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The World Society for Virology (WSV) is a non--profit organization established in 2017 to connect virologists around the world with no restrictions or boundaries, and without membership fees. The WSV brings together virologists regardless of financial resources, ethnicity, nationality, or geographical location to build a network of experts across low-, middle- and high-income countries. The WSV's aims include fostering scientific collaboration, offering free educational resources, advancing scientists' recognition and careers, and providing expert virology guidance. To facilitate global interactions, the WSV makes extensive use of digital communication platforms. By fostering cross-sectional collaboration between experts who study viruses of humans, animals, plants, and other organisms, as well as leaders in the public health and private sectors, the WSV strongly supports the One Health and One World approach. The WSV is a steadily growing society with currently more about 1,700 members from 90 countries across all continents. Members include virologists at all career stages including leaders in their field as well as early career researchers and postgraduate students interested in virology. The WSV has established partnerships with The International Vaccine Institute, the Elsevier journal Virology (the official journal of the WSV) and an increasing number of other organizations including national virology societies in Brazil, China, Colombia, Finland, India, Mexico, Middle East, Eurasia and Africa Network, Morocco, South Korea, Spain and Sweden.

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Keynote speakers

SARS-COV-2 SARS-COV-2 DERIVED RNA REPLICONS AS VACCINE CANDIDATES

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Propagation-deficient coronavirus-derived RNA replicons are safe and promising candidates as vaccines. Our laboratory developed MERS-CoV RNA replicons by deleting genes 3, 4a, 4b, 5 and E. These replicons, after intranasal administration of a single dose, induced strong humoral and cellular immune sterilizing responses in the K18-DPP4 transgenic mouse model. These replicons were safe because were propagation-defective and were derived from attenuated genomes by the deletion of several virus genes (1). The same strategy was applied to the engineering of SARS-CoV-2 derived RNA replicons. Nevertheless, both coronaviruses have a similar but distinct genome composition, and the set of genes that had to be deleted for the construction of a propagation-deficient and attenuated RNA replicon were different in the SARS-CoV-2 derived replicons. The generation of a SARS-CoV-2 RNA replicon as a human vaccine candidate, has been based in the deletion of several combinations of non-essential genes located at domains mapping at distal positions of the genome, including: a domain located at the 5'-end of the genome, another one in the S gene domain, and two additional ones by deleting different non-essential genes affecting either RNA replicon dissemination, or its attenuation. These deletions made very unlikely RNA replicon reversion to a virulent virus. The combined deletion of accessory genes significantly attenuated SARS-CoV-2. The attenuation was associated with a decrease of IFNβ and pro-inflammatory responses in the lungs of infected mice. The RNA replicons expressed S proteins from former SARS-CoV-2 strains and from Omicron, and induced humoral and cellular immune responses providing full protection in mouse models with a single intranasal dose.

Keywords: SARS-CoV-2, replicon vaccine, coronaviruses, attenuation

Gutiérrez-Álvarez, J., Honrubia, J.M.,…, Enjuanes, L., 2021. Middle East respiratory syndrome coronavirus vaccine based on a propagation-defective RNA replicon. Proc. Natl. Acad. Sci. USA **118**, e2111075118.

ZIKA VIRUS VACCINE DEVELOPMENT: EXPERIENCE IS NOT PREPAREDNESS

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In 2016, the World Health Organization (WHO) declared Zika virus (ZIKV) a Public Health Emergency of International Concern in response to the association of ZIKV infection with an increased frequency of infants born with microcephaly and cases of Guillain-Barre syndrome in adults. In response to this declaration and informed by significant experience with related flaviviruses, vaccine development for ZIKV proceeded quickly. We developed DNA vaccines encoding two ZIKV structural proteins sufficient for producing non-infectious virus-like particles. Both DNA vaccines were evaluated in phase I clinical trials and the most promising candidate was studied further in a phase II trial conducted at sites throughout the Americas. To identify vaccine-elicited correlates of protection, we conducted vaccine dose de-escalation studies in animals. These studies revealed neutralizing antibodies as an imperfect correlate of vaccine-elicited protection and identified qualitative characteristics of the neutralizing antibody response critical for protection. Furthermore, we demonstrated that virus-like particles inefficiently elicit the most protective antibodies when compared to natural infection. To address these limitations, we investigated features of ZIKV structural proteins that define the antigenic structure of virus-like particle vaccine antigens and strategies to manipulate these antigens to elicit the most protective responses. A more complete understanding of the complex interplay between antibody repertoires, immunogenicity, and viral antigen structures may accelerate vaccine development to the degree required to impact short-lived but explosive arbovirus epidemics.

Keywords: Zika virus, flavivirus, DNA vaccine, virus-like particle vaccine

INFLUENZA VIRUSES AT THE ANIMAL-HUMAN INTERFACE

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Outbreaks of influenza viruses continue to cause morbidity and mortality in mammals and birds worldwide. In addition to seasonal human influenza virus strains, it is critical that influenza virus evolution and emergence be monitored in other species, particularly swine and birds. A wide range of avian influenza virus (AIV) strains have been associated with mammalian infections causing ongoing risks to human and animal health. This includes the highly pathogenic avian influenza (HPAI) goose/Guangdong (Gs/GD) lineage H5NX viruses that are now circulating endemically throughout the world. These 2.3.4.5b lineage viruses have an increased host range including infections in fox, coyote, bear, marine mammals, and dogs as examples, as well as humans. The ability of AIVs to cross the species barriers is associated with a constellation of changes in several viral genes that allow replication in mammalian cells. The molecular traits identified as important for interspecies transmission include hemagglutinin (HA) receptor binding and fusion stability and changes in internal genes supporting increased replication, to highlight a few. In this talk, we will discuss the current state of the H5 outbreak, risk assessment pipelines used to identify viruses with increased risk to mammals and conducting studies at the human-animal interface. The interface between humans and wildlife is changing and, with it, the potential for pathogen introduction into humans has increased. Hypothesis-based field research at critical interfaces is critical to understand this and other zoonotic threats. Only by monitoring dynamic viral populations and defining their biology *in situ* can we gather the information needed to ensure effective pandemic preparation.

Keywords: influenza A virus, H5N1, zoonotic viruses, avian infleunza.

MARBURG VIRUS ECOLOGY WITH SPECIAL REFERENCE TO NATURAL TRANSMISSION MECHANISMS

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The majority of filovirus cases result from human to human transmission. However, the initial spillover events occur when humans encounter either the natural reservoir of the filovirus or secondary amplifying hosts, typically through hunting or utilizing bush meat. Despite more than the half a century of filovirus research and increasing number of outbreaks in the last 20 years, the reservoirs of filoviruses, and in particular of ebolaviruses remain elusive. Current evidence points to bats. There are numerous challenges in reservoir studies of filoviruses, including their erratic occurrence in remote and poorly resource locations, the biological diversity, and the abundant opportunities for their transmission from animals to humans in Africa. As of April 2023, seventeen outbreaks of Marburg virus disease (MVD) have been reported, most occurring in sub-Saharan Africa. Recently West and East Africa have recorded the first emergence of MVD; Guinea in 2021, Ghana in 2022, Equatorial Guinea in 2023 and Tanzania in 2003. To date the largest MVD outbreak occurred in Angola during 2004-205 and had a case fatality rate of 90%. Outbreaks of MVD have been associated with persons entering caves or mines, and results of outbreak investigations, ecologic and experimental studies implicate the Egyptian rousette bat (Rousettus aegyptiacus; ERB)) as the natural and prime reservoir of Marburg virus (MARV). However, the mechanisms by which MARV is maintained and transmitted among ERBs remain elusive. The results of experimental infections, modes of shedding demonstrated in both wild caught and experimentally infected captive-bred ERBs, the role of birth pulses and herd immunity, possibility of a prolong incubation period and/or chronic infection, the role of blood-sucking ectoparasites and results of vector competence studies, as well as the potential role of co-housing insectivorous bat species (one host versus multiple hosts – reservoir ecosystem) in maintenance and/or re-introduction of MARV infection will be discussed.

Keywords: Marburg virus, ecology, transmission, outbreak investigations, experimental studies, reservoir ecosystem.

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SARS-COV-2 IN ANIMALS: PUBLIC HEALTH IMPLICATIONS

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2) is the causative agent of Coronavirus Disease 2019 (COVID-19) originated in China and spread across the globe leading to a pandemic causing over 765 million confirmed cases and approximately seven million deaths in humans as of May 2023. The virus belongs to the family *Coronaviridae*, genus *Betacoronavirus* which includes several other coronaviruses that infect humans and animals which originated from bats. Human-to-animal transmission of SARS-CoV-2 (reverse Zoonosis/anthropozoonosis) has been testified, and the virus has been detected in at least twenty different animal species from ten families (*Felida, Viverridae, Hyaenidae, Canidae, Mustelidae, Procyonidae, Cervidae, Hippopotamidae, Hominidae, and Cricetidae*) of four animal orders (*Carnivora, Artiodactyla, Primates, and Rodentia*). The susceptible animal species consist of pets (dog, cat, ferret, and hamster), farmed (mink), and zoo/captive (tiger, lion, snow leopard, cougar, lynx, fishing cat, binturong, hyena, otter, coatimundi, hippo, and gorilla) and wild (deer, wild otter, feral mink, raccoon dog and big cats) animals. As of May 2023, there have been only 404 reported cases of SARS-CoV-2 infections in animals in the US, and in addition, 18 mink farms and wildlife (mink, mule deer, and white-tailed deer) in 29 states reported SARS-CoV-2 infections. The criterion used to classify the animal species of concern for SARS-CoV-2 epidemiology was their ability to shed infectious virus and to transmit SARS-CoV-2 to other animals (e.g., mink-to-mink and mink-to-cat transmission) and humans. However, currently, there is no evidence that animals play a significant role in spreading SARS-CoV-2 to people. Under experimental conditions, horses, cattle, sheep, pigs, or poultry are not susceptible to SARS-CoV-2. The presentation will be focused on the SARS-CoV-2 natural and experimental infections in domestic and wild animals and the possibility of the emergence of viral variants that can spill over into humans, and the potential implications on public health.

Keywords: SARS-CoV-2, animal coronaviruses, public health, Betacoronaviruses.

THE CONTINUING THREAT OF CLADE 2.3.4.4B H5N1 HIGH PATHOGENICITY AVIAN INFLUENZA VIRUS: WHAT HAVE WE LEARNT FROM THE EPIZOOTIC?

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Since 2020, the global impact of High pathogenicity Avian Influenza has escalated with the virus spreading across a broader geographical range than ever before. Within the UK over 320 infected poultry premises (IPs) have been confirmed with the virus affecting multiple sectors. Further, the impact on wild populations has been severe with over 2500 dead positive wild birds detected in Great Britain (GB) alone against an extraordinary number of deaths across different bird species resulting in significant impacts on avian populations. The virus has decimated bird populations across many regions. Alongside this, in the last 12 months, numerous spill over events have occurred into mammalian species that have increased the interest in these viruses as a potential pandemic zoonotic threat. Following the emergence of H5Nx, with H5N8 dominating in 2020 before the emergence and escalation of cases of H5N1 during following seasons, we have undertaken reactive research to both understand factors associated with the increasing impact of these viruses and try and answer key questions. Studies have centred on susceptibility, transmission, the risk to different commercial sectors, virus survival, impacts on wild bird populations and the impact of human behaviours on infected premises and in environmental settings on the exacerbation of virus dissemination and spread. Outputs from collaborative efforts across a broad range of partners will be used to overview our current understanding for these viruses and gaps in knowledge that are primary targets for future assessment.

Keywords: H5N1, avian influenza, HPAI, poultry

UNMET NEEDS OF ANIMAL VACCINES - OPTIONS TO APPROACH THEM.

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Commercialized vaccines of different types are effective against infectious diseases in humans and animals. Current vaccines are characterized by high safety and efficacy. The vaccine antigens are usually directed against one antigenotype of one pathogen. A challenge in veterinary medicine is to develop vaccines which are cost effective, do protect against antigenic variants of the same pathogen, and do have a fast onset and a long duration of immunity. Another challenge is early detection and fast reaction to an emerging disease in the veterinary field as human medicine has learned during the crisis caused by a zoonotic coronavirus SARS-COV-2. An important unmet need is that many pathogens, although bearing the same name, are different in antigenicity that either no or limited cross-protection is achieved after vaccination against one pathogen. An approach is using biomathematical methods to generate so-called broad-spectrum antigens inducing cross-protective immunity by analyzing amino acid sequences of the different antigenotypes relative to one another. In addition, mutations of certain amino acids within a hemagglutinin led also to such broad-spectrum protectotype. In a further step, it is now possible to generate three dimensional structures necessary to determine whether this linear amino acid sequence also assumes the three-dimensional structure necessary for optimum protection. This can help to further select candidate antigens for animal testings. The early detection of disease outbreaks and monitoring their relevance and spread in real time is an unmet need. This applies to both emerging and transboundary diseases. Systems based on Artificial Intelligence using Natural Language Processing can analyze daily millions of messages and thousands of scientific articles to detect early disease outbreaks and track them in real time. Boehringer Ingelheim Animal Health has partnered with LifeBit to establish such a system which shows that real-time outbreak identification and spread is possible.

Keywords: vaccines, broad-spectrum, antigen, outbreaks

WEST NILE VIRUS: A VIRTUAL BRIDGE THAT LINKS AFRICA AND EUROPE

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West Nile virus (WNV) is the most widely spread zoonotic arbovirus in the World. Member of the *Flavivirus* genus within the *Flaviviridae* family, it is responsible for neurological symptoms in humans and animals and is currently considered as a serious public health problem worldwide. The virus is maintained in nature in an enzootic cycle involving competent mosquitoes and a wide variety of reservoir host bird species. First identified in Africa in 1937, WNV has been spreading worldwide by migratory birds. Europe represents an important resting/ transit area and/or final destination of most long and short distance migratory birds coming from the African continent. The annual movements of migratory birds have contributed and are contributing to the introduction and spread of WNV strains, particularly those belonging to the lineages 1 (WNV L1) and 2 (WNV L2), the most widespread among the eight lineages described until now. In the last few years, we have tried to uncover the spatial and temporal viral dynamics of the two lineages between Africa and Europe. By integrating epidemiological studies with molecular, phylogenetic, and phylogeographic analyses, it has been shown that WNV L1 clade 1A originated in the 1900s between North and West Africa. The study also revealed that the common ancestor of the WNV L2 strains, now circulating in Europe and Africa, likely originated between the 18th and the 19th century in South Africa. From South Africa, it was then introduced into Hungary in 2004 before spreading throughout Europe. The study also demonstrated that WNV L1 and L2 strains move through two ideal corridors connecting Africa and Europe, which perfectly overlap the two most important Afro-Palearctic migratory flyways. These different ways of spreading likely influenced the two lineage's evolutionary history underlying the fundamental role played by the different bird species in WNV trans-continental diffusion.

Keywords: WNV, Europe, zoonotic arbovirus, flavivirus

ONE WORLD, ONE HEALTH.....ONE VIROME?

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Mammalian virome consists of viruses that infect cells, viral elements present in episomes or chromosomes, and viruses that infect microorganisms. Members of the virome fill different ecological niches within the host with significant effects on physiology that go beyond the issue invasion and destruction paradigm on which traditional virology has focused. Our knowledge of the virosphere is still limited and focused on high socio-economic impact viruses responsible for diseases of humans or domestic animals and modestly of wild animals. It is estimated that mammals and birds harbor 1.67 million unknown viruses that may potentially infect humans. This is the basis for the Global Virome Project (GVP), launched in 2018, with the aim of gaining new insights into the biodiversity of animal virome to prevent future pandemics. The financial investment will amount to USD 3.4 billion over 10 years, testifying to the strategic importance of acquiring data on animal virome. There are conflicting voices on the approach taken by the GVP. The main one argues that, in the absence of data on the mechanisms leading to species jumps, it is difficult to build predictive models based on animal virome diversity alone. Moreover, RNA viruses, most often responsible for species jumps, are constantly evolving and would require continuous monitoring with unsustainable costs. Zoonotic risk analyses, only based on the knowledge of the biodiversity of animal virome, would therefore be ineffective without taking into account the human-animal interface. Instead, an approach based on the risk of occupational exposure to animals in areas subject to changes in land use, in live animal markets, in places where wild animals are hunted and slaughtered and in densely populated areas with high mobility flows would be necessary. In addition, the immense virome of certain invertebrate species, vectors of diseases for humans and animals should not be underestimated.

Keywords: one health, interface, virome, emerging diseases

FROM THE RESPIRATORY TRACT TO THE BONE MARROW AND BEYOND. RECEPTOR SWITCHING AS PARVOVIRUS INVASION STRATEGY

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The family *Parvoviridae* comprises an exceptionally diverse group of viruses infecting a wide range of hosts. Relevant members of this family include human parvovirus B19 (B19V), a highly prevalent human pathogen, and adeno-associated virus, a leading platform for gene therapy. B19V causes the childhood rash *erythema infectiosum* and a broad range of syndromes in adults varying in severity. The virus has a remarkably restricted tropism. Following entry through the respiratory route, B19V targets and infects exclusively erythroid precursor cells (EPCs) in the bone marrow. The lytic infection in EPCs accounts for the hematological disorders associated with the infection. B19V exploits two distinct receptors and local pH conditions to enter through the airway epithelium and infect the distant target cells in the bone marrow. Under the natural acidic environment of the nasal mucosa, the virus binds the glycosphingolipid globoside to enter the respiratory epithelium by transcytosis. Under the neutral pH conditions of the bone marrow, the virus changes the receptor and binds an erythroid-specific surface molecule to internalize in EPCs. Inside the acidic endosomes, the incoming virus swaps receptors again and binds globoside leading to membrane deformation, vesicle budding, and retrograde transport of the virus to the Golgi. The environment of the Golgi enables the activity of a capsid-associated phospholipase enzyme that mediates virus escape into the cytosol. The pH-dependent receptor switching represents an evolutionary adaptation facilitating B19V entry through host cellular barriers, efficient cell targeting, and intracellular trafficking.

Keywords: Parvovirus B19 / receptors / virus entry / intracellular trafficking

EMERGING VIRUS PSEUDOTYPES – A ONE VIROLOGY APPROACH

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Although pseudoviruses were first described over 50 years ago, it is only in the last decade and particularly since the start of the COVID-19 pandemic, that pseudotyped viruses (PVs) have become widely accepted as valuable tools to study emerging and zoonotic RNA viruses. This presentation will outline different approaches taken in our laboratory to develop PVs against viruses in the *Orthomyxoviridae*, *Bunyaviridae* and *Flaviviridae* families. Generation of PVs using HIV and vesicular stomatitis virus systems will be compared and steps taken to improve yields of difficult-to-pseudotype viruses described. The use of PVs to determine the entry requirements of different viruses will be illustrated using PVs expressing the haemagglutinin esterase fusion (HEF) proteins of influenza C and D proteins. Data will also be presented on the application of influenza A, D and Rift Valley fever virus pseudotypes to measuring neutralizing antibody responses. Finally, the challenges that remain to be addressed in some aspects of developing and applying PVs will be discussed.

Keywords: pseudotyped virus; neutralization test

MECHANISMS OF HERPESVIRAL CAPSID MOBILITY AND TRANSPORT THROUGH THE CHROMATIN LABYRINTH

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Herpesviruses are not only significant pathogens but are also promising candidates in oncolytic and gene therapy. As herpes simplex virus type 1 infection proceeds, the nuclear expansion of replication compartments leads to the compaction of chromatin toward the nuclear periphery. Our analyses using a combination of microscopy techniques e.g. fluorescence imaging, EM, and soft x-ray tomography (1) combined with machine learning demonstrated virus-induced changes in chromatin organization and local density. The movement of viral capsids within the nuclear chromatin prior to the export through the nuclear envelope is crucial for the viral life cycle. Live-cell imaging and single-particle tracking of capsid motion indicated that the marginalized chromatin was a restrictive barrier to the capsids. Later in the infection, the chromatin became more permissive and the probability of capsids entering the chromatin increased. Thus, although chromatin marginalization initially restricted capsid transport to the nuclear envelope, a structural reorganization of the chromatin counteracted that to promote capsid transport later. Analyses of capsid motion revealed that it was subdiffusive and that the diffusion coefficients were lower in the chromatin than in regions lacking chromatin. In addition, the diffusion coefficient in both regions increased during infection. Finally, analysis of chromatin motion by photoactivated fluorescent histones and telomeres in living infected cells revealed virus-induced alteration of local chromatin fluctuations and chromatin accessibility. Our studies suggest that viral capsids have evolved mechanisms of trafficking within the nuclear chromatin environment to reach the nuclear envelope prior to nuclear egress. Our studies extend the frontiers of virus-cell interactions by elucidating the principles of viral capsid motion in the nucleus.

Keywords: Herpes simplex virus type 1, nuclear capsid mobility, reorganization of chromatin, diffusion

DO PLANT VIRUSES HAVE A ROLE IN THE 'ONE HEALTH' APPROACH?

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Viruses, the most abundant life forms on earth, have coevolved with their hosts through millions of years. It is no wonder that the human genome contains nearly 0.1 million viral sequences. Some viral sequences are also known to have integrated in plant genomes. A recent finding has also shown the possibility of integration viral sequences in non-putative hosts. The transmission of viruses between animals and humans leading to zoonotic diseases is a major challenge to 'one health' approach that aims at optimizing human, animal and environmental health; the recent COVID-19 pandemic that resulted in over two million deaths and huge losses to world economy is a glaring example of the challenge. Fortunately, plant viruses (PVs) are not known to replicate in mammals, although some PVs also infect insects. The PVs adversely impact both human and animal health indirectly through reduction in the supply and availability of food and feed. The areas in the Global South are most vulnerable to PV epidemics in important staple-, oilseed-, protein-, vegetable-, fruit-, and fibre crops. The list of the destructive PVs is long but the most threatening are the tospo-, begomo- and poty-viruses. There is an urgent need to strengthen global plant disease surveillance and management systems to optimize crop productivity by minimizing the losses caused by various biotic stresses including viruses. On a positive side, the PVs are important tools to express desired sequences or proteins for use as therapeutics, vaccines, or targeted treatments. The phage therapy is also emerging as a promising approach in managing bacterial diseases. It is obvious that PVs and phages have an important role in the 'one health' programme. There is urgent need for greater investments to promote faster development and utilization of these valuable natural genetic resources for optimizing human, animal and environmental health.

Keywords: tospoviruses, begomoviruses, potyviruses, plant viruses

MOLECULAR MECHANISM OF DIFFERENTIAL NEUROAIDS BY HIV-1 B AND C CLADES

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Human immunodeficiency virus type 1 (HIV-1) is commonly associated with immune dysfunctions and the suppression of antigen-presenting cells. This results in immune alterations, which could lead to impaired neuronal functions, such as neuroAIDS. The neurotoxic factor kynurenine (KYN), the rate limiting enzyme indoleamine 2,3-dioxygenase (IDO), serotonin (5-HT), and serotonin transporter (5-HTT) may play a role in tryptophan deficiency and serotonergic dysfunction in neuroAIDS. HIV-1 transactivator regulatory protein (Tat) is known to play a major role in immune dysfunction. Previous studies suggest that HIV-1 B and C clades differentially manifest neuronal dysfunctions in the infected host. In this study we examined the effect of HIV-1 B and C clade–derived Tat on IDO and 5-HTT, to understand the molecular mechanism of differential neuroAIDS by HIV-1B and C clades. In the present study we examined the effect of HIV-1 B and C clade–derived Tat on IDO and 5-HTT gene and protein expressions by dendritic cells as studied by quantitative polymerase chain reaction (qPCR) and Western blot. In addition, the intracellular IDO expression, IDO enzyme activity, and the levels of 5-HT and KYN were also measured. Our results indicate that HIV-1 clade B Tat up-regulates IDO and down-regulates 5-HTT gene and protein expressions. Further, HIV-1 clade B Tat caused a reduction of 5-HT with simultaneous increase in KYN levels as compared to HIV-1 clade C Tat. These studies suggest that HIV-1 clade B and C Tat proteins may play a differential role in the neuropathogenesis of HIV-associated dementia (HAD) or HIV-associated neurocognitive disorder (HAND).

Keywords: Medical-neurovirology, HIV, neuroAIDS, HAND

Submitted Talks

Human Virology

HUMAN PARVOVIRUS TISSUE PERSISTENCE

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Human parvovirus B19 (B19V) and human bocavirus 1 (HboV1) are the first human-pathogenic parvoviruses. B19V shows extreme tropism to erythroid progenitor cells, resulting in erythema infectiosum, anemias, and fetal death. HboV1 replicates in human airway epithelial cells, causing mild to life-threatening respiratory tract infections, while HboV2-4 are enteric. Of the recently discovered human protoparvoviruses, bufavirus (BuV) has been associated with gastroenteritis and cutavirus (CuV) with cutaneous T-cell lymphoma, while tusavirus (TuV) is extremely rare. After acute infection, viral genomic DNA can persist life-long in various human tissues, but their host-cell tropisms and the impact of their tissue persistence are poorly studied. We have determined the host-cell tropism, virus activity, and impact of persisting human parvoviruses in non-permissive host cells. In tonsillar tissues, we have discovered the persistence site of HboV1 DNA to be lymphoid germinal centers (GCs), and the host cells to be B cells and monocytes. In tonsillar B-cell cultures, the cellular uptake of both HboV1 and B19V occurred through antibody-dependent enhancement (ADE). For HboV1 this resulted in viral mRNA transcription, but without detectable productive replication. In biopsy specimens of paired diseased/healthy intestinal mucosa of 130 individuals, only three individuals exhibited HboV (one each of HboV1, 2 and 3), and eight had CuV, whereas none harbored TuV or BuV DNA. Conversely, B19V DNA was detected in intestinal biopsy specimens of ~50% of cancerous or inflamed mucosa and in 27% of healthy individuals. By RNAscope-ISH and immunohistochemistry, we located the B19V DNA to vascular endothelial cells and mucosal B cells of lymphoid follicles. With RNA-seq, we further identified 272 B19V-modulated differentially expressed genes in normal ileum specimens, and B19V persistence was shown to activate cell viability and inhibit apoptosis. Lifelong B19V DNA persistence thus seems to modulate host gene expression, which may lead to clinical outcomes in predisposed individuals.

Keywords: Parvovirus, bocavirus, cutavirus, tissue persistence

BACULOVIRAL COVID-19 DNA VACCINE CROSS-PROTECTS AGAINST SARS-COV2 VARIANTS IN K18-ACE2 TRANSGENIC MICE

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After severe acute respiratory syndrome coronavirus-2 (SARS-CoV2) made the world tremble with a global pandemic, several vaccines were developed and fast-tracked to the market. However, owing to the intrinsic nature of the coronavirus, new variants emerged such as Delta and Omicron, which were refractory to the vaccines derived using the original Wuhan strain (prototype). We previously developed an HERV-enveloped recombinant baculoviral DNA vaccine against SARS-CoV2 (AcHERV-COVID19). A non-replicating recombinant baculovirus that delivers the SARS-CoV2 S prototype strain gene showed a protective effect against the homologous challenge in a K18-hACE2 Tg mice model; however, it offered only a 50% survival rate against the virulent SARS-CoV2 Delta variant. Therefore, we further developed the AcHERV-COVID19 Delta vaccine (AcHERV-COVID19D). Cross-protection experiments revealed that mice vaccinated with the AcHERV-COVID19D showed 100% survival upon challenge with Delta or Omicron variants and 71.4% survival against prototype SARS-CoV2. These results support the potential of the viral vector vaccine, AcHERV-COVID19D, in preventing the spread of coronavirus variants such as Omicron and SARS-CoV2 variants.

Keywords: Baculoviral DNA vaccine, SARS-CoV2, Concern of variants, Vaccine

ONE-YEAR FOLLOW-UP ON IMMUNOGENICITY OF TWO-DOSE BBIBP-CORV (SINOPHARM) COVID-19 VACCINE IN MOROCCO: RESULTS OF THE NEUTRALIZING ACTIVITY AGAINST SARS-COV-2 VARIANTS OF CONCERN

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We aimed to assess the immunogenicity against SARS-CoV-2 BBIB-CorV (Vero cells) Sinopharm vaccine after two dose vaccination during one-year follow-up. The second objective was to evaluate the capacity of sera from vaccine recipients to neutralize SARS-CoV-2 variants of concern. We did an observational study among healthy individuals (n=129) aged 18-23 years and negative for serum-specific neutralizing antibodies (Nabs) against B.1 Wuhan/D614G by the micro-neutralization (MN) assay. Individuals received two doses of BBIB-CorV Sinopharm vaccine. The study was conducted at Military Hospital of Rabat, from February 2021. Collected sera were used to measure Nabs at different times, ~ 28, ~180 and ~365 days from two-dose vaccination (dpv) against B.1, B.1.1.7 (Alpha), B.1.617.2 (Delta), and/or BA.5.2.2 (Omicron) VOCs. The overall seroconversion rates in vaccine recipients against B.1 were more than 72% and between 54.1% -67.1% against Alpha variant over the study period. However, lower immunogenicity percentage for delta and Omicron VOCs (45.5%- 48,5% and 26,1%-29,3% on 49 and 180 dpv were obtained. A total of 13, 22 and 12 elicited-sera conserved Nabs against all variants at 49, 180 and 365 days post vaccination respectively. Two-dose immunization by the inactivated SARS-CoV-2 vaccine Sinopharm achieved higher Nabs titres against SARS-CoV-2 B.1 and B.1.1.7 (Alpha) in vaccine recipients on day 49 and six months post vaccination. However, lower immunogenicity was observed against the newly emerging VOCs. These data indicated low neutralization potency of two-dose BBIBP-CorV (Sinopharm) COVID-19 vaccine against SARS-CoV-2 VOCs and pointed out recommendation for additional boost vaccinations.

Keywords: immunogenicity, two-dose bbibp-CoV (20lcyone20a) , sars-cov-2, variants of concern

SIGNIFICANT ASSOCIATION WITH HIGH PREVALENCE AND ACTIVITY OF CUTAVIRUS IN PARAPSORIASIS SKIN

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In 2016, metagenomic studies in diarrheic stool and skin of cutaneous T-cell lymphoma (CTCL) revealed a novel human protoparvovirus; cutavirus (CuV). Soon after, we and others detected CuV DNA in CTCL lesions while all skin-biopsy specimens of healthy subjects were CuV-DNA negative, revealing an association of CuV with CTCL. This has led to the hypothesis that chronic antigen stimulation by a pathogen could have a role in CTCL carcinogenesis. Hence, in the current study we aim to identify the prevalence, activity and cell tropism of CuV in skin of CTCL and parapsoriasis, a preform of CTCL. We studied 27 fresh-frozen skin-biopsy specimens obtained from 26 patients, 19 with CTCL and 7 with parapsoriasis: (Group A, Age: 32 – 83 years) as well as 24 FFPE skin-biopsy and 52 skin-swab samples from 13 parapsoriasis patients (Group B, Age: 37 – 86 years). CuV was detected and quantified with qPCR and RT-qPCR, and localized and host-cell typed with RNAscope ISH and IHC. In group A, CuV DNA and spliced mRNA were detected in fresh-frozen skin biopsy specimens from 6/26 (23%) individuals: 4 had parapsoriasis (4/7, 57%) and 2 CTCL (2/19, 10.5%). In group B, CuV DNA was detected in swabs from 6/13 (46%) and in FFPE samples from 8/12 (66%) parapsoriasis individuals, compared to only 1/51 (1.96%) swabs of healthy controls ($p<0.001$). Overall, among the 11 CuV-positive parapsoriasis individuals from both groups, 4 progressed to CTCL. CuV was observed primarily in T cells, but also in some keratinocytes and macrophages. We found an association of CuV with parapsoriasis, a preform of CTCL, with a 57-66% CuV-DNA prevalence in skin. CuV also actively transcribed mRNA. Hence, it is imperative to clarify if skin-persistent CuV leads to T-cell stimulation and the development of CTCL and if CuV presence could serve as a biomarker for developing CTCL.

Keywords: parvovirus; cutaneous T-cell lymphoma; parapsoriasis; cutavirus

HANTAAN VIRUS GLYCOPROTEIN Gc INDUCES NEDD4-DEPENDENT PTEN UBIQUITINATION AND DEGRADATION TO ESCAPE THE RESTRICTION OF AUTOPHAGOSOMES AND FACILITATE VIRAL PROPAGATION

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Hantaan virus (HTNV) infection causes severe hemorrhagic fever with renal syndrome (HFRS) in humans and the infectious process can be regulated by autophagy. The phosphatase and tensin homolog (PTEN) protein has antiviral effects and plays a critical role in the autophagy pathway. However, the relationship between PTEN and HTNV infection is not clear and whether PTEN regulated autophagy involves in HTNV replication is unknown. The effect of HTNV infection on the expression of PTEN was determined by quantitative real-time polymerase chain reaction, immunohistochemistry and western blot in HK-2 cell lines and mice kidneys. The interaction of HTNV and PTEN was determined by pull-down and co-immunoprecipitation assay. The ubiquitination by NEDD4 on PTEN was validated by *in vitro* ubiquitination assay with HTNV Gc induction. *In vitro* and *in vivo* assays were performed to investigate the inhibition of PTEN on the production of progeny HTNV through promoting autophagy by western blot, confocal analysis, transmission electron microscopy, immunoelectron microscopy, IFA. We identified that HTNV infection inhibits PTEN expression *in vitro* and *in vivo*. HTNV glycoprotein Gc interacts with the C2 domain of PTEN, which promotes PTEN ubiquitination and degradation through 26S-proteasome pathway *via* the E3 ubiquitin ligase NEDD4. In addition, knockdown of PTEN prevents autophagy and increases HTNV production. While, overexpression of PTEN induces autophagosome formation which can wrap HTNV particles, thus leading to restrain the release of progeny viruses. This study reveals the role of PTEN in HTNV infection by autophagy, highlighting the potential importance of PTEN and autophagy in the treatment of HFRS diseases.

Keywords: Hantaan virus; PTEN; Ubiquitination; Autophagy

NATURAL KILLER CELLS IN SARS-COV-2-VACCINATED SUBJECTS WITH INCREASED EFFECTOR CYTOTOXIC CD56DIM CELLS AND MEMORY-LIKE CD57+NKG2C+CD56DIM CELLS.

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As other European countries, Italy launched its SARS-CoV-2 vaccination campaign on 27 December 2020. The analysis of SARS-CoV-2 vaccination impact on host immune system of healthy subjects might elucidate the potential impact on COVID-19 outcomes. We evaluated whether mRNA-based anti-SARS-CoV-2 vaccination (Comirnaty) elicited a robust protective innate immune response. PBMC were obtained form whole blood obtained by donors who received three doses of mRNA-based anti-SARS-CoV-2 vaccination (Comirnaty). NK (Natural Killer) cells immunophenotype and cytotoxicity have been tested after stimulation with SARS-CoV-2 spike antigen (Wuhan, Alpha B.1.1.7, Delta B.1.617.2, Omicron B1.1.529 variants) by FACS assay and related with the anti-SARS-CoV-2 antibody production, in order to evaluate the relevance of innate immune response to SARS-CoV-2 vaccines. We reported the presence of specific effector cytotoxic CD56dim, characterized by high levels of CD107a and granzyme production, and memory-like CD57+NKG2C+CD56dim phenotype of NK cells exposed to SARS-CoV-2 spike antigen (Wuhan, Alpha B.1.1.7, Delta B.1.617.2, Omicron B1.1.529 variants), in association with specific anti-SARS-CoV-2 antibody production, especially after the booster dose. We found that the booster dose caused early NK CD56dim subset activation and memory-like phenotype, confirming the relevance of innate immune response in the efficacy of SARS-CoV-2 vaccination.

Keywords: SARS-CoV-2; vaccination; NK cells

GESTATIONAL COVID19: MORPHOLOGICAL ALTERATIONS AND DECREASED HLA-G EXPRESSION CAUSED BY SARS-COV-2 INFECTION

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The evaluation of the effect of SARS-CoV-2 infection during pregnancy has raised interest. Even if virus vertical transmission is still controversial, several researchers have focused on the possible distinctive markers associated with different susceptibility to SARS-CoV-2 infection during pregnancy. Morphological alterations were assessed in the placental / chorionic villi, chorionic plate, basal plate, and umbilical cord tissues obtained from 7 subjects with symptomatic respiratory SARS-CoV-2 infection and compared with those in 7 non-COVID control subjects. The expression of SARS-CoV-2 Nucleoprotein (NP) and Human Leukocyte Antigen-G (HLA-G) was estimated by the use of immunohistochemistry. The 57%, 42,8%, and 28,6% of placental / chorionic villi, chorionic plate, and basal plate, respectively, were found positive for NP antigen ($p<0.01$), while none of the umbilical cords stained for NP. Placental / chorionic villi samples showed the highest positivity for NP. The presence of NP positivity correlated with high levels of the fibrinoid component in placental / chorionic villi samples and leukocyte infiltration in basal plate. All placental / chorionic villi samples were found positive for HLA-G, independently from NP staining. All the NP positive chorionic plate and half of the NP positive basal plate samples expressed HLA-G. On the contrary, the placental / chorionic villi, chorionic plate, and basal plate of all non-COVID subjects were positive for HLA-G, with a higher H-score in comparison to pathological samples ($p<0.05$). The presence of SARS-CoV-2 NP expression in gestational tissues correlates with morphological alterations and a decreased HLA-G expression compared to the control group. These data suggest a possible implication of SARS-CoV-2 infection in morphological and protein expression modification during pregnancy, which might impact ~~on~~ infection susceptibility, pregnancy complications, and vertical transmission.

Keywords: COVID-19; pregnancy; HLA-G; immunohistochemistry

CORONAVIRUS ENVELOPE PROTEINS INTERACT WITH HOST PROTEINS TO REGULATE PATHOGENICITY

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SARS-CoV, MERS-CoV, and SARS-CoV-2 are reported to be more virulent than HcoV-229E, HcoV-NL63, HcoV-OC43, and HcoV-HKU1. The envelope I protein is a structural protein found in all CoVs, and has been linked to the pathogenesis of SARS-CoV and SARS-CoV-2. The PDZ-binding motif (PBM) of the E protein facilitates its binding to host proteins, allowing the virus to exploit certain host cell processes. Several host proteins have been identified as targets of the E protein, but this has only been demonstrated for the three virulent hCoVs. To understand the disparity in virulence between the hCoVs, we generated homology-based full-length 3D models of the E proteins for SARS-CoV, SARS-CoV-2, MERS-CoV, HcoV-229E, and HcoV-NL63. These models were then docked to host proteins which have previously been shown to interact with E. The PBMs of the SARS-CoV, SARS-CoV-2, and MERS-CoV E protein models each adopted a flexible coil secondary structure, whereas the HcoV-229E and HcoV-NL63 models showed a less flexible α-helix conformation. Interestingly, all the docked E peptides occupied the same binding site on the host proteins PALS1, MLLT4, and LNX2, while HcoV-229E and HcoV-NL63 showed fewer interactions with host proteins compared to SARS-CoV, SARS-CoV-2, and MERS-CoV. The E peptides of the less virulent hCoVs notably lacked certain hydrophobic contacts and the requisite ionic interactions compared to the E peptides from the more virulent hCoVs. We propose that the greater flexibility of the PBMs of SARS-CoV, SARS-CoV-2 and MERS-CoV allows for increased stable binding to host proteins, resulting in increased virulence.

Keywords: coronavirus; envelope protein; pathogenesis; PDZ-binding motif

MUTATIONS IN STRUCTURAL PROTEINS DURING CELL CULTURE PROPAGATION OF SARS-COV-2 VARIANTS

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JLG/ To investigate the efficacy of available vaccines against COVID-19, it is necessary to propagate the newly emerging SARS-CoV-2 variants. This study 26lcyone26a2626es the amino acids of the viral structural Spike proteins of the SARS-CoV-2 variants (AY.33 and BA.5.2.20) and specifies the frequency of these mutations in the viral stocks produced in culture. Viral strains AY.33 and BA.5.2.20 were isolated on cell lines Vero-E6 and 293T-ACE2- TMPRSS2, respectively. Repeated passages (P) on Vero cells were used to propagate the viruses up to P3 with a multiplicity rate of 0.001. Whole genome sequencing of the viral stocks was performed using Ion Torrent technology. Analysis of reads was performed by LoFreq software (version 2.1.8) in comparison to SARS-CoV-2 wild-type (Wuhan-Hu-1) strain. The spike mutations of the parental isolate were retained during cell culture passages with major frequencies of ≥97%. However, adaptation changes appeared scattered throughout the structural proteins during passages. For instance, V642G was stable in delta subpopulations with a frequency of 11-13%, while the frequency of other mutations increased such as for T572I (from 3% to 38%), N343H (from 14% to 24%), and V213G (from 26% to 53%) in BA.5.2.20 subpopulations. The parental strains predominated in the cell cultures during the viral passages. However, other mutations acquired during viral passages appeared that were similar to those detected in naturally circulating isolates. The propagation of these variants does not affect furin cleavage sites, making them relevant for SARS-CoV-2 immunogenicity and antiviral studies.

Keywords: Mutations, spike protein, cell propagation, SARS-CoV-2 variants.

JC POLYOMAVIRUS INFECTION AND REPLICATION IN HUMAN iPS CELL-DERIVED GLIAL CELLS INDEPENDENT OF SV40 T ANTIGEN

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Non-pathogenic (archetype) JC polyomavirus (JCPyV) persists in kidney tissue and the pathogenic virus is identified in patients with progressive multifocal leukoencephalopathy (PML) of the brain. Both archetype and PML-type of JCPyV replicate efficiently in COS-7, but data analysis is complex because COS-7 carries the gene of SV40 T antigen. In this study, we established a culture system in which human iPS cell-derived glial cells were infected with JCPyV. Human iPS cell line 253G1 was used to differentiate into astrocytes and oligodendrocytes. M1-IMRb strain was used as the JCPyV. The amount of viral DNA in the culture supernatant was quantified by TaqMan real-time PCR. Viral replication and viral protein1 (VP1) production were assessed by HA assay and immunostaining in astrocytes and oligodendrocytes. Human iPS cells were differentiated through the neural stem cell stage into astrocytes or oligodendrocytes in the medium containing adequate growth factors, as confirmed by immunostaining for GFAP and O4, respectively. These human iPS cell-derived astrocytes and oligodendrocytes were infected with JCPyV and cultured for three weeks. Viral DNA, VP1 antigen, and HA were detected in both JCPyV-infected astrocytes and oligodendrocytes. Scanning electron microscopy also confirmed the extracellular appearance of the virus-like particles. JCPyV can infect and replicate in COS-7 cells that produce SV40 T antigen. SV40 T antigen efficiently promotes JCPyV replication. In this study, we were able to infect and replicate JCPyV in astrocytes and oligodendrocytes differentiated from human iPS cells. Lacking the SV40 T antigen gene, which may be a useful system for analyzing JCPyV replication in the pathogenesis of PML.

Keywords: JC polyomavirus, iPS cell, glial cell, PML

ISOLATION OF SARS-COV-2 IN CELL CULTURES HAS HELPED IN BETTER MANAGEMENT OF COVID-19 PATIENTS DURING HOSPITALIZATION

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Since the onset of COVID-19 in Wuhan in December 2019 to March 2020, negative RT-PCR results in the respiratory tract samples were required for the patient hospital discharge. In the present study, we sought to establish the contribution of viral infectivity endpoint in the management of patients' COVID-19 hospitalization. We recruited 50 male patients with a median age of 46 years old at the medical department of Virology Center for Infectious and Tropical Diseases between the May and June 2020. The median hospitalization of participants was 9 to 15 days. Nasopharyngeal (NPS) and oral (OS) swab specimens were simultaneously collected every three days. SARS-CoV-2 testing was performed by the GeneFinder IVD kit (South Korea) and Ct values were registered at each time. Immediately after sample collection, NPS and OS were subjected to virus isolation using the Vero-E6 cell line. The t student test was used for comparative analyses. Higher positive load was observed in nasopharyngeal swabs than in oropharyngeal swabs, especially in symptomatic patients ($p<0.05$). The Ct value of the N gene in the initial sample decreased during the first cell passage with 0.6 log (D ct of 2.5) in samples with Ct values ≤ 32 ($p= 0.006$) indication the presence of infectious viral particles. 17% and 3% of NPS and OS samples were successfully isolated on cell culture respectively defining therefore, people with infectious swabs. Only 5% of NSP and OS were infective at the same time ($p < 0.05$). A positive correlation between lower Ct values in the initial samples and infectivity of SARS- was found. Infectivity assay was effective in the management of hospitalized patients with COVID-19 and was established as the best strategy to define the duration of the quarantine defining potentially people still capable of transmitting the virus to others.

Keywords: COVID-19, Cell Culture, Infectivity, Nasopharyngeal swab.

ENTEROVIRUS D68 VP3 D18E INFLUENCE ON CAPSID ASSEMBLY

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Structural protein VP4 is a small protein residing on the internal enterovirus D68 (EV-D68) capsid surface. VP4 is critical for capsid stability, viral breathing, host cell entry, and uncoating due to its dynamic transient N-terminus. As part of ongoing research on EV-D68 interprotomer interactions and capsid dynamics, a VP4 lysine 33 (K33) and VP3 aspartic acid 18 (D18) interprotomer interaction was discovered. Surprisingly, conservative mutation of VP3 D18 to glutamic acid (VP3 D18E) completely halts EV-D68 infection. Due to the non-infectious phenotype of this mutant, we investigated the effect of VP3 D18E on EV-D68 assembly and maturation. Following rhabdomyosarcoma (RD) transfection, cell lysates as well as supernatants were analyzed by plaque assay for VP3 D18E infectivity and western blot for the presence of precursor protein VP0 or mature protein VP2. To further test for the presence of intact viral particles, electron microscopy was used along with small scale purification and fractionation of a discontinuous 10-50% sucrose gradient. Fractions were concentrated with a 100kDa cutoff protein concentrator and analyzed by SDS-PAGE and western blot for presence or absence of VP2. Electron microscope imaging suggests that VP3 D18E can form viral particles. In fact, western blot analysis shows that VP3 D18E does not produce VP2, indicating any assembled particles do not undergo maturation. This was further supported by sucrose fraction analysis where SDS-PAGE gels and western blots demonstrated that VP3 D18E VP0 was found in 20-30% sucrose fractions while native WT EV-D68 VP2 was present in 40-50% sucrose fractions.
EV-D68 VP3 D18E is not infectious and does not mature as evidenced by VP0 not being cleaved. Furthermore, fractionation separated VP3 D18E at a lower density compared to WT EV-D68. Taken together, our data suggests that VP3 D18E can initiate the capsid assembly process potentially forming empty particles.

Keywords: Enterovirus; assembly; dynamics, maturation

PREVALENCE SCREENING OF RESPIRATORY VIRUSES AMONG SYMPTOMATIC ILLNESS PEOPLE IN THE MEXICAN POPULATION FROM JULY 2021 TO JANUARY 2023

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Respiratory viral infections represent a public health problem worldwide. Some of the most common viruses that may cause respiratory infections are Influenza Virus, Respiratory Syncytial Virus, Rhinovirus, Parainfluenza Virus (PIV), and recently, SARS-CoV-2, among others. Herein, we determined the prevalence of different respiratory viruses among symptomatic illness patients from July 2021-January 2023 in the Mexican population. Firstly, a naso-oropharyngeal swab was retrieved from people with viral-like respiratory symptoms visiting the Laboratorio de Diagnostico de Enfermedades Emergentes y Reemergentes for molecular diagnosis. The RT-qPCR kit BlueFinder22 was employed to screen the respiratory pathogen aetiological agent. Moreover, to molecular characterize the circulating respiratory virus in Mexico, we recovered the nucleotide sequence from a public database, and a phylogenetic analysis was carried out. Viral respiratory infections were diagnosed in 4017 individuals, 3865 (96 %) were identified as infected was SARS-CoV-2, 141 with Influenza virus (3.4%; subtyping of some of those showed that 77 were H3N2 and 1 Influenza B-Victoria). Coinfection were observed mainly with H3N2/SARS-CoV-2 and H3N2/Adenovirus. Other viruses detected were Rhinovirus, Bocavirus, HKU, and BPP. Seasonally, no-SARS-CoV-2 respiratory viruses reappeared at the end of 2021 with a low prevalence, restarting their circulation in 2022 fall. Furthermore, phylogenetic analysis showed that for SARS-CoV-2, Omicron sub-variants were prevalent in 2022; the for the influenza variant, H3N2 predominated circulating in the years pre-pandemic, we lack recent sequencing information. Type 3 and 1 of Parainfluenza virus were barely reported in the country. Genotype ON1 of the Syncytial virus is scarcely found in public databases. This study provides additional information related to the reappearance of respiratory viruses after the SARS-CoV-2 pandemic, exhibiting areas that require special attention for epidemiological description to determine the genotypes and variants circulating in the country that might impact public health.

Keywords: respiratory virus, epidemiology, phylogenetic analysis, Mexican population

NETWORK ANALYSIS OF POTENT SIGNALING OF COVID-19, MPOX, PROSTATE CANCER, AND MENTAL HEALTH

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SARS-CoV-2 entry requires host cell proteins ACE2/TMPRSS2, which are related to androgen regulation. Recent clinical investigations suggest prostate cancer (Pca) patients receiving anti-androgen therapy are less vulnerable to COVID-19, with possible mechanisms related to the androgen regulated TMPRSS2 and ACE2/TMPRSS2 co-expression in lung and prostate. Recently, we showed androgen receptor as a potent immune respondent in SARS-CoV-2 spike binding through spike LXXL motif, which is a cofactor binding motif of androgen receptor for interactions. Single cell analysis suggests the potent prostate cancer related signaling may involve the gender effect of the infection, which mpox also shows the gender effect. In addition, mental health is highly concerned due to the pandemic. First, using network analysis and molecular docking via database search, we explored potent intersections' gene lists among COVID-19, prostate cancer and mental health. Networks for enriched pathways were analyzed by GO, KEGG. Molecular docking was analyzed for the prostate cancer therapy drug's effect on SARS-CoV-2 target by simulation. Mpox protein sequences were analyzed by alignment with LXXLL, LXXL motifs. We found the intersections of 321 targets with cytokine storm, EGFR, MAPK kinase signaling, which may be the common signaling of infection of COVID-19, and prostate cancer with some target which are poor prognostic. C-reactive protein (CRP) was found in the intersection targets of depression. Moreover, the molecular docking suggests that potent anti-androgen therapy drugs might bind strongly to SARS-CoV-2 targets. Mpox showed the similar mechanisms of possible androgen signaling regulation as mpox virus proteins exhibited motifs of LXXLL, or LXXL. Our data suggest the potent anti-androgen therapy in anti-SARS-CoV-2, or mpox and CRP signaling, might be a target for prevention of depression due to the pandemic.

Keywords: Mpox, COVID-19, Prostate cancer, One health

THE PRESENCE OF COMMON HERPESVIRUSES (EBV, HHV-6, AND HHV-7) IN THE THYROID GLAND – IMPLICATIONS FOR DISEASE DEVELOPMENT

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Several human herpesviruses have been implicated in autoimmunity development or exacerbation in such autoimmune diseases as multiple sclerosis, scleroderma, rheumatoid arthritis, and others. Strong evidence has been presented for the association between HHV-6 and autoimmune thyroiditis (AIT). Incidental results have also linked other herpesviruses, such as HHV-7 and EBV. Here we aim to investigate the prevalence of common herpesviruses in AIT tissue samples and to elucidate the potential of their involvement in autoimmune thyroiditis development. Post-operative thyroid tissue and peripheral blood samples were collected from 81 AIT patients. DNA was isolated from thyroid tissues and used for PCR with specific primers to detect HHV-6, HHV-7, and EBV. HHV-6 load was determined by qPCR with a commercially available kit by Saccace. RNA isolated from thyroid tissues and peripheral blood mononuclear cells (PBMC) was used for HHV-6 active infection marker detection by synthesizing cDNA and using it as a template for nPCR. From the three human herpesviruses investigated in this study – HHV-6 was found most frequently in patient samples and specifically most frequently in thyroid tissues. In contrast, EBV and HHV-7 DNAs were found more frequently in peripheral blood samples as opposed to thyroid tissues. None of the patients' PBMC samples were positive for HHV-6 active infection markers, yet over 50 % of thyroid tissues were. In addition, HHV-6 viral load was found to be significantly higher in thyroid gland tissue in comparison to peripheral blood samples.Higher frequency of HHV-6 DNA, mRNA, and higher viral load in AIT patients' thyroid glands points to a strong association of HHV-6 with AIT development. Even though relatively rarely detected in the thyroid gland, other herpesvirus infections should be investigated further to evaluate their impact on autoimmunity development on their own or together with HHV-6.

Keywords: EBV, HHV-6, HHV-7, autoimmune thyroiditis.

HUMAN HERPESVIRUS 6B INFECTION AND THYROID AUTOIMMUNITY – MOLECULAR AND IMMUNOLOGICAL INVESTIGATION OF THE ASSOCIATION.

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Human herpesvirus 6 (HHV-6) is an ever-present human pathogen that can persist in its host indefinitely. Even though the primary infection is most often mild, HHV-6 has been linked with several disease development later in life, especially autoimmune. HHV-6 is considered an environmental autoimmunity-triggering factor, and associations between the virus and several autoimmune conditions have been made. We aimed to demonstrate the link between HHV-6 and thyroid autoimmunity via molecular and immunological investigations of samples obtained from patients with autoimmune thyroiditis (AIT). Postoperative thyroid tissues and whole blood from 119 AIT patients were collected. Nucleic acids were isolated from thyroid tissue samples and whole blood and used for HHV-6 DNA, active infection marker detection, and load determination. Blood plasma from 81 patients was used for HHV-6 protein-specific antibody detection. FFPE tissues from 4 patients were used for immunofluorescent microscopy. All patients harbored HHV-6 in their thyroids with a median viral load of 558,3 HHV-6 copies/10^6 cells, in one patient reaching 1883419,8 HHV-6 copies/10^6 cells. Twenty patients had detectable HHV-6 in the blood, yet with extremely low viral loads – 4,1 HHV-6 copies/10^6 cells. In 55 thyroid tissues, markers of active infection were found. We evaluated the potential role of two poorly studied HHV-6 proteins (U12 and U51) in thyroid autoimmunity – 36 tissues expressed the proteins, and in tissues expressing U12/U51, viral loads were significantly higher (1399 vs 238 HHV-6 copies/10^6 cells). U12 and U51 antibodies could be detected in patient plasma and microscopy revealed the colocalization of HHV-6 glycoproteins and U12/U51. The high prevalence of HHV-6 in thyroid glands points to its role in AIT development or disease exacerbation. HHV-6 U12/U51 may help HHV-6 evade immune responses and persist in the thyroid.

Keywords: HHV-6; autoimmunity; autoimmune thyroiditis

INVESTIGATION OF THE INVOLVEMENT OF HHV-6 ENCODED VIRAL CHEMOKINE RECEPTORS IN AUTOIMMUNE THYROIDITIS DEVELOPMENT

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Human herpesvirus-6 (HHV-6) contains two genes (U12 and U51) that encode putative homologues of human G-protein-coupled receptors like CCR1, CCR3, and CCR5. It has been shown that these viral proteins can be expressed on the surface of epithelial and some peripheral blood mononuclear cells, suggesting that they could potentially induce autoimmunity. The aim of this study was to investigate the possibility of HHV-6 encoded viral chemokine receptors (U12 and U51) involvement in autoimmune thyroiditis (AIT) development by detecting viral peptide specific antibodies in AIT patient samples. Seventy-nine AIT patients whose thyroid tissues were shown to be positive for HHV-6 and 32 blood donors were enrolled in this study. Twenty-eight synthetic peptides derived from HHV-6 U12 and U51 proteins' amino acid sequences, as well as recombinant human CCR1, CCR3, and CCR5 proteins were used in suspension multiplex immunological assay to detect specific IgG and IgM antibodies. HHV-6 peptide specific IgG and IgM antibodies were found in patients' samples. AIT patient group's samples were more frequently positive for HHV-6 peptide IgGs in comparison to control group's. Significantly (using Chi square test) more frequent IgG antibody presence was found against 5 HHV-6 synthetic peptides: three corresponding to HHV-6 U12 and two corresponding to HHV-6 U51. On other hand, Mann-Whitney test showed significantly ($P < 0.05$) higher levels of IgMs against 3 out of 10 synthetic peptides in control group's samples. AIT patients' and blood donors' plasma pools did not show any significant signal changes after preabsorbtion with either of the human recombinant proteins (CCR1, CCR3, and CCR5), showing absence of cross-reactivity between the linear epitopes analyzed in this study with human CCRs. Even though peptide antibody cross-reactivity with human CCRs was not demonstrated, our results show a new immunogenic HHV-6 antigen-a possible new player in the HHV-6 induced autoimmunity exacerbation.

Keywords: G protein-coupled receptors; HHV-6; antibodies; autoimmune thyroiditis.

OCCURRENCE OF HUMAN HERPESVIRUS-6A/B AND HUMAN HERPESVIRUS -7 IN MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME

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Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a multifactorial disorder with undefined cause and virus infection is believed to be one of ME/CFS potential triggers. The aim of the study was to determine occurrence of human herpesvirus (HHV)-6A/B and HHV-7 in patients with ME/CFS. 200 patients with clinically diagnosed ME/CFS and 150 apparently healthy individuals (controls) were included in the study. Single-round polymerase chain reaction (PCR), nested PCR, and quantitative real-time PCR were used to determine extracted DNA quality, detect the presence and load of HHV-6A/B and HHV-7. HHV-6A/B genomic sequences in DNA isolated from peripheral blood were detected in 53% of patients and 29% controls (persistent infection), in DNA isolated from cell free plasma (active infection) – in 11% of ME/CFS patients and none of controls ($p < 0.0001$), in 42% of patients and 29% of controls ($p = 0.0133$) infection was in latent phase. In patients with latent HHV-6A/B infection median (IQR) HHV-6 load was 279 (1022–54.5) copies/10^6 cells, with active infection – 1927 (6732–348.5) copies/10^6 cells ($p = 0.0019$). Six patients' HHV-6A/B load was 1209033 (1464421–808183) copies/10^6 cells. Markers of persistent HHV-7 infection were revealed in 92% of ME/CFS patients and 75% of controls ($p = 0.0766$), active HHV-7 infection in – 34% of patients and 8% of controls ($p < 0.0001$), though in 58% of ME/CFS patients and 67% controls ($p = 0.0766$) infection was in latent phase. In ME/CFS patients with latent HHV-7 infection load was 196.7 (533–132) copies/10^6 cells and with active infection – 238.6 (410.6–80.2) copies/10^6 cells ($p = 0.3502$). In one patient with ME/CFS HHV-7 load was 1140127.6 copies/10^6 cells. Persistent HHV-6A/B and HHV-7 infection in an active phase is presented significantly more frequently and with a higher viral load among patients with ME/CFS than controls.

Keywords: Myalgic encephalomyelitis, human herpesvirus-6, human herpesvirus-7, PCR

HHV-6 AND AUTOANTIBODIES TO MUSCARINIC ACETYLCHOLINE RECEPTORS AS POTENTIAL BIOMARKERS IN THE DIAGNOSTIC ALGORITHM OF MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME

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Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is a complex disease that is mainly diagnosed based on its clinical symptoms. Biomarkers that could facilitate the diagnosis of ME/CFS are not yet available; therefore, reliable and clinically useful disease indicators are of high importance. The aim of this work was to analyze the association between ME/CFS clinical course severity, presence of HHV-6A/B infection markers, and plasma levels of autoantibodies against muscarinic acetylcholine receptors. A total of 134 patients with ME/CFS and 33 healthy controls were analyzed for the presence of HHV-6A/B using PCRs, and antibodies against muscarinic acetylcholine receptors (M3 AchR and M4 AchR) using ELISAs. HHV-6A/B U3 genomic sequence in whole-blood DNA was detected in 19/31 patients with severe, in 18/73 with moderate, and in 7/30 with mild ME/CFS clinical course. Severity-related differences were found among those with the HHV-6 load of more than 1,000 copies/106 PBMCs. A significant difference of antibodies against M4 AchR median concentration was found between ME/CFS patients (8.15 ng/ml) and healthy controls (6.45 ng/ml) ($p = 0.0250$). The levels of anti-M4 plotted against disease severity did not show any difference; however, increased viral load correlated with the increase in anti-M4 level. ME/CFS patients with high HHV-6 load had a more severe course of the disease, thus confirming that the severity of the disease depends on the viral load – the course of the disease is more severe with a higher viral load. An increase in anti-M4 AchR levels was detected in all ME/CFS patient groups in comparison to the control group not depending on ME/CFS clinical course severity. However, the increase in HHV-6 load correlated with the increase in anti-M4 level. Elevated levels of antibodies against M4 receptors in ME/CFS patients support their usage as clinical biomarkers in the diagnostic algorithm of ME/CFS.

Keywords: ME/CFS; HHV-6; M3 AchR; M4 AchR

SARS-COV-2 INFECTION TRIGGERS MORE POTENT ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC) RESPONSES THAN COVID-19 VACCINES

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Protective and lasting immunity to viral infections or vaccines are usually achieved through the combined actions of both cellular and humoral immune responses. Although there exists clear evidence supporting the importance of neutralizing antibodies (Nabs) and T-cell mediated immunity against SARS-CoV-2, these responses appear to be less effective against emerging variants. Also, little is known about the extent to which different COVID-19 vaccines can induce NK-cell dependent ADCC activity compared to natural infection. We analyzed the ADCC activity targeting SARS-CoV-2 spike (S) and nucleocapsid (N) antigens in 255 samples collected from infected patients (*n*=91), and people vaccinated with mRNA-based vaccine (BNT162b2 or mRNA-1273, *n*=77), vector-based vaccine (*n*=41), and inactivated virus vaccine (*n*=46). Correlations between ADCC, binding and neutralizing antibody titers were reported. ADCC was elicited within the first week post-infection and full vaccination with the four COVID-19 vaccines and it remained detectable for up to 6 months. Patients with symptomatic disease had significantly higher ADCC levels against the S protein than asymptomatic and vaccinated individuals. Also, no difference in ADCC levels against the nucleocapsid (N) proteins was shown between symptomatic and asymptomatic patients, but the levels were still higher than ADCC levels induced by COVID-19 vaccines. Notably, no significant difference in the ADCC activity was observed between the four vaccines, despite that Nabs and binding antibody titers were significantly higher in mRNA-vaccinated individuals. The findings of our study show that vaccination, regardless of the type, is inferior in inducing ADCC activity compared to SARS-CoV-2 infection. . This suggests that ADCC could be used to estimate the extra-neutralization level against COVID-19 and provide evidence that vaccination should not only focus on Nabs but also on other Fc effector functions.

Keywords: DENV, vaccine, recombinant vaccinia virus, Dis

DEVELOPMENT OF SAFE RECOMBINANT DENGUE VIRUS VACCINE TARGETING NON-STRUCTURAL PROTEINS USING ATTENUATED VACCINIA VIRUS DIS STRAIN

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Dengue virus (DENV) is resident mainly in the tropics and infects about 400 million people annually, of which about 100 million develop dengue fever. Moreover, currently a safe and effective vaccine that does not induce antibody dependent enhancement (ADE) is lacking, of which development of safe and effective DENV vaccine is urgently needed for dengue prevention and control. We have chosen vaccinia virus Dis strain as a vector, as it was highly attenuated and could replicate only in primary chicken fibroblast. Also, vaccinia vaccine can give long lasting immunity, which is expected to give life-long immunity by single shot. Here we constructed recombinant DENV vaccine which have inserted nonstructural protein regions of DENV in order to avoid ADE and induce cellular immunity(rDIs-DENV2C-NS25). We confirmed that rDIs-NS25 can induce antibody production and cellular immunity through mice experiments. We are also evaluating protection effect of rDIs-N25 against DENV-1~4 serotypes in AG129(Interferon-alpha and gamma receptor double knockout mice)-DENV infection system. We also confirmed that rDIs-DENV2C-NS25 is safer than inactivated (IA)-DENV vaccine or the tetravalent dengue yellow fever chimeric vaccine (CYD-TDV). Comprehensive analysis of cytokines suggested that IA-DENV more enhanced humoral immunity than cellular immunity. Since rDIs-DENV2C-NS25 of the present invention does not contain the structural protein region of DENV involved in ADE and the vaccinia virus vector Dis strain has no replication activity in humans and is highly attenuated. Thus, it is a safe and effective DENV vaccine which are effective for all serotypes through cell-mediated immunity.

Keywords: DENV**,** vaccine**,** recombinant vaccinia virus, Dis

EMERGENCE OF DENGUE VIRUS IN THE SAVANNA REGION, KANO NORTHWESTERN NIGERIA

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Dengue is an emerging mosquito-borne viral disease with high propensity for pandemic spread. This was a multicentre hospital-based prospective study conducted in Kano. Kano State is a crowded city and is the second largest industrial and commercial center in Nigeria. It is located at latitude $12^0$3' north and longitude $8^0$31'east, northwestern Nigeria. Sera from 200 febrile patients were screened for Dengue by NS1 ELISA and then subjected to Nested RT PCR using serotype-specific primers for DENV. Also haematological parameters as well as malaria parasitaemia were investigated. The mean age of the participants was 29.24 ± 10.41 and adults (17-41 years) constitutes the majority (87.5%: 175/200). Eighty-one-point five percent (81.5%) presented fever as a major complaint, headache (67.5%: 135/200), Joint pain (38.5%: 77/200), muscle pain (24.5%: 49/200), nausea (8%: 16/200), vomiting (8%: 16/200) and diarrhoea (6%: 12/200), respectively. Overall seroprevalence of the DENV infection was 11% (22/200), however, only DENV-II was detected. Similarly, the mean total lymphocytes, neutrophils, and haemoglobin concentration were statistically significantly lower among seropositive subjects compared to their counterparts ($p < 0.05$). Although not statistically significant, the mean total lymphocytes count was relatively higher among seropositive individuals (2.67±1.29 versus 2.37±1.30). Only 7/22 showed malaria positive test results as against 73/178 with seronegative DENV test results. Interestingly, the PCR result further portrays the point that DENV-II infection was higher among male adults. Haematological indices shows, mean total Lymphocytes count was relatively higher among individuals with DENV-II infection and there was statistically significant leucopaenia ($p = 0.027$). Other important haematological findings were lymphopaenia (2.54±1.59 versus 2.78±1.03) and neutrophilia (3.66±2.51 against 3.42±1.42). Our finding indicated that DENV-II has emerged in Kano northwestern Nigeria and with high circulation rate of public health concern.

Keywords: Dengue virus, Savanna Region**,** Kano, Nigeria.

PRESENCE OF HUMAN HERPESVIRUSES INFECTION MARKERS IN HOSPITALIZED COVID-19 PATIENTS IN LATVIA

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Herpesviruses share ability of persistence in latent state after primary infection which can switch to reactivation due to factors including other infections and altered state of immune system. Some studies have associated the reactivation of human herpesviruses with the severity and length of COVID-19. Viruses may under certain conditions escape the immune system response and hide in tissue reservoirs while maintaining possibility to reactivate, thus facilitating the entry of the SARS-CoV-2 into cells, enhancing viral load and aggravating the severity of symptoms. The aim of this work was to determine the presence of human herpesviruses infection markers in COVID-19 patients. The study included 86 hospitalized COVID-19 patients aged 18 – 93 years. Presence of human herpesviruses infection markers were determined by multiplex real-time and nested PCRs using peripheral blood and cell-free blood plasma DNAs as the templates. For 48/86 patients, repeated samples in dynamics were available at two or three timepoints. Levels of anti-EBV-CA IgG, anti-EBV-EA-D IgG and anti-HHV-6 IgG was determined by ELISAS. 37.2% of blood but none of the plasma DNA samples were positive for the presence of EBV genomic sequences; 10.47% of blood DNA samples were positive for HHV-6A/B, and 20.9% blood and one plasma DNA sample were positive for HHV-7 genomic sequences. In dynamics, EBV genomic sequences were detected in 31.25% of blood DNA samples, HHV-6A/B sequences – in 8.3% of blood and in 4.2% of plasma DNA samples, HHV-7 genomic sequences – in 16.7 % of blood and in 6.25% of plasma DNA samples. Preliminary results show that anti-EBV-CA IgG, anti-EBV-EA-D IgG and anti-HHV-6 IgG are present among COVID-19 patients. This study shows that human herpesviruses infection markers are present in COVID-19 patients and could indicate to the reactivation of these viruses that can affect the course and recovery of COVID-19.

Keywords: COVID-19, herpesviruses, EBV, HHV-6A/B, HHV-7

ROLE OF PRM AND E INTERACTIONS IN VIRUS RELEASE AND CELLULAR TROPISM OF POWASSAN VIRUS

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Powassan virus (POWV) is a tick-borne flavivirus causing fatal neuroinvasive disease in humans. Although several cell-specific receptors have been implicated in flavivirus entry, receptor binding determinants of tick-borne flaviviruses are not yet defined. There are two circulating lineages of POWV in North America, Lineage I POWVLB and Lineage II POWV-Spooner (deer tick virus). Here we sequenced several isolates of lineage II POWV from black-legged ticks (Ixodes scapularis) collected from Pennsylvania and characterized the growth kinetics and tropism in mammalian and insect cell lines in comparison with POWV-LB and POWV-Spooner strains. We identified amino acid variations in the envelope protein I associated with large, medium, and small plaque phenotypes of the isolates. To study the role of these E amino acid variations in virus attachment and entry, we generated chimeric cDNA clones of Zika virus (ZIKV), substituting the prM and E proteins with POWV. In addition, we selected surface-exposed E residues and E residues interacting with pr domain based on structural comparisons between tick-borne and mosquito-borne flaviviruses for mutagenesis in the chimeric virus. Mutant chimeras were evaluated for infectivity in different cell types to produce infectious virus. Defects in entry and replication were determined using immunofluorescence assay, western blot, and qRT-PCR. Key glycoprotein mutations were subsequently generated on the cDNA clone using a two-plasmid system encoding full-length POWV lineage II, and virus titers in different cell lines were obtained. We show mutations of POWV at positions E 157-161 and E 170-171, in the domain I of E inhibited entry. Our chimeric system provides a platform for the analysis of POWV envelope glycoprotein in a BSL-2 lab, guiding targeted studies in BSL-3. We identified surface-exposed POWV residues critical to receptor-mediated entry and cell-line specificity, which helps define differences between tick-borne and mosquito-borne flavivirus tropism.

Keywords: Flavivirus, Tick-borne, Powassan, assembly

THE PRESENCE OF HUMAN HERPES VIRUS – 6 INFECTION MARKERS IN THE BRAIN TISSUE OF PATIENTS WITH ALZHEIMER'S DISEASE: A PILOT STUDY

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Human herpesvirus-6 (HHV-6) belonging to the *Betaherpesvirinae* subfamily of herpesvirus exhibits lymphotropic and neurotropic affinity and persists in a variety of tissues and body cells. Recent reports have linked HHV-6 infection to the development of a number of CNS disorders. The probable involvement of infectious agents in the development of Alzheimer's disease (AD) has drawn increasing attention. In this study, the major objective was to determine the presence of HHV-6 and its target cells in the post-mortem brain tissues of AD individuals. Brain specimens of two AD patients were acquired from the McGill Brain Bank. The morphological assessment was performed on the posterior cingulate, the inferior parietal, and the lingual cerebral tissues of the right hemisphere. Monoclonal mouse anti-HHV-6 antibody was used for immunohistochemical detection of HHV-6. A quantitative analysis has further been performed to assess viral antigen expression. The immunopositivity for HHV-6A and B antigens was confirmed in all specimens studied. There were more HHV-6-positive cells in the grey matter (5.95%) than in the white matter (0.07%). In particular, neurons exhibited the greatest HHV-6 positivity, up to 26.44% across brain regions and patients. Astrocytes were the second-most often HHV-6-affected cells, up to 25.95% across brain regions and patients. In some cases, studied AD patients demonstrate up to a quarter of HHV-6 positive neurons and astrocytes. Less prevalent antigen immunostaining has been shown in oligodendrocytes up to 1.58%. No microglial cells were shown to be positive for the HHV-6 antigen. HHV-6, primarily located in neurons and astrocytes, can have a strong impact on neural homeostasis in AD. This study is a proof of concept and provides a framework for further, more detailed research on the link between HHV-6 and AD.

Keywords: Immunohistochemistry; HHV-6; Alzheimer's; neuromorphology

EBV INFECTION PROMOTES MIGRATION OF THE HOST B CELLS VIA UP-REGULATION OF THE INFLAMMATORY CHEMOKINE RECEPTORS CCR1 AND CCR2

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Chemokines and their receptors control migration of immune cells and mediate dissemination of malignant cells. The chemokine receptors, CCR1, CCR2, CCR3, and CCR5, belong to one family of a single protein homology cluster and share responses to the same inflammatory chemokines. Earlier, we demonstrated that CCR1 and CCR2 were up-regulated in *ex vivo* peripheral blood B cells upon Epstein-Barr virus (EBV) infection and in established non-malignant lymphoblastoid B-cell lines (LCL) with the EBV latency III program. EBV latency III is found in B-cell lymphomas of immunosuppressed patients. EBV-positive Burkitt lymphoma (BL) tumors are mostly associated with latency I, but BL cell lines drift towards latency III during culturing *in vitro*. Up to now, the role of EBV in pathogenesis of B-cell lymphomas is not fully understood. We analyzed the mRNA and protein expression of four known inflammatory chemokine receptors, CCR1, CCR2, CCR3, and CCR5, and the expression of the EBV latent genes, EBNA2, LMP1, and LMP2, in LCLs and in the isogenic EBV-carrying and EBV-negative BL cell lines. We also assessed the migration of LCL and BL cells stimulated by the chemokine CCL2 (MCP1). *Results.* Both CCR1 and CCR2 are expressed at mRNA and protein levels in LCLs and in BL cell lines with the co-expression of the EBV latent genes EBNA2, LMP1, and LMP2. The CCR3 and CCR5 transcripts were hardly detectable. We detected the migration of LCL and BL type III cells toward CCL2. Our data suggest that *in vivo*, the up-regulation of CCR1 and CCR2 may contribute to the enhanced migration of the EBV-infected B cells into compartments enriched with inflammatory chemokines. CCR1 and CCR2 can be involved in the selection of BL cells with restricted EBV gene expression programs.

Keywords: EBV; CCR1, CCR2; lymphoblastoid cell lines; Burkitt lymphoma cell lines

ROLE OF HUMAN PAPILLOMAVIRUS AND VACCINATION: A WEB-BASED SURVEY

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Human papillomaviruses (HPV) form a large family of DNA viruses that infect only human skin keratinocytes and mucosal epithelial cells, subsequently cells react by proliferation that can result in different clinical manifestations ranging from common warts to malignant changes. Currently available vaccine has enormous potential value in protecting against the HPV infection. We aimed to evaluate Latvian general population's knowledge about the human papillomavirus and vaccination. A survey–based cross–sectional study was performed February to October 2021. Participants completed an anonymous survey, which included 19 questions, assessing age, gender, education, and knowledge about HPV and vaccination. Obtained results were statistically analyzed using IBM SPSS 28. Our survey was completed by 277 participants, 188 females and 89 males. 82.3% of participants had heard of HPV and there was a statistically significant difference between genders – females had a higher tendency to know about the virus than men ($p<0.001$). 82% of respondents were aware, that HPV infection can proceed without symptoms. 74.4% of participants considered, that there is a treatment for this disease. The opinion on whether the partner should be vaccinated had statically significant differences between vaccinated and unvaccinated participants – the vaccinated respondents 44lcyone44a their partner vaccination, but for unvaccinated participants it did not matter ($p<0.001$). Vaccinated and unvaccinated individuals have different opinions and education on HPV. To increase vaccination coverage and knowledge of HPV, it is important to organize wider educational campaigns and introduce new ways to deliver information.

Keywords: human papillomavirus; vaccination; survey; general knowledge.

A RAPID REVIEW OF EFFICACY AND PAIN INFLICTION AMONG NEWEST TREATMENT MODALITIES FOR CUTANEOUS VIRAL WARTS

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Warts are prevalent benign cutaneous lesions caused by the human papillomavirus. In recent years new treatment methods have emerged as promising alternatives to common destructive and often painful modalities. A rapid review was performed in a PubMed bibliographic database using a search string "Warts/therapy"[MAJR]. The search was limited to articles published from 2013 onwards and articles with a study population of more than 10 patients. Only the first 101 articles were examined. The efficacy was defined as clearance of all visible warts for a single patient. In total, 40 articles were included with 30 different treatment modalities compared in terms of efficacy and pain infliction. The highest efficacy of 95.6% was seen with intralesional Vitamin D3 in a study by Zainab Z et al. In total, 8 studies assessing intralesional Vitamin D3 were identified showing efficacy rate from 20% to 95.6%. As a comparison, cryotherapy was studied in 13 articles with efficacy rate from 23.7% to 76.7%. From the most effective treatment modalities most were intralesional methods – intralesional vitamin D3 (up to 95.6% efficacy), intralesional zinc sulphate (up to 93.4% efficacy for injected wart only), intralesional combined furosemide and digoxin (up to 92.5% efficacy) and intralesional tuberculin purified protein derivative (up to 86.7% efficacy). The least painful modality with highest efficacy was photodynamic therapy with Eosin-Trans studied in one article. The efficacy of this modality was shown 86.4% with no pain in any of the participants. Intralesional Vitamin D had the highest efficacy rate and is therefore a very promising alternative to current destructive treatment modalities and furthermore has also the advantage of targeting multiple distant viral warts. Photodynamic therapy using Eosin-Trans had the highest efficacy together with least pain, which is very practical for the treatment of children, which are the main patient population.

Keywords: viral warts, intralesional vitamin D, photodynamic therapy, cryotherapy

IMMUNO-THERAPY OF HEPATITIS C VIRUS INFECTION BY HETEROLOGOUS PRIME/BOOST VACCINE

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Chronic hepatitis C caused by infection with the hepatitis C virus (HCV) is a global health problem. We aimed to address how HCV expression causes chronic liver diseases and to provide new options for HCV vaccine development. Using a mouse model of hepatitis C, we examined the therapeutic effects of a prim/boost vaccine using a HCV-cDNA vaccine and a recombinant vaccinia virus (rVV) that encodes an HCV protein. We generated immunocompetent mice expressing HCV structural proteins or whole HCV proteins through the Cre/*loxP* switching system, mice expressing HCV structural proteins (MxCre/CN2-29) and mice expressing HCV whole proteins (MxCre/RzCN5-15). We constructed the DNA vaccines and attenuated rVV strain, which are expressing an HCV structural protein (rVV-CN2), non-structural protein (rVV-N25). Furthermore, we examined homologous-antigen for prime/boost vaccination (N25-cDNA + rVV-N25) or heterologous-antigen prime/boost vaccination (N25-cDNA + rVV-CN2 or CN2-cDNA + rVV-N25). We found that within 28 days after immunization the HCV core protein levels were significantly lower in livers of the homologous-antigen prim/boost using DNA vaccine and rVV-N25-treated mice expressing HCV structural proteins (MxCre/CN2-29). However, the homologous-antigen prim/boost using DNA vaccine and rVV-N25 could not reduce the HCV core protein levels in mice expressing HCV whole proteins (MxCre/RzCN5-15). We found that HCV core protein levels were significantly reduced by the heterologous-antigen prim/boost with DNA vaccine and rVV-N25 treatment in HCV whole proteins transgenic mice (MxCre/RzCN5-15). Collectively, we showed that the significant therapeutic effect of heterologous-antigen prim/boost using DNA vaccine and rVV method. We propose that the heterologous-antigen prim/boost vaccination (N25-cDNA + rVV-CN2 or CN2-cDNA + rVV-N25) could become an effective therapeutic vaccine.

Keywords: HCV, 46lcyon-therapy, heterologous, prime/boost

PLANTARICIN NC8 *αβ* RAPIDLY AND EFFICIENTLY INHIBITS FLAVIVIRUSES AND SARS-COV-2 BY DISRUPTING THEIR ENVELOPES

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Potent broad-spectrum antiviral agents are urgently needed to combat both existing and emerging viral infections. Antimicrobial peptides, such as bacteriocins, could be attractive candidates as antiviral agents against enveloped viruses. One of these bacteriocins is PLNC8 αβ, which consists of amphipathic peptides with positive net charges that display high affinity for negatively charged pathogen membrane structures, including phosphatidylglycerol rich bacterial membranes and phosphatidylserine rich lipid membranes of viral envelopes. Due to the morphological and physiological differences between viral envelopes and host cell membranes, PLNC8 αβ are thought to have high safety profile because they specifically target viral envelopes with 47lcyon, if any, effect on cellular membranes. In this study, we tested the antiviral effects of PLNC8 αβ using cell lines infected by various types of viruses that include the flaviviruses Langat and Kunjin, the coronavirus SARS-CoV-2, the Influenza A virus, and HIV. The concentration of PLNC8 αβ that is required to eliminate all the infective virus particles is in the range of nanomolar (nM) to micromolar (µM), which is surprisingly efficient considering the high content of cholesterol (8-35%) in their lipid envelopes. We demonstrated that viruses that use the ER/Golgi complex pathway, e.g., SARS-CoV-2 and *Flaviviruses*, are considerably more susceptible to PLNC8 αβ, compared to viruses that acquire their lipid envelope from the plasma membrane, such as Influenza A virus and HIV-1. PLNC8 αβ can thus be developed into effective and safe antiviral agents that target the viral envelopes and should not be affected by the problem of virus antigenic mutations which faces many antiviral drugs and vaccines.

Keywords: Antiviral agents, plantaricin NC8 αβ, flaviviruses, SARS-CoV-2

IS HEPATITIS E VIRUS A NEGLECTED OR EMERGING PATHOGEN IN EGYPT?

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Though Egypt ranks among the top countries for viral hepatitis and death-related liver disease, Hepatitis E virus (HEV) is a neglected disease. Living in villages and rural communities with low sanitation, use of underground well water and contact with animals are the main risk factors for HEV infection. Domestic animals, especially ruminants and their edible products, are one source of infection. Contamination of water by either human or animal stools is the main route of infection. In addition, HEV either alone or in coinfection with other hepatotropic viruses has been recorded in Egyptian blood donors. HEV seropositivity among Egyptian villagers was 60–80%, especially in the first decade of life. Though HEV seropositivity is the highest among Egyptians, HEV infection is not routinely diagnosed in Egyptian hospitals. The initial manifestations of HEV among Egyptians is a subclinical infection, although progression to fulminant hepatic failure has been recorded. With the improvement in serological and molecular approaches and increasing research on HEV, it is becoming clear that HEV represents a threat for Egyptians and preventive measures should be considered to reduce the infection rate and possible complications.

Keywords: HEV, Egypt, zoonoses, emerging pathogen

THE SLIPPERY SLOPE: HOW METABOLISM CONTROLS VIRUS-HOST INTERACTIONS AND PATHOGENESIS

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Flaviviruses actively modify cellular communication pathways such that the host cell is focused on viral replicative needs. By rearranging the intracellular membrane environment to form complex 'viral cobwebs' these viruses increase membrane contact sites for optimized or altered signaling and transport of raw materials required for virion biogenesis. During this process significant perturbations are observed in lipid anabolism and catabolism presumably driven by the expression of viral gene products. We have explored the role of enzymes within the fatty acid and sphingolipid metabolic pathways involved in both biosynthesis and lipolysis and determined their impact on flavivirus genome replication and infectious virus assembly. Specifically, we have determined that the axis of fatty acid synthesis driven by fatty acid synthase, stearoyl CoA desaturase and elongases are required for viral genome replication and also greatly impact the membrane composition and architecture of the virion envelope altering its capacity to produce structurally optimized infectious particles. We have also determined that a temporally controlled delicate balance between lipid biosynthesis and lipolysis occurs to benefit a replicative advantage. Based on the hypothesis that viral proteins strictly control the expression, localization and activity of these enzymes we are now exploring the mechanism of control to identify avenues for intervention. Interestingly, we have also identified how metabolic disruptions observed intracellularly translate to measurable alterations in the metabolic profile in the serum lipidome of human patients providing a link to possible disease states. An update on these studies will be presented.

Keywords: Virus, metabolism, pathogenesis, arboviruses

A COMBINATION OF TWO RESISTANCE MECHANISMS IS CRITICAL FOR TICK-BORNE ENCEPHALITIS VIRUS TO ESCAPE A BROADLY NEUTRALISING HUMAN ANTIBODY THAT TARGETS THE ENVELOPE PROTEIN

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Tick-borne encephalitis virus (TBEV) is a flavivirus that causes human neuroinfections in much of Europe and Asia and represents a growing health problem. Analysis of human antibody response to TBEV infection or vaccination revealed that expanded clones of memory B cells expressed closely related anti-envelope domain III (EDIII) antibodies in both cohorts, but the most potent neutralizing antibodies were found only in individuals who recovered from natural infection. These antibodies also neutralized other tick-borne flaviviruses. Structural analysis revealed a conserved epitope near the lateral ridge of EDIII adjoining the EDI-EDIII hinge region. Prophylactic or early therapeutic antibody administration was effective at low doses in mice lethally infected with TBEV. TBEV escape mutants, however, evolved rapidly in vitro in the presence of these monoclonal antibodies, but the escape was not observed in vivo. The in vitro TBEV escape resulted in virus variants of reduced pathogenicity and characterized by distinct sets of amino acid changes in EDII and EDIII that were jointly needed to confer resistance. The EDIII substitution K311N impaired the formation of a salt bridge critical for antibody-epitope interaction. The EDII substitution E230K was not on the epitope but likely induced quaternary rearrangements of the virus surface due to repulsion of positively charged residues on the adjacent EDI. Combination of two distinct monoclonal antibodies prevented virus escape and synergized for neutralization.

Keywords: flavivirus, tick-borne encephalitis virus, monoclonal antibody, therapy

VIRAL INFECTION AND Th17 CELLS: A DOUBLE-EDGED SWORD WITH DIAGNOSTIC AND THERAPEUTIC POTENTIAL

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The present research summarizes recent findings on the involvement of Th17 cells and cytokines that they generate in the course of viral infections. Virus-induced cancers are not caused only by viral infections, but both genetic and host safety factors have a role in their development. Acquired immune responses, through the differentiation of Th17 cells, form the novel components of the Th17 cell pathway when reacting with viral infections all the way from the beginning to its final stages. As a result, rather of inducing the appropriate immunological responses, these events depress the immune system. In addition, the secretion of IL-17 and other cytokines by Th17 cells during persistent viral infections produces chronic inflammation, which promotes tumor growth and progression. Additionally, these cells have been implicated in tumor growth and metastasis throughout the last decade. However, further research is needed to fully comprehend Th17 cells' immunological processes in a wide range of viral infections. The aim of this study is to understand the roles and impacts of the immune system, particularly Th17 cells, in the development of vir'l infections, which might be extremely useful in the diagnosis and treatment of these infections.

Keywords: Viral infection, Th17 cells, Cytokines, Virus-induced cancers

E6-siRNA IN COMBINATION WITH OXALIPLATIN: A NEW STRATEGY FOR TREATMENT OF HPV-RELATED CERVICAL CANCER

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Human papillomaviruses (HPV's) early proteins are expressed in infected cells following viral persistent infection and are associated with disorders. E6 has been implicated in immortalization and uncontrolled cell proliferation, interfering with cell cycle regulation and signaling pathways for apoptosis of infected cells. Alteration of E6 oncogene expression by E6 specific small interference RNA (siRNA) sequences, in combination with Oxaliplatin, and the effect of this combination on the recovery and restoration genes involved in apoptosis and metastasis; which owing to increased apoptosis and plummeted proliferation in cervical cancer cells. We used CaSki cell line, 48 hours after transfection of E6-siRNA and treatment with Oxaliplatin, the cellular genes such as *P53*, *MMP9*, *Nanog* and *caspases* gene expression were assessed by quantitative real-time PCR. Cell death rate, cell cycle, and cell viability were examined by Annexin V/PI staining, flow cytometry, and MTT test, respectively. Besides, the stemness ability and cell metastasis were determined by colony formation assay and scratch test, respectively. Among the various dosage of siRNA, 100 picomoles of siRNA for 48 hours had a sharp effect on the knockdown of the target gene, followed by an increase in apoptosis to 44.2% in cervical cancer cells. Combination therapy also increased the re-expression of genes involved in the P53-dependent apoptosis pathway and reduced stemness and metastasis ability compared to either siRNA or Oxaliplatin monotherapy. We showed that simultaneous use of both siRNA and Oxaliplatin increased the susceptibility of cervical cancer cells to Oxaliplatin and decreased the ability to survive, proliferate, metastasize, and consequently escalate apoptosis rate and arrested cell cycle in the sub-G1. Overall, combination therapy reduces both drug chemo-resistance and the medicine dosage, so it prevents cancer progression and healthy tissue destruction.

Keywords: Human papillomavirus, E6 oncoprotein, Cervical cancer, Oxaliplatin, siRNA

HUMAN PAPILLOMAVIRUS AND ANTIGEN PRESENTING CELLS: THE AMOUNT OF CD MARKER'S TYPE IN CERVICAL CANCER PROGNOSIS

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Human papillomaviruses (HPV), an oncogenic DNA virus, can cause squamous intraepithelial lesions and cervical cancer. Oncoproteins from the early HPV virus have been linked to cancer initiation and progression, as well as tumor-enhancing activity. Cervical cancer progresses slowly from pre-invasive to invasive, requiring years or even decades depending on variables including HPV infection, inflammation, and immune system status. HPV infection can cause immunological tolerance and lead to the development of cervical cancer by altering the immune system. The release of immune-regulating factors from HPV-infected cells is thought to cause the recruitment of tumor suppressor cells, tumor-associated macrophages (TAM), myeloid-derived suppressor cells, regulatory T cells (T-reg), and dendritic cells (DCs) differentiation, resulting in the activation of acquired immune response via cytokine-dependent inflammatory pathways. Furthermore, HPV can also evade the immune system due to its oncoproteins, causing cancer in epithelial cells. Certain forms of HPV-infected cells may employ distinct immune-evasion pathways, making the virus unidentifiable to immune cells, particularly antigen-presenting cells (APCs). In addition to viral oncoproteins, antigen-presenting cells (APC) and the number of clusters of differentiation (CD) markers expressed on their surface play an essential role in the disease progression or tumorigenesis inhibition. Here, we will discuss the function of CD markers in the interaction between APCs and cancer cells, immune cells' function in the infection process, and finally infected cells' malignancy. Moreover, in the current research, we have shown antigen-presenting-cells position in ectocervix by laser scanning confocal microscopy. Furthermore, we suggest that targeting these markers as a novel insight to create a new therapeutic or diagnosis strategy to prevent cervical cancer progression.

Keywords: Human papillomaviruses, antigen presenting cells, clusters of differentiation, cervical cancer

HUMAN PAPILLOMAVIRUS AND PROSTATE CANCER: ONCOPROTEINS AND APOPTOSIS PATHWAYS

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Human papillomaviruses (HPV), is well-established oncoviruses, which can initiate various human carcinomas. Prostate cancer is one of the most frequent cancers in men and it is reported that about one in nine men experience the disease in their lifetime. Researchers have suggested that viral infections such as HPV may influence prostate cancer development pathway. However, the possible role of high-risk-HPV infection in prostate cancer progression has not been identified. Likewise, a few types of HPV (6, 11, 15, 16, 18, and 33) are associated with this cancer. The effect of oncogene proteins of HPV on apoptosis pathways in prostate cancer need to be investigated in order to shed light on the role of the virus in prostate cancer. The virus activates numerous pathways, permitting the infected cells to avoid extrinsic and intrinsic apoptosis pathways. The inability of prostatic epithelial cells to induce apoptosis leads to the invasive development of prostate cancer. Various risk factors have been reported for the pathogenesis of prostate cancer. High-risk-HPVs cause malignancy by interfering with the apoptotic and inflammatory pathways; these viruses, such as HPV16 and HPV18, block apoptotic pathways and result in prostate cancer. The current study is presenting a summary of oncogenes (E5, E6, and E7) HR-HPVs' functions on signaling pathways, inflammation in prostate tumorigenesis, and emphasizing the link between these oncogenes with apoptosis and prostate cancer.

Keywords: Human papillomaviruses, Oncoproteins, Apoptosis, Prostate cancer

INNOVATIVE AND AFFORDABLE HIV-1 DRUG RESISTANCE TESTING FOR RESOURCE LIMITED SETTINGS

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As use of dolutegravir (DTG) becomes more common in resource limited settings (RLS), affordable methods for genotyping all relevant HIV-1 *pol* genes (i.e., *protease* (PR*), reverse transcriptase* (RT) *and integrase* (IN)) are required to guide choice of future antiretroviral therapy (ART) regimens. We designed an in-house HIV-1 drug resistance (HIVDR) genotyping method that is affordable and suitable for use in RLS. We obtained remnant plasma samples from CAPRISA 103 study and amplified HIV-1 PR, RT and IN genes, using an innovative PCR assay. We genotyped samples by Sanger sequencing and assessed HIVDR mutations using the Stanford University HIV drug resistance database. We compared PR and RT mutations to previous sample genotypes, calculated method cost-estimates, and performed phylogenetic analysis. From 96 samples processed, we obtained sequence data for 78 (81%), of which 75 (96%) had a least one HIVDR mutation, with no major-IN mutations observed. When compared to previous genotypes, 18/78 (23%) had at least one discordant mutation, but only 2/78 (3%) resulted in different phenotypic predictions that could affect choice of subsequent regimen. Overall genotyping cost per sample was estimated at ~US$43, with a processing time of ~2 working days. All sequence pairs clustered together in phylogenetic analysis. We successfully designed an in-house HIVDR method that is suitable for genotyping HIV-1 PR, RT and IN genes, at an affordable cost and shorter turnaround time. This HIVDR genotyping method accommodates changes in ART regimens and will help guide HIV-1 treatment decisions in RLS.

Keywords: HIV-1, pol gene, genotypic drug resistance, Resource limited settings

ACCELERATING VACCINE DEVELOPMENT: CEPI'S 100-DAY MISSION TO COMBAT FUTURE PANDEMICS

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The rapid development and deployment of vaccines against emerging pathogens are critical in mitigating the devastating impact of future pandemics. The Coalition for Epidemic Preparedness Innovations (CEPI) has embarked on an ambitious and transformative initiative known as the "100-Day Mission." This mission aims to revolutionize vaccine development timelines by ensuring the availability of effective vaccines within 100 days of identifying a pandemic threat. CEPI's 100-Day Mission leverages strategic partnerships, innovative technologies, and agile manufacturing processes to expedite the entire vaccine development process. Through accelerated research, rigorous clinical trials, and streamlined regulatory pathways, CEPI strives to overcome the historical challenges and bottlenecks associated with vaccine development. By significantly shortening the time required for vaccine development, this initiative holds immense potential in saving lives, reducing morbidity, and curbing the socio-economic impact of future pandemics.

Keywords: Vaccines development; 100-Days Mission; Pandemic; Viruses

LONG COVID-19: THE DEVIL IN THE DETAILS

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To date more than 767 million laboratory confirmed SARS-CoV-2 infection were recorded worldwide. Long COVID-19 refers to post-acute symptoms, that cannot be explained by an alternative diagnosis. The syndrome occurs in considerable huge numbers of people who recovered acute form of SARS-CoV-2 infection and may persist for months after recovery from the initial acute SARS-CoV-2 infection. More than 200 signs are associated with long-COVID that attributed to affections in various organs of the body organs. Cognitive dysfunction and fatigue are among the most common reported signs. Risk factors associated with developing of long COVID-19 include female sex, environmental, and economic stressors, smoking, obesity and a wide range of comorbidities. The long COVID is too complex to provide exact pathogenesis of this syndrome. However, the are many potential overlapping mechanisms and hypotheses underlying the possible causes that lead to long COVID-19. These hypotheses include neurotropic tendency of the SARS-CoV-2, persistence of SARS-CoV-2 infection, neuroinflammation, autoimmunity and reaction of other latent viruses. Although there is no current approved treatment protocol to handle long COVID, many clinical trials are in progress in continuous attempts to find out suitable treatments. One of the most important pillars that could help in long COVID care is psychological and social determinants of public health that have a great impact on the outcomes and disease course.

Keywords: Long COVID, global health, post-viral syndromes, SARS-CoV-2.

One Health / One World

MAPPING THE POTENTIAL DISTRIBUTION OF THE PRINCIPAL VECTOR OF CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS *HYALOMMA MARGINATUM* IN EUROPE

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Crimean-Congo haemorrhagic fever (CCHF) is the most widely distributed tick-borne viral disease in humans caused by the Crimean-Congo haemorrhagic fever orthonairovirus (CCHFV). The virus is widely expanded across western China, South Asia, and the Middle East to southeastern Europe and Africa. The historical known distribution of the CCHFV vector *Hyalomma marginatum* in Europe included most of the Mediterranean and the Balkan countries, Ukraine, and southern Russia. Further expansion of its potential distribution is possibly occurred in and out of the Mediterranean region. This study updated the map of the principal vector of CCHFV, *H. marginatum*, in the Old World by using an ecological niche modeling approach based on a modeling environment that uses the Maxent algorithm, named *kuenm*, capable to manage diverse settings to better estimate the potential distribution of this tick species. The model estimated the environmental suitability of *H. marginatum* in the Old World, including Europe. On the continental European scale, the model anticipated a widespread potential distribution, covering southern, western, central, and eastern Europe, as far north as southern parts of Scandinavian countries. The distribution of *H. marginatum* also covered the countries across the central part of Europe where the species is not autochthonous. All models were statistically robust and performed better than random ($p < 0.001$). Based on the results of the model, climatic conditions could hamper the successful overwintering of *H. marginatum* and their survival as adults in many areas of the region. Regular updates of the models, using updated occurrence, current, and future climatic data are recommended to regularly assess the areas at risk.

Keywords: Crimean-Congo haemorrhagic fever orthonairovirus; *Hyalomma marginatum*; *kuenm*; ecological niche modeling

FIRST DETECTION OF SARS-COV-2 IN WHITE RHINOCEROS DURING CORONAVIRUS SURVEILLANCE IN THE BANDIA RESERVOIR, SENEGAL

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The SARS-CoV-2 pandemic has heightened interest in monitoring and surveillance of coronaviruses in wildlife. Testing for the virus in animals can provide valuable insights into viral reservoirs, transmission, and pathogenesis. In this study, we present the results of the molecular surveillance project focused on coronaviruses in in Senegalese wildlife. During the project, we screened fecal samples of the wild animals living in the Bandia Reserve (ten non-human primates, one giraffe, and two white rhinoreos samples) and the free-living urban population of African four-toed hedgehogs in Ngaparou. The results showed absence of coronaviruses in hedgehogs, non-human primates, and a giraffe. A single positive sample was obtained from a white rhinoceros (*Ceratotherium simum*). The sequencing results showed that the detected virus was SARS-CoV-2. As all the experiments were performed without any positive control, the persons performing the screens were negativelly tested for presence of SARS-CoV-2, and all negative controls were always negative through the whole surveillance project, we can exclude a possibility of any cross-contamiantion. Therefore, we can assume that this study represents according to our best knowledge the first documented instance of molecular detection of SARS-CoV-2 in white rhinoceros and therefore extend our knowledge about possible SARS-CoV-2 hosts.

Keywords: SARS-CoV-2, Coronavirus, Wildlife, Surveillance.

ANTI-SARS-COV-2 ACTIVITY OF BACTERIOPHAGE-DERIVED DOUBLE-STRANDED RNA IN GOLDEN SYRIAN HAMSTERS

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SARS-CoV-2, a member of the coronavirus family, is the causative agent of the COVID-19 pandemic. Despite vaccination, searching for new efficient antiviral drugs that could be used preventively or on an outpatient basis is still relevant. This study aimed to evaluate the antiviral effect of bacteriophage-derived double-stranded RNA (Larifan), a nationally well-known broad-spectrum antiviral medication against SARS-CoV-2 in golden Syrian hamsters. Hamsters were intranasally infected with 2 x10^4 $TCID_{50}$ of SARS-CoV-2 and treated subcutaneously (s.c.) or intranasally (i.n.) using Larifan dosage of 5 mg/kg either before and post-infection or only before and only post-infection. Clinical signs, appearance and body weight changes were monitored daily for 14 days. On days three and five post-infection and at the end of the experiment, four hamsters from each group were humanely 60lcyone60a60, and tracheal lavages and lungs were collected for virus quantification and histological observations. We demonstrated that Larifan treatment reduced virus numbers in infected hamsters' tracheal lavages and lungs when animals received Larifan repeatedly before and after infection. A reduction in virus numbers was also observed when Larifan was administrated before infection. However, Larifan administration post-infection did not show signs of virus inhibition. A more pronounced effect of Larifan was observed after its i.n. administration versus s.c. administration. Improvements in the infection- induced pathological lesion severity in the lungs of animals treated with Larifan were also demonstrated at the peak of infection. The reduction in the viral load in the lungs of infected hamsters treated with Larifan suggests a potential use of Larifan in controlling the COVID-19 disease, primarily when used as a preventive measure.

Keywords: SARS-CoV-2, Larifan, Bacteriophage-derived double-stranded RNA, Golden Syrian hamsters

RESEARCH ON INSECT VIRUSES IN LATVIA: PAST AND FUTURE

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Research on insect viruses in Latvia began at the Institute of Biology in the 60s of the last century. The aim was to gain new knowledge on insect viruses and clarify their role in regulating pest populations in Latvia. The main tasks were to monitor significant forest pest populations; to obtain new virus isolates and describe their properties; investigate occurrence and natural variability of baculoviruses in pest populations and. After 1990s, the focus was on the development of experimental strains with high virulence; development novel viral insecticide preparations and determination their efficacy and assess perspectives for use in pest control; the interaction of baculoviruses in the plant-pest-pathogen system and the study genome of baculoviruses. Monitoring of outbreaks and natural epizootics of forest pests have been done since 1965 on a regular basis. Living insects were laboratory-reared in isolators under optimal conditions. Viruses were purified using the methods described by Evans & Shapiro (1997). The presence of baculoviruses in larval tissue was detected by direct examination of larval tissue smears. The viral 61lcyone61a61 or granules were observed in electron microscope. Method of DNA amplification by specific primers were used for identifying latent and persistent viral infections. Biotests were carried out for determining the virulence and efficacy of isolates (Huber, Hughes, 1984). Virulent experimental strains were obtained from wild isolates, using passages through host organisms under stress factors. The majority of entomopathogenic viruses were isolated from dangerous forest and agricultural pests. Nucleopolyhedroviruses (NPVs) were isolated from 18 pest species, Granuloviruses (GVs) from six species, and Cypoviruses (CPVs) from three species. On the basis of experimental strain, a new viral insecticide were developed. The methods of obtaining dry and liquid preparative virus insecticide forms were developed. In next years the role of microorganism associations in development of NPV infection will be clarified.

Keywords: Nucleopolyhedroviruses, Granuloviruses Cypoviruses, biological control

DEEP SEQUENCING AT THE ZOONOTIC INTERFACE UNCOVERS NOVEL, EMERGING AND HIGH CONSEQUENCE VIRUSES

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Zoonotic and vector-borne virus infections are a major cause of acute febrile illness in sub-Saharan Africa. Available diagnosis options are limited to a few pathogens, resulting in a risk of onward transmission of high-consequence viruses. Next generation sequencing and serology are proven adaptable tools for the detection of infection in patients and potential reservoirs. In partnership with the Uganda Virus Research Institute, we sequenced a total of 458 plasma samples from clinically undiagnosed patients from the Uganda Acute Febrile Illness (AFI) and ArboViral Infection (AVI) studies using metagenomic next generation sequencing (mNGS). With a One-Health focus, we also reviewed the environment of the patients and sampled potential animal reservoirs (200 rodents, 100 bats) and vectors (10,026 mosquitos, 2754 ticks). In addition, we did serosurveillance for high consequence viruses amongst high-risk occupational groups (cattle farmers, abattoir and healthcare workers). We identified potential haemorrhagic fever viruses: Crimean-Congo haemorrhagic fever virus (CCHFV), Rift Valley Fever virus (RVFV), dengue virus and yellow fever virus; from human patient cohorts; among other respiratory, gastrointestinal and blood borne pathogens. We also detected two emerging viruses: Le Dantec virus, a rhabdovirus last reported in 1969 and a novel chaphamaparvovirus from patients. mNGS on vector and animal samples yielded over 200 viral species of which 50% were novel, spanning 23 viral families, many of which have known human and animal pathogen members. Serosurveillance of occupational groups identified high exposure to Zaire ebolavirus in healthcare workers, RVFV in abattoir workers and CCHFV in cattle farmers. Our study highlights an ongoing significant risk to public health and a need for improved vigilance as well as an extensive estimation of the true disease risk/burden in the region. Advanced genomic and antibody assays such as broad targeted capture NGS and multiplexed serology are potential high-throughput applications to achieve this.

Keywords: sub-Saharan Africa, febrile illness, zoonosis, diagnostics

TICK-BORNE FLAVIVIRUS NS5 ANTAGONIZES INTERFERON SIGNALING BY INHIBITING THE CATALYTIC ACTIVITY OF TYK2

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While mosquitoes are the major vectors of pathogens in tropical regions, ticks are the leading vectors in temperate climates. In Europe, they carry significant pathogens such as the closely related tick-borne encephalitis virus (TBEV) and Louping Ill virus (LIV). These viruses cause severe central nervous system disease in humans and sheep, respectively. Several thousand human cases are reported each year, with recent increases attributed to climate changes, population dynamics, the range of permissive ticks and shifts in land usage. A common feature of Flaviviruses is their efficient strategy to evade immune signaling. The utilized mechanisms, however, are varied and incompletely understood. The NS5 of several mosquito-borne Flaviviruses display functional convergence in antagonizing the JAK-STAT signaling pathway, albeit by virus-specific mechanisms. Using virological approaches, biochemical assays and mass spectrometry analysis, we report here that the NS5 proteins of TBEV and LIV, antagonize JAK-STAT signaling through interactions with the tyrosine kinase 2 (TYK2). Co-immunoprecipitation (co-IP) experiments, yeast gap-repair assays, computational protein-protein docking and functional studies identified a stretch of 10 residues of the RNA dependent RNA polymerase domain of tick-borne Flavivirus NS5, but not mosquito-borne NS5, that is critical for interaction with the TYK2 kinase domain. Additional co-IP assays performed with several TYK2 orthologs revealed that the interaction was conserved across mammal species. Furthermore, *in vitro* kinase assays showed that TBEV and LIV NS5 reduced the catalytic activity of TYK2. Our results illustrate that NS5 proteins of tick-borne Flaviviruses are employing a unique mechanism to antagonize IFN signaling. This work highlights the variety of strategies employed by ecologically diverse Flavivirus NS5 to counteract the IFN-induced JAK/STAT pathway. It also emphasizes the pleiotropic function of the Flavivirus NS5 polymerase domain.

Keywords: Flavivirus, innate immunity, JAK-STAT, TYK2

NOROVIRUS DETECTION USING A BIOLOGICAL NANOPORE SENSOR

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Human noroviruses are the leading cause of foodborne illness globally. Numerous properties of norovirus make them difficult to control, including low infectious dose, stability, and diversity making rapid detection and subtyping crucial. Outer membrane protein (OmpG)-based nanopore sensor has numerous advantages such as high sensitivity, tolerance of inhibitors, rapidity, portability, and the ability to subtype; however, it has not been used for virus targets. Norovirus capsid protein was cloned and expressed in *E.coli*. Norovirus affinity peptides were presented on OmpG in two different ways; tethering or sequence replacement of peptide in an OmpG loop. Initial work resulted in detectable signal of norovirus target; however, signal needed to be improved for more repeatable and sensitive signal. Thus, optimization of bait peptide location in OmpG was performed using a FLAG tag and 3 anti-FLAG antibodies. The electrical current of peptide-tethered OmpG after adding target norovirus protein exhibited a 20pA drop in signal, showing the potential of OmpG to detect norovirus, however aforementioned optimization was needed to increase sensitivity and subtyping ability. We thus optimized the binding motif presentation in OmpG with a FLAG tag. As a result sequence replacement in OmpGQ222FLAG not only detect multiple target antibodies, but also was capable of discriminating different isotypes of monoclonal antibodies, and different isotypes of antibodies in a polyclonal antibody, each with characteristic signal at the tested antibody concentration of 17nM. We further tested the antibody detection in 10% FBS, and it generated target unique signals.This work reports the first successful detection of norovirus by OmpG nanopores, and further optimization demonstrates the potential of the OmpG nanopore sensing to rapidly, sensitively, and portably detect target protein, as well as sensitively discriminate even closely related proteins and mixture thereof.

Keywords: Norovirus, Nanopore sensor, Virus detection, Outer membrane protein G

EVALUATING EXTRACTION OF NON-ENVELOPED VIRUS AND VIRAL RNA USING MAGNETIC IONIC LIQUIDS FOR EVENTUAL ONE-TUBE CAPTURE, CONCENTRATION, AND GENOMIC EXTRACTION

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Magnetic ionic liquids (MILs) are a class of hydrophobic solvents that have proven effective as capture reagents for foodborne bacterial pathogens; however, data on viruses is limited. Since their binding is charge-based, it is important to evaluate their affinity for intact viral particles versus free nucleic acid, especially when used with endpoint detection methods such as PCR. In this study, we evaluated the capture and purification of bacteriophage MS2, a human norovirus surrogate, versus free RNA using MILs. Intact MS2 or purified RNA was diluted into 1X PBS (pH 7.2) and extracted from suspension using MILs. Briefly, MIL was added to the suspension, vortexed to disperse the MIL droplets and separated using a magnet. Supernatant was removed and samples were washed to remove unbound target. Captured target was then eluted by vortexing with modified Luria broth and quantified by RT-qPCR. MILs with transition metal-based anions showed similar capture (4.35±6.93%-18.5±7.03%) and recovery (2.71±0.43%-7.33±3.13%) efficiency for intact MS2. One MIL with a rare earth metal-based anion showed much greater capture (92.6±0.35%) but gave comparable recovery (6.90±0.91%) to the others. All MILs showed higher capture and recovery with free RNA, but trends were similar. A plaque assay confirmed that MIL extraction did not damage the virus capsid. Adjusting MIL volume led to no significant changes in capture or recovery, and reducing elution volume led to a slight increase in recovery, indicating MILs could be used for target enrichment after further optimization. MILs demonstrated favorable affinity for both intact virus and free RNA, and could potentially be optimized for recovery of either target. If combined with a wash and lysis step, this could facilitate development of a one-tube viral concentration and RNA purification method without the need for heavy instrumentation, which would inform future work on portable sample preparation for in-field pathogen detection.

Keywords: Sample preparation; virus detection; magnetic ionic liquids; foodborne viruses

ENHANCED INACTIVATION OF FOODBORNE VIRUSES BY CINNAMALDEHYDE NANOEMULSIONS REQUIRE A LIPID ENVELOPE

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The inability to successfully inactivate viruses can be attributed to their high levels of stability in comparison to bacteria and more specifically in the case of human norovirus as it is a non-enveloped virus, a feature that can make them more stable and more resistant than enveloped viruses. Previous work has demonstrated that restructuring disinfectants into charged nanoemulsions can enhance inactivation of bacteria and fungi, but their effect on viruses is unknown. The purpose of this study was to conduct comparative inactivation studies of cationic cinnamaldehyde nanoemulsions on norovirus surrogate phage MS2 and enveloped viruses such as SARS-CoV-2 surrogate coronavirus 229E. MS2 bacteriophage, and coronavirus-229E were treated with different concentrations of cationic cinnamaldehyde nanoemulsion (5.55 µg/mL and 27.7 µg/mL) by suspension assay. Significantly less reduction of MS2 was observed when treated for 1 hour with cationic cinnamaldehyde nanoemulsion. For instance, 4.02 ± 0.102 PFU/mL and 2.78 ± 0.34 PFU/mL log reductions when treated with 27.7 µg/mL and 5.55 µg/mL of cinnamaldehyde alone, respectively. Whereas 1.54 ± 0.08 PFU/mL log reduction with 27.7 µg/mL and no reduction with 5.55µg/mL of cinnamaldehyde in nanoemulsion was observed. Alternatively, significant reduction of coronavirus-229E was observed with treatment of cinnamaldehyde nanoemulsions, 6 log reduction was observed with 27.7 µg/mL concentration for a treatment time of just 1 minute. For a lower concentration of about 5.55 µg/mL there was a log reduction of 2±0.3 PFU/ml after treatment of 5 minutes. These data suggest that one potential reason for the reduced efficacy against MS2 bacteriophage in comparison to coronavirus-229E could be the high proportion of carrier oil and nonionic surfactant relative to cinnamaldehyde required for spontaneous emulsification, which resulted in sequestering of the cinnamaldehyde. Further, it may simply be that the nanoemulsions are too lipophilic for nonenveloped viruses regardless of the presence of hydrophobic patches on the viral capsid protein, and thus only offer benefit with enveloped viruses.

Keywords: antiviral; bioactive phytocompounds; SARS-CoV-2; nanoemulsion.

DETECTION AND DIVERSITY OF ENTERIC VIRUSES CAUSING HUMAN GASTROENTERITIS IN WASTEWATER IN CARACAS, VENEZUELA, 2021-2022

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Enteric viruses are the main gastroenteritis causal agents among infants and young children, especially in developing countries. Very limited information is available about the epidemiological burden of gastrointestinal viruses in Venezuela. Such viruses may be present in domestic effluents, as they generally originate from human faeces. Wastewater samples from 6-8 urbanized areas from Caracas were collected monthly directly from domestic effluent discharge points for 12 months (October 2021-October 2022). Virus concentration was accomplished by polyethylene glycol/sodium chloride precipitation. Viral DNA/RNA was extracted from a total of 91 sample concentrates by commercial spin-column procedure. Two carefully optimized multiplex reverse transcription-PCR formats were developed to reduce the number of assays needed to detect enteric viruses for each sample, one for rotavirus/norovirus/astrovirus and another for enterovirus/klassevirus/cosavirus. Adenovirus and Aichi virus genomes were searched individually by PCR and RT-PCR, respectively. Molecular characterization of the isolates was performed. A total of 47 (51.6%) samples were positive for at least one of the enteric viruses studied, and 13 for two or more. The viral detection rate varied from 25% to 75%, peaking during the 2022 rainy season. Adenovirus was the most frequently detected virus group (47.5%), followed by norovirus (26.2%), klassevirus (9.8%), rotavirus (8.2%), cosavirus (4.9%), astrovirus (1.6%) and enterovirus (1.6%). Aichi virus was not detected. Norovirus was present during the rainy months, and adenovirus all year long. Norovirus/adenovirus co-detection was the most frequent. Type F-41 prevailed (64.3%) among the human adenovirus of species F. Evidence of virus occurrence by rapid screening in wastewaters allows for setting an early warning of epidemics that need to be adequately addressed to avoid community transmission and track the environmental persistence of a public health risk. A wastewater-based epidemiological investigation should be adopted as a complementary strategy to know the burden of gastrointestinal disease in the population.

Keywords: Gastroenteritis viruses, Wastewater-based epidemiology, molecular characterization, community transmission

DEVELOPMENTS IN DETECTION AND CONTROL OF HIGHLY TRANSMISSIBLE VIRAL PATHOGENS

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Highly transmissible animal viruses continue to exact a considerable public health burden globally. Human noroviruses are the leading cause of foodborne illnesses in the U.S, and SARS-CoV-2 continues to disrupt food production and service by infecting workers. Being able to portably detect such viruses is critical to stopping their spread. Our lab is investigating multiple promising rapid viral concentration and detection methods, including: a novel upstream virus concentration method for effectively and cheaply concentrating low numbers of viruses from food and environmental samples using bacteria and other novel reagents; and a portable nanopore-based sensing platform with potential to rapidly detect and subtype pathogens. With this work, we have observed the ability to capture viruses with bacteria achieving capture efficiencies higher than 75%, and have demonstrated proof-of-concept that nanopore sensing is capable of sensitively discriminating subtypes of a common target both in isolation and in mixture with high confidence. In addition to detection, the ability to develop better inactivation agents is also crucial to stopping viral spread. We are currently working to improve control of these viruses by restructuring and delivering antiviral natural disinfectants in a number of ways to improve their efficacy both on virus and in feasibility of application. With this work, we observe inactivation of norovirus by greater than 2 logs using nature-derived compounds in a system that leaves little residual active ingredient and is safe for use in the presence of humans and food contact surfaces. These results suggest promising technologies for controlling the spread of highly transmissible eukaryotic viruses that can be transmitted through foods and/or food production workers.

Keywords: Norovirus, inactivation, detection, nanopore

COMPARATIVE STUDY OF INNATE IMMUNE GENE LANDSCAPE IN BAT CELLS

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Bats are natural reservoirs for numerous emerging zoonotic viruses, including the potential ancestor of SARS-CoV-2. Immune features facilitating antiviral responses and tolerance towards viral infections are believed to enable bats to harbor viruses without pathogenesis. The vast species diversity of bats is, however, often underestimated and findings generalized. Mapping innate immune effectors in different bats may thus provide a broader understanding of their ability to sustain viral infection. Bat cell lines belonging to six species (*Eptesicus fuscus, Eidolon helvum, Myotis myotis, Nyctalus 69lcyone, Rhinolophus 69lcyone* and *Rhinolophus ferrumequinum*) were stimulated with a synthetic dsRNA ligand to activate innate immune pathways. RT-qPCR analysis of conserved mammalian immune genes confirmed that all cells responded to stimulation. A comparative polyA+ RNA-Seq transcriptomic study of stimulated bat cells, as well as one human cell line for comparison, was then performed. Hundreds of differentially expressed genes (DEGs) were identified in each cell line. Several bat DEGs were not previously reported as innate immune genes in humans or other vertebrate species and may thus be bat-specific immune genes. RT-qPCR analyses to validate the levels of expression of promising DEGs across all stimulated bat cells were performed. Loss-of-function studies to decipher the role of these genes in bat cells infected with bat flaviviruses and bat coronaviruses are on-going. These newly identified genes represent promising targets to decipher bat-specific immune mechanisms and might be potential antiviral effectors. This study reveals the diversity of innate immune mechanisms in place in bat cells and opens up new lines of research to characterize the molecular mechanisms by which bat cells control viral replication.

Keywords: Bat, innate immunity, interferon stimulated gene, transcriptomic

SENSITIVE AND NOVEL DIAGNOSTICS KITS DEVELOPMENT FOR BANANA VIRUSES FOR ENSURING QUALITY BANANA PLANTS TO ENHANCE PRODUCTION IN INDIA

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Banana is significantly contributing to food security and the basis of livelihood of millions of people. India is the largest producer of banana in the world. In banana and plantains, banana bunchy top virus (BBTV), banana streak MY virus (BSMYV), cucumber mosaic virus (CMV), and banana bract mosaic virus (BBrMV) are reported to cause yield loss amounting to 50-100 million US$ in India. The use of virus-free quality planting material is the only option for clonally propagated crops like banana. In this study, an attempt was made to develop accurate, sensitive, specific, low cost and simple diagnostic for banana viruses to use for certification of virus-free tissue culture (TC) banana plants. The coat protein gene of BBrMV, CMV, and viral associated protein gene of BSMYV were cloned and expressed in a bacterial system. The fusion proteins were used to raise polyclonal and monoclonal antibodies. For BBrMV and CMV, serological tests like DAC-ELISA, TAS-ELISA and lateral-flow Immunoassay methods were developed following standard protocols. For BBTV and BSMYV, novel primers targeting the viral genomes using different versions of polymerase chain reaction (PCR) were developed following the standard protocols. The diagnostic kits developed in this study were highly sensitive and very specific In detection and the results could be obtained within 6-7 hrs. This TAS- ELISA kit developed for BBrMV and CMV are highly sensitive than DAC-ELISA kits. For BBTV, the primers developed targeting the DNA-N component was more sensitive than others. These ELISA and PCR based diagnostic kits are validated by testing 1.93 lakh tissue culture samples from tissue culture industries under the certification program. The kits developed in this study were highly sensitive, specific, and useful in certifying the TC banana in India. We certified a total of 295.98 million TC plants under NCS-TCP system being operative in India.

Keywords: Banana; Diagnostics; ELISA; Certification

Animal Viruses

STRUCTURAL STUDIES OF FLAVIVIRUS – ANTIBODY INTERACTIONS

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We have been studying flavivirus – antibody complexes using cryo-electron microscopy. Extensive analyses of the assembly and maturation of the virus particles has previously been described although the dynamic properties of these viruses remain to be completely described. A comprehensive analysis of the structural landscape of the humoral immune response against flaviviruses is crucial for understanding the role of antibodies in controlling virus infection. The structures of several new flavivirus – antibody complexes will be shown and interpreted. Antibodies against flaviviruses have been shown to have multiple diverse interactions and have revealed multiple mechanisms for antibody-induced virus neutralization. The presentation will compare multiple flaviviruses and their antibody complexes to demonstrate mechanisms of action with some common and some unique binding modes. This fundamental knowledge of antibody-mediated neutralization may be useful in the design of immunogens for future vaccines. Importantly, parallel studies of alphavirus – antibody interactions are providing additional insights that collectively will aid in the understanding of antibody-mediated virus control.

Keywords: antibodies, cryo-electron microscopy, flavivirus, viruses

BASIC VIROLOGICAL AND TRANSLATIONAL DIAGNOSTICS AND VACCINOLOGY RESEARCHS LED TO ERADICATION OF RINDERPEST AND CONTROL OF MANY OTHER DISEASES IN INDIA

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The microbiology researches in India started with the establishment of Imperial Bacteriology Laboratory (IBL) in 1889 at Pune. Initially, the focus of the IBL was Trypanosomosis in equines, Tuberculosis, and rinderpest. However, large-scale cattle deaths prompted shift in the strategy and the researches were diverted towards rinderpest with full focus to develop immunotherapy regimens. Wide scale of immunotherapy protocols were developed in early 1900s and practiced in field with part success. In later years, the Goat Tissue Vaccine (GTV) for Rinderpest was developed in 1927. This vaccine was used for national Rinderpest control programme till early 1960s which was then replaced with live attenuated "Tissue Culture Rinderpest Vaccine (TCRV) which ultimately led to global eradication of Rinderpest in 2011. Thus, rinderpest became the first animal disease to have been eradicated next to smallpox – a disease of humans. The presentation will give elaborate details on basic virological and translational virological work including researches on immunotherapy protocol, vaccine development, and understanding the disease pathobiology. Presentation will further throw light on establishment of necessary infrastructure for research, teaching and training of Virology (including microbiology), developing Acts and Regulations, handling many animal disease epidemics like African Horse sickness (ANS) Equine Infectious Anaemia (EIA), Equine Influenza (EI) for controlling and eradicating them through emergency development of diagnostics and vaccines and using them in field.

Keywords: rinderpest virus, animal diseases, vaccine, virus eradication

EXPERIMENTAL AND "NATURAL" INFECTION OF SHEEP AND MOSQUITOES WITH RIFT VALLEY FEVER PHLEBOVIRUS

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Rift Valley fever phlebovirus (RVFV), a mosquito-borne enveloped RNA virus, can cause severe illness in ungulates and humans. Sheep were infected with RVFV by inoculation or mosquito transmission then analyzed for viral variants by next-generation sequencing (NGS). Experimental infection, sheep were needle-inoculated with the RVFV Kenya-06 strain. "Naturally" infected by allowing Kenya-06 blood fed *Culex tarsalis* mosquitoes to feed on the sheep. Transmission to *Culex* species was investigated by allowing naïve mosquitoes to feed on infected sheep at the peak of viremia. Clinical signs and body temperature were recorded daily, and various clinical samples and tissues were collected. Virus positive samples, such as the inoculum, serum, and mosquitoes, were sequenced using NGS and then analyzed for viral quasispecies. Our results showed that 100% of the experimentally infected sheep and 50% of the "naturally" infected sheep were productively infected and developed clinical signs of Rift Valley fever. Experimental infection of mosquitoes resulted in an average infection rate of 34%. Naïve mosquitoes that were fed on RVFV-infected sheep had average infection rates of approximately 40%. A comparative analysis of viral variants in experimental and "natural" infected sheep and mosquitoes showed significant differences in variant diversity and frequency in "natural" infected sheep when compared to the experimental infected mosquitoes.

Keywords: RVFV, mosquito, sheep, quasispecies

RIFT VALLEY FEVER VACCINES: CHARACTERIZATION OF THE NEXT GENERATION MP-12 LIVE-ATTENUATED VACCINE, RVAX-1

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Rift Valley fever (RVF) is a virus transmitted by mosquitoes that poses a significant public health and economic threat to humans and ruminants. The virus causes severe symptoms such as hemorrhagic fever, encephalitis, and retinitis in humans, while livestock experience high rates of spontaneous abortion during outbreaks. The RVF endemic region has expanded from eastern Africa to sub-Saharan Africa, the Middle East, and Indian Ocean islands since the first reported outbreak in Kenya in the early 20th century. The RVFV virus belongs to the family Phenuiviridae of the order Bunyavirales and has a tripartite negative-stranded or ambi-sense RNA genome named Large (L), Medium (M), and Small (S) segments. Vaccination is likely the only effective approach to preventing future RVF outbreaks. The live-attenuated MP-12 vaccine has been conditionally licensed for veterinary use in the U.S. and tested for immunogenicity and safety in phase 1 and 2 clinical trials. However, the MP-12 strain is attenuated mainly via the M segment, while still retaining the capability to disseminate in mosquito vectors. To address these limitations, the next generation MP-12 vaccine candidate, RVax-1, has been generated. RVax-1 was designed to retain the strong immunogenicity of the original MP-12 vaccine while abolishing mosquito-borne vaccine virus transmission by deleting the 78kD gene. It confers the attenuation phenotype to reassortant RVFV encoding RVax-1 RNA segment(s) and encodes unique genetic markers to distinguish vaccine strains from wildtype RVFV strains. Further characterization of RVax-1 candidate vaccine for safety and immunogenicity will lead to the development of an effective RVF vaccine for humans and animals.

Keywords: Rift Valley fever virus, Live-attenuated vaccine, MP-12; RVax-1

IMPLICATIONS FOR FLAVIVIRUS NUCLEOCAPSID CORE-ENVELOPE GLYCOPROTEIN INTERACTIONS

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Although flaviviruses have been widely studied for several decades, the mechanisms by which the core and envelope of these viruses assemble to form infectious virions remain poorly understood. Virus assembly is an essential step in the life cycle of flaviviruses and represents a target for antiviral therapeutics. Single particle Cryo-EM reconstruction has shown that the nucleocapsid core (NC) of immature Zika virus (ZIKV) is found in proximity with the envelope glycoproteins on the inner side of the virus's lipid bilayer. We hypothesized that the capsid proteins (CP) interact with the envelope glycoproteins during virus assembly via interactions between the CP of the NC and transmembrane helices of the precursor membrane (prM) protein or the envelope (E) protein. Two strategies to identify NC-prM/E interactions are being explored using pseudo-infectious ZIKV reporter virus particles (RVPs), infectious ZIKV and dengue virus serotype 2 (DENV2) molecular clones. Firstly, amino acids within the prM/E transmembrane helices posited to be key in the viral assembly process were mutated in all three systems to investigate their role in promoting particle assembly. The ability of these mutants to form infectious particles was experimentally determined using viral plaque assays, ELISA assays, fluorescent cell imaging, and qRT-PCR. Secondly, the ability of the prM and E transmembrane helices to interact with CP is being examined using reconstituted prM and E proteins within styrene-maleic acid lipid nanoparticles. Our results identified mutations within the prM and E protein transmembrane helices that disrupt infectious flavivirus particle formation. However, the molecular mechanism for the reduction in particle infectivity remains under study. Due to the high structural similarity among flaviviruses, the techniques, results and mechanisms identified in this study have the potential to be applied to other flavivirus infections.

Keywords: Flavivirus, assembly, Zika, Dengue

COMPARATIVE PATHOLOGY OF SARS-COV-2 INFECTION IN DOMESTIC/WILD ANIMALS AND LABORATORY RODENTS

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Animal models are crucial to enhance our understanding of SARS-CoV-2 pathogenesis and assessing therapeutics/vaccines. Since 2020, we have characterized histological changes, virus tropism and entry factors (ACE2 [virus receptor] and TMPRSS2 [protease]) in various animal models including cats, pigs, ruminants (white-tailed deer [WTD], sheep), and laboratory rodents (humanized ACE2 and wild-type mice, and Syrian hamsters). These exhibit variable susceptibility to intranasal SARS-CoV-2 infection, and remarkable differences in virus tropism and pathological changes. Cats and WTD were highly susceptible, efficiently transmitting virus to sentinels. Cats displayed neutrophilic/erosive rhinitis and lymphohistiocytic tracheobronchoadenitis with viral RNA/antigen within nasal and gland epithelia, and hyperplasia of bronchus-associated lymphoid tissue but no viral RNA/antigen after re-infection. SARS-CoV-2 showed no tropism for respiratory epithelia elsewhere and did not induce pneumonia, despite abundant ACE2/TMPRSS2 epithelial co-expression. WTD developed lymphohistiocytic/neutrophilic rhinitis, erosive/suppurative tracheitis/bronchitis and peribronchiolitis, with viral antigen in respiratory epithelia particularly within sites of high ACE2/TMPRSS2 co-expression. Sheep showed low susceptibility with little pathological alterations and low viral antigen in mucosal-associated lymphoid tissue. Pigs were not susceptible; no histological changes were noted. Regarding laboratory rodents, K18-hACE2 mice developed interstitial pneumonia with various SARS-CoV-2 strains; interestingly, despite high bronchiolar expression of human ACE2, tropism for bronchiolar epithelia was a feature of mouse-adapted or N501Y-bearing SARS-CoV-2 but not ancestral Wuhan-like strains. Moreover, mouse-adapted strains induced higher viral load, pneumonia, and lethality in K18-hACE2 compared to C57BL6/J and BALB/c mice. Infection with Beta and Delta VOCs resulted in airway tropism with prominent pneumocyte hyperplasia. Syrian hamsters developed necrotizing bronchointerstitial pneumonia with viral spread from bronchiolar to alveolar epithelia followed by exuberant reparative responses with bronchiolar and alveolar hyperplasia. In summary, SARS-CoV-2 pathology and tropism vary across the animal models studied by our group; these models can be exploited in a complementary manner to understand viral pathogenesis, ecology/transmission, and evaluate therapeutic/vaccine candidates.

Keywords: Animal models, SARS-CoV-2, pathology, tropism

ACQUISITION OF NORTH AMERICAN TICK-BORNE BANDAVIRUS AND FLAVIVIRUS BY INVASIVE HAEMAPHYSALIS LONGICORNIS DURING TICK CO-FEEDING

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The Asian Longhorned Tick, *Haemaphysalis longicornis*, is an invasive species from East Asia that recently established populations in 18 states in the United States and continues to expand its geographic range. In its native range, *H. longicornis* is the main vector of Dabie bandavirus, which is genetically closely related to Heartland virus (HRTV), an emerging North American tick-borne bandavirus. The other emerging tick-borne virus in North American is Powassan virus (POWV), a tick-borne flavivirus. Results from our previous studies suggest that the invasive *H. longicornis* tick is a competent vector for both HRTV and POWV. In the United States, invasive *H. longicornis* have been repeatedly detected feeding simultaneously on the same vertebrate hosts as two native tick species, *Amblyomma americanum* and *Ixodes scapularis*, which are the main vectors for HRTV and POWV, respectively. Therefore, interspecies co-feeding could serve as a mechanism for invasive *H. longicornis* to acquire and transmit North American tick-borne viruses. Using our *in vivo* tick transmission models, we assessed whether *H. longicornis* larvae and nymphs are capable of acquiring HRTV or POWV when they are co-fed on the same host as infected *A. americanum* or *I. scapularis*, respectively. HRTV and POWV RNA was detected in engorged *H. longicornis* larvae and nymphs that had co-fed with the HRTV or POWV-infected native ticks. Interestingly, *H. longicornis* co-feeding acquisition of HRTV occurs in the absence of host viremia, while *H. longicornis* co-feeding acquisition of POWV occurs on both viremic and non-viremic hosts. These findings demonstrate that interspecies tick co-feeding transmission of HRTV and POWV occurs, even in the absence of host viremia. Due to this mode of virus transmission, HRTV and POWV infection rates in invasive *H. longicornis* ticks and in native ticks could increase, thus raising the risk of human infection.

Keywords: tick-borne virus, bandavirus, flavivirus, tick co-feeding

BAT SADS-RELATED CORONAVIRUSES SHOW POTENTIAL RISK OF CROSS-SPECIES INFECTION

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The bat-origin swine acute diarrhea syndrome coronavirus (SADS-CoV) has a wide tissue tropism in different host, including primary human cells, thus posing a significant risk of cross-species spillover. A large diversity of SADS-related CoVs (SADSr-CoVs) circulating in *Rhinolophus* bats have been identified in China and Southeast Asia since the outbreak of SADS-CoV in 2016. However, none of these bat SADSr-CoVs have been successfully isolated *in vitro*, and their functional receptor has not been identified, which makes it difficult to evaluate their risk of cross-species transmission and further develop effective medical interventions to control potential spillovers. To understand the genetic diversity of bat SADSr-CoVs, we amplified, analyzed and classified genes encoding the spike (S) protein of a number of bat SADSr-CoVs. Using one-step assembly of viral cDNA clones by transformation-associated recombination in yeast, we rescued synthetic SADS-CoV and chimeric SADS-CoVs each expressing a S derived from these bat SADSr-CoVs. We performed *in vitro* infections in various cell lines and organoids, as well as challenge experiments in suckling mice, to access their ability of causing cross-species infection and pathogenicity. The S protein of SADSr-CoVs can be divided into four genotypes (clade1-4) according to their variations at the N-terminal and C-terminal amino acid sequences. Wild-type SADS-CoV and Chimeric SADS-CoVs expressing S of clade1-4 were all successfully rescued in Huh7 cells. Strikingly, almost all of the recombinant SADSr-CoVs were able to replicate efficiently in intestinal and respiratory cell lines as well as organoids derived from human and swine. Moreover, mortalities and tissue damage of different degrees were observed after intragastrical infection of these recombinant SADSr-CoVs. Our results show that SADSr-CoVs circulating in *Rhinolophus* bats pose potential risk of cross-species infection and causing severe illness.

Keywords: SADS-related coronavirus; Spike; Risk assessment; Pathogenicity

WEST NILE VIRUS, A MISSED CAUSE OF ACUTE FEVER OF UNKNOWN CAUSE AND NEUROLOGICAL INFECTIONS AMONG HOSPITALIZED PATIENTS THROUGH THE COVID19 PANDEMIC IN SOUTH AFRICA

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West Nile virus (WNV) is endemic to South Africa and an important emerging arbovirus in the Northern hemisphere. Human and animal cases are diagnosed annually and associated with lineage 2 WNV in South Africa. The incidence in hospitalized patients with acute fever of unknown cause with or without neurological signs remains unknown in Africa. During the COVID19 pandemic, etiologies of fever of unknown cause (AFDUC) in hospitals in Africa and elsewhere other than COVID were neglected, especially due to arboviruses. To define the contribution of WNV to neurological and fever cases in hospitals in South Africa before and during the COVID19 pandemic we investigated actively enrolled AFDUC cases with or without neurological signs from 3 hospitals in 2 provinces in South Africa (2019-2021). Blood, serum and CSF samples were randomly selected through the arbovirus season (January-June,2019-2021) from patients enrolled in 3 hospitals with AFDUC with or without neurological signs in hospitals in the Gauteng and Mpumalanga Provinces, South Africa. Samples were screened with a flavivirus real-time RT-PCR and WNV IgM ELISA followed by serum neutralization, sequencing and phylogenetic analysis. WNV was identified in 40/441 (9,07%) AFDUC patients, 72.50% with neurological signs (2019-2021). All cases identified through IgM serology were confirmed to have serum neutralizing antibodies to WNV. This consisted of 16/187 (8,55%) in 2019, 12/144 (8,33%) in 2020 and 12/110 (10,90%) (2021). Cases peaked in January-March. Immunocompetent females, adolescents, and residents of the Mpumalanga province had a higher likelihood for WNV infection compared to WNV negative cases. Neurological signs were present in 72% of cases. This study suggests that WNV contributed significantly to AFDUC and neurological infections in hospitals through the pandemic during late summer and autumn months in South Africa. The disease burden associated with arboviruses may have been largely missed through the pandemic in Africa.

Keywords: Arboviruses, pandemic, West Nile, under reported

IMPORTANCE OF HOST FACTORS DURING ALPHAVIRUS REPLICATION

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All positive-strand RNA viruses (viruses whose genome acts directly as mRNA) replicate their genome in association with cytoplasmic membranes. This vast group of viruses includes many important pathogens, e.g. dengue, Zika, chikungunya, rhinoviruses and coronaviruses. These viruses induce extensive alterations of the membranes, hijacking them for the purpose of replication. Alphaviruses encode only four replication proteins, the nonstructural proteins nsP1-nsP4. We have carried out proteomic analyses of host proteins interacting with alphavirus nsP1-nsP3 during infection, to identify host cell components involved in membrane alteration and virus replication. siRNA transfection followed by virus infection was used to study the importance of the identified proteins. Altogether, our dataset includes 720 host proteins, of which 109 are partners with all the three alphavirus replication proteins analyzed, and additional 152 proteins are partners with two nsPs. Associated with nsP3, we find the membrane-bending protein amphiphysin-2 (BIN1), the adapter protein CD2AP and the stress granule protein G3BP, as well as additional membrane-active proteins and proteins of unknown function. Associated with nsP1 and nsP2 we find multiple novel proteins not connected with virus replication previously, surprisingly including several putatively nuclear proteins involved in RNA processing. We have verified with siRNA that several of the identified proteins are proviral (siRNA inhibits virus replication), but only few are antiviral. The proviral proteins include, surprisingly, the major cellular mRNA exoribonuclease XRN1. With the host protein knock-downs and knock-outs, we are conducting mechanistic assays of spherule formation and RNA replication to understand their precise functional and structural roles. Conclusions: We expect that multiple host proteins are required to facilitate membrane remodeling during virus infection. The essential host proteins identified can provide novel and possibly more general targets for antiviral drug development, and thus we are testing known inhibitors of the interacting proteins and pathways.

Keywords: alphavirus; host factor; proteomics; antiviral

IMMUNOGENICITY OF A RECOMBINANT H2 HEMAGGLUTININ (HA) PROTEIN FROM AVIAN INFLUENZA VIRUS H2N6 ISOLATED IN PERU

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Influenza virus is the most important avian pathogen and represents a major concern for animal and public health. As hemagglutinin (HA) is a potential candidate for control and prevention, we employed a baculovirus-based system to produce the recombinant H2 HA protein using the genetic information of an avian Influenza A virus H2N6 obtained as part of an Influenza surveillance program in Peru. We selected this isolate due to the unique amino acid changes identified in this gene compared to its closest relatives described to date. Hence, we produced the recombinant H2 HA protein in mg quantities, using a baculovirus expression vector system (BEVS). We tested the activation of innate immune responses using an intra-abdominal inoculation model in three-week-old broiler chickens. In brief, we identified multiple parameters of macrophage activation, such as reactive oxygen species and nitric oxide production. Moreover, a large proportion of infiltrating leukocytes were detected 24 hours post inoculation, which were mainly composed of heterophils. Following 72 hours of inoculation, most parameters were returning to the homeostatic levels. Our results show that the recombinant baculovirus-expressed H2 protein has an immunogenic capacity to promote a self-resolving immune response. Thus, this recombinant protein can be used as a potential candidate for vaccine development. Further studies are required to assess whether these responses develop into long-term protective responses.

Keywords: avian influenza; recombinant H2; baculovirus expression; innate immune response

CHARACTERIZATION OF FLAVIVIRUS STRUCTURE AND INFECTIVTY AFTER NON-IONIC DETERGENT TREATMENT

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Flaviviruses are enveloped vector borne RNA viruses such as dengue virus, that infect over 400 million people each year. There are limited vaccines that confer protection against flaviviruses, making new vaccine strategies imperative to combat emerging flavivirus related disease. The assembly and structure of flaviviruses are intensely studied to determine therapeutic targets with the majority focusing on structural proteins with little attention on the lipid membrane. Flaviviruses rely on host cell membranes to form a lipid envelope for encapsulation of the nucleocapsid and integration of structural glycoproteins Membrane (M) and Envelope (E). We hypothesized that treatment of infectious virus particles with nonionic detergents would disrupt the lipid membrane without altering the structural conformation of the immunodominant protein, E. Kunjin virus (KUNV) and Zika virus (ZIKV) were treated with 10-fold dilutions of Tween 20 and analyzed using infectivity assays, qRT-PCR, Western Blotting, and Cryo-Electron Microscopy (CryoEM). Our results demonstrated a significant reduction in viral titer after Tween 20 treatment. Moreover, titer reduction was exacerbated by detergent treatment at 37°C. CryoEM comparison of treated vs. untreated particles showed that treated particles displayed highly distorted morphologies. Two general structures dominated the Tween-treated particles: a smaller round structure approximately 30nm wide lacking inner density, and a 50nm structure with large protrusions. Western blot results suggest that the observed lack of inner density corresponds to an absence of capsid protein. Quantitative RT-PCR has further shown that while genome is still present within the less infectious treated samples, they display a two-log reduction of RNA when compared to untreated samples. The two observed morphologies suggest the formation of two distinction viral particle populations. These results indicate that changing the native composition of the lipid membrane impairs flavivirus infectivity, making membrane focused inactivation a novel strategy for vaccine development.

Keywords: Flavivirus; Vaccines; Membrane; Cryo-Electron Microscopy

ASSESSING THE ZOONOTIC POTENTIAL OF BAT INFLUENZA-A LIKE VIRUSES

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Bats are notorious reservoirs of diverse, potentially zoonotic viruses, exemplified by the evolutionarily distinct, influenza A-like viruses H17N10 and H18N11 (BatIVs). Unlike classical influenza A viruses (IAVs), the surface glycoproteins [haemagglutinin (H) and neuraminidase (N)] of BatIVs neither bind nor cleave sialic acid receptors, which suggests that these viruses employ unconventional cell entry mechanisms. Identifying the cellular factors that mediate entry and determine susceptibility to infection will help assess the potential host range of BatIVs. We investigated a range of cell lines from different species for their susceptibility to pseudotype viruses bearing bat H17 and/or N10 envelope proteins. A transcriptomic comparative analysis between susceptible and unsusceptible cells coupled with overexpression/knock-down studies identified that a human MHC class-II surface receptor influences the host susceptibility range for H17-pseudotypes and is an essential mediator of H17 entry into target cells. So far, bats in Central and S. America, but not in Central Europe, have been found seropositive for H17N10. We recently identified H17-seropositive samples in non-Neotropical *Eidolon Helvum* bat species raising questions about the geographical range of BatIVs. The scientific evidence so far indicates a limited spillover risk for BatIVs, but data is not conclusive enough to dismiss entirely the possibility of zoonotic transmission.

Keywords: bat viruses, influenza virus, Major Histocompatibility Complex (MHC) class II, zoonosis

EMERGENCE OF AFRICAN SWINE FEVER VIRUS: EPIDEMIOLOGY, DIAGNOSTICS AND VACCINE SITUATION UPDATES FROM INDIA

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African swine fever (ASF) is a deadly viral disease of swine, caused by ASF virus (ASFv), a double stranded DNA virus of *Asfarviridae* family. It has recently emerged as a threat to Indian pig industry. As per the Indian livestock census (2019), there are 9.06 million pigs in the country which are at potential risk by ASFv. Since, its first appearance in the North-Eastern states of India *i.e.,* Assam and Arunachal Pradesh states in early to mid 2020, the disease has been reported from almost 15 Indian states during mid 2022, especially, to the Northern states like Uttarakhand, Punjab and Haryana as well as Jharkhand, Bihar, Madhya Pradesh and the southern state Kerala. It is being reported in domestic pigs and wild boars. Research initiatives have been taken in India on indigenous diagnostics for diagnosis of ASF. In the line, probe-based diagnostics have been developed to test for ASFv. Assays detect structural genes (singleplex assay) and simultaneously structural gene plus non-structural genes (duplex assay) for increased sensitivity and accuracy of diagnosis. The probe-based assays detect the Asian genotypes, probe-based and produce results in short span of time. In addition, a polymerase spiral reaction (PSR) based assay has been developed for early diagnosis of the ailment. Apart from the above nucleic acid-based diagnostics, the serological diagnostics i.e., p30 and p54 recombinant protein based ELISAs have also been developed. Similarly, as there are no appropriate commercial vaccines available at present, research has also been initiated for development of viral vector-based vaccine platforms. The fowl pox and Newcastle disease-based vectors are being explored for incorporation of ASF virus genome. However, safety efficacy and potency of the vaccine through the clinical trials are yet to be explored for the vaccine.

Key words: African swine fever, epidemiology, diagnostics, emerging, India

DEVELOPMENT OF REVERSE GENETIC SYSTEM AND INFECTIOUS MOUSE MODEL FOR AKABANE VIRUS

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Akabane virus (AKAV) is classified in the genus *Orthobunyavirus* in the family *Peribunyaviridae.* AKAV leads to reproductive disorders and congenital abnormalities in ruminants. In South Korea, the outbreak of AKAV was first reported in 1980 and a large-scale outbreak of Akabane viral encephalomyelitis in adult cows reported in 2010. The neurotropic AKAV-7/SKR/2010 (AKAV-7) strain was isolated from the brain of the affected cow. However, the mechanism of AKAV-7 infection has hardly been studied. Reverse genetic systems are important tools for understanding pathogenesis of RNA viruses. Herein, we successfully rescued infectious AKAV-7 from full-length cloned cDNAs based on a T7 RNA polymerase-driven plasmid. The rescued AKAV-7 (rAKAV-7) showed cytopathic effects (CPE) in vero cells. Although several AKAV showed mortality in suckling mice, there are no suitable small animal models for pathogenesis studies of AKAV. Adult mice with gene knockouts of the interferon α/βreceptor ($IFNAR^{-/-}$) have been used as a model of arbovirus infections such as Schmallenberg, Rift Valley Fever, Crimean Congo fever and Dengue virus. However, no pathogenesis study of AKAV had been conducted in $IFNAR^{-/-}$ mice. Therefore, we investigated the utility of $IFNAR^{-/-}$ mice as AKAV animal model. Four groups of mice (AKAV-7- C57BL/6, AKAV-7- $IFNAR^{-/-}$, K0505- C57BL/6, K0505- $IFNAR^{-/-}$) were inoculated with 100 μℓ of 10^6 $TCID_{50}$/ml by intraperitoneal routes. AKAV-7- C57BL/6, K0505- C57BL/6 and K0505- $IFNAR^{-/-}$ mice showed no significant clinical sings and mortality. However, AKAV-7- $IFNAR^{-/-}$ mice showed steady weight loss, anorexia, depressed, ruffled fur, hunched posture and 100% mortality on 3dpi, suggesting $IFNAR^{-/-}$ mice are susceptible to AKAV-7 infection. Our results can improve the studies of AKAV pathogenesis and developing of antivirals and vaccines against AKAV infection.

Keywords: Akabane virus; $IFNAR^{-/-}$ mice; reverse genetics; Infectious clone

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FIRST DETECTION AND GENETIC CHARACTERIZATION OF NEUROTROPIC ASTROVIRUSES IN BRAIN TISSUE OF WILD RACCOON DOGS

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Astroviruses (AstV) infect the gastrointestinal tract of animals and are known to be associated with gastroenteritis. Recently, neurotropic astroviruses (NT-AstVs) have been reported to cause non-suppurative encephalitis and neurological signs in the brains of some mammals. This study investigated AstV infection in raccoon dogs in Korea and the possibility of NT-AstV infection in the brain. A total of 133 wild raccoon dog intestine and brain tissue samples, collected between 2017 and 2019, were tested for AstVs. AstVs were detected by PCR following RNA extraction and cDNA synthesis. In tissue samples from raccoon dogs, six AstVs were detected in the intestine (17-148I, 17-153I, 17-162I, 17-165I, 18-026I, 18-038I) and four AstVs in the brain (17-148B, 17-153B, 17-157B, 18-038B). The capsid protein amino acid (aa) sequences of raccoon dog AstVs detected in Korea were significantly different from those of raccoon dog AstVs identified in China (13.1-59.1%). Blast analysis of the capsid protein aa sequences revealed that most of the raccoon dog AstVs are close to the canine AstVs identified in Hungary (74.9-91.4%) and, in the case of 18-026I, close to the chicken AstV (96.3%). These results show that the raccoon dog AstVs identified in South Korea are similar to canine and chicken AstVs, unlike previously reported raccoon dog AstVs. Phylogenetic analysis revealed that the 17-153B, 17-153I 17-157B, 17-162I, 17-165I, 18-038B and 18-038I AstVs belong to *Mamastrovirus 5*. The 17-148B and 17-148I AstVs belong to the *Unclassified Mamastrovirus* in HMO clade, to which most of NT-AstVs belong. The 18-026I AstV was shown to belong to *Avastrovirus*. These results indicate the potential of different genotypes of AstVs in the raccoon dog. And it shows that the 17-148B AstVs are more likely to be NT-AstVs. This is the first report to identify and molecularly analyze NT-AstVs in raccoon dogs.

Keywords: Astrovirus, neurotropic astrovirus, raccoon dog, brain

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MULTIPLE-LINEGE PORCINE ASTROVIRUS INFECTION OF PORCINE BRAIN IN SOUTH KOREA

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Since 2010, association of neurological symptoms with astroviruses (AstVs) were reported in human, minks, cattle, pigs, sheep, muskoxen and alpacas, sequently. Five genotypes of Porcine astroviruses (PAstVs) have been reported in worldwide, neuro-invasive (Ni) PAstV 3 was detected in the central nervous system (CNS) of pigs with encephalomyelitis in Hungary and the USA in 2017. To investigate the PAstV genotypes and Ni-PAstV in South Korea, fourteen tissue samples including brain and intestine were collected from 5 pigs with neurological symptoms in three farms in 2020. In the type differential multiplex RT-PCR, PAstV 1-4 types were detected and cases of co-infection with two or more PAstV types in single pig was observed. Additional PCR was performed with partial RNA-dependent RNA polymerase (RdRp) gene and the complete capsid protein-coded open reading frame 2 (ORF2) gene. Histopathology revealed meningitis, neuronal vacuolation and gliosis of the CNS. The *in situ* hybridization results showed that PAstV infection occured in the brain tissues of pigs. All PAstV genotypes (1–5) were detected in brain samples of Korean pigs. Phylogenetic analyses were performed, partial RdRp gene showed a minimum of 74.0% nucleotide sequence identity within a single lineage and PAstV 3 showed 67.9%–69.2% identity with the Ni-PAstV 3 reported in the USA and Hungary in 2017 (USA/IA/7023/2017 and NI-Brain/HUN), on BLASTn. ORF2 gene of PAstV 3 in the KOR/1295/2020/brain1 sequence showed the highest amino acid identity (91.8%) with Uganda/U460 (KY933399.1), and 58.7%–59.2% and 59.1%–60.4% identity with Ni-PAstV 3 strains of the USA and Hungary, respectively. To our knowledge, this is the first study to report the identification of a multiple-genotype Ni-PAstV infection in South Korea. Expression of recombinant capsid proteins for ORF2 gene of PAstV 3 and virus isolation are tried to vaccine development.

Keywords: neuro-invasive porcine astroviruses, multiple-lineage
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UNDERSTANDING THE MOLECULAR MECHANISMS OF ATTENUATION IN CODON DEOPTIMIZED FMDV STRAINS

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Codon-pair deoptimization (CPD) of viral RNA genomes can result in the production of viruses with significant reduction in viral fitness. Use of CPD as a strategy to develop live-attenuated vaccine (LAV) candidates against foot-and-mouth disease virus (FMDV) is considered a promising approach to control FMD. We have previously demonstrated that FMDV can tolerate CPD in almost all coding regions of its genome resulting in different degrees of attenuation *in vitro* and *in vivo*. Interestingly, the level of attenuation varies depending on the length, location and even the FMDV strain/serotype. In this study CPD of the capsid region in FMDV serotypes A24 and Asia1 resulted in attenuation in swine. However, vaccine efficacy studies showed that humoral responses were not strong enough to provide protection. We think that CPD of the P1 region in FMDV serotypes A24 and Asia1 resulted in over-attenuation in the natural host of FMDV. In order to understand the underlying molecular mechanisms of attenuation of FMDV CPD strains, we examine the molecular changes induced by CPD in the structural region of FMDV. Our results demonstrate that CPD in FMDV P1 regions resulted in disruption of secondary RNA structures, unintentional increased in CpG dinucleotide frequencies and significant delay in viral expression and inefficient targeting of cellular proteins involved in translation and antiviral responses (i.e., eIF4G, G3BP1/2, PKR). Furthermore, detection of double-stranded RNA as a marker of active replication was severely delayed in CPD viruses when compared to parental strains. Further adjustment of the CPD strategy in FMDV to induce the right level of attenuation will improve the development of successful vaccine candidates that have the ideal balance between attenuation and immunogenicity to provide effective protection.

Keywords: FMD, foreign animal diseases, vaccines, picornavirus

DEVELOPMENT OF A BEAK AND FEATHER DISEASE VIRUS VACCINE CANDIDATE IN N. BENTHAMIANA.

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Psittacine beak and feather disease (PBFD) is a psittacine viral disease caused by the beak and feather disease virus (BFDV): this includes the endangered Cape parrot that is endemic to South Africa. The virus can cause significant physical and immunological damage, resulting in a weakened state that leads to death. BFDV is highly contagious and is transmitted through contact with contaminated faeces, crop secretions, and feather and skin dander. To date, there is no licensed vaccine or cure available for BFDV. Therefore, the development of an effective vaccine is of the utmost importance. The production of an effective vaccine depends on having an expression system and purification platform that are both easy to use and scalable, with the end product eliciting an effective immune response. Thus, the aim of this study was to produce a plant-based BFDV vaccine candidate and to test the immune response in *Coturnix japonica* (quails). To the best of our knowledge, there are no studies that have shown the accumulation of IgY specific to recombinant plant-produced antigens in quail eggs. The BFDV capsid protein (BFDV CP) was transiently expressed in *Nicotiana benthamiana* plants. The recombinantly produced BFDV CP was subsequently purified using a two-step purification method. Western blotting and SDS-PAGE analysis were used to confirm the expression and purity of the BFDV CP. Female quails were inoculated with 10 µg of purified BFDV CP and additional boosters were given on day 14 and 28. Eggs were collected six weeks post-immunization and the yolk-derived IgY was purified by water dilution and salt precipitation. Western blot analysis was used to confirm the specificity of serum and yolk-IgY to BFDV CP. In conclusion, this study demonstrated that plant-produced BFDV CP elicited a significant immune response in quails and that antibodies were successfully detected in the serum and the yolk.

Keywords: BFDV CP, *N. benthamiana*, quails, western blotting, antibodies (IgY).

CONCURRENT ANTIBODY DETECTION IN DROMEDARY CAMELS WITH MIDDLE-EAST RESPIRATORY SYNDROME CORONAVIRUS, SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS -2 AND INFLUENZA A VIRUS, NORTH-WEST NIGERIA

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The Middle-East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus -2 (SARS-CoV-2) and Influenza A virus (IAV) are well-known high-impact zoonotic pathogens responsible for epi- and pandemics with severe public health and animal health consequences. In Nigeria, limited data exist on the epidemiology of the diseases caused by the viruses. In this study, we examined camels at slaughter slabs for possible exposure to multiple infections with selected respiratory viruses. Over a six-month period (May - October 2022), 424 serum samples were collected from dromedary camels (*Camelus dromedarius*) at the Kano abattoir, North-West Nigeria. The sera were screened for MERS-CoV and IAV antibodies using commercially available kits (IgG competition ELISA), while antibodies to SARS-CoV-2 was determined using an in-house indirect ELISA based on the receptor binding domain of the spike gene. Overall detectable antibodies to MERS-CoV, SARS-CoV-2 and IAV are 79.5% (337/424), 3.3% (14/424) and 0.63% (2/320) respectively. Concurrent presence of antibodies against MERS-CoV and SARS-CoV-2 was detected in 2.1% (9/424) of the animals, while antibodies against both MERS-CoV and IAV were detected in 0.94% (3/320) of the camelids sera. None of the camels had antibodies against all three viruses. Here, we report a high seroprevalence for MERS-CoV and, for the first time, concurrent antibody detection for SARS-CoV-2 and IAV with MERS-CoV in Nigeria. Further confirmative serological and virological assays are required to verify infections. Co-infections of different coronaviruses hold the possibility for recombination which may confer possible deleterious properties with dire public health consequences on the virus.

Keywords: Camelids, ELISA, MERS-CoV/SARS-CoV-2/IAV, Nigeria

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