaminated water and remain infectious for long periods in aquatic environments. The use of treated water is increasingly necessary due to the decrease in freshwater availability. That is why, in the present study we determined the diversity of phytopathogenic viruses in fresh and wastewater samples through metagenomic analysis. We detected 38 virus families; among them, *Virgaviridae* was the most relatively abundant. Within this family we detected 15 different phytopathogenic viruses that belonged to the genus *Tobamovirus*. Pepper mild mottle virus (PMMoV) and tomato brown rugose fruit virus (ToBRFV) had the largest relative abundance. We assembled 24 coat protein (CP) sequences. Phylogenetic analysis of the CP amino acid sequence revealed a close relationship between the solanaceous plant-infecting viruses, and the reference sequences found in GenBank. However, CP amino acid sequence of viruses infecting *Fabaceae* and *Cucurbitaceae* plants showed less identity to reference sequences. These indicate that the detected viral sequences may be new strains of viruses related to local plants. On the other hand, since most *Solanaceae* plants, seeds, and products as well as derivates are imported we see less variation and thus more identity to the reference sequences. *Fabaceae* and *Cucurbitaceae*-infecting viruses did not show such a close relationship when compared with GenBank reference sequences.

Keywords: Freshwater; Metagenomic; Tobamovirus; Viral ecology; Wastewater.

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Vol. 11 | Special Supplement

Virome Analysis of the Cardon *Pachycereus pringlei* in Baja California Sur (BCS), Mexico

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RNA viruses (Alphaflexiviridae, Betaflexiviridae, Puribunyaviridae, Tombusviridae, and Virgaviridae) and DNA viruses (Geminiviridae, Nanoviridae, and Caulimoviridae) have been documented in cacti. Symptoms of viruses, such as chlorosis, mottling, brown spots, and morphological alterations, have been observed in the columnar cactus (P. pringlei), endemic to BCS. However, there are no reports of viruses in this species. Hence, we analyzed the virome of cardon distributed in different regions of the peninsula. DNA extraction was performed on seven symptomatic cardons, and genomic libraries were constructed. Metagenomes were assembled using MEGAHIT, and Virsorter2 was employed for searches, with quality assessment using checkv. Annotations were 2 conducted using blastn and MEGAN databases. Relative abundances at the family and genus levels were obtained. Additionally, taxonomic identification was conducted using Viptree. Results revealed that Cardon harbor ubiquitous viruses from the Caulimoviridae family, specifically from the genera Badnavirus, Soymovirus, and Caulimovirus. Notably, Badnaviruses exhibited a size range of 7 to 8 kbp, forming a distinct monophyletic clade, implying the potential existence of new virus species within this group.

Keywords: Gigant; Columnar; Arid biome.

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Molecular Characterization of Omicron Subvariants of SARS-CoV-2 in Patients with Severe and Fatal Outcomes, Winter Season 2022-2023

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In the COVID-19 pandemic, the emergence of variants and subvariants of the SARS-CoV-2, especially the Omicron variant, remains a matter of great concern. Mutations in the Spike protein of these variants have significant implications for their infectivity, immune evasion, and disease severity. To address this situation, the National Institute of Respiratory Diseases (INER) and CoViGen-Mex, conducts constant surveillance of these variants by analyzing genomic sequences, aiming to identify specific mutations with relevant biological implications. During the period from November 2022 to May 2023, a comprehensive study was conducted using advanced sequencing technologies such as Illumina and Oxford Nanopore. In 456 analyzed samples, we identified several omicron subvariants, with XBB being the most prevalent (50.7 %), followed by BQ (26.8 %), BA (7.7 %), and BW (4.4 %). Additionally, the GD.1 lineage (0.9 %) was detected, which carries F453Y mutation in the receptor-binding domain (RBD). Divergence of the omicron subvariants indicate a continuous evolution of the virus and the need for special surveillance. Understanding the dynamics of these variants is essential for improving responses and implementing appropriate public health measures. Genomic surveillance enables proactive and effective management to control and minimize the impact of SARS-CoV-2 variants on public health.

Keywords: Genomic surveillance; Omicron; Variants; Receptor-Binding Domain (RBD); Mutations.

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Analysis of the expression of miRNAs 145-5P and 195-5P in high-risk HPV positive patients

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Infection by human papillomavirus (HPV) is the main factor associated with the risk of developing some form of cervical intraepithelial neoplasia (CIN) and eventually the development of cervical cancer (CCU). More than 200 types of HPV are known, of which at least 13 are oncogenic or also called high-risk virus, including mainly the genotypes 16, 18, 35 and 45. miRNAs have an important role in the oncological area, due to their important regulatory task of the expression of some genes of the cell cycle and their implication in several types of cancer, among them CCU. Therefore, in the last years, differences in their levels of expression have been related with a remarkable role as possible biomarkers. The deregulation of miRNAs 145-5p and 195-5p has been implicated in multiple studies with the development of CCU. In the present study a total of 236 women who attended in the Sinaloa Cancer Institute in Culiacán Sinaloa were included. When detecting HPV, 172 were positive for infection. When genotyping all the samples, 64 were positive for some of the analyzed high-risk genotypes (16,18,35 and 45), with genotypes 16 and 45 predominating. The expression levels of miRNAs 195-5p and 145-5p were analyzed of 25 tissue samples using a qRT-PCR, observing a statistically significant difference between the presence of the virus and the overexpression of miRNA-145-5p ($P = 0.001$).

Keywords: HPV; CIN; miRNAs; Expression.

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Vol. 11 | Special Supplement

Evaluation of the in vitro antiviral activity of new organic compounds against Zika virus

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Antiviral drugs are invaluable tools for the control of epidemic or pandemic outbreaks of pathogenic viruses in the human population; however, their high adaptive capacity, mainly of those with RNA (Ribonucleic Acid) genomes, compromises their effectiveness. Therefore, the demand for new organic molecules with antiviral activity has increased significantly in recent years. With this in mind, our objective in this study is to evaluate whether organic compounds derived from phenol, cinnamic acid, and carbamic acid, had antiviral activity against Zika virus by immunofluorescence and flow cytometry. The results suggest that used candidates with antiviral activity evidenced by a reduction of viral antigen-positive cells from ZIKV-infected cell cultures were identified.

Keywords: Antiviral; Zika; Phenol; Cinnamic acid; Carbamic acid.

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Vol. 11 | Special Supplement

Antiviral effect in vitro of the alga Spirulina Maxima (Arthrospira) against the chikungunya virus

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Chikungunya fever is an acute viral disease caused by the Chikungunya Virus (CHIKV), which is transmitted to humans through infected Aedes aegypti and Aedes albopictus vectors. Spirulina (formerly Arthrospira) maxima (SP) is a filamentous cyanobacterium containing different molecules, some of which per se have shown antiviral activities. It has been reported that SP has a great participation in the viral replication cycle, a property that is very advantageous for therapeutic use, in addition to reducing the appearance of drug-resistant viruses. The purpose of this study was to analyze the antiviral effect and cytotoxicity of SP algae against the CHIKV virus in Vero cells. The determination of SP cytotoxicity was performed by the method based on the metabolic reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazole (MTT) bromide. The evaluation of SP antiviral activity against CHIKV, was observed in the reduction percentage was estimated by counting the plates. All assays were carried out in triplicate. Untreated infected cells were used as a positive control and dimethyl sulfoxide (DMSO), SP diluent, was added as a negative control. In the present study, the antiviral activity of SP against the CHIKV virus obtained ≥ 50 % inhibition of virus in cell culture, presenting a reduction of plaques (≥ 48.3 %). The results suggest that SP is an algae with great antiviral potential against CHIKV.

Keywords: *Spirulina Maxima* (*Arthrospira*); Antiviral effect; Chikungunya virus.

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Vol. 11 | Special Supplement

Quality of life during postpartum of women with HIV/AIDS receiving antiretroviral therapy in Mexico City, Mexico

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Adherence to antiretroviral therapy is essential during pregnancy to avoid vertical HIV transmission. However, pregnancy and postpartum could influence perceptions of quality of life among women living with HIV/AIDS, modifying their adherence to therapy. Here, we estimate the association of quality of life with sociodemographic, clinical and related sexuality variables of HIV-infected women on antiretroviral treatment during postpartum. A cross-sectional study was conducted in a tertiary care institution in Mexico City from April 2020 to September 2022, to evaluate the quality of life of 75 HIV-infected women who received antiretroviral therapy during postpartum with the WHOQOL-VIH-BREF instrument. Sociodemographic, clinical, and sexual variables were obtained from their clinical records. The total quality of life score was intermediate (15). The physical health and independence domains received the highest scores (16). The domains of psychological health, environment and personal beliefs obtained the lowest scores (14). There was a significant correlation between four out of six domains with the CD4+ account. Other variables also obtained a significant correlation with at least one of the domain scores. Despite poverty, stigma, and discrimination, this group of HIV-infected women obtained an intermediate total quality of life. An acceptable CD4+ count is associated with a good quality of life and could be a relevant marker for antiretroviral adherence.

Keywords: 90-90-90 targets; LATAM: HIV pregnancy quality of life; Combination antiretroviral therapy; WHOQoL-HIV.

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Vol. 11 | Special Supplement

Role of importin-β3 and NS5 in acute and persistent infection with Dengue virus 2 in C6/36 cells

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Dengue virus (DENV) is able to establish long-term infections in its vector. NS5 is the viral polymerase and importin-β3 is a cellular nuclear transport protein. To assess the level of expression and localization of importin-β3 and NS5, C6/36 infected with DENV-2 and persistently infected C6-L55 cells were analyzed. C6/36 cells were infected with DENV-2 at a multiplicity of infection (MOI) of 0.1, 2, and 10. C6-L55 cells were incubated with brain extract of uninfected mice. At 12, 24, 36, 48, 72, and 120 h the culture media was harvested for virus titration and RNA and total proteins were purified from cells for evaluation of importin-β3 expression and viral genome copy number by RT-qPCR and Western blot assays. Additionally, the localization of importin-β3 and NS5 was determined by immunofluorescence. Copy number of viral genome, viral titers and level of importin-β3 mRNA do not vary over time in C6-L55 cells, while the highest level was observed in acutely infected cells; this correlates with higher levels of NS5 in the nucleus. Importin-β3 and NS5 protein levels were different in both cells. DENV-2 infection produced a marked difference in the expression levels of importin-β3 and NS5 between C6/36 cells acutely and persistently infected.

Keywords: Dengue virus; Persistent infection; C6/36 cells; Mosquitoes; Nuclear transport.

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Anti-viral activity of *Euphorbia sp* resin against the Chikungunya

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In 2015, almost 1 million cases of Chikungunya virus (CHIKV) transmission were reported in the American continent, making this one of the emerging infections on which multiple researches have been focused; however, there is no approved vaccine or drug to combat this virus. One of the most used strategies in the search for new molecules with antiviral activity is obtain them from natural sources by biodirected assays. In the present research, chromatography was used to obtain an active fraction from the resin of Euphorbia sp. MTT assay showed a minimum toxic concentration (MTC) of 20 µg/mL in the HaCat cell line, decreasing cell viability to 88.13 %. In addition, its anti-CHIKV activity was demonstrated at concentrations below the MTC, 4 µg/mL it´s the concentration in which the greatest anti-CHIKV effect was observed, obtaining a titer of 60 PFU/mL, which represents a 99.9957 % decrease of the viral titer of the control group. About the possible mechanism of action, an antiviral and non-virucidal effect was determined by an addition time assay, acting in the early stages of viral replication, since only a significant decrease in viral titer was only observed when the treatment was added 2 hours post infection.

Keywords: ChikV; *Euphorbia sp*; Antiviral activity; Cytotoxic.

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Vol. 11 | Special Supplement

SARS-CoV-2 Persistence Among Healthcare Workers: Exploring Rarity and Occasional Recurrence

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SARS-CoV-2 is the causative agent of COVID-19 and RNA persistence in the nasopharynx is typically limited to < 2 weeks after symptom onset in otherwise healthy subjects. Between 2020 and mid-2022, the National Institute of Respiratory Disease (INER) provided care for severe COVID-19 patients with over 3 600 healthcare workers (HCW). A robust occupational health service was established to detect, diagnose, and follow up on COVID-19 cases in HCW. During this period, 125 HCW cases were detected lasting 16 days or more with positive RT-PCR results for SARS-CoV-2. Persistence averaged 30.1 days (SD 10.1), median 29 days, mode 22 days. Interestingly, the longest persistence (50 - 74 days) occurred in 2020 cases. While many cases had recurrent COVID-19 episodes, most tested negative 7 days post-symptom onset. Just two instances demonstrated recurrent persistence across separate years. Here we showed that SARS-CoV-2 persistence in HCW is a rare event (up to 2.1 % of all HCW in 2021) and that the recurrence of persistence in those patients is even more rare, suggesting that other factors beyond the host might be involved.

Keywords: SARS-CoV-2; persistence; recurrence; HCW; COVID-19.

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Vol. 11 | Special Supplement

Study of respiratory viruses in oncopediatric patients

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Around 1 % of children are SARS-CoV-2 asymptomatic carriers, and up to 7 % of SARSCoV-2 cases are also positive to other respiratory viruses. The frequency of asymptomatic COVID-19 cases, reinfections, and coinfections are unknown in oncopediatric patients. In this work, we investigated the frequency of infections with SARS-CoV-2, Respiratory Syncytial Virus, Influenza A, and Influenza B viruses, in 18 oncopediatric patients followed from 2021 to 2022, for a total of 38 samples. RT-PCR was performed for the viruses identification, meanwhile antibodies against SARS-CoV-2 were evaluated by lateral flow tests. The main condition of the participants was acute lymphoblastic leukemia (67 %). 16 % of the samples were positive to one virus and 14 % to two or three viruses. 25 % of the cases positive to one or more viruses, did not show respiratory symptoms; meanwhile, 46 % of the samples from patients with respiratory symptoms, were negative to all the viruses tested. 18 % of the samples tested positive for SARS-CoV-2 IgG, but the patients did not refer to have had COVID-19 neither received the vaccine. So far, no COVID-19 fatal cases 3 have been reported in the studied population. This work offers a general view of viral respiratory infections in oncopediatric patients.

Keywords: Virus; Respiratory symptoms; SARS-CoV-2; Leukemia; Oncopediatric patients.

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Expression and function of the viroporin SH of the Respiratory Syncytial Virus in A549 cells

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The SH protein is located in the envelope of the Respiratory Syncytial Virus (RSV). The protein monomers interact with each other, which make a pentamer that forms a pore (viroporin). This facilitates for ions to pass through. Furthermore, it has been observed that it participates in the life cycle and pathogenesis Due to these and other characteristics, it has been considered a pharmacological target. Our objective is to generate a model of expression and function of the viroporin SH of the RSV in the A549 cell line. Using the gene sequences of the SH protein of RSV subgroups A and B a multiple alignment was made. The construction of the expression plasmid was carried out with the SH gene in the plasmid pVITRO1-neo-mcs to transfect cells. We obtained that SH protein sequences have 100 % intra-subgroup identity and 64 % inter-subgroup identity. The plasmid with the SH gene subgroup A and B was obtained to subsequently transfect cells. Therefore, it is considered that the SH gene has been conserved over time and around the world. The transfection and expression of the SH protein was achieved. As perspectives once the cells are transfected, they will be evaluated with qRT-PCR and Western blot. Subsequently, the functionality of the viroporins will be evaluated too.

Keywords: Viroporin; SH protein; RSV.

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Vol. 11 | Special Supplement

Dynamics Of Evolution and Sequencing Of SARS-CoV-2 In Mexico

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SARS-CoV-2 is a new virus in the coronavirus family that causes the disease COVID-19 and is responsible for the 2020 pandemic. SARS-CoV-2 is characterized by its high transmissibility between humans, the origin of which is believed to be a zoonotic event. Mexico has made a major effort to monitor SARS-COV -2 variants, through the association of CONAHCYT, various public agencies. This has resulted, for example, in the Genomic Surveillance Consortium for SARS-COV-2 (CoviGen), composed IBT-UNAM, INER, LANGEBIO-CINVESTAV and CIAD-CONAHCYT, within the framework of the National Strategic Project for Research and Consultancy in Virology of CONAHCYT. The objective of this research is to investigate and collect information about coronaviruses, with a particular focus on the SARS-COV-2 virus, addressing the evolutionary processes, the origin of the virus, the variants circulating during the main epidemiological waves in México, as well as the vaccines and drugs available for the prevention. After analyzing the distribution of variants during the first wave of the SARS-CoV-2 pandemic, the dominance of the autochthonous variants B.1.222 and B.1.519 was observed. These variants circulated together with variant B.1.1.7 (alpha), which did not prevail nationwide but had a higher incidence in northern Mexico. Similarly, variant P.1 (gamma) became dominant in the south of the country during this phase. In the third wave of the pandemic, variant B.1.617.2 (Delta) emerged, displacing all previous variants, and maintaining its dominance for much of 2021. It was not until the end of that year that the variant B.1.1.529 (Omicron) arrived and displaced all other variants, dominating to this day. The laboratory that has sequenced the largest number of genomes of the SARS-CoV-2 virus is INMEGEN, with a total of 11,701 sequences. This laboratory has made a significant contribution in terms of genomic sequencing of the virus in Mexico. On the other hand, there are several laboratories that have sequenced only one or a few sequences of the SARS-CoV-2 virus. These laboratories include CIADJ, LABOPAT, LESP-H, NHRC, UASLP, UUSMD_CRUZ-ROJA, CICESE, HGM, IMSS, INCMNSZ, INER-INR, and UGA_HRAEI_SSA. Each of them has sequenced less than 50 genomes.

Keywords: Covid-19; SARS-CoV-2; Variants; Whole genome sequencing.

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Antiviral activity of *Pentalinon andrieuxii* against Influenza H1N1

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Respiratory viral infections such as influenza, continue to be a global health problem causing annual seasonal epidemics. In 2009, the first influenza pandemic of the XXI century was caused by a new influenza A(H1N1) virus, responsible for more than 200,000 deaths in more than 214 countries. Currently, even though there exist vaccines and antivirals on the market, the multiple mutations of the viruses make it necessary to continue the search for new and more effective antiviral agents. *Pentalinon andrieuxii* (Apocynaceae) is a vine found in the Yucatan Peninsula, where it is used in traditional Mayan medicine to alleviate lesions derived from cutaneous leishmaniosis. Currently, the phytochemical knowledge of *P. andrieuxii* includes trinorsesquiterpenes, triterpenes, steroidal derivatives, and sterols; however, to date, no antiviral activity has been reported from extracts or secondary metabolites obtained from this species. Here we describe the preliminary results that demonstrate the capacity of semi-purified fractions from the crude extract of the root of the plant to inhibit the cytopathic effect in post and co-treatment against the H1N1 influenza virus strain A/Yucatan/2370/09 (H1N1). Future studies will identify those metabolites present in the semi-purified fractions as the potential compounds responsible for the antiviral activity.

Keywords: Influenza A(H1N1); Antiviral; Cytopathic effect; Semipurfied fractions; *Pentalinon andrieuxii*.

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Generation of the monoclonal antibody against the nonstructural protein 1 of the Chikungunya virus

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Chikungunya virus (CHIKV) is the most transmitted alphavirus nowadays. CHIKV is transmitted by *Aede aegypti* and *Aedes albopictus*, these mosquitoes can adapt to different environments, this feature facilitates the spread of CHIKV outside the tropical and subtropical areas where it is usually transmitted. Chikungunya is a positive-sense RNA virus that codes for six structural and four non-structural proteins. NSP1 is a non-structural protein that is essential for the protection of viral genetic material and the anchoring of the viral replication complex to the plasma membrane of the infected cell. Due to the function of this protein, it could be used as a marker of active infection with CHIKV. Antibodies are the most widely tool used to detect proteins and other molecules inside the cell. Due to its specificity, a monoclonal antibody is very useful for various studies of pathogenicity and modulation of viral replication, for the transmitting vector as well as in animal and cellular models. It's worth to mention that there is no commercial monoclonal antibody against the CHIKV NSP1 protein. In this work we produced and purified the NSP1 protein of CHIKV. This protein was used to immunize BALB/c mice and thus carry out the production of monoclonal antibodies using hybridoma technology. Both polyclonal and monoclonal antibodies with enough specificity for recognition of the native NSP1 protein in Chikungunya-infected cells were obtained. As a result, the production of these antibodies from the recombinant NSP1 protein provides us with an essential tool for studies on the pathogenicity of the CHIKV.

Keywords: Chikungunya virus; Monoclonal antibody; Hybridoma; NSP1.

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Transcriptomic analysis of mosquito cells infected with a Mexican isolate of Zika virus

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Zika virus (ZIKV) is a mosquito-borne virus from the genus flavivirus, which emerged in the Americas during the 2015 epidemic. Although the symptoms of this infection are generally mild, it can pose a risk of severe complications. As with other arboviruses, studies aimed to elucidate the molecular mechanisms of ZIKV infection in its mosquito vector are limited. This study compares the transcriptome of mosquito C6/36 cells infected with an isolate of ZIKV of Mexican origin. The resulting RNAseq data was processed to identify differentially expressed mRNAs in infected cells. Then, an ontology analysis was carried out to identify cellular processes most affected by ZIKV infection. Interestingly, some of the mRNAs identified in this experiment, or their homologs, have been previously reported as being involved in the replicative cycles of several viruses. These results will be helpful in identifying the pathways that ZIKV uses to successfully infect mosquito cells and apply this knowledge to strategies for controlling this infection.

Keywords: Zika; Mosquito; Aedes; Arbovirus; Transcriptomic.

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Evaluation of immune response induced by a plasmid that codifies O-SN SARS CoV-2 fusion protein in a mouse model

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New generation of COVID-19 vaccines have focused on the evaluation of more than one SARS CoV-2 antigen to induce a long-lasting immune response. We design a plasmid that codify for fusion protein and evaluated their immune response in a mouse model. Most immunogenic regions from Spike and Nucleocapsid Omicron SARS CoV-2 strain were determined by Insilco approaches. Once the sequences were selected, we generate the structure of this fusion protein and determined physicochemical and immunogenic properties. Docking molecular was performed to predict the capacity of fusion protein to be recognized by innate receptors such as TL3 and TLR4. The sequence was cloned in pcDNA3.1 and named pcDNA3.1/O-SN, expression of the plasmid was evaluated. BALB /c mice were immunized with 10 µg, 20 µg, 40 µg of pcDNA3.1/O-SN. Three doses of DNA were performed at interval of 20 days. Mice immunized with parental vector pcDNA3.1 were used as control. After immunization, bleedings were performed, and serum samples were obtained. Docking molecular showed capacity of binding TLR3 and TLR4 to O-SN. Specific antibody response of IgM and IgG against N and S1 proteins from SARS CoV-2 were observed in immunized mice. Our results indicate that DNA vaccination with pcDNA3.1/O-SN generated by our working group is capable to inducing a specific humoral immune response.

Keywords: SARS CoV-2; DNA Immunization; Preclinical studies; Fusion proteins; Immune response.

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Seroprevalence and Antibody Responses to SARS-CoV-2 in Adults and Children: Implications for Vaccination Strategies

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This study assessed the seroprevalence of SARS-CoV-2 and antibody responses in a high-risk adult population and hospitalized children in the state of Morelos. An ELISA test utilizing the SARS-CoV-2 spike protein's RBD domain was employed to detect IgG, IgM, and IgA antibodies. Among adults, before vaccination campaigns (n = 114), 26.3 % tested positive for IgG, while IgM and IgA were detected in 19.3 % and 13.2 %, respectively. The impact of Pfizer-BioNTech (BNT162b2) vaccination was analyzed, revealing significant increases in antibody levels following the second dose. Subsequently, participants receiving third doses from different vaccines exhibited comparable or higher IgG and IgM levels. IgA levels varied, with a subset reaching high levels only after receiving the booster dose. Children, before vaccination campaigns (n = 115), displayed 56.5 % IgG seropositivity, despite minimal reported COVID-19 cases. Elevated MIS-C/Kawasaki syndrome diagnoses (15 %) prompted a need for pediatric diagnosis focus. Comparative analysis of antibodies' levels in seropositive adults and children showed higher responses in adults both after infection (IgG: $r = 0.42$, $P < 0.001$; IgM: $r = 0.63$, $p < 0.001$) and after application of two vaccine doses (IgG: $r = 0.52$, $p < 0.001$; IgM: $r = 0.57$, $P < 0.001$; IgA: $r = 0.26$, $p < 0.001$). These findings underscore the necessity for continued antibody response monitoring post-vaccination in children and emphasize the importance of tailored vaccination strategies and diagnostic prioritization to address SARS-CoV-2 immunity in diverse populations.

Keywords: SARS-CoV-2; Seroprevalence; Antibody responses; Vaccination; Pediatric population.

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Environmental samples are key for monitoring viruses for human health and agriculture on the south border of Mexico

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Mexico's southern border is a complex region due to migration flows and social dynamics, whose effects impact the human population and plant health, the ecosystems, and the biodiversity of the border-sharing countries. But what about the viruses? In 2020, due to the COVID-19 pandemic, our research team proposed a strategy for SARS-CoV-2 virus monitoring via the wastewater-based epidemiology approach (using wastewater from the treatment plants) and evaluating the viability of implementation using urban rivers and sewer water samples. We selected strategic points for collecting the samples from Tapachula (Chiapas), a border city with complex population dynamics due to the human migratory phenomenon. We measured physicochemical parameters to determine the water samples' pollution level. We optimized a low-cost viral particles concentration method to detect the SARS-CoV-2 by RT-qPCR. We demonstrated that the method effectively detects viral particles from urban rivers despite the pollution level and the environmental temperature prevailing in this tropical region and could strengthen the monitoring of epidemiologic strategies. Also, by metagenomic sequencing and bioinformatic analysis, we determined the virus diversity in the water samples from the all-sampling points. The results revealed a high quantity of plant-infecting viruses (around 80 % of all detected) belonging to the Virgaviridae family, specifically of the *Tobamovirus* genus, that infect solanaceous like tomato, potato, or chili. Secondly, we detected bacteria-infecting phaguses and interesting animal-infecting viruses diversity (including insects or vertebrates) and fungi. This opens the possibility of developing monitoring strategies for viruses of human, ecological, and agricultural health relevance on Mexico's southern border.

Keywords: Wastewater-based epidemiology; Viruses diversity; Viral metagenomics; Plant-infecting viruses; Urban rivers.

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Genotyping of feline leukemia virus and feline immunodeficiency virus in naturally infected domestic cats

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Feline leukemia and immunodeficiency viruses (FeLV and FIV) are retroviruses with worldwide distribution that infect different species of felines, including domestic cats. Infections caused by feline retroviruses can cause various syndromes that can lead to the death of the host. The identification and segregation of infected cats is the most important measure for the control of infections caused by feline retroviruses, with viral genetic variability and the phase of infection being key points for the use of different diagnostic tools. The objective of this study was to genotype feline retroviruses present in naturally infected domestic cats. Detection of retroviral infections was performed with lateral flow immunochromatography and endpoint PCR assays in 50 domestic cats with different clinical-haematological characteristics. The FeLV genetic sequences corresponded to subgroup A and were shown to be related to sequences previously reported in America. Phylogenetic analysis of the FIV sequences revealed the presence of the B subtype in the infected cats.

Keywords: Feline leukemia virus; Feline immunodeficiency virus; Genotyping; Provirus; Retroviridae.

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Role of aspartate aminotransferase-to-lymphocyte ratio index (ALRI) in COVID-19

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COVID-19 has a mortality toll exceeding 5.4 million worldwide. Early identification of patients at high mortality risk is essential to save their lives. AST-to-lymphocyte ratio index (ALRI) is a novel biomarker of survival in patients with hepatocellular carcinoma, an organ susceptible to SARS-CoV-2 infection. The prognostic value of ALRI as a marker of COVID-19 mortality was evaluated. For this purpose, ALRI was compared with main biomarkers for COVID-19 mortality (neutrophil-to-lymphocyte ratio [NLR], systemic immune-inflammation index [SII], plate-let-to-lymphocyte ratio [PLR], lactate dehydrogenase (LDH)/lymphocyte ratio [LDH/LR]). A retrospective cohort of 225 patients with SARS-CoV-2 infection and without chronic liver disease was evaluated. In non-survival group, the ALRI, NLR, SII, and LDH/LR were significantly higher than in survival group ($P_{corrected} < 0.05$). ALRI had an area under the curve (AUC) of 0.81, sensitivity 70.37 %, and specificity 75 %, with a best cut-off value > 42.42. COVID-19 patients with high ALRI levels had a mean survival time of 7.8 days. Multivariate Cox regression revealed that ALRI > 42.42 (HR = 2.32, 95 % CI: 1.35-3.97; $P_{corrected} = 0.01$) was a prognostic factor of COVID-19 mortality. These findings prove that ALRI is an independent predictor of COVID-19 mortality and may help identify high-risk subjects with SARS-CoV-2 infection at admission.

Keywords: COVID-19; SARS-CoV-2; Aspartate aminotransferase-to-lymphocyte ratio index (ALRI); Biomarker; Mortality