power OF VIRUSES 2024

BOOK OF ABSTRACTS

September 25-28, 2024

Zadar, Croatia

Power of Viruses

PROGRAMME AND ABSTRACTS

September 25–28, 2024
Zadar, Croatia

Publisher

Croatian Microbiological Society

Pierottijeva 6, HR-10000 Zagreb, Croatia

www.hmd-cms.hr

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ISBN 978-953-7778-20-0

Organised and hosted by

Croatian Microbiological Society

Supported by

Federation of European Microbiological Societies (FEMS)

The European Molecular Biology Organization (EMBO)

Organised in cooperation with

Croatian Institute of Public Health

Croatian Veterinary Institute

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Slovenian Microbiological Society

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PRESIDENTS' FOREWORD

Dear Colleagues and Friends,

it is our great honor to welcome you to the second international symposium, *Power of Viruses 2024*, taking place at the hotel Kolovare in Zadar, Croatia, September 25-28, 2024.

The inaugural *Power of Viruses* conference, held in Poreč in 2018, addressed key topics in virology comprehensively. This year, we are once again gathering experts from Croatia and across Europe to discuss a wide range of virus-related issues, exchange knowledge and encourage the ongoing development of virology, with a particular focus on Southeastern Europe. We also aim to foster international collaboration among virologists in diverse fields, including medical, plant and veterinary virology.

Building on the success of our previous meeting, we are committed to strengthening scientific ties among research groups in neighboring countries and beyond. The symposium is organized by the Croatian Microbiological Society in collaboration with the Croatian Society for Clinical Microbiology – Virology Section, the Slovenian Microbiological Society and the Hungarian Society for Microbiology. It is supported by the Federation of European Microbiological Societies (FEMS), the Croatian Institute of Public Health, the Croatian Veterinary Institute and the European Molecular Biology Organization (EMBO).

This year's program features three full days of keynote and invited lectures, oral presentations grouped in eleven sessions, and two poster presentation sessions. We are delighted to welcome renowned international experts as keynote and invited speakers, including some of Croatia's leading researchers in virology. The program also includes ample opportunities for oral presentations of submitted abstracts and the active participation of young researchers.

In the aftermath of the COVID-19 pandemic, we are especially eager to create a welcoming and friendly atmosphere for socializing, exchanging ideas and establishing new connections. The meeting will take place in Zadar, a welcoming city rich in history and natural beauty, providing an ideal backdrop for both scientific discussions and informal networking.

We hope to continue the tradition of *Power of Viruses* as a recurring series of meetings organized by the Croatian Microbiological Society. On behalf of the *Power of Viruses* Organizing Committee, we wish you a productive, enriching symposium and hope you enjoy the scenic charm of Zadar County and the hospitality of Croatia!

Warm regards,

Irena Tabain
President of the Symposium

Roberto Antolović
President of Croatian Microbiological Society

PROGRAMME

Wednesday – September 25, 2024

13.00-14.00		*Registration of participants*
14.00-14.30		*Opening ceremony*
14.30-15.00	KL1	Mart Krupovic (France): A kaleidoscope of archaeal viruses
15.00-15.30	KL2	Santiago F. Elena (Spain): Harnessing the Caenorhabditis elegans/Orsay virus pathosystem as a new model for experimental virus evolution
15.30-16.00		*Coffee break*

SESSION I: Virus-host interactions

CHAIRPERSONS: Beatriz Navarro, Tina Uršič

16.00-16.30	IL1	Beatriz Navarro (Italy): Molecular interplay between viroids and plants
16.30-17.00	IL2	Tina Uršič (Slovenia): Respiratory viruses and the stories behind the scene
17.00-17.15	OP1	Ilana S. Fratty (Israel): The aftermath of COVID-19: Exploring its impacts on influenza patterns in Israel
17.15-17.30	OP2	Marina Prišlin Šimac (Croatia): *In vitro* interaction between canid alphaherpesvirus 1 and canine adipose-derived mesenchymal stem cells: impact on cell gene expression and secretome profile
17.30-17.45	OP3	Ivona Viduka (Croatia): Cytomegalovirus secondary envelopment and its release from infected cells is mediated by Rab15-positive membrane organelle

20.00 *Welcome reception (supported by KEFO)*

Thursday – September 26, 2024

8.00-9.00	*Registration of participants*	

SESSION II: Veterinary virology

CHAIRPERSONS: Lorena Jemeršić, Dragan Brnić

(in parallel with session III)

9.00-9.30	IL3	Lorena Jemeršić (Croatia): A large virus causing a large problem – African swine fever virus
9.30-10.00	IL4	Sandra Blome (Germany): African swine fever vaccination – concepts and status quo (*online*)
10.00-10.15	OP4	Margarita Božiković (Croatia): First detection of porcine lymphotropic herpesviruses 1,2 and 3 in domestic pigs in Croatia
10.15-10.30	OP5	Ivana Giovanna Zupičić (Croatia): Seawater Outbreak of Infectious Haematopoietic Necrosis (IHN) in Rainbow Trout (*Oncorhynchus mykiss*)
10.30-10.45	OP6	Jelena Maksimović Zorić (Serbia): Establishment of the European Swine Influenza Network (ESFLU) for improvement of the One Health perspective

SESSION III: Plant viruses

CHAIRPERSONS: Dijana Škorić, Éva Várallyay

(in parallel with session II)

9.00-9.30	IL5	Éva Várallyay (Hungary): The legacy of classical virologists explored by HTS can lead to new discoveries
9.30-10.00	IL6	Sebastien Massart (Belgium): "Into the wild": from plant virus ecology in wild ecosystems to plant virus epidemics in crops
10.00-10.15	OP7	Dijana Škorić (Croatia): Discovery of a previously unknown virus in ancient olives remains
10.15-10.30	OP8	Lana Vogrinec (Slovenia): High-throughput sequencing reveals hidden viral diversity in aquatic plants
10.30-10.45	OP9	Karima Ben Mansour (Czech Republic): Where do viruses come from, and when? Case studies of watermelon mosaic virus and grapevine Pinot Gris virus
10.45-11.00	*Coffee break*	

SESSION IV: Immune response to virus infection

CHAIRPERSONS: Vanda Juranić Lisnić, Sanda Ravlić

(in parallel with session V)

11.00-11.30	IL7	Vanda Juranić Lisnić (Croatia): Pathogenesis of cytomegalovirus infection in ovaries and adrenal glands
11.30-12.00	IL8	Sanda Ravlić (Croatia): Understanding differences in immune status between COVID-19 survivors and vaccinated individuals
12.00-12.15	OP10	Maja Bogdanić (Croatia): Seroepidemiology of cytomegalovirus infections in childbearing-aged and pregnant women in Croatia
12.15-12.30	OP11	Jasmina Kučinar (Croatia): Prevalence of herpes simplex virus type 1 and type 2 antibodies in risk populations, Istria County, 2020-2023

SESSION V: Environmental virology

CHAIRPERSONS: Sebastien Massart, Denis Kutnjak

(in parallel with session IV)

11.00-11.30	IL9	Eeva Vainio (Finland): Evolutionary links and lifestyle of mycoviruses in forest ecosystems (*online*)
11.30-12.00	IL10	Denis Kutnjak (Slovenia): Assessing virome diversity across ecosystems: from plants to water samples
12.00-12.15	OP12	Anna S. Speranskaya (Russia): Metavirome analysis of oral and faecal swabs from hedgehogs in central Russia: Coronaviruses, Mammarenaviruses and differences in virus composition in active and hibernating hedgehogs
12.15-12.30	OP13	Dorotea Grbin (Croatia): First virome analysis of severely symptomatic zucchini in Croatia reveals high prevalence and diversity of potyviruses

12.30-14.00 *Lunch break (optional, not included in registration fee)*

POSTER SESSION I

HOSTED BY: Marina Drčelić

14.00-15.30	Poster session
14.00-14.15	Poster teaser

17.00-20.00 *Social event-excursion*

Friday – September 27, 2024

SESSION VI: Emerging and neglected viruses

CHAIRPERSONS: Tatjana Vilibić Čavlek, Pavle Jeličić

(in parallel with session VIII)

9.00-9.30	IL11	Tatjana Vilibić Čavlek (Croatia): Emerging trends of arbovirus infections in Croatia
9.30-9.45	OP14	Dario Sabadi (Croatia): Clinical characteristics of neuroinvasive flavivirus infections in Croatia
9.45-10.00	OP15	Barbara Igriczi (Hungary): First report of porcine parvovirus 8 in Europe: widespread detection and genetic characterization on commercial pig farms in Hungary and Slovakia
10.00-10.15	OP16	Pavle Jeličić (Croatia): Epidemiology of hepatitis E virus in Croatia
10.15-10.30	OP17	Iva Pem Novosel (Croatia): Assessment of West Nile virus transmission risk in Croatia
10.30-10.45	SP1	Golden sponsor presentation – *Altium* Ines Topalović Piteša: Emerging viruses – from another perspective
10.45-11.10	*Coffee break*	

SESSION VII: One Health

CHAIRPERSONS: Tamaš Petrović, Ljubo Barbić

(in parallel with session IX)

11.10-11.40	IL12	Tamaš Petrović (Serbia): Some of zoonotic viruses that have circulated in Serbia over the last 20 years
11.40-12.10	IL13	Ljubo Barbić (Croatia): A veterinary perspective on One Health in Croatia
12.10-12.25	OP18	Gašper Grubelnik (Slovenia): Investigation of Feline Infectious Peritonitis Virus during SARS-CoV-2 pandemic
12.25-12.40	SP2	Golden sponsor presentation – *NOACK* Gordana Rožić: Different solutions in the diagnosis of emergent animal diseases

SESSION VIII: Virus diversity and evolution

CHAIRPERSONS: Snježana Židovec-Lepej, Francesco di Serio

(in parallel with session VI)

9.00-9.30	IL14	Francesco di Serio (Italy): The expanding world of novel viroid-like RNAs
9.30-10.00	IL15	Snježana Židovec-Lepej (Croatia): Molecular diversity of EBV
10.00-10.30	IL16	Leticia Botella (Czech Republic): The use of mycoviruses as potential markers to track the invasion pathways of their hosts
10.30-10.45	OP19	Iro Skopa (Greece): Genomic surveillance in the era of SARS-CoV-2 Omicron variant of concern, from December 2021 to March 2024, in Greece
10.45-11.10	*Coffee break*	

SESSION IX: Virus and cancer

CHAIRPERSONS: Beata Halassy, Jasmina Vraneš

(in parallel with session VII)

11.10-11.40	IL17	Beata Halassy (Croatia): Oncolytic virotherapy of a localized breast cancer recurrence in neoadjuvant setting – a case study
11.40-12.10	IL18	Jasmina Vraneš (Croatia): HPV-associated cancers and HPV testing
12.10-12.25	OP20	Anita Szalmás (Hungary): The role of the interaction between cytoplasmic protein phosphatases and HPV E7
12.25-12.50	SP3	Golden sponsor presentation – *Sartorius BIA Separations* Maja Leskovec: Leveraging chromatographic tools for isolation and purification of viruses and viral vectors
13.00-14.30	*Lunch break (optional, not included in registration fee)*	

POSTER SESSION II

HOSTED BY: Sanda Ravlić

14.30-16.30	Poster session
14.30-14.45	Poster teaser
20.00-23.00	*Gala dinner*

Saturday – September 28, 2024

SESSION X: Human virology

CHAIRPERSONS: Sunčanica Ljubin Sternak, József Kónya

9.00-9.30	IL19	Igor Jurak (Croatia): RNA Editing-Dependent and -Independent Roles of Adenosine Deaminases Acting on RNA Proteins in Herpesvirus Infection
9.30-9.50	IL20	Sunčanica Ljubin Sternak (Croatia): Contemporary Insights into Rhinoviruses: Exploring Global Trends and Local Perspectives
9.50-10.20	IL21	József Kónya (Hungary): Assessment of the benefit of co-PCR-testing SARS-CoV-2 virus with influenza and RSV viruses on archived specimens
10.20-10.35	OP21	Ivana Ferenčak (Croatia): Genomic surveillance of respiratory viruses in Croatia
10.35-11.15	*Coffee break*	

SESSION XI: Antivirals and vaccines

CHAIRPERSONS: Jelena Ivančić Jelečki, Nataša Lindič

11.15-11.45	IL22	Dragomira Majhen (Croatia): Adenovirus vectors: when bad guys become the good ones
11.45-12.15	IL23	Nataša Lindič (Slovenia): Cysteine cathepsins as potential targets for treatment of viral infections
12.15-12.45	IL24	Maja Jagušić (Croatia): Guinea pig as a model for mumps virus infection
12.45-13.00	OP22	Filip Glavač (Croatia): Intensive care unit treatment in people living with HIV in Croatia: a single Centre 25-year experience
13.00-13.30	*Closing remarks*	

Legend

KL – Keynote lecture
IL – Invited lecture
OP – Oral presentation
SP – Sponsor presentation

Disclaimer

The accuracy of scientific content and language in the abstracts is the sole responsibility of the authors.

KEYNOTE AND INVITED LECTURES

KL-1

A kaleidoscope of archaeal viruses

Mart Krupovic

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Viruses of archaea represent a distinct part of the virosphere and are characterized by remarkable morphological and genetic diversity. Some archaeal virus groups are evolutionarily related to viruses of bacteria and are traceable to the virome of the last universal cellular ancestor (LUCA). However, most of the characterized archaeal viruses, especially those infecting extremophilic hosts, are archaea specific and have apparently originated in Archaea. Environmental studies have shown that archaeal viruses are not a marginal component of the global virosphere, but play prominent roles in influencing microbial communities, especially in the oceans. In this talk, I will give an overview of what we currently know (and do not know) about the archaeal virosphere. In particular, I will highlight recent advances in exploring the archaeal virus diversity across extreme and moderate ecosystems using both culture-dependent and culture-independent (metagenomics) approaches. I will then present some of the unusual properties of archaeal virus particles and their interactions with the host cells. Finally, I will examine the place of archaeal viruses in the global virosphere and discuss why these viruses left no apparent imprint on the composition of the eukaryotic virome.

KL-2

Harnessing the Caenorhabditis elegans/Orsay virus pathosystem as a new model for experimental virus evolution

Santiago F. Elena (1), Victoria G. Castiglioni (1), Rubén González (2), Dominik Herek (1), Celso Iniesta (1), Esmeralda G. Legarda (1), Susana Martín (1), Izan Melero (1), Juan C. Muñoz-Sánchez (1), María J. Olmo-Uceda (1), Zheliana Radilova (1), Ana Villena-Giménez (1)

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The discovery of the Orsay virus (OrV), the first virus infecting wild populations of Caenorhabditis elegans, has catalyzed studies of viral immunity pathways in this nematode. Given the numerous advantages that C. elegans offers for fundamental research in host-pathogen interactions, this pathosystem holds great potential to serve as a model system for experimental virus evolution studies. However, the evolutionary constraints operating in this pathosystem have received scant exploration.

Here, we delineate a series of evolutionary experiments aimed at probing various aspects of virus-host interactions. Initially, we have subjected OrV to evolutionary pressures within worm populations exposed to diverse environmental stresses such as temperature fluctuations, radiation, and simulated absence of gravity. Our findings indicate that, on the whole, infection mitigates the detrimental effects of these stresses on nematode fitness. Moreover, we have pinpointed candidate genes associated with the virus-induced enhancement of tolerance and susceptibility to abiotic stresses.

Subsequently, we delved into the three-way interplay among nematode development and senescence, the vigor of innate immune responses to infection, and the evolution of virus diversity. Our investigation unveiled differential activation of various immune factors across developmental stages, revealing a damping-wave-like pattern of infection progression. Specifically, we observed an initial acute infection in young larvae transitioning into a persistent low-level infection, punctuated by several rebounds as the animals age and senesce.

IL-1

Molecular interplay between viroids and plants

Beatriz Navarro

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Viroids are non-protein coding, circular RNAs, ranging in size from 230 to 450 nt, and able to infect plants, wherein they replicate in the nucleus (family *Pospiviroidae*) or in the plastids (family *Avsunviroidae*). Despite their small size, viroids replicate and move autonomously in the infected host without the requirement of a helper virus. In some viroid/plant host combinations, they can cause disease. Plants react to viroid infection activating RNA silencing targeting the viroid RNA. Such defensive response has been also involved in viroid pathogenesis through viroid-derived small-RNAs (vd-sRNAs) which direct AGO1-mediated cleavage of cognate host mRNAs. In the case of the chloroplastic replicating peach latent mosaic viroid and chrysanthemum chlorotic mottle viroid, this mechanism may generate the primary molecular lesion that ultimately elicits the expression of chlorotic symptoms. It has been recently shown that this chlorosis is associated with segregating pathogenic variant sub-populations with the ability to colonize certain leaf sectors and excluding other non-pathogenic variants, likely through superinfection exclusion mechanisms, thus linking viroid pathogenesis to the evolutionary pathways of the infecting viroid variants in the host. For nuclear viroids, a number of host mRNA targeted by vd-sRNAs have been also identified. However, the expression of macroscopic symptoms could derived from the contribution of different pathways, such as the activation of other plant defense responses, the interference with translation machinery, and/or induction of epigenetic changes in the host DNA. Recently, several studies focused on the potential interference of viroid RNAs with host DNA methylation, opening new perspectives in the study of viroid/plant interactions, will be here presented and discussed.

IL-2

Respiratory viruses and the stories behind the scene

Tina Uršič

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Countries in the Northern Hemisphere face annual winter epidemics caused by respiratory viruses like SARS-CoV-2, influenza, and respiratory syncytial virus. Several other co-circulating viruses such as human metapneumovirus, seasonal human coronaviruses, parainfluenza viruses, adenoviruses, rhinoviruses, enteroviruses, and human bocavirus 1 also contribute to respiratory illnesses globally. Some of these pathogens circulate year-round, while others show seasonal patterns, varying in prevalence annually. All these viruses strain healthcare systems, leading to increased primary care consultations and hospitalizations, especially among those with comorbidities and severe disease risk factors.

Twenty years ago, the technology and diagnostics we now take for granted were nearly science fiction. Methods like direct immunofluorescence were then used to detect certain viruses. Since then, extensive research has enhanced our understanding of these pathogens' epidemiology, clinical impact, and molecular characteristics. Technological advances have also led to high-quality, rapid, high-throughput molecular diagnostics for respiratory viruses, which may better prepare us for future epidemics.

In addition, the quality of microbiological diagnostics for respiratory viruses relies heavily on meticulous pre-analytical and analytical processes. In the pre-analytical phase, it is crucial to collect the correct sample from the appropriate anatomical site, using the right technique, at the optimal time, and to ensure proper transportation to the laboratory for immediate processing. In the analytical phase, employing methods with the highest sensitivity and specificity is essential to minimize the risk of false negative and false positive results. Furthermore, the SARS-CoV-2 pandemic has highlighted the dangers of using low-quality samples and tests with poor sensitivity and specificity in diagnostics and what problematic consequences this can have. The SARS-CoV-2 pandemic has also exposed errors and gaps in knowledge that might otherwise have remained hidden, and It has also made it clear that future epidemics are not a question of if, but when. While valuable lessons should have been learned from this experience, the critical question remains: have we truly understood and applied them?

IL-3

A large virus causing a large problem – African swine fever virus

Lorena Jemeršić (1), Jelena Prpić (1), Magda Kamber (1), Margarita Božiković (1), Marica Lolić (2), Mario Škrivanko (2), Dragan Brnić (1), Ivana Lojkić (1)

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(2) Croatian Veterinary Institute, Branch Vinkovci, Vinkovci, Croatia

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African swine fever (ASF) was recognized in Kenya in 1921. It entered Europe for the first time in 1957, after which it spread to Central and South America. However, the current pandemic caused by the entry of ASF virus in 2007 into the Caucasus region and its spread into Eastern Europe in 2010, the European Union in 2014, and China in 2018, is certainly the most widespread and dramatic one. It has also anthropogenically entered remote islands of the Atlantic and Pacific oceans. The resulting losses are counted in millions of euros, and the long-term damages hit small and medium-sized pig producers the worst. Croatia was not spared from the disease, either. ASF was confirmed in domestic pigs on June 26th, 2023. By the end of the year, 1124 outbreaks were recorded. The infection was detected in 28 municipalities, of which 85.7% were in Vukovar-Srijem County, while a few outbreaks were recognized in Brod-Posavina and Osijek-Baranja counties. The virus was also detected in eight dead wild boars found along the border with Bosnia and Herzegovina. However, at the end of 2023 and in 2024 ASF was found in 19 remains of wild boar corpses in Vukovar-Srijem County indicating virus spillover from the domestic pigs. The causative agent of ASF is a large virus with a diameter of 260 to 300nm from the order *Asfuvirales*, family *Asfarviridae*. It contains a DNA of 170-190kb that codes for 150 to 200 proteins. DNA mutations are rare due to the efficient DNA polymerase resulting in only 24 identified genotypes to date. The current pandemic is caused by genotype II, while subtype 19, identified in Bulgaria, Greece, Serbia and northern Italy was isolated in Croatia as well. Presently, there is scarce knowledge of the properties of some viral proteins, including the recognition of virulence carriers or post-infection immune modulators in infected pigs, which makes it difficult to develop an efficient vaccine against ASF. Therefore, the prevention and control of ASF is based on the application of strict biosecurity measures on pig farms, specific and prompt diagnosis of the disease, and depopulation. Given the variety of the localities where the infection has been recognized, there are no standardized measures that could be applied globally, which is an additional challenge in combating the infection. Therefore ASF remains a reality provoking the interest of scientists worldwide.

IL-4

African swine fever vaccination – concepts and status quo

Sandra Blome

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African swine fever (ASF) is one of the most serious and complex viral diseases of pigs and has an enormous socio-economic impact. It is caused by a large and very complex DNA virus that belongs to the genus *Asfivirus* in the *Asfarviridae* family (acronym for African swine fever and related viruses).
The development of a vaccine is made more difficult by the complexity of the pathogen. So far, all attempts to produce an inactivated, vectored or subunit ASFV vaccine have failed.
However, in recent years, several promising live ASF vaccine candidates have been reported to provide complete or near-complete protection against challenge infection under experimental conditions. In addition to naturally occurring variants, these are mainly genetically engineered (by homologous recombination) deletion mutants, which in particular lack genes coding for factors that bypass the host's immune system (immune modulation/evasion). These variants have been developed and tested by several international groups, albeit not uniformly in terms of dose, route of administration and challenge infection. Approaches with good protection against the currently circulating ASFV genotype II strains include, for example, the US vaccine candidates "ASFV-G-I177L", "ASFV-G-Δ9GL/UK and "ASFV-G-ΔMGF" as well as the Chinese vaccine candidate "HLJ/18-7GD". The naturally occurring, non-haemadsorbing virus "Lv17/WB/Rie1" and its derivatives have also been discussed and tested as vaccine candidates. In addition, CD2v deletion mutants have been investigated with some success.
There are now several live vaccine candidates that could be shortlisted for a potentially licensable vaccine and in Vietnam three of these vaccines are now licensed for controlled use in the field. Headlines from China pointing to chronic disease progression with increased respiratory and reproductive symptoms after the large-scale use of (illegal) live vaccine candidates, but also negative reports from Vietnam, urge caution in the use of live vaccines in the field before sufficient clinical trials have been conducted. A vaccine that is to be used in Europe must undergo a central authorisation procedure at the European Medicines Agency. Efforts to this end will be made as part of an EU-funded project that will take some of the above-mentioned candidates through the required efficacy and safety studies, assess them in computer-aided models and develop corresponding vaccination concepts. The focus of the project work will primarily be on an oral immunisation concept for wild boar, as the vaccination of domestic pigs in Central Europe is not expedient or necessary from today's perspective.

IL-5

The legacy of classical virologists explored by HTS can lead to new discoveries

Éva Várallyay

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Classical virology flourished in the second half of the XX century. Investigation of symptomatic plants revealed the presence of viruses impacting plants with agricultural or horticultural interest, describing their host range. The purified virions were used to produce virus-specific antibodies for serological diagnostic methods. The rise of recombinant DNA techniques delivered sequence information and established nucleic-acid-based diagnostic methods. The time and labor-intensive nature of the recombinant techniques allowed the research to focus on viruses with economic importance. High throughput sequencing (HTS), allowing the description of the virome of the investigated plant revolutionized virus discovery and opened the possibility of supplementing the previously described viruses without sequence information. Responsible biobanking of the classical virologists allows us now to reinvestigate these plant materials, provide sequence information, and make it possible to map their host range and geographical distribution on a bigger scale.

In my talk, I would share two stories when the legacy and finding of classical virology was reexplored using HTS. This is the story of the grapevine line pattern virus (GLPV) and Prunus virus I (PrVI).

GLPV was described in Hungary as a virus that causes line patterns in some grapevine cultivars. It has been maintained in a pathologic garden on a vine. HTS of this vine revealed that GLPV is an *Anulavirus*, present on hop and Paeonias in China suggesting its oriental origin. However, recently it has been also found in hemp in the US and France raising new questions about its original host and origin.

PrVI has been described in Greece, infecting sweet cherries and peaches, and has been detected in ornamental Clematis in Russia. Investigation of symptomatic *Clematis vitalba* plants using HTS in Hungary and Slovakia revealed the presence of PrVI. Its sequence showed high similarity to a PrVI that was sequenced in the German virus collection in an archive *C. vitalba* originating from Croatia. Recently PrVI has been described from *C. vitalba* from Italy, suggesting that its original host could have been this plant in Central Europe.

Our research shows that investigation of the legacy of great classical virologists using HTS can deliver important information about the origin and spread of plant viruses.

This work was supported by the grant of NKFIH K119783, K127951 and the Flagship Research Group Programme of the MATE.

IL-6

"Into the wild": from plant virus ecology in wild ecosystems to plant virus epidemics in crops

Sebastien Massart

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Historically, the study of plant viruses has focused on epidemic viruses causing crop losses. These crops occupy ~4% of the earth's surface and account for ~2% of total plant biomass. They also represent a tiny portion of plant diversity.
Recently, a novel approach has emerged in the study of plant viruses, focusing on the viromes of wild plants and their interactions with hosts. This shift in perspective has been made possible by the advent of high-throughput sequencing techniques. More specifically, virus ecology extends the scope of epidemiological studies to include an understanding of the distribution patterns and dynamics of viruses in a given environment, their effects on the plant community (cultivated and wild) and the properties of ecosystems, as well as the reciprocal effects of the environment on the dynamics and evolution of viruses.
The presentation will show how differences between wild and cultivated plant communities (inter- and intraspecific diversity, spatial distribution, selection pressures...) majorly impact viral diversity and plant-virus interactions. Several recently published works will illustrate these impacts, focusing on 6 critical questions in viral ecology: (0) are the viruses infecting wild plants well known already? (1) Is viral infection the rule in wild plants? (2) Does the plant community's spatial structure influence viruses' prevalence and diversity? (3) Do viruses tend to co-infect wild plants? (4) Can viruses contribute to host plant resistance to abiotic stresses? (5) Is there a continuum from pathogenicity to symbiosis between viruses and their host plants?
The findings from these studies will demonstrate the importance of stepping up the study of the ecology of plant viruses in wild ecosystems in order to gain a better understanding of plant-virus interactions and promote the development of sustainable solutions to limit viral epidemics in cultivated plants.

IL-7

Pathogenesis of cytomegalovirus infection in ovaries and adrenal glands

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Cytomegalovirus (CMV) is a highly relevant and widespread human pathogen and an excellent model virus for studying antiviral immune responses. Due to its wide tissue tropism, CMV infects nearly every tissue, making it an excellent model for studying organ and tissue-specific immunity. However, our knowledge about immune responses to viral infection is often restricted to large and readily accessible organs, such as the spleen, liver, and lungs. We have recently shown that murine CMV readily and strongly infects smaller organs, such as ovaries and adrenal glands (AGs), with the peak of infection at day 4-6 and virus clearance by day 8. In ovaries, a highly tissue-specific infection has been observed, with widespread infection of corpora lutea resulting in progesterone insufficiency and pregnancy loss and complete absence of infection in the ovarian follicles. Multiple layers of protection, from microanatomical barriers to innate immune responses, guard the ovarian follicles from infection and preserve fertility. We have recently observed similar, widespread, and oftentimes site-restricted infection in adrenal glands. In my talk, I will present our recent progress in virus-host interactions and pathogenesis in female reproductive and adrenal glands.

IL-8

Understanding differences in immune status between COVID-19 survivors and vaccinated individuals

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Understanding the SARS-CoV-2-specific immunity generated by infection, vaccination, or both is crucial for managing and predicting COVID-19 outcomes. This study presents a comprehensive analysis of neutralizing antibody (NAb) responses in different cohorts using a standardized wild-type SARS-CoV-2 neutralization assay calibrated to the WHO standard. A comparison of NAb responses induced by four vaccines used in Europe in 2021 (BNT162b2, mRNA-1273, ChAdOx1 nCoV-19, Ad26.COV2.S) and natural infection shows that while the quantity of NAbs was similar, those from natural infection were of superior quality.
Widespread immune responses induced by the vaccines were equaled by mRNA booster vaccinations, reaching peak NAb levels. Convalescents achieved an equally high NAb plateau with a single vaccine dose. Notably, disease-induced NAbs were better at activating the complement system than those induced by vaccination. Spike-specific IgGs contributed less to overall SARS-CoV-2 neutralization in convalescents, suggesting that recovered individuals possess antibodies of various specificities and classes enhancing virus neutralization.
These findings highlight the need to re-evaluate certain epidemiological measures and underscore the importance of standardized approaches in assessing humoral immunity with comprehensive assays in future epidemics.

1. Ravlić S, Kurtović T, Cvetko Krajinović L, Hećimović A, Miloš M, Mateljak Lukačević S, Markotić A, Halassy B. What can neutralizing antibodies tell us about the quality of immunity in COVID-19 convalescents and vaccinees? Hum Vaccin Immunother. 2023 Dec 15;19(3):2270310. doi: 10.1080/21645515.2023.2270310. Epub 2023 Oct 31. PMID: 37905722; PMCID: PMC10760325.

IL-9

Evolutionary links and lifestyle of mycoviruses in forest ecosystems

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Fungal viruses (mycoviruses) are intracellular and infect their hosts persistently. They are transmitted though cellular contacts between different fungal strains (horizontally) or through spores (vertically), and most have high species specificity. Their genomes are mostly simple with coding capacity for only few genes. Recent studies based on high-throughput sequencing have revealed that positive and negative sense single-stranded RNA viruses are much more common and diverse among fungal species than considered earlier. Moreover, new information on mycovirus diversity and genome organization suggests that many viral taxa previously considered to occur exclusively in plants or insects have related viruses in fungi, which supports the hypothesis that occasional cross-kingdom virus transmission takes place between these ecologically closely associated eukaryotic kingdoms. Our studies have addressed these topics in the context of forest-dwelling fungi such as the root rot pathogens *Heterobasidion* spp., *Armillaria* spp., as well as other wood decay fungi and tree root symbionts.

IL-10

Assessing virome diversity across ecosystems: from plants to water samples

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Virome approaches, based on high-throughput sequencing, allow to study viral diversity of a wide array of sample types, spanning from host tissues to environmental samples. Moreover, accelerated use of high-throughput sequencing has led to an increased amount of publicly available sequencing data, which represents a rich information source about the presence of viruses in diverse environments. We have used virome approaches for discovery and untargeted detection of viruses in a variety of sample types, including crops, wild and ornamental plants, invertebrates, and water samples and extended our research by using data mining approaches to better understand the occurrence and diversity of viruses on different geographical scales.
Virome investigation encompassing diverse sample types (tomato, wild plant species, irrigation, and surface water samples) from 14 tomato farms in Slovenia revealed a large diversity of previously unknown viruses, most of which were found in the viromes of wild plants and in water samples. These results showed that viral diversity is still poorly understood, even in relatively well-characterised systems such as the tomato agroecosystem. Combining information obtained from water samples and plants enabled us to obtain surprisingly extensive insights into the distribution and diversity of some of the newly discovered plant viruses, which would not be possible if analysing plants only.
Using data mining, we extended the knowledge about the distribution and diversity of some of the detected viruses. We have also established an automated pipeline to search for known and novel tobamoviral sequences in a global set of RNA shotgun sequencing datasets originating from various sources, thus enriching the knowledge about the diversity and distribution of the members of this plant virus genus, which contains important plant pathogens.
Using integrative analysis of virome data from different sample types, we now aim to elucidate possible movement of viruses between different fractions of an ecosystem, including wastewater, river water, aquatic and terrestrial wild plants and crops.

IL-11

Emerging trends of arboviral infections in Croatia

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The most common arboviruses of medical importance in Europe include flaviviruses (tick-borne encephalitis virus; TBEV, West Nile virus; WNV, Usutu virus; USUV) and bunyaviruses (Toscana virus; TOSV). Although Tahyna orthobunyavirus (TAHV) and Bhanja bandavirus (BHAV) are also documented in European countries, human clinical infections are rarely reported. In Croatia, arboviral etiology was confirmed in 189 hospitalized patients with neuroinvasive disease in the period from April 2017 to June 2024. TBEV (92/48.7%) and WNV (85/44.9%) were most frequently detected, while other arboviruses were recorded sporadically: USUV 3/1.6%, TOSV 5/2.6%, TAHV 2/1.1%, and BHAV 2/1.1%. TBEV is endemic in northwestern and northeastern regions with a seasonal distribution (April-August and October-November). Two clusters of TBE were recorded in the Gorski Kotar in 2019 and 2022. Males predominated (69.6%) with a male-to-female ratio of 2.3:1. The main clinical presentations of TBE were meningitis (54.9%) and meningoencephalitis (30.9%), while the abortive form „febrile headache" was present in 13.2% patients. WNV infections are detected in almost all continental counties, mainly in August and September. Clinical presentations of WNV included meningitis (44.8%), meningoencephalitis (38.3%), and meningoencephalomyelitis (11.2%). In addition, some rare manifestations such as retinitis in a patient with WNV encephalitis, cauda equina arachnoiditis, opsoclonus-myoclonus syndrome, and cerebellitis were also observed. TOSV infections occurred only in the Croatian littoral (Split-Dalmatia County). Interestingly, four of five patients with neuroinvasive TOSV infection were young people aged 17 to 22 years. USUV, TAHV, and BHAV were reported in continental regions. Sporadic infections caused by dengue, Zika, and chikungunya virus were also continuously recorded in travelers returning from endemic areas. In addition to human cases, arboviral infections were detected in animals: horses (TBEV, WNV, USUV, TAHV), birds (WNV, USUV), goats (TBEV), sheep (TBEV, WNV, USUV), poultry (WNV), pet animals (TBEV, WNV, USUV, TAHV) and mosquitoes (USUV). Continuous detection of neuroinvasive flaviviruses suggests endemization in continental Croatia. Therefore, arboviruses should be included in the routine diagnostic algorithm in patients presented with the neuroinvasive disease who develop symptoms during the arbovirus transmission season.

Acknowledgment: Croatian Science Foundation, project IP 2016-06-7456: Prevalence and molecular epidemiology of emerging and re-emerging neuroinvasive arboviral infections in Croatia; CRONEUROARBO, and the Next Generation EU project supported by Ministry of Science and Education of the Republic of Croatia, grant number NPOO 1 of the Croatian Veterinary Institute: Epidemiology of avian flaviviruses in the "'One Health"' context; FLAVIR.

IL-12

Some of the zoonotic viruses that have circulated in Serbia in the last 20 years

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The first recent data on the presence of West Nile virus (WNV) in Serbia was obtained by serological testing of horses sampled from 2009/2010 to 2013. WNV circulation was also confirmed by 5/92 WNV antibody-positive blood sera and 8/82 WNV RNA-positive tissues of wild birds collected during 2011/2012, and by WNV RNA-tested positive mosquito samples from 2010 and 2013. Human WNV clinical outbreaks were recorded each year, starting from 2012, when the first human epidemic was recorded. The biggest human epidemics were detected in 2013 (302 cases / 35 deaths), in 2018 (415 cases / 36 deaths), and in 2022 (226 cases / 12 deaths).
In the last years, tick-borne encephalitis virus (TBEV) circulation has continuously been confirmed by seropositive findings in sporadic testing of humans. Recently, the presence of TBEV was determined in 2% (1/50) and 6.6% (30/450) of tested *Ixodes ricinus* ticks in 2 of 17 localities collected between 2014 and 2015. In 2017, the clinical outcome of TBE was detected in one horse in the central part of the country. For Usutu virus (USUV) presence in Serbia, only recent data are available. USUV RNA was confirmed in 0.4%, 0,93%, 2.75% and 9.73% of tested Culex pipiens mosquito pools in 2014, 2015, 2017, and 2018. The obtained results confirmed the first findings of specific antibodies in human blood (2015), horses (2010), and wild boars (2011/2012). Research on the presence of hepatitis E virus (HEV) in pigs in Serbia began in 2007 when HEV was detected in 9 (30%) feces samples from 4 of 5 tested farms. The first serological examination in pigs was conducted in 2006/2007 when the presence of HEV IgG antibodies was detected in 34.6% (109/315) samples. The HEV-specific antibodies were detected in 34.33% of blood samples, and the HEV genome in 9.4% of liver samples of wild boars hunted in 2010/2011. In 2010, the presence of HEV antibodies was detected in 15% of blood samples from voluntary donors. HEV genome was also detected in slaughter lines in samples of domestic pigs. Since 2019, research has been started on the presence of hemorrhagic fever viruses in mouse-like rodents in both urban and forest/field environments near Novi Sad. The presence of DOBV was confirmed in 10 out of 137 individuals (*A. agrarius* (6), *A. sylvaticus* (2), A. flavicollis (1) and *M. minutus* (1)).
More recent research on the presence of coronaviruses in bats began in 2016/2017. The coronavirus was detected in 22.24% of tested feces samples, and in 7 of the 14 analyzed bat species.

IL-13

A veterinary perspective on One Health in Croatia

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In recent decades, we have observed an increasing trend in the emergence of new and the spread of existing infectious diseases. This trend is influenced by the development of modern society, including globalization, socio-demographic changes, and climate change. In response, a new One Health approach has been introduced. This approach has evolved from the original idea only two decades ago to a universally accepted health policy supported by all relevant global organizations. The One Health approach is built on the principle of global cooperation, uniting various disciplines to tackle the challenges posed by emerging infectious diseases. The strong involvement of the veterinary profession is a key component of this approach. This is underscored by the fact that most infectious diseases in humans are zoonoses, a trend that is even more pronounced for emerging diseases. The recent COVID-19 pandemic serves as a stark reminder of the global impact of such emerging zoonoses, highlighting the need for a united effort under the One Health approach.

Unlike other professions, veterinarians have been at the forefront of the One Health approach for years. Their work extends beyond animal health to encompass food safety and the control of zoonoses, which are the foundation of the One Health approach. This is evident in the rigorous veterinary inspection of all animal products and the successful implementation of programmes to combat various zoonoses, such as rabies. In recent years, a new activity has been added under the influence of emerging zoonoses, namely collecting epidemiological data on the occurrence and spread of emerging pathogens by sentinel animals. Although such programmes were previously conducted as part of scientific activities, they are now becoming an effective public health tool, confirmed in Croatia with the WNV control programme. Due to the proven effectiveness of such an early warning system for human infectious risks and the ease of implementation, it will become even more integrated into global health. In addition, research into potential zoonotic pathogens in animals, in which veterinarians play a key role, will be intensified, as it is now recognized that the prevention of emerging diseases is essential for the development of modern society.

All this requires strengthening the veterinary profession, which must continue to work according to its basic principles, which today bear the generally accepted name of the One Health approach.

Acknowledgment: Croatian Science Foundation, project IP 2016-06-7456: Prevalence and molecular epidemiology of emerging and re-emerging neuroinvasive arboviral infections in Croatia; CRONEUROARBO, and the Next Generation EU project supported by Ministry of Science and Education of the Republic of Croatia, grant number NPOO 1 of the Croatian Veterinary Institute: Epidemiology of avian flaviviruses in the "'One Health"' context; FLAVIR.

IL-14

The expanding world of novel viroid-like RNAs

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The discovery of viroids, which are subviral infectious agents consisting of small, non-protein coding and circular RNAs, dates back to the beginning of the seventies of last century when they were identified as pathogens of plants. Completely relying on host proteins and/or on catalytic activities mediated by ribozymes endowed in their own RNA viroids represent the smallest infectious agents known so far. Later a few other viroid-like RNAs were discovered, such as viroid-like satellite RNAs of certain plant viruses and hepatitis delta virus (HDV) and delta-like agents infecting humans and animals, which differ from viroids due to their functional dependence from a coinfecting virus and/or their protein-coding capability (in the case of HDV and delta-like agents). Due to the circularity and the catalytic activity of their RNA genome, viroids and viroid-like RNAs have been considered relics of an RNA world preceding the current cellular life based on DNA and proteins. However, the question of why these hypothetical very ancient infectious agents did not colonize other living entities such as fungi and bacteria remained unanswered for about fifty years. The screening of metagenome/metatranscriptome data available in databases using powerful bioinformatics tools aimed to identify circular and ribozyme-containing RNAs, allowed the discovery of more than 20000 coding and non-coding viroid-like RNAs in different geographic areas and ecological niches, including fungi, thus unveiling the existence of a variegated and surprisingly wide world of circular, catalytic and potentially infectious RNAs remained largely unknown so far. Structural and biological features, of these novel infectious agents – including non-coding RNAs of relatively small size (several hundreds of nt) or of very large sizes (up to about 5000 nt, such as hybrids of viroid-like elements and RNA viruses replicating in fungi)- will be presented. Infectious capability of several representative members of this novel group of biological entities, together with their potential application as biological control agents of fungi, will be shown and discussed.

IL-15

Molecular diversity of EBV

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Epstein–Barr virus (EBV) or Human gammaherpesvirus 4 is a prototypic oncogenic virus that enables a transformation of B-cells into B-lymphoblastoid cell lines in vitro by using complex molecular mechanisms mediated by oncogenic proteins and microRNAs (miRNAs). Malignant and non-malignant diseases associated with EBV infection cause significant morbidity and mortality. However, efficient antiviral therapy as well as EBV vaccines are currently not available. EBV genome is double-stranded DNA 172 kb in size and contains approximately 86 open reading frames (ORFs) which, besides proteins, can be transcribed in non-coding EBV miRNAs. Along its genome, EBV contains 4 major internal repeats (IR1-IR4) and a high number of terminal repeats. EBV is classified into two genotypes that exhibit distinct immunobiological features in vitro and in animal models as well as specific geographical distribution patterns. Based on the sequence of the LMP1 gene, the virus is classified into several variants including Alaskan (AL), China1, China2, China3, Mediterranean with (Med+) or without (Med-) deletions, and North Carolina (NC), SEA1, SEA2 and CAO. Additionally, EBV can be classified into 12 EBV phylopopulations of monophyletic and paraphyletic origins. Distribution of multiple genetic EBV variants has been described in a variety of benign and malignant diseases but the data on their biological and clinical relevance is still insufficient. A better understanding of EBV molecular diversity is exceptionally important for the development of innovative therapeutic strategies as well as for prophylactic and therapeutic vaccine development.

IL-16

The use of mycoviruses as potential markers to track the invasion pathways of their hosts

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Virus research in forest sciences has historically aimed to understand hypovirulence in tree pathogens (fungi and oomycetes) and their application as biological control agents (BCAs). While many mycoviruses cause cryptic infections without phenotypic changes in hosts, their influence on host behavior and populations likely reflects adaptations for modulating transmission rates and complex environment-host-mycovirus interactions within ecosystems. Studying tree pathogen and virus populations in natural forests can enhance our understanding of virus effects, coevolution patterns, and biotic and abiotic impact on virus distribution. Mycoviruses, replicating only in host cytoplasm and transmitted intracellularly, may mirror host evolution and provide insights into the history of pathogen dispersion. For instance, *Cryphonectria hypovirus* 1 (CHV1) was introduced to Europe with its fungal host, the chestnut blight fungus (*Cryphonectria parasitica*), and rapidly colonized the expanding host population. Similarly, the conifer pathogen *Gremmeniella abietina* (biotype A) was introduced to the United States with its gammapartitivirus from Europe. *Hymenoscyphus fraxineus mitovirus 1* (HfMV1) supported the hypothesis that only two mitovirus-carrying individuals of *H. fraxineus* were brought to Europe from Japan, and the panglobal pathogen *Phytophthora cinnamomi* has carried several of its viruses from its native environment into new territories. These cases highlight the potential of mycoviruses in tracking pathogen invasion history and their use in sustainable forest management.

IL-17

Oncolytic virotherapy of a localized breast cancer recurrence in neoadjuvant setting - a case study

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Intratumoral oncolytic virotherapy may have promise as a means to debulk and downstage inoperable tumours in preparation for successful surgery. Here we describe the unique case of a 50-year-old female patient with locally recurrent muscle-invasive breast cancer who was able to proceed to simple, non-invasive tumour resection after receiving multiple intratumoral injections of viruses, first an Edmonston-Zagreb measles vaccine strain (MeV) then vesicular stomatitis virus Indiana strain (VSV). The intratumoral virus therapy was well tolerated and led to transient pseudoprogression followed by partial tumour remission. Frequent imaging studies and regular clinical observations documenting size, consistency and mobility of the injected tumour demonstrate that both MeV and VSV contributed to the overall favourable response. Two months after the start of the virus injections, the shrunken tumour was no longer invading the skin or underlying muscle and was surgically excised. The excised tumour showed strong lymphocytic infiltration, with increase in CD20-positive B cells, CD8-positive T cells and macrophages. PD-L1 expression was detected in contrast to the baseline PD-L1-negative phenotype. The patient completed one-year trastuzumab adjuvant therapy, remains well and recurrence free 43 months post-surgery. Although an isolated case, it encourages consideration of oncolytic virotherapy as a neoadjuvant treatment modality.

IL-18

HPV-associated cancers and HPV testing

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Cancer-related diseases represent the second overall cause of human death. Human papillomavirus (HPV) infection is the most common sexually transmitted infection in the world and a necessary cause for virtually all cervical cancers and an attributable cause for variable proportions of anal, oropharyngeal, vaginal, vulvar, and penile cancers worldwide. The advent of HPV vaccines and the introduction of HPV testing as a new screening approach have created the opportunity to eliminate cervical cancer, a recognized public health problem, by the end of the 21st century. Cervical cancer is the first cancer deemed amenable to elimination through prevention, and thus lessons from the epidemiology and prevention of this cancer can provide information on strategies to manage other cancers. Whereas 20th-century prevention efforts were dominated by cytology-based screening, the present and future of HPV-associated cancer prevention relies mostly on HPV vaccination and molecular screening tests. Current clinical trials including patients with HPV-associated cancers focus on finding optimal testing for all HPV-related cancers as well as improving the currently applied treatments. The epidemiology of HPV-associated cancers, their disease burden, how past and contemporary preventive interventions have shaped their incidence and mortality, and the potential for elimination with a particular focus on the cofactors that could have the greatest effect on prevention efforts should be well studied, and elimination efforts are unlikely to succeed unless prevention efforts focus on both primary and secondary prevention.

IL-19

RNA Editing-Dependent and -Independent Roles of Adenosine Deaminases Acting on RNA Proteins in Herpesvirus Infection

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ADAR family proteins catalyze the conversion of adenosines to inosines in dsRNA. A-to-I editing is the most common post-transcriptional RNA modification in mammals that prevents aberrant immune responses to cellular dsRNAs. In viral infections, ADAR proteins have been found to exert proviral and antiviral activity through editing-dependent and editing-independent functions. While much is known about the function of ADAR proteins in RNA virus infections, the role of these proteins in herpesvirus infections is largely unknown. However, recent studies have shown that ADAR proteins are critical for efficient viral infection and that ADARs have very different functions in both productive and latent herpesvirus infection.
We have recently demonstrated post-transcriptional editing of HSV-1-encoded miRNAs in latently infected human tissues, strongly suggesting a role for ADAR proteins in the regulation of latency. Furthermore, our recent work suggests that ADAR1 has a critical function in attenuating the activation of dsRNA sensors that enable efficient viral replication and spread.

IL-20

Contemporary Insights into Rhinoviruses: Exploring Global Trends and Local Perspectives

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A decade-long neglect of human rhinovirus (RV) as a significant disease agent in humans stemmed primarily from its association with mild respiratory ailments such as the common cold. However, the emergence of molecular diagnostic techniques has led to an increasing number of reports identifying RV as a pathogen in the lower respiratory tract and recognizing its importance as a risk factor for asthma-related issues in childhood.
Our investigation focused on RV prevalence over a two-year period, involving 182 adult patients from 2016 to 2018 and 590 children from 2017 to 2019, all hospitalized with acute respiratory infections in northwestern and central parts of Croatia. Nasopharyngeal swabs were collected from each patient and analyzed using multiplex RT-PCR.
In adults, RV was the fifth most prevalent virus detected, with a prevalence of 11.4%, of which 79% were monoinfections. Among children, RV was the most frequently detected virus, found in 33.4% of cases, with 60.4% detected as monoinfections. The median age of HRV-infected adults was 70 years, while for children, it was 2.25 years. More than half of hospitalized adults and children infected with RV (63.3% and 55.8%, respectively) presented with lower respiratory tract infection (LRTI).
Furthermore, we identified RV species in a subset of positive children. Sequence analysis of the 395 base pairs of the 5'UTR region revealed 69 distinct genotypes, with RV-C being the most prevalent (47.4%), followed by RV-A (44.7%) and HRV-B (7.9%). Strains belonging to group C exhibited the highest diversity (41.6% identity among strains), while group B was the most conserved (71.5% identity among strains). Despite this genetic diversity, the clinical presentation of infected children was largely similar.
Our findings, along with similar studies worldwide, emphasize the importance of RV, especially when recognized as the sole pathogen in patients with LRTIs, particularly in infants and the elderly.

IL-21

Assessment of the benefit of co-PCR-testing SARS-CoV-2 virus with influenza and RSV viruses on archived specimens

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Simultaneous multiplex testing for respiratory viruses SARS-CoV-2, influenza, and respiratory syncytial virus is generally recommended for patients requiring hospitalization with acute respiratory symptoms. An in-patient clinic has its triage algorithm upon admitting a new patient and medical indications for diagnostic testing support both early decisions on patient care and definitive diagnosis on etiology. This retrospective analysis was performed in a tertiary care hospital between December 2022 and March 2023 with a purpose of assessing whether or not the current combinations of multiplex respiratory virus testing should be maintained. During this period SARS-CoV-2 epidemic was still going on and an influenza epidemic appeared in the population served by the hospital. For triage reasons at admission, patients were tested for SARS-CoV-2 infection using a rapid antigen test. The current diagnostic respiratory virus PCR panel (Seegene Allplex Respiratory Virus Essential Assay) used to be ordered for patients with acute respiratory symptoms and negative antigen test for SARS-CoV-2 and the PCR panel included epidemic influenza A and B subtypes, RS virus, adenovirus and minor respiratory viruses. The objective of the analysis was to assess the efficacy of the rapid antigen testing at admission compared to including SARS-CoV-2 testing in the PCR panel. The study was designed to retest 460 respiratory PCR panel specimens for SARS-CoV-2. Of these 460 specimens, 79, 69, and 33 tested positive for influenza, RS viruses, and SARS-CoV-2 viruses, respectively. However, only 12 of the 33 SARS-CoV-2 PCR positives had Ct values below 30 and seven of them had been identified as positives even by rapid antigen test. The outcomes are discussed.

IL-22

Adenovirus vectors: when bad guys become the good ones

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The field of adenovirology is undergoing rapid change in response to increasing appreciation of the potential advantages of adenoviruses as the basis for new vaccines and as vectors for gene and cancer therapy. Substantial knowledge and understanding of adenoviruses at a molecular level has made their manipulation for use as vaccines and therapeutics relatively straightforward in comparison with other viral vectors. Since they mimic natural infection, recombinant adenovirus vectors have been proven as ideal shuttles to deliver foreign transgenes aiming at inducing both humoral and cellular immune response. In addition, a potent adjuvant effect can be exerted due to the adenovirus inherent stimulation of various elements of innate and adaptive immunity. Very important milestone has been achieved when European Medicines Agency recently approved three adenovirus-based vector vaccines for use in humans, two of them based on human adenovirus type 26 (HAdV-D26). Yet, there are still many unknowns regarding the basic biology of this low seroprevalent adenovirus. In our work we examined the role of integrins in HAdV-D26 cell entry and we observed that HAdV-D26 colocalizes with αvβ3 integrin and that increased αvβ3 integrin enhances internalization of HAdV-D26, thus leading us to conclude that HAdV-D26 uses αvβ3 integrin as a receptor for infecting epithelial cells. Further on we provided insight into the interesting nature of the HAdV-D26 infection pathway suggesting that depending on the receptor status this virus can enter the cell in different manner which can be independent of dynamin-2, clathrin and/or caveolin-1. Finally, we observed that the HAdV-D26-induced IL-6 gene expression in epithelial cells is αvβ3 integrin dependent and NF-κB mediated. Altogether, our findings give new data regarding HAdV-D26 receptor usage and cell entry, pro-inflammatory cytokine and chemokine expression in HAdV26-infected epithelial cells, as well as details concerning HAdV26-induced host signaling pathways.

IL-23

Cysteine cathepsins as potential targets for treatment of viral infections

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To prepare for future pandemics and effectively control viral infections, new therapeutic approaches are essential. Therapeutic drugs offer a crucial advantage over vaccines by directly targeting the infected population. However, the development of antiviral drugs is often delayed by lengthy discovery and synthesis processes, thus, drug repurposing represents a viable and expedited strategy.
In response to the emergency situation in 2020, we aimed to identify inhibitors targeting the main protease (M^{pro}) of SARS-CoV-2 virus. We conducted a comprehensive drug repurposing screen using high-throughput X-ray crystallography. We tested over 5000 compounds, including approved drugs and those in clinical trials, for their ability to bind to M^{pro}. We confirmed binding for 37 compounds and identified allosteric sites on M^{pro} with potential for therapeutic targeting. Seven compounds showed antiviral activity in cell-based assays, with calpeptin emerging as a promising candidate (Günther et al., 2021). Interestingly, calpeptin exhibited moderate activity against M^{pro} in kinetic assays, but high potency in cell-based assays, suggesting an off-target effect. Our kinetic studies revealed that these calpeptin targets are cysteine cathepsins. Cysteine cathepsins are host proteases, mainly expressed in endosomes, and they play a crucial role in the entry of SARS-CoV-2 and other viruses like Ebola, Nipah, Hendra and dengue. We found that in SARS-CoV-2-infected golden Syrian hamsters, sulfonated calpeptin significantly reduced viral load in the trachea. Altogether, our results suggest that cysteine cathepsins hold a strong potential for antivirals development (Reinke et al, 2023; Tušar et al., 2023). Building on these findings, we continue our exploration of the structural basis of protease inhibition to improve inhibitor efficacy and reduce side effects in antiviral therapies (Falke et al., 2024). We believe that targeting host proteases holds advantage over viral targets, as we can prepare therapies before new viral epidemics emerge.

IL-24

Guinea pig as a model for mumps virus infection

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Mumps is a highly contagious viral disease preventable through vaccination. However, in the past two decades, repeated outbreaks of mumps have occurred in highly vaccinated populations, raising concerns about the effectiveness of existing vaccines. Understanding virus-host interactions is crucial for development of new vaccines and studying viruses like the mumps virus (MuV), which naturally infects only humans, presents a specific challenge.
Our study investigated the interaction between MuV and guinea pigs. We demonstrated that guinea pigs of the Hartley strain can be infected *in vivo* through intranasal and intratesticular inoculation. Significant viral replication was observed in infected tissues up to 5 days post-infection along with the histopathological changes in the lungs and testicles. Despite the asymptomatic nature of the infection, MuV infection strongly activated both humoral and cellular immune responses, providing protection against virus challenge. Viral transmission through direct contact between animals was not observed.
To further explore the stability of guinea pigs as a model for MuV infection, we conducted successive *ex vivo* virus passages in target organs. We monitored how this process influences the genetic diversity of viral populations and viral phenotype. Our results showed no evidence of virus-host adaptation or changes in virus pathology.
Overall, our findings offer perspectives on the use of guinea pigs in research on MuV pathogenesis, antiviral response, and vaccine development and testing.

ORAL PRESENTATIONS

OP-1

The aftermath of COVID-19: Exploring its impacts on influenza patterns in Israel

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The seasonal resurgence of influenza, subsequent to the decline in SARS-CoV-2 cases, especially with the emergence of the Omicron variant, has remained a notable contributor to global morbidity and mortality.
This study aimed to characterize the circulation of influenza among hospitalized patients in Israel following the decline of COVID-19. For this purpose, 15,833 nasopharyngeal samples from hospitalized patients were examined using RT-PCR during two winter seasons, 2022-2023 and 2023-2024. Additionally, hemagglutinin sequencing was performed to compare the strains identified during each winter season with the corresponding vaccine strains.
Our findings reveal the predominance of influenza A(H1N1)pdm09 (60.9%), alongside influenza A(H3N2) (17.1%) and Influenza B/Victoria (16.3%) during the 2022-2023 season. The subsequent winter season experienced a late peak in influenza cases, (February 2024) likely due to mitigation measures imposed during the war in Israel and the delayed onset of winter. In the 2023-2024 season, influenza cases were predominantly caused by influenza A(H3N2) (83.8%), with lesser proportions of influenza A(H1N1)pdm09 (10.1%) and Influenza B/Victoria (1.3%). Comparisons with the USA and Europe revealed varying patterns of strain circulation. In the 2022-2023 season, influenza A(H3N2) emerged as the predominant strain, whereas in 2023-2024, influenza A(H1N1)pdm09 took precedence, reaching its peak in December 2023.
Sequencing of the hemagglutinin of circulating influenza strains revealed mutations that were not observed in the vaccine strains, including A186T, Q189E, and E224A. The following year, when influenza A(H3N2) dominated in Israel, amino acid substitutions such as E66K, N112S, I140K, S156H, and N186D were predominant in circulating influenza among hospitalized patients. To conclude, the prevalence of influenza during 2022-2024 returned to levels reminiscent of the pre-SARS-CoV-2 pandemic and was incompatible with the vaccine strain recommended. Moreover, the epidemiological profile in Israel diverged from global trends in influenza in timing and type due to mitigation efforts during the war and the late arrival of winter.

OP-2

In vitro interaction between canid alphaherpesvirus 1 and canine adipose-derived mesenchymal stem cells: impact on cell gene expression and secretome profile

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Canine adipose-derived mesenchymal stem cells (cAD-MSCs) exhibit significant potential for tissue repair and regeneration. However, harvesting and preserving these cells or their secreted factors for therapeutic use carries the risk of viral contamination, an area that has not yet been adequately explored. Therefore, this study aimed to investigate the impact of canid alphaherpesvirus 1 (CHV) on the functional properties of cAD-MSCs, focusing on gene expression profiles and secretome composition. Abdominal adipose tissue was harvested from twelve healthy dogs to isolate cAD-MSCs. These samples were screened for CHV contamination before a wild-type CHV strain was introduced by serial passages. After infection, RT-PCR arrays and LC-MS/MS analyses were used to assess gene expression and secretome proteomic profile, respectively. The initial cAD-MSC populations were found to be free of CHV. Upon exposure to the wild-type CHV strain, the cAD-MSCs showed susceptibility, as reflected by significant gene expression and secretome composition changes. These changes affected fundamental intrinsic properties, including stemness, differentiation potential, structural integrity, proliferative capacity, survival, directional migration, and immunomodulatory functions, ultimately compromising their regenerative efficacy. Despite the absence of pre-existing CHV contamination, these findings highlight the critical need for routine viral screening prior to therapeutic application. To our knowledge, this is the first study to investigate the effects of viral infection on gene expression and secretome composition in cAD-MSCs. Furthermore, it provides new insights into the pathogenic mechanisms of CHV and lays the foundation for future comprehensive studies on stem cell-virus interactions in dogs.

OP-3

Cytomegalovirus secondary envelopment and its release from infected cells is mediated by Rab15-positive membrane organelle

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The replication of cytomegalovirus (CMV) ends with the nuclear production of new capsids and their final (secondary) envelopment on cytoplasmic membranous organelle of unknown origin. The enveloped virions are collected in large multiviral bodies (MViBs) and released from the cell as a bulk pulse by poorly understood mechanisms.
We investigated cytoplasmic egress using recombinant murine CMV (MCMV) with fluorescently labeled small capsid protein (S-mCherrry-SCP-MCMV) for confocal analysis and long-term live imaging with digital holotomographic microscopy (DHTM) in NIH3T3 cells. Digital holotomographic microscopy is a label-free imaging technique providing information about fine changes in a cell's refractive indices in three dimensions at high spatial and temporal resolution. We used 50 membrane compartment markers to identify the compartment for MCMV egress from the cell.
Fluorescent S-SCP accumulated in the nuclei of approximately 50% of infected cells, and fluorescent capsids were visualised in large cytoplasmic structures corresponding to MViBs. The release of MViBs began at 20-24 hours post-infection (hpi) and continued over the next two days. Static confocal images showed a small proportion of infected cells with cytoplasmic accumulations of S-mCherrry-SCP, consistent with the heterogeneity of cellular responses to DNA virus infection and the temporal extent of virion release. Live recordings of 330 cells over the period of 16-72 hpi showed release events in 40-68% of infected cells. Colocalization analysis showed that cytoplasmic S-mCherry-SCP accumulations were positive for Rab15 (>80% overlap for Mander's coefficient M1), viral envelope glycoproteins (pM55 and pM74), the major tegument protein of MCMV (pM25) and to a lesser extent for Vps35, Rab8a, Rab35, CD63, Lamp1, p62, Vti1a and Golgin97.
These results suggest that Rab15-positive organelles can be utilized for secondary envelopment and egress of CMV.

Supported by the Croatian Science Foundation (IP-2020-02-2916, IP-2020-02-1323, IP-2019-04-3582) and by UNIRI (uniri-biomed-18-180, 18-88, 18-229).

OP-4

First detection of porcine limphotropic herpesviruses 1, 2 and 3 in domestic pigs in Croatia

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Porcine lymphotropic herpesviruses 1, 2 and 3 (PLHV-1, PLHV-2 and PLHV-3) are DNA viruses belonging to the genus Macavirus and the subfamily Gammaherpesvirinae within the family Herpesviridae. Infections of domestic pigs with PLHV-1 and 2 were described in 1999, whereas PLHV-3 was recognized and isolated from pigs in 2003. PLHV have a tropism for peripheral blood mononuclear cells, especially B lymphocytes. A pronounced tropism for other lymphoproliferative organs such as tonsils, liver, spleen, kidneys and lungs is also observed. According to previous studies, natural infections with PLHV in domestic pigs do not cause clinical signs of disease. However, PLHV-1 is found to cause lymphoproliferative disorders in domestic pigs, similar to those described in humans infected with human herpesvirus 4 (HHV-4) derived from subjects after organ transplantation. HHV-4 is the causative agent of mononucleosis and is the first virus in which oncogenic potential has been described. HHV-8 causes Kaposi's sarcoma and contributes to the development of lymphoproliferative diseases in humans, such as primary effusion lymphoma and multicentric Castleman disease. Although herpesviruses are generally species-specific, the close genetic relationship of PLHV with HHV-4 and HHV-8 may be an indicator of a possible zoonotic potential, particularly in immunosuppressed human recipients following xenotransplantation. Given the impact of PLHV on the immune response of pigs and their potential zoonotic transmission, the results of our pilot study are essential for further assessment of public health risks. PLHV have been detected in pigs in Germany, Spain, Italy and Ireland, so this was the trigger for our preliminary trial with the aim of investigating their presence in Croatian pig herds. In our pilot study, blood and spleen samples of pigs were analyzed by real-time polymerase chain reaction according to a previously published protocol that proved to be an excellent method for the detection of PLH viruses as it is rapid, highly specific and sensitive. The presence of PLHV in domestic pigs in Croatia was confirmed for the first time in pig herds regardless of the breeding conditions. Further investigation of PLHV will contribute to a better knowledge of their importance in maintaining pig health and include genotyping to identify potential risks associated with public health.

OP-5

Seawater Outbreak of Infectious Haematopoietic Necrosis (IHN) in Rainbow Trout (*Oncorhynchus mykiss*)

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Infectious hematopoietic necrosis virus (IHNV), a pathogen from the Salmonid novirhabdovirus genus within the *Rhabdoviridae* family, was isolated and identified in diseased rainbow trout (*Oncorhynchus mykiss*) farmed experimentally in the Adriatic Sea. Higher mortality rates were recorded among the affected fish. Specimens, weighing between 50 to 300 grams, exhibited clinical signs such as hemorrhages on the opercula, vent, fin bases, and mouth, along with darkened gills and liver, hemorrhages in the pyloric caeca and liver, an enlarged spleen, and an affected swim bladder.

Kidney swabs from the affected fish were analyzed in the laboratory. Viral RNA was extracted and RT-q-PCR for detection of Viral hemorrhagic septicemia virus (VHSV) according to Commission decision 2015/1554 tested negative. However, analyses of the same RNA using a modified RT-q-PCR method confirmed the presence of IHNV. Further amplification and sequencing of the "mid-G" region of the G gene verified IHNV identification and showed genetic similarity to the CRO/05 strain in the GenBank database.

Comparative analysis of sequences from the GenBank database and unpublished Croatian outbreaks from 2005, 2013, and 2014 indicated that the tested isolate closely matched CRO/05 and was related to other national isolates. This study underscores the persistent threat of IHNV to salmonid aquaculture, both in fresh water and in the Adriatic Sea and highlights the need for ongoing surveillance and molecular characterization of viral pathogens in marine environments.

OP-6

Establishment of the European Swine Influenza Network (ESFLU) for improvement of the One Health perspective

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Background: Swine influenza (SI) is one of the most important infectious respiratory diseases in pigs within the pig farming sector across Europe. The causative agent, swine influenza A virus (swIAV), is continuously mutating, occasionally giving rise to zoonotic variants. Despite the evident risk to swine and human health and the economic burden it may cause, the surveillance, diagnostic examination, and vaccine coverage of this infection in swine across Europe are inconsistent, and even completely absent in some countries.
Methods: The multidisciplinary European Swine Influenza Network (ESFLU) was funded by the European Union (COST CA21132) with the goal of developing a comprehensive approach to SI during the 2022-2026 period. The objectives of ESFLU are to facilitate data sharing across all scientific fields, improve pandemic preparedness, build expertise in the detection and characterization of swIAVs, compile surveillance, prevention, management, and control practices, connect with the OFFLU network, and promote awareness of this potential zoonosis among all interested parties.
Results and Discussion: Through cooperation and the exchange of knowledge and experience, during its first year of existence, ESFLU managed to collect and analyze different swIAV laboratory procedures and surveillance programs in Europe, making them publicly available (https://swineflu.eu). Capacity building was accomplished through a training school related to the detection and characterization of swIAVs, and short-term scientific missions for young researchers focused on phylogenetic and phylogeographic studies of swIAVs. This facilitated networking, the conducting of reviews, and surveys. Cooperation with OFFLU was established, and the first report containing sequences of swIAVs circulating in swine populations within Europe was published.

OP-7

Discovery of a previously unknown virus in ancient olives remains

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Olive (Olea europaea L.) or its wild relatives have been a part of the Mediterranean diet and trading routes. The shallow Pupak was on the ancient trans-Adriatic waterway and the site of a Roman shipwreck where a large number of olive stones and well-preserved fruits were found in two amphorae. The biological material was confirmed to be 2200 years old by carbon-dating and used for detection of viral sequences.
Total nucleic acids extracted from olive embryos were DNase treated. RNA reverse transcription and DNA preamplification was performed prior to shotgun high-throughput sequencing (Illumina HTS). An in-house developed bioinformatic pipeline was used for the detection of viruses.
De novo assembly of short reads enabled the construction of a putative new virus near-complete genome. A short gap was closed by RT-PCR and Sanger sequencing. Newly designed primer pairs were also used to confirm the amplification of viral genomic RNA in several 2200-year-old olive samples. Phylogeny confirmed the virus provisionally named Olive alphacarmovirus 1 belongs to the genus Alphacarmovirus, family Tombusviridae. Its sequence (or closely related sequences) cannot be found in published short read data sets included into Serratus search.
This virus sequence is novel and probably originating from ancient olives. If substantiated, this would be the oldest record of a plant virus and provide an unprecedented insight into the virome of olives traded in antiquity.

OP-8

High-throughput sequencing reveals hidden viral diversity in aquatic plants

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Aquatic plants are a taxonomically diverse group of organisms inhabiting numerous marine and freshwater habitats. Due to their variety and widespread presence, they represent a considerable pool of potential viral hosts. Viruses released from aquatic plants might spread through water over longer distances, potentially reaching agricultural areas or sources of irrigation water. Despite their potential significance, the viromes of aquatic plants remain largely unexplored.
The aim of this study was to expand the currently limited knowledge of viruses associated with aquatic plants. We explored the viromes of diverse aquatic plant species by utilizing two approaches. Initially, we mined the transcriptome data from the 1000 Plant Transcriptome Initiative to search for possible viral sequences. In the second phase, we sequenced ribosomal RNA depleted total RNA of 17 ornamental freshwater aquatic plants, acquired through online aquaristics shops. In both approaches, we utilized various bioinformatic tools to develop a multi-step virus detection pipeline. Reads were first trimmed and assembled into contigs, followed by a homology search against the NCBI non-redundant protein database and subsequent taxonomical classification. We manually analyzed the datasets, specifically focusing on the annotation of contigs from putative plant viruses.
We have found that aquatic plant species from different taxonomic groups contain sequences of several known plant viruses. These include viruses previously found only in wild plants, as well as those that are known to infect economically important plants and such that are listed as quarantine pests. Additionally, we have assembled multiple near-complete genome sequences with moderate similarity to known plant viruses, which represent putative novel species. An example of the latter is a potyvirus, which was detected in two different species of plants from the genus Sagittaria with both approaches.
Our findings demonstrate the presence of diverse viral sequences in aquatic plants, including some from putative novel viral species. The detection of viral contigs in ornamental aquatic plants is particularly important given their global trade, as it could facilitate the emergence of novel viruses in previously unaffected environments. Additional experimental work is needed to validate the infectivity of these viruses and assess their potential transmission through water.

OP-9

Where do viruses come from, and when? Case studies of watermelon mosaic virus and grapevine Pinot Gris virus

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Phylogenetics, the study of the evolutionary history of organisms, first flourished almost two centuries ago as a result of the insights of Charles Darwin and Alfred Wallace. They realized that each living organism evolves and produces a tree of bifurcating lineages. Initially phylogenies were inferred by observing and comparing superficial characters of organisms. However the sequences of, first, amino acids in proteins, and then nucleotides in nucleic acids of organisms were shown to evolve through time in a similar way, and it became possible to calculate phylogenetic trees mathematically from gene sequences alone. The development of cheap and fast gene sequencing methods over the past three decades allows phylogenies of viruses to be produced. Dates can now be added mathematically to the phylogenies, especially if the viruses are evolving at measurable rates, and if their population has been sampled on different occasions. Knowledge of the likely dates of the nodes in virus phylogenies, combined with the searchable resources of the Internet, may point to the factors that altered the branching pattern of their phylogenies, such as 'new encounters' resulting from seed trading, or changes in cropping methods forced by pest invasions. I will illustrate these possibilities for two viruses I have studied.

I thank Dr Petr Kominek, Crop Research Institute, Prague, and Professor Adrian Gibbs, Emeritus Faculty, Australian National University, Canberra.

OP-10

Seroepidemiology of cytomegalovirus infections in childbearing-aged and pregnant women in Croatia

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While in immunocompetent children and adults, cytomegalovirus (CMV) is a common cause of asymptomatic or mild mononucleosis-like disease, congenital CMV infection represents a public health concern that affects 0.67% of live births. This study aimed to analyze the seroprevalence of CMV infections in Croatian childbearing-aged and pregnant women. A total of 1032 women (16-45 years) tested consecutively during ten years (2014-2023) were included. Detection of IgM and IgG antibodies was performed using a commercial enzyme-linked immunoassay (ELISA; Vircell Microbiologists, Granada, Spain). IgM/IgG positive samples were further tested for IgG avidity (Euroimmun, Lübeck, Germany). The mean age of study participants was 31.9±5.1 years. CMV IgG antibodies were detected in 728 (70.5%, 95%CI=67.7-73.3) women, while 65 were IgM positive (6.3%, 95%CI=4.9-7.9). Using IgG avidity, current/recent CMV infection was confirmed by a low/borderline avidity index in only two women. There were no significant differences in the IgG seroprevalence between age groups (62.5%-72.4%), urban and suburban/rural areas of residence (69.1% vs 73.9%), as well as continental and coastal regions (70.1% vs 71.8%). In women with unfavorable obstetric history, higher CMV IgG seropositivity was found (75.2%) compared to non-pregnant women (69.4%) and women with normal pregnancy (69.9%), however, these differences were not statistically significant. Analyzing seroprevalence rates by year, the seropositivity ranged from 66.2% (2020) to 77.3% (2015), but the differences were not significant. However, the overall seroprevalence rate in the period 2014-2023 was significantly lower compared to a previous Croatian study (2005-2009) conducted in the same population group (70.5% vs 78.7%). A comparable declining trend in the CMV seropositivity, as seen in other European countries, was expected in Croatia. The increased proportion of seronegative women who are at risk of primary infections during pregnancy should be kept in mind.

OP-11

Prevalence of herpes simplex virus type 1 and type 2 antibodies in risk populations, Istria County, 2020-2023

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Herpesvirus infections are caused by two types of herpes simplex viruses (HSV): HSV-1 and HSV-2. The seroprevalence rates vary in Europe. This study aimed to analyze the prevalence of HSV-1 and HSV-2 in different risk populations in Istria County. During four years (2020-2023), 276 consecutive serum samples were tested at the Microbiology Service of the Istria County Teaching Institute of Public Health for the presence of HSV-1 and HSV-2 IgG antibodies. The risk population groups were defined as newborns, childbearing-aged/pregnant women, transplant candidates, and patients with autoimmune diseases. Serologic tests were performed using type-specific commercial enzyme-linked immunoassays (ELISA). Equivocal results as well as HSV1/HSV-2 simultaneously positive samples were confirmed by the western blot. Significant differences between age groups ($p<0.001$) were observed: childbearing-aged/pregnant women median 32 (IQR=27-36) years; transplant candidates median 59 (IQR=48-65) years; patients with autoimmune diseases median 42 (IQR=32-60) years. The overall IgG seroprevalence rates were 74.3% (95%CI=68.7-79.3) for HSV-1 and 7.2% (95%CI=4.5-10.9) for HSV-2. According to the population group, statistically significant differences in HSV-1 IgG seropositivity were found ranging from 57.6% in newborns to 85.2% in transplant candidates ($p=0.010$). In the adult population, there was no significant difference between females and males neither for HSV-1 (76.6% vs. 76.4%, $p=0.969$) nor for HSV-2 (8.4% vs. 7.9%, $p= 0.874$). A significant increase in both HSV-1 ($p=0.008$) and HSV-2 IgG ($p=0.047$) seropositivity with age was observed. HSV-1 seropositivity was lower in participants less than 40 years (70.9 and 65.8%) compared to those above 40 years (87.0-88.0). HSV-2 seropositivity increased progressively with age from 1.8% in the age group ≤29 years to 17.4% in the age group 50-59 years. Risk analysis showed that transplant candidates were at the highest risk of being HSV-1 IgG positive (OR=4.25, 95%CI=1.58-11.44, $p=0.018$). In addition, an association between older age (>60 for HSV-1 and >50 for HSV-2) and seropositivity was found (HSV-1 OR=3.00, CI=1.07-8.44, $p=0.036$; HSV-2 (OR=11.36, 95%CI=1.19-108.16, $p=0.034$). The presented results showed that age is the most significant risk factor for the HSV-1 and HSV-2 seropositivity. Transplant candidates showed the highest HSV-1 and HSV-2 seropositivity, probably due to older age compared to other groups.

OP-12

Metavirome analysis of oral and fecal swabs from hedgehogs in central Russia: Coronaviruses, Mammarenaviruses, and differences in virus composition in active and hibernating hedgehogs

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Wild animals living around towns and villages carry a number of potentially zoonotic viruses. Hedgehogs are small animals that live close to human settlements and often come into contact with people. An important characteristic of hedgehogs is their ability to hibernate at low temperatures. Two closely related species live in Europe (*Erinaceus roumanicus* and *Erinaceus europaeus*) and interbreed in the territory of European Russia. We investigated the prevalence of viruses in hedgehogs (*Erinaceus sp*., most likely *E. roumanicus* or *E. roumanicus* x *E. europaeus*) sampled in 2022-2023a. We characterized the virome of 7 active animals captured in central Siberia and 14 animals from European Russia (7 active and 7 hibernating). High-throughput metaviromic analysis of total RNA/DNA extracted from oral and anal swabs was used for virome analysis. The most frequently identified viruses were putative vertebrate viruses. In European Russia, 21% (3/14) of hedgehogs were found to be carriers of betacoronaviruses and 28% (4/14) were found to be carriers of arenaviruses. The complete genomes of several betacoronaviruses and arenaviruses were assembled. The coronaviruses belong to the same phylogenetic clade as others from European hedgehogs (EriCoVs). The mammarenaviruses were represented by novel viruses related to MEMV (from Hungary). This is the second report of mammarenaviruses in European hedgehogs. The composition of the virome from animals from Siberia differs from that from European Russia. A number of plant/fungal viruses, insect/invertebrate viruses, amoeba/protists and bacteriophages were identified and complete genomes were assembled for some viruses (as collateral results).
One animal was co-infected with two different strains of EriCoVs and two arenaviruses (MEMV-like or Alxa-like). We propose that the concept of a 'superspreader' can be applied not only to humans but also to wild animals. We had the opportunity to compare the viromes of hedgehogs active in the wild immediately after capture with those hibernating in a rehabilitation center: the virus communities observed in the hibernating captive animals were significantly lower than those active in the wild. We hypothesize that a systematic analysis of the interaction between viruses and the hedgehog organism during the physiological changes that accompany the hibernation process may shed light on issues such as the long-term persistence of viruses in the mammalian organism.

OP-13

First virome analysis of severely symptomatic zucchini in Croatia reveals high prevalence and diversity of potyviruses

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During the vegetative seasons of the years 2021 and 2022, cucurbit fruits and/or leaves exhibiting virus-like symptoms were collected from the surroundings of Pitomača (Virovitica-Podravina County), Croatia. Symptoms ranged from leaf deformations, mosaic, and vein yellowing to mottling, deformations, and lumpiness of zucchini fruits. High throughput sequencing (HTS) using Illumina and Oxford Nanopore Technologies (ONT) identified the presence of potyviruses, including Watermelon mosaic virus (WMV), Zucchini yellow mosaic virus (ZYMV), and Moroccan watermelon mosaic virus (MWMV). Nanopore sequencing of a zucchini sample (T3) yielded 80,200 reads, covering 2.94% of the viral genomes and resulting in 238 reads assembled to the MWMV reference. This enabled us to obtain the partial polyprotein gene sequence of MWMV (GenBank accession number OQ729739). Conversely, Illumina sequencing of a zucchini sample (T5) generated 10,559,780 reads, covering 15.45% of the viral genomes, resulting in 2,049,672 reads assembled to the MWMV reference. This allowed us to assemble the complete genome of MWMV (GenBank accession number OQ729737). A Kraken algorithm-based bioinformatic analysis revealed the dominant presence of genus Potyvirus reads with a significant proportion of 42,85% and 50.5%, within T3 and T5 samples, respectively. RT-PCR and Sanger sequencing confirmed that all zucchini plants were co-infected with MWMV and WMV, while ZYMV was additionally detected in one out of six zucchini plants. Symptom analysis suggested that MWMV is the primary cause of the observed symptoms, with WMV and ZYMV contributing to a lesser extent. Phylogenetic analysis revealed that Croatian MWMV isolates cluster with other Mediterranean isolates in the Mediterranean group, regardless of the analysis type (NIb/CP region or whole genome). Interestingly, Croatian isolates in the NIb/CP genomic region form a distinct branch within the Mediterranean group, with 99% support. Based on the CP region, Croatian WMV isolates were assigned to Group 3, subgroup EM3, which stands for emerging isolates (EM). NIb/CP sequence of Croatian ZYMV zucchini isolate was grouped in a subgroup A1 under Group A, with most of other European isolates, representing no-emerging strains. This study represents the first report of MWMV in Croatia and highlights the utility of comprehensive virome analysis, providing insights into the viral diversity and epidemiology of potyviruses affecting cucurbit crops in Croatia.

OP-14

Clinical presentations of neuroinvasive flavivirus infections

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Human flavivirus infections have been a significant public health concern in eastern Croatia. Flaviviruses are a group of RNA viruses transmitted primarily by mosquitoes and ticks, with some species causing diseases in humans. The incidence of tick-borne encephalitis (TBE) in eastern Croatia has shown a rising trend, corresponding with the broader geographic spread of the disease in Europe. Since 1973, TBE has been confirmed in 104 patients treated at the Clinic for Infectious Diseases at the Clinical Hospital Center Osijek. The first human clinical cases of West Nile virus (WNV) infection in eastern Croatia were identified during an outbreak in August and September 2012. By the end of 2023, a total of 36 patients with neuroinvasive WNV infections were treated at the Clinic for Infectious Diseases at the Clinical Hospital Centre Osijek. Although most infections are asymptomatic or mild, some patients, especially those who are immunocompromised or elderly, may develop neuroinvasive diseases such as meningitis, encephalitis, or myelitis. This study aimed to analyze the clinical characteristics and laboratory parameters of patients with flavivirus infections in eastern Croatia. Neuroinvasive flavivirus infection was confirmed in 44 patients hospitalized from April 2017 to October 2023 (WNV: n=32; TBEV: n=11; USUV: n=1). Patients with WNV infection were older (median 65, IQR=6-89 years) than patients with TBEV infection (median 36, IQR=14-58 years) ($p<0.001$). Comorbidities were more frequently detected in patients with WNV (median 1, IQR=0-4). Comparing the main clinical presentations in patients with WNV and TBEV infections, there was no significant difference in the frequency of meningitis (59.4% vs. 63.6%, $p=0.999$) or meningoencephalitis (21.9% vs. 27.3%, $p=0.864$). Sporadic cases of cerebellitis, retinitis, myelitis, and polyradiculitis caused by WNV were also reported. Initial neutrophil pleocytosis in the cerebrospinal fluid was higher in patients with WNV infection (median 48.5%, IQR=25.3-58.5) compared to patients with TBE (median 10%, IQR=7.0-18.0, $p=0.008$). Conversely, initial lymphocyte pleocytosis was higher in patients with TBE (median 90%, IQR=82.0-93.0) compared to those with WNV infection (median 51.5%, IQR=40.8-74.7, $p=0.008$). Other laboratory findings were not significantly different between the two groups of patients. In 2018, the first case of neuroinvasive Usutu virus infection in eastern Croatia was identified in an immunocompromised patient, with a fatal outcome.

OP-15

First report of porcine parvovirus 8 in Europe: widespread detection and genetic characterization on commercial pig farms in Hungary and Slovakia

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Porcine parvoviruses (PPVs) are small DNA viruses that are widespread in swine populations. The first known PPV (PPV1) was identified in the 1960s and remains a major cause of reproductive losses in pigs. Recent advances in metagenomic technology have led to the identification of seven novel PPVs (PPV2–8) worldwide. However, their pathogenic roles and clinical relevance remain undefined. PPV8 was first detected in 2022 by high-throughput sequencing of lung tissue samples from PRRSV-positive pigs in China. Retrospective analyses indicates that PPV8 has been circulating in China since 1998, but it had not been reported in other countries until now. Like other PPVs, the single-stranded, linear DNA genome of PPV8 consists of two major open reading frames (ORFs): ORF1 encodes the non-structural protein (NS) and ORF2 encodes the capsid protein (VP). It shows a close genetic relation to PPV1 and is classified within the Protoparvovirus genus. The current study aims to detect the presence of PPV8 in large-scale pig herds in Hungary and Slovakia.
For prevalence estimation, a total of 2230 serum samples from different age groups, 233 oral fluid and 115 processing fluid samples were collected in a systematic way from 23 Hungarian and 2 Slovakian large scale swine herds between 2020 and 2023. A real-time quantitative PCR method was developed to detect the viral genome. In some cases, sequencing of the VP2-gene and genetic analysis were performed. PPV8 was present on 65% of the Hungarian farms and on both Slovakian farms included in our study, marking its first detection in Europe. The detection rates varied across sample types, with oral fluids showing exceptionally high positivity, reaching up to 100% in certain herds. The viral genome was successfully detected in serum and processing fluid samples as well, however with significantly lower prevalence rates of 4% and 5%, respectively. Genetic analysis of 11 partial VP2 sequences demonstrated high similarity to the original Chinese strain (GDJM2021) but with unique amino acid mutations, suggesting possible local evolution of the virus.
Our study confirms the presence of PPV8 in Europe. In the examined herds, the virus seems to circulate subclinically, as no overt clinical disease was reported in the herds during the period of the samplings. Further research is needed to assess the virus's prevalence in Europe and to understand its impact on swine health.

OP-16

Epidemiology of hepatitis E virus in Croatia

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The hepatitis E virus (HEV) is an emerging virus that poses a threat to global health. The seroprevalence rates of HEV vary significantly depending on the population group and geographic area. This study analyzed the HEV seroprevalence in different populations in Croatia testing between October 2016 and December 2021: transplant patients, animal-related professions, and nonexposed populations. The exposed population was represented by forestry workers (n=93), hunters (n=74), and veterinarians (n=151). The control group consisted of 118 pregnant women and 126 people from the general population. Hematopoietic stem cell transplant recipients (HSCTR; n=39), kidney transplant recipients (KTR; n=43), and liver transplant recipients (LTR; n=83) were among the transplant patients. The enzyme-linked immunosorbent assay was used to detect HEV immunoglobulin G antibodies, and the immunoblot test was used to confirm the results. Significantly different seroprevalence rates were observed between population groups: pregnant women 1.7%, forestry workers 6.5%, general population 7.1%, hunters 14.9%, and veterinarians 15.2%. LTR had the highest seropositivity in transplant patients (19.3%), while the seroprevalence in KTR and HSCTR was comparable to the general population (6.9% and 5.1%, respectively). Age-related increases in seropositivity were observed, rising from 2.9% in people under 30 to 23.5% in people over 60. HEV seropositivity was not correlated with sociodemographic factors (sex, education, area of residence, and number of household members), eating patterns (consumption of game meat, offal, and pork products), or housing and environmental factors (drinking water supply, type of water drainage/sewer, waste disposal, domestic animals). In conclusion, the results of this study showed that professionally exposed individuals and LTR are at higher risk of HEV infection. Furthermore, older age is a significant risk factor for HEV seropositivity.

OP-17

Assessment of West Nile virus transmission risk in Croatia

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West Nile virus (WNV) is one of the most widely distributed arboviruses. The epidemiological characteristics, as well as the impact of weather conditions (temperature, precipitation, humidity) and landscape patterns (flooded area, altitude above sea level, urbanization level, distance from forests and rivers) on WNV epidemiology, are still not fully understood. We analyzed the prevalence, epidemiological characteristics, and abiotic variables associated with WNV infections in Croatia from 2011 to 2014. The relationship between climatological, geomorphological, geographical, and demographic characteristics and the prevalence in horses was investigated in the counties with the highest number of acute infections and the highest seroprevalence rates. The WNV IgG seroprevalence rates ranged from 6.9% to 12.9%, while acute asymptomatic infections (IgM positive) were detected in 0.1-1.4% of horses. These results were consistent with the incidence of acute human infections in 2012 and indicated a more intense virus circulation, leading to an increased number of infections. A significant correlation of IgG seroprevalence with temperature was observed in 2011, 2013, and 2014 and with humidity in 2012. In contrast, a negative correlation of WNV infections with temperature was observed in 2011 and 2014 as well as the amount of precipitation in 2013. At the univariate level, the flooded area and the distance from forests were statistically significant predictors of WNV IgG seroprevalence in horses, while the distance from the water surface was a negative predictor. Only altitude and forest distance were significant at the multivariate level. In a few selected counties, the human WNV seroprevalence rates were 4.2% in 2013 and 0.9% in 2014. Seroprevalence in humans and horses correlated significantly in four of the six analyzed counties: the City of Zagreb, Zagreb County, Osijek-Baranja County, and Vukovar-Srijem County.

OP-18

Investigation of Feline Infectious Peritonitis Virus during SARS-CoV-2 pandemic

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During the SARS-CoV-2 epidemic in Slovenia, an abrupt increase in the number of cats with feline infectious peritonitis (FIP), caused by the feline coronavirus (FCoV) infection, was observed. Previous research has shown that cats are susceptible to infection with SARS-CoV-2 and that humans can be infected with FCoV. In line with these facts, the question arose as to whether a possible recombination event leading to specific mutations between the two viruses had taken place in cats, which could explain the increased number of cases of FIP during the epidemic and whether specific mutations could be associated with the clinical presentation of FCoV infection.
A total of 14 FCoV-positive domestic cats treated at the Small Animal Clinic, Veterinary Faculty, University of Ljubljana, were included in the study. The first group of 6 cats had clinically confirmed FIP and the second group of 8 cats had confirmed FCoV infection, but no clinical signs of FIP. We analyzed multiple samples taken from the first group of cats and stool samples from the second group of animals. Total nucleic acids were isolated for testing by metagenomics next-generation sequencing using the Pan-Coronavirus Enrichment Panel with Illumina RNA Prep. Results were analyzed using a custom in-house bioinformatics pipeline.
In all but one of the six clinically affected cats, the FCoV sequence was detected in different samples. Raw reads and contigs corresponding to FCoV showed the highest proportion in analyzed samples among other putative viral reads. Cats from the first group had nearly complete (47,5 – 91,7%) FCoV genomes assembled. The highest diversity was found in the spike gene when analyzing the sequence alignment of the assembled FCoV genomes. No SARS-CoV-2 genetic elements were found in the assembled FCoV genomes. Phylogenetic analyses showed that all FCoV genomes analyzed were relatively similar, yet differed significantly from SARS CoV-2 reference sequence. Finally, no significant differences were observed in the mutational profile of FCoVs from the two groups of cats.
The results of our study showed no recombination between FCoV and SARS-CoV-2 indicating that a recombination event between FCoV and SARS-CoV-2 is highly unlikely, as no SARS-CoV-2 genetic elements were detected in the FCoV sequences isolated from cats diagnosed with FIP. No specific mutational profile was detected that could predict the clinical outcome of FCoV infection when comparing the two groups of cats.

OP-19

Genomic surveillance in the era of SARS-CoV-2 Omicron variant of concern, from December 2021 to March 2024, in Greece

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Background: This study aims to describe the Omicron variant evolution in Greece from December 2021 to March 2024, as monitored by the Central Public Health Laboratory (CPHL) through genomic surveillance to inform public health actions.
Materials & Methods: 53.741 SARS-CoV-2-positive nasopharyngeal community- and hospital-acquired samples were pre-screened for S gene target failure (SGTF) using the TaqPath COVID-19 CE-IVD RT-PCR Kit. A subset of 22.878 samples have been sequenced with the Illumina NextSeq2000 platform and analyzed for lineage/clade classification using PANGO and Nextclade.
Results: Amid No-SGTF Delta variant dominance during December 2021, a surge in SGTF was observed, implying a new variant introduction. This was confirmed with WGS, identifying the first Omicron BA.1 case, with Omicron rapidly outplacing Delta over the next weeks. The SGTF surge peaked on week 7_2022, followed by a decrease until week 22_2022. Accordingly, WGS showed the predominance of BA.1 followed by the introduction and further spread of BA.2. From week 23_2022, the SGTF increased until week 7_2023, reflecting the emergence and further spread of BA.4 and BA.5. The BA.5 subvariants BQ.1-BQ.1.1 occurred in early October and had dominated BA.5 strains by week 14_2023. No cases of BA.3 have been identified so far. A gradual increase of No-SGTF was noticed from week 43_2022 due to BA.2 subvariants of interest BA.2.75 and CH.1.1. Finally, the XBB.1.5-like recombinant variants, firstly isolated in mid-December 2022, became dominant four weeks later in the BA.2 clade and kept No-SGTF cases high until week 51_2023, where SGTF cases started outnumbering them, and the latter have been dominating ever since. According to the NGS data, the mentioned weeks align with the emergence and sharp increase of BA.2.86 and BA.28.6+ variants, including the closely monitored JN.1.
Conclusions: The alignment of SGTF pre-screening with WGS results verifies it as a robust and useful pre-screening tool. Mass public testing and monitoring of hospital admissions allowed for efficient epidemiological and genomic surveillance, and timely identification of the newly circulating variants for public health monitoring.

OP-20

The role of the interaction between cytoplasmic protein phosphatases and HPV E7

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Prophylactic vaccines have the potential to have a major impact on the global burden of human papillomavirus (HPV)-)-associated malignancies; however, they lack therapeutic potential. Therefore, there is still a pressing need to understand the pathomechanism of HPV-associated diseases for the development of therapeutic interventions. A hallmark feature of HPV-attributable cancers is the continued and high-level expression of viral early oncoproteins. These HPV proteins, particularly E7, play a significant role in the induction of malignancy by targeting critical cell control pathways. Using mass spectrometry analysis, PTPN14 and MYPT1 were identified as novel interactors of high-risk HPV E7. We aimed to characterize these associations during our experiments to clarify the role of PTPN14 and MYPT1 regulated proteins in the life cycle of the virus and in the development of HPV-associated diseases.

First, we studied protein expression levels in several HPV positive and negative cell lines by using Western blot. Next, we investigated the effect of HPV E7 proteins from different genotypes (HPV-11, HPV-16, HPV-18, HPV-31) on the steady state expression level of the studied phosphatases and their target proteins, and we performed HPV E6/E7 specific siRNA treatment in HPV-18 positive HeLa and HPV-16 positive CaSki cells to investigate the effect of gene silencing on protein expression. Moreover, we confirmed the interactions between E7 and cellular proteins by using pull-down experiments.

We show that the presence of HPV E7 proteins leads to altered expression levels and cellular localization of the studied protein phosphatases and regulatory proteins; thereby possibly affecting their function. Moreover, we observed an association between HPV E7, PTPN14, MYPT1, and certain mitotic kinases, indicating that these interactions might be important for the function of HPV E7 to enhance host cell proliferation and promote malignant transformation.

OP-21

Genomic surveillance of respiratory viruses in Croatia

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The HERA2 project, supported by the European Union's Health and Digital Executive Agency (HaDEA), aims to enhance whole genome sequencing (WGS) and polymerase chain reaction (PCR) methods and infrastructure for surveillance and outbreak investigation. One of the objectives is to improve national and European surveillance systems by enhancing sentinel surveillance of influenza-like illness and/or acute respiratory infections using PCR and WGS methods. To achieve this, we focused on implementing genomic surveillance of Influenza A and B viruses and respiratory syncytial virus (RSV) alongside the existing SARS-CoV-2 surveillance.

Genomic monitoring using next-generation sequencing data is crucial in modern surveillance strategies, aiding in the characterization of viruses to identify strains and variants and conducting antiviral resistance testing. This allows for more efficient monitoring of trends, patterns, and outbreaks, especially cross-border outbreak detection.

In Croatia, SARS-CoV-2 genomic surveillance commenced in early 2021. Since then, 46,374 SARS-CoV-2 sequences have been sequenced, analyzed, and uploaded to GISAID database. Monitoring of variants helped in tracking transmission and regional spread utilizing county-based surveillance and data visualization through the Nextstrain tool.

Influenza surveillance assists in identifying new subtypes and resistance to major antiviral drugs. The data submitted contribute to joint WHO and ECDC characterization reports which are crucial for vaccine composition planning and tracking spread and genomic shifts of the virus. To date, 157 influenza viruses have been sequenced, including 155 influenza A, and two influenza B. RSV, a leading cause of lower respiratory tract infections in young children with high morbidity and mortality rates is another focus of our genomic surveillance. This monitoring helps track seasonality and identify viral clades, and from the last RSV season, we sequenced, analyzed, and published 47 viral sequences.

For genomic laboratory surveillance we use high throughput, short and long read sequencing devices. A robust bioinformatics pipeline supports this infrastructure, encompassing quality control, alignment, genome assembly, variant calling, and phylogenetic analysis. All collected data are available in publicly accessible databases, ensuring transparency and facilitating global collaboration and early response.

OP-22

Intensive care unit treatment in people living with HIV in Croatia: a single Centre 25-year experience

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HIV/AIDS remains a challenge for intensive care unit (ICU) physicians, and the outcomes of critically ill HIV/AIDS patients are understudied. We retrospectively reviewed persons living with HIV (PLHW) treated in the ICU at the University Hospital for Infectious Diseases, Zagreb, Croatia, from 1996 to 2021. Follow-up ended in December 2021. We assessed mortality, length of stay (LOS), and modified Rankin Scale (mRS) at discharge and follow-up (mRS 0-2 was considered favorable). Kaplan-Meier analysis was done to examine factors related to mortality. Out of 8484 patients treated in the ICU, 98 (1.2%) had HIV/AIDS. Of 92 evaluable PLWH median age was 48.5 (25 - 71) years, 83 (90.2%) were male. In 53 (57.6%) HIV infection was recently diagnosed (within 3 months of admission), while 39 (44.4%) had been previously diagnosed. There were three patterns of admission to ICU: newly-diagnosed patients (N= 53), ART non-adherent patients (N= 14) and ART adherent patients (N= 25). The most common condition in newly diagnosed patients (23, 39.7%) and ART non-adherent (8, 42.1%) was *Pneumocystis jirovecii* pneumonia, while sepsis (4, 18.1%.) was most common in adherent ones. Mechanical ventilation (MV) was used in 64 (69.6%), VV-ECMO in 8 (8.7%), and renal replacement therapy in 22 (23.9%) PLWH. ART was administered to 60 (65.2%); ten (16.7%) had an immune reconstitution inflammatory syndrome. Of 92 PLWH, 48 (52.2%) died during hospital stay (figure 1). The median ICU and hospital LOS were 8 (1–143) and 33 (3–232) days, respectively. At discharge, 14 (15.2%) patients had moderate to severe disability (mRS 3-5), while 30 (32.6%) had mRS 0-2. The median follow-up of survivors was 64 (1-230) months. Nine patients (22.5%) died, 7 (17.5%) had moderate to severe disability (mRS 3-5), and 28 (70.0%) had mRS 0-2 (figure 2). The probability of survival at 30 days after admission was lower in those on MV, whereas there was no difference regarding viral load, CD4+ count and age (figure 3).
More than half of critically ill PLWH were newly diagnosed and treated for opportunistic infections. In-hospital mortality was high, and was also a significant during follow-up.

SPONSOR PRESENTATIONS

SP-1

Different solutions in the diagnosis of emergent animal diseases

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SP-2

Monolithic Chromatography in Viruses and Viral Vector Applications for Purification and Analysis

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Viral vectors are among the most effective means of gene transfer for modifying specific cell types or tissues and can be manipulated to express therapeutic genes. Currently, various viral vectors, including adenoviruses (Ad), adeno-associated viruses (AAV), retroviruses (γ-retroviruses and lentiviruses), and others, are employed in thousands of clinical trials. Applications such as gene therapy, oncolytic therapy, and vaccines frequently use viral vectors to deliver target genes to patients. Additionally, wild-type viruses, like bacteriophages that infect and replicate only in bacterial cells, are regaining importance due to antibiotic-resistant pathogenic bacterial strains.
Monoliths are highly porous, single-piece chromatographic media used for the purification and separation of biomolecules, including viruses, proteins, and nucleic acids. Their unique structure allows for high flow rates, high binding capacities and low shear forces, making them ideal for such applications. Monolithic chromatography as a tool to concentrate viruses was first described 20 years ago. Since then, the number of scientific publications has grown significantly, and there are already therapeutic products on the market that use monoliths in production process.
Recent applications will be discussed in the presentation, including:

- Purification of lentiviral vectors using anion-exchange monoliths, where more than 60% yield was achieved with significant impurity removal.
- Removing 7 logs of endotoxins from bacteriophage lysate with two-step monolithic purification.
- Simultaneous analysis of Orf-virus concentration and purity with chromatography analytical methods employing multi-angle light scattering detector (MALS).
- Gene therapy and the main contributions of monoliths to the field – purification of AAV with cation-exchange chromatography and separating different AAV species (empty, full, partial capsids) with anion-exchange chromatography.

SP-3

Emerging viruses – from another perspective

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The latest advancements in viral-based gene therapies are redefining how we use viruses to treat various diseases. Viruses like Adeno-Associated Viruses (AAVs) and Lentiviruses are increasingly harnessed as delivery tools for genetic material, offering new possibilities for tackling genetic disorders, cancers, and other complex conditions. New approaches are enhancing the quality, consistency, and safety of these viral vectors, which are essential for effective gene therapy. Streamlining the production and purification of viral vectors is key to achieving high purity and stability, reducing contamination risks, and improving clinical outcomes. In this lecture, we will explore how these processes are being optimized and why they are crucial for advancing gene therapies. Precise characterization techniques also play a critical role, with methods to measure viral genome copies, monitor capsid integrity, and assess aggregation, ensuring that each therapy meets stringent regulatory standards and is both safe and effective. As the field moves toward more personalized treatments, maintaining the stability and functionality of viral vectors from production to patient delivery becomes increasingly vital. We will cover innovative techniques for monitoring virus stability across various formulations and conditions to preserve their therapeutic potential. These advancements are paving the way for safer, more effective, and more personalized solutions to treat genetic disorders, cancers, and other challenging diseases, marking a transformative step in modern medicine.

POSTER PRESENTATIONS

PS1-1

Setting up a monitoring of highly pathogenic avian influenza virus in environmental waters at migratory bird gathering sites

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Highly Pathogenic Avian Influenza (HPAI) poses significant risks to wild birds and farmed poultry. Outbreaks and spillovers caused by the Eurasian strain of H5N1 (2.3.4.4b clade) have been occurring since 2021 in both terrestrial and marine mammals, including recent ones in cattle farms in the USA, with the consequent increased zoonotic pressure and potential risk for human health. This calls for advanced monitoring strategies to track the presence and introduction of known and new strains of avian flu virus into new regions. Wastewater-based epidemiology has proven to be a valuable tool for monitoring the presence of viruses, such as SARS-CoV-2, in the population. Analysis of environmental irrigation waters has also helped to investigate plant viruses occurring in crops and wild plants.
Here, in the frame of the project IAVSur – BME Influenza A Viruses Surveillance in Birds, Mammals, and Environment, GA 101132734, financed by EU4H/HADEA, coordinated by the Slovenian Administration for Food Safety, Veterinary Sector and Plant Protection, we will use our expertise gained during previous analysis of wastewater and environmental waters to set up a pilot monitoring of environmental water bodies frequented by migratory birds. This study focuses on optimizing methods for detecting the HPAI virus in lakes and other water bodies where migratory bird flocks congregate, thereby providing critical insights for early warning systems and outbreak prevention. The monitoring will run from 2024 to 2027, 6 months per year and will include water and sediment samples. We will combine comprehensive field sampling, advanced molecular diagnostics (qPCR, dPCR, and targeted HTS), and innovative concentration methods to maximize virus recovery and detection sensitivity. In this presentation we will describe the selection of qPCR/dPCR assays and comparison of different methods for concentration of large water volumes, using water samples inoculated with thermally inactivated HPAI H5N1 strain. We will also show the first results obtained in the first sampling time points, which started in July 2024.

PS1-2

Investigation of miRNA modulation during infection with potato spindle tuber viroid (PSTVd) upon treatment with 2,6-dichloroisonicotinic acid (INA)

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One of the pathogens that causes significant yield losses in potato (Solanum tuberosum L.) by leading to stunted growth and spindle-shaped tubers is potato spindle tuber viroid (PSTVd). The molecular mechanisms underlying PSTVd infection involve complex interactions between the viroid and the regulatory pathways of the host plant, in particular the microRNA (miRNA) machinery. PSTVd triggers the disease through viroid-driven small RNAs (sRNAs), altering the defense signaling pathway and hormone synthesis and is closely linked to the role of plant microRNA (miRNA). These miRNAs play a crucial role in regulating plant development, response to abiotic and biotic stresses and also have a role in the modulation of plant immunity. The signaling pathways of various hormones, like salicylic acid (SA), can directly influence the transcription of certain miRNA genes. SA is crucial for basal defense mechanisms, the establishment and maintenance of systemic acquired resistance (SAR) and the regulation of the severity of viroid infections. Exogenous treatment with 2,6-dichloroisonicotinic acid (INA), a functional analog of SA, was used to investigate the role of miRNAs in hormone-mediated defense responses against PSTVd infection. This study investigated the changes in miRNA expression profiles in PSTVd-infected potatoes and evaluated the potential effects of INA. Using RNA sequencing (RNA-seq), we identified a comprehensive set of miRNAs whose expression levels were significantly modulated in response to PSTVd infection. Key miRNAs were found to be up- or down-regulated, suggesting their role in host defense mechanisms and viroid pathogenesis. To validate the RNA-seq results, stem-loop real-time polymerase chain reactions (RT-qPCR) were performed, which confirmed the differential expression of the selected miRNAs. Further analysis showed that the application of INA led to changes in the miRNA expression of PSTVd-infected potatoes, indicating the potential of the compound to modulate the regulatory networks of host genes in favor of resistance. In addition, target gene analysis revealed that the expression levels of genes associated with stress responses, signal transduction and metabolic processes were significantly affected by the changes in miRNA expression. This study provides new insights into miRNA-mediated regulatory mechanisms during PSTVd infection and highlights the potential of INA as a therapeutic agent to modulate these signaling pathways.

PS1-3

Molecular characteristics of Polish watermelon mosaic virus isolates

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Watermelon mosaic virus (WMV) is a member of the *Potyvirus* genus infecting over 170 plant species, belonging to 27 different families. WMV is one of the most prevalent viruses in cucurbit crops, including zucchini. Symptoms affected on infected plants can vary from mild (or even the presence of the virus in the plant can be asymptomatic) to severe and include vein bindings, chlorotic mosaic, leaf deformation, and characteristic chlorotic rings on fruits. Zucchini (Cucurbita pepo var. giromontiina) is one of the major cucurbit species cultivated in Poland, and previous years have shown that zucchini crops in Poland are infected by different virus species, also in mixed infections. In 2022-2023, two regions of Poland, Wielkopolska and Kujawsko-Pomorskie, were subjected to survey of the presence of WMV. Over 1000 asymptomatic and symptomatic zucchini samples as well as weeds growing near zucchini crops were collected and combined in groups of 10. Subsequently, from pooled samples the total RNA was isolated and sequenced using high throughput sequencing. The obtained results were analyzed on the CLC Genomic Workbench platform (Qiagen, Germany). Among over 100 obtained WMV sequences the representants of the Polish WMV population were chosen. Subsequently, coat protein (CP) coding sequences of the Polish isolates and sequences deposited in GenBank (https://www.ncbi.nlm.nih.gov) were used to detect recombinants with RDP4. The selective pressure acting on particular codons was investigated using www.datamonkey.org platform. The phylogenetic analysis was performed in the MEGA X program. The analyses revealed that recombination is pervasive in the WMV population. Recombinants were found both among the Polish isolates, as well as those originated from different part of the world. The Polish population of WMV is diverse, however among all mutations, synonymous are prevailed. The identity of nucleotide and amino acid CP sequences is 93.2 %-100 % and 96.8 %-100 %, respectively. Based on the analysed sequences, no codons under positive selective pressure were found. Despite the one WMV isolate obtained in 2022 in Kujawsko-Pomorskie, which is grouping with isolates from the phylogenetic G1 group, the rest of the Polish WMV isolates belong to different subgroups in group G3 of WMV.

This study was supported from the project 2021/41/B/NZ9/03574 from National Science Centre of Poland.

PS1-4

Extraction-free method for citrus leaf blotch virus RT-PCR detection from sweet orange samples

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Citrus leaf blotch virus (CLBV) is the first discovered virus of the genus Citrivirus (subfamily Trivirinae, family Betaflexiviridae). It is known to be the causal agent of bud union disorder of trifoliate orange rootstock, vein clearing, chlorotic blotching, and stem pitting in various citrus plants. CLBV has been recorded in citrus all over the world, including Mediterranean countries. It is primarily transmitted via grafting and, to a lesser extent, through seeds. As CLBV has been linked to economically important citrus diseases, various molecular assays have been successfully applied for its detection. Nonetheless, most methods require nucleic acid extraction as the first step in the protocol. This can be impractical for testing remotely located trees because the tissue, and the viral RNA therein, need to be cold preserved prior to the analysis. One solution to this problem is a fast extraction-free method using FTA cards (QIAcards, Qiagen) for nucleic acid immobilization in the field. Using this method, tissue prints of rolled leaves cross sections with midveins, petioles and fruit columella were collected from a sweet orange (Citrus sinensis (L.) Osbeck 'Washington Navel') orchard in Trogir. Cards were air-dried for 30 minutes and stored at room temperature for several months prior to analysis. Two simple extraction-free methods were tested to release immobilized RNA from the cards into a buffer and thus provide a template for CLBV RT-PCR detection with specific primers. A couple of squares (3x3 mm) cut out from each FTA card were treated with 5 % Triton X-100, rinsed with TE buffer and dried provided reliable results in CLBV RT-PCR detection. Notably, the cards with imprints from fruit columella enabled more sensitive CLBV detection than leaf vein and petiole imprints. CLBV was thus detected from symptomatic orange previously confirmed to be virus infected with several other methods and CLBV-negative trees were consistently negative. FTA cards provide fast and practical method for field CLBV screening, collection of its small-scale RNA samples, the amplification-based detection and other downstream applications.

PS1-5

First report and complete genome of citrus vein enation virus infecting Satsuma mandarin in Montenegro

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The cultivation of citrus crops in Montenegro is of significant economic importance. The geographic location of the country provides an optimal environment for the growth of cold-tolerant citrus varieties, such as the Satsuma mandarin (Citrus unshiu Marc.), which accounts for 85% of the total citrus production. In 2018, a single non-symptomatic Satsuma mandarin mother tree from the Herceg-Novi municipal district was subjected to high-throughput sequencing (HTS) analysis. The generated dataset included 7,646,702 raw reads, which were assembled de novo using Geneious Prime software (version 2023.1.1) and subjected to BLASTn analysis. The alignment showed the assignment of the obtained contigs to three distinct pathogens, namely citrus vein enation virus (CVEV), citrus tristeza virus (CTV), and hop stunt viroid (HSVd), and complete genome sequences could be reconstructed. In this work, infection of CVEV was confirmed by RT-PCR using primers VE5f/VE15r (Vives et al., 2013). A multiple alignment of the obtained CVEV genome and full-length genome sequences from GenBank showed ~97-99% nt identity among each other, and phylogenetic analysis revealed that the CVEV isolate from Montenegro clustered with isolates from the USA, rather than with European ones. Furthermore, the isolate from Montenegro exhibited the same four amino acid residues at positions 83, 104 and 113 of ORF2 and 41 of ORF5 common to the majority of non-European CVEV isolates. In light of the substantial economic consequences of this virus for the citrus industry, further investigation is essential to ascertain the prevalence of the virus in citrus crops within the country. To the best of our knowledge, this is the first report of CVEV in Montenegro.

PS1-6

Occurrence and population structure of viruses infecting zucchini in Poland

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The occurrence of viral diseases in crops hamper the sustainability of food production, often leading to economically significant losses. Additionally, ongoing climate changes favor the emergence of new viruses and the expansion of ecological niches of existing ones i.e. by increasing the population of their vectors. Climate change has various effects on vectors such as the modification of vector phenology, vector over-wintering, density, migration, and its predators. It is estimated that currently aphids are the most common and economically important vectors of plant viruses, transmitting hundreds of different species. A warm and long autumn allows for the free development of aphids and the spread of a viral infection in cultivated fields of plants, including those from the Cucurbitaceae family. The aim of our research was to conduct comprehensive analyzes of the occurrence and population structure of viruses infecting zucchini crops in Poland. In 2022-2023, over 900 zucchini samples were collected in spring and summer in the Wielkopolska and Kujawsko-Pomorskie regions. Both symptomatic and asymptomatic samples were collected and combined in groups of 10. Symptomatic plants were characterized by different disease symptoms such as chlorosis, deformations, mosaic and discolorations on leaf blades, and deformations and discoloration of the fruits. RNAs were then isolated from the pooled samples and sequenced using HTS on the Illumina platform. The obtained results were analyzed on the CLC Genomic Workbench platform (Qiagen, Germany). The preliminary analyzes showed the in both studied years and regions, watermelon mosaic virus (WMV, Potyvirus genus, Potyviridae family) was the predominant species and occurred in 98% of pooled samples. In the majority of samples, the mixed infections have been observed with other viruses such as zucchini yellow mosaic virus (ZYMV, *Potyvirus* genus, Potyviridae family) and cucumber mosaic virus (CMV, Cucumovirus genus, Bromoviridae family). Mixed infections can cause disease synergism due to an increase in viral replication, viral movement, and interference with host defense mechanisms. It is suspected that the presence of these viruses is related to early invasions of aphids on agricultural fields, which transmit the virus from nearby weeds and wild plants. The research will be continued this year.

PS1-7

Viral Treasure Hunt: Unearthing Novel Tobamoviruses in Public Databases

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Members of Tobamovirus have caused significant crop losses worldwide, with new species emerging over the last decade. Tobamoviruses are characterized by highly stable virions and are efficiently disseminated via mechanical vectors, as well as passively through aqueous environments, soil and seeds. The recent emergence of at least two tobamoviruses with significant agricultural impacts emphasizes the need for proactive surveillance and discovery of yet unknown tobamoviruses in diverse plant species and environmental samples. This could accelerate the development of diagnostic tests and provide insights into the potential points of origin and future spread of such viruses.

Data mining is one of the promising approaches to increase our knowledge about these emerging plant pathogens. In our study, an extensive collection of datasets that could potentially harbor known and potentially unknown tobamoviruses was obtained from public repositories, including samples from wastewater, human and animal guts, and different plant species. A pipeline for automated processing of large datasets was established. Using this approach both known and potentially novel tobamoviral sequences were obtained from a variety of sources.

Several challenges were encountered during the examination of the data obtained from analyzed datasets, e.g. reliable classification of short and incomplete virus-like sequences. To reduce the rate of both false positive and false negative classification of short sequences, different strategies were developed depending on their length and presence of open reading frames. This research not only contributes to our understanding of the diversity and distribution of tobamoviruses but also highlights the importance of continuous innovation in data analysis techniques to keep pace with the rapidly expanding volume of genomic data. As we continue to refine our methods and explore new datasets, we look forward to uncovering further insights into the world of plant pathogens.

PS1-8

Water and soil contaminated with emerging tobamoviruses are the source of plant infections

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The recent emergence of tomato brown rugose fruit virus and tomato mottle mosaic virus (ToBRFV and ToMMV, Tobamovirus, Virgaviridae) poses a significant threat to global tomato and pepper production. Tobamoviruses have highly stable virions and are mainly transmitted through infected seeds, planting material, and mechanically, but other transmission routes should not be neglected. Our objective was to determine the possible transmission routes of emerging tobamoviruses via infested water or soil. Results of our study indicate that ToBRFV can remain infectious in water stored at room temperature for up to four weeks, while its RNA can be detected in water for at least four months. We have shown that ToBRFV-contaminated water used for irrigation in hydroponic or soil-based cropping systems can infect tomato plants through the roots after one to six months of exposure. In addition, soil infested with ToBRFV or ToMMV was confirmed to be an inoculum source for planted tomato seedlings and seedlings grown from seeds. These results indicate a new epidemiological pathway of ToBRFV and ToMMV via water and soil. The results fill existing knowledge gaps and point to the need to control the presence of emerging tobamoviruses and to disinfect irrigation water and soil in tomato and pepper production if contamination occurs.

PS1-9

Virome analysis reveals the presence of hop latent virus in hops in Brazil

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In Brazil, there has been a significant increase in the hop (Humulus lupulus) cultivated areas. To investigate the presence of viruses and viroids, we collected leaf samples of hop varieties in commercial yards located in Southeast Brazil. Total RNA was extracted and processed with the Ribo-Zero rRNA removal kit (Illumina, USA), followed by cDNA library construction using TruSeq RNA library preparation kit (Illumina) and sequencing by Illumina HiSeq 2000 paired-end method. Raw reads underwent quality trimming and de novo assembly using the CLC Genome Workbench 6.5.2. Contigs associated with viruses and viroids were identified through BLASTx and BLASTn analysis, respectively, against a viral/viroid RefSeq databases from the NCBI and implemented within the Geneious program. Trimmed reads were mapped back to the respective viral genomes using Geneious 11.1.5 software (Biomatters). Viral genome annotation was performed, wherein open reading frames were assigned through BLASTx searches against the NCBI non-redundant protein database. Analysis of the sequencing data unveiled the presence of hop latent viroid (HLVd), the genome of the carlavirus hop latent virus (HpLV), and new viruses belonging to the genera Polerovirus and Potyvirus. To confirm the presence of HpLV and HLVd, RT-PCR with specific primers were performed, with positive results for the presence of both pathogens in the original samples of 'Cascade' and 'Comet' varieties from Minas Gerais. Phylogenetic analyses were performed using the coat protein amino acid sequences through the maximum likelihood method. The Brazilian HpLV formed a distinct branch alongside other HpLV variants, as well as sequences from hop mosaic virus and yam latent virus. In a phylogenetic tree analysis featuring 20 HpLV sequences from several countries, the Brazilian isolate clustered within a branch containing isolates from China (ABN11180, ABN11181, ABN11182) and Italy (QKY12183). This is the first report of HpLV in hop in Brazil. A more comprehensive survey will still be carried out on the incidence of this virus in hop yards in different regions of Brazil, and the infection status of other hop-cultivated varieties.

This work has been performed in the frame of the CNR(Italy)/FAPESP(Brazil) joint project "Comparative analyses of hop- and citrus-associated virome in Brazil and Italy and studies on viroid-induced pathogenesis in hop (2024-2025)" (2023/07807-2). ME is supported by CNPq research fellowship (401753/2023-9).

PS1-10

Pathogen Profiling of Petunia hybrida Using High-Throughput RNA Sequencing and Multilocus Sequence Typing Analysis

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Petunia hybrida, a widely cultivated ornamental bedding plant, can host a wide range of viruses, viroids, and bacteria. Our primary objective was to identify the diversity of these pathogens in selected petunia populations of Bulgaria. This is crucial for understanding the pathogens' global dispersal due to trade, their impact on horticulture, and the associated phytosanitary risks of their potential spread to neighboring crops.
In the current study, we have conducted a real-time PCR-based diagnostic analysis of petunias showing phytoplasma-like symptoms. Triplex real-time PCR assay targeting map gene revealed the presence of 'Candidatus Phytoplasma solani' in one of the analyzed petunia plants exhibiting symptoms such as shortened internodes, small leaves, and yellowing. Furthermore, amplification of the 16S rRNA gene fragment in direct and nested PCR followed by Sanger sequencing confirmed the association with 'Ca. P. solani'. Multilocus sequence typing analysis (MLST) of non-ribosomal genes was subsequently used for phytoplasma subtype identification. The use of MLST provides detailed information on the specific strain of the phytoplasma, which is valuable for managing the disease and preventing its spread.
To achieve a comprehensive viral profile, pooled RNA samples were subjected to high-throughput RNA sequencing. High-quality reads were classified using Kraken2 with PlusPFP DB, and the metatranscriptomics viral data were further analyzed and visualized with the Pavian Shiny tool. Viral prevalence and diversity analysis identified mainly plant viral species in P.hybrida, including petunia vein clearing virus, pepper chlorotic spot virus, and soybean mosaic virus. Additionally, we detected the insect virus Choristoneura fumiferana granulovirus of seed-borne origin. The obtained data will be validated further by targeted RT-PCR. These findings revealed the seed-invading viruses of petunia, promoting the need for strict phytosanitary control and better propagation practices. Furthermore, this study demonstrates the effectiveness of RNA sequencing in characterizing complex pathogen populations that can be further exploited to study host-virus interactions.

Acknowledgements: The work was funded by the Bulgarian National Fund grant number КП06 Н76/10. The authors are thankful to Dr. W. Menzel and Dr. P. Margaria (Leibniz Institute DSMZ) for the support in the frame of the EVAg Trans-National Access program.

PS1-11

Detection of norovirus and hepatitis A in berries

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In recent years, there has been a growing recognition of viruses as significant pathogens that can be transmitted through contaminated food, particularly in minimally processed items like fresh fruits and vegetables. Serbia, being the largest exporter of raspberries in the EU, holds a crucial position in this context. Raspberries are considered perishable and are consumed both fresh or minimally processed, as well as being used as frozen ingredients in various food products. According to the European Food Safety Authority noroviruses (GI/GII) and hepatitis A virus are among the most important viruses that can be transmitted through the food chain. Berry contamination can occur at various stages of the production chain, highlighting the need for thorough monitoring and control measures to ensure food safety.

Based on above mentioned, the objective of the study was to gather data on the presence of noroviruses and hepatitis A virus in raspberry, blackberry, and strawberry samples grown in Serbia. Samples were collected from various independent producers between January 2019 and January 2024. The analysis focused on Norovirus (NoV) genogroups I (GI) and II (GII) as well as Hepatitis A Virus (HAV). The methodology employed followed ISO 15216-2:2019 standards for microbiology of the food chain, specifically the horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR. Out of 2034 raspberry samples analyzed, 11 (0.54%) showed an unsatisfactory positive result for NoV genogroup I (GI). Among 611 blackberry samples, 3 (0.49%) tested positive for NoV genogroup I (GI) and 1 (0.16%) for NoV genogroup II (GII). None of the 164 strawberry samples yielded an unsatisfactory positive result. Regarding HAV, no detections were reported out of 2398 analyzed berry samples.

The findings indicate that the cultivation and processing of berries in Serbia have reached a commendable standard concerning the safety and hygiene of the final products. However, there remain opportunities for further progress and enhancement, achievable only through sustained and rigorous monitoring efforts. This ongoing vigilance is crucial to continually improve practices and ensure the highest levels of food safety for consumers.

PS1-12

Development and application of Apple stem grooving virus as VIGS vector

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The Virus-Induced Gene Silencing (VIGS) method remains a highly suitable technique for studying gene function in plants. Although there are several VIGS vectors available, but they have their own limitations. In this study, we have developed Apple stem grooving virus (ASGV) as VIGS vector that has broad natural and experimental host range with no obvious symptoms on plants. The developed agroinfectious clone of ASGV was able to infect plants of the family Solanaceae, Fabaceae, Cucurbitaceae, and Amaranthaceae. The virus recombinant clone was also able to infect woody hosts like citrus. In order to develop it into a VIGS vector, the coat protein (CP) gene minimal promoter sequence (-70/+15) harboring full promoter activity was duplicated after the CP gene. The resulting plasmid showed instability that was subsequently stabilized with the introduction of a stop codon in ORF1 at nucleotide position 5621. The recombinant ASGV-based VIGS vector was able to silence the phytoene desaturase (pds) gene of bean and cucumber. This study successfully demonstrates the application of ASGV as a VIGS vector for future biotechnological and virological studies.

PS1-13

Investigation of RNAi Suppressor Coding Capacity of Prunus Virus F and Cherry Virus A in Solo and Mixed Infections

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In woody plants, multiple virus infections are common. Although infection with viruses individually is typically latent, their combined presence can have a synergistic effect. Surveying virus infection of cherry and sour cherry trees, the frequent and simultaneous presence of Prunus virus F (PrVF) and Cherry virus A (CVA) have been detected. During virus infection, the plant's defence system, the RNA interference (RNAi), is induced. To evade this mechanism, viruses encode proteins that act as viral suppressors of RNAi (VSR). Our research aimed to investigate whether PrVF and CVA encode proteins with VSR activity, and if so, how their activity is altered when they are present alone or in co-infection. For this, an Agrobacterium-mediated transient gene expression assay was performed. The movement protein (MP), large coat protein (LCP), and small coat protein (SCP) of PrVF, and the MP coding region of CVA were cloned into a BinHA binary plasmid using the In-fusion method. The recombinant constructs were transformed into Agrobacterium tumefaciens (strain C58C1) and infiltrated into leaves of wild-type Nicotiana benthamiana together with a GFP-expressing construct. To evaluate local VSR activity, GFP fluorescence signals in the infiltrated patches were visually examined under UV light at 3.5 days post-inoculation (dpi). The level of GFP protein expression was determined by western blotting, while GFP mRNA expression was determined by real-time RT-PCR. For the systemic VSR activity assay GFP transgenic N. benthamiana plants (line 16c) plants were used. The spread of the mobile silencing signal was monitored visually under UV light over 20 days. Our results show that LCP and SCP of PrVF act as local and systemic suppressors of RNAi, while MP of CVA exhibited weak local but strong systemic suppressor activity. Based on our preliminary data, in mixed infection (co-infiltration with LCP of PrVF and MP of CVA) the local suppressor activity was not enhanced, while the systemic spread of the silencing signal was slowed down more efficiently compared to the single infection.

Our work was supported by NKFIH K134895, the Únkp-23-3 New National Excellence Program Of The Ministry For Culture And Innovation from the source of The National Research, Development And Innovation Fund, the Erasmus Mundus Master Program in Plant Breeding and the Flagship Research Group Programme of the MATE. F.V. is a PhD student of the Doctoral School of Biological Sciences of MATE.

PS1-14

Exploring viroids within the extra-cellular space of plants and their potential implications in cross-kingdom trafficking

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The concept of cross-kingdom RNA trafficking via plant extracellular vesicles has emerged as an intriguing area of study in plant-microbe interactions. Extensive research highlights that plant extracellular vesicles (EVs) contain a diverse repertoire of RNA molecules, including small RNAs, fragmented and intact mRNAs, ribosomal RNAs (rRNAs), long non-coding RNAs (lncRNAs), and circular RNAs, among others. Viroids, classified as circular non-coding RNAs, may exploit these EVs for transport, offering a dual advantage of protection from the host's potentially hostile cellular environment and facilitating inter-cellular and intra-cellular movement by hitchhiking within EVs. Apple scar skin viroid (ASSVd) RNA is a non-coding RNA of size 330nts. ASSVd RNA has the ability to move cross-kingdom from plants to whiteflies and fungi, individually. In this study, we investigated the presence of ASSVd RNA in plant extracellular vesicles. The detection of ASSVd RNA in the extracellular space suggests that EVs could serve as a potential pathway for the cross-kingdom movement of viroid RNA. Notably, in mammals certain sorting factors (EXOmotif) are known to assist in RNA loading into extracellular vesicles (EVs). Interestingly, ASSVd RNA exhibits an intriguing pattern when subjected to sequence-specific screening for exomotif presence, revealing an enrichment of various exomotifs in its genome. Given that viroid RNAs have been documented to overcome barriers between different biological kingdoms, it would be an engrossing endeavor to ascertain whether the presence of exomotifs contributes to viroids loading into EVs and their subsequent inter-kingdom transport.

PS1-15

Molecular characteristics of barley virus G isolates from Poland

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Barley virus G (BVG) classified within the *Polerovirus* genus in the Solemoviridae family is a new threat to cereal crops in Poland. It was identified for the first time in 2023 using high-throughput-sequencing (HTS) technology in pooled barley samples showing leaf yellowing and stunting and then confirmed by RT-PCR with diagnostic primers from literature. A positive result was obtained for 3 out of 11 samples. Amplicons (394 bp in size) were purified and directly sequenced. The results of Sanger sequencing and HTS were consistent with each other (99% similarity). A Nucleotide BLAST search of the NCBI database revealed the highest similarity (99.83%) with BVG-18-326 (ON419456) and BVG-POR19SW (OL472215). The final results showed mixed infections of BVG and barley yellow dwarf virus-PAS (BYDV-PAS). In 2024, BVG was detected in 4 new locations in western, southern, south-eastern, and eastern Poland, in co-infection with BYDV-PAS in barley and oat , and with wheat dwarf virus in wheat. Due to the mixed nature of BVG infection, a set of total RNA samples, isolated previously from BYDV-infected plants, was also used. RT-PCR results confirmed BVG/BYDV-PAS co-infections in samples collected in 2021, 2019 and in 2015. This data indicate that BVG has been present in Poland for at least 9 years. Molecular characterization of the Polish BVG isolates was performed based on the full sequence of coat protein (CP) gene (594 nt long). RT-PCR reactions were carried out with newly developed primers. The oligonucleotides were designed by Primer3software based on the consensus sequence from HTS. For the study, 12 selected RT-PCR amplicons, from plants originating from various regions of Poland and collected in different seasons (2015-2024), were purified and sequenced. Resulted sequences were analysed and edited in BioEdit software and used in phylogenetic studies with 22 other BVG isolates from the GenBank database. Multiple sequence alignments were performed using ClustalW and sequence identity matrices were displayed by BioEdit and SDTv1.2. The phylogenetic relationships were analysed by ML algorithm, with chosen model (K2+G), bootstrap 1000, implemented in MEGA11. CP gene sequence of MaYDV-RMV (MH205607) served as an outgroup. Comparative analysis of obtained nucleotide sequences showed high level of nt identity between the Polish isolates ranged from 99.3% to 100%. Phylogenetic analysis indicated slight differentiation of the studied BVG population. The Polish isolates belong to a common group.

PS1-16

A novel bunya-like virus discovered in *Aphanomyces laevis*, an opportunistic oomycete pathogen of fishes

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Oomycetes are fungal-like microorganisms that are responsible for disease outbreaks in plant and animal populations worldwide and threaten natural biodiversity and food safety. In recent years, high-throughput sequencing (HTS) has enabled the discovery of a growing number of oomycete viruses. The documented effects on the host range from more or less asymptomatic infections to hypovirulence, i.e. a reduction in the ability of oomycetes to infect their multicellular hosts. However, all oomycete viruses reported so far have been detected in plant-associated oomycetes. In freshwaters, oomycetes from the genera *Saprolegnia* and *Aphanomyces* are important pathogens of fish and crayfish. Our aim was therefore to screen a collection of Saprolegnia and Aphanomyces isolates for viruses. Total RNA from 24 Aphanomyces spp. and 14 Saprolegnia parasitica cultures was isolated, pooled and sequenced on the Illumina platform for virus detection. The bioinformatics pipeline included filtering out low-quality reads from the raw data, followed by alignment of the samples with viral and host reference genome sequences. Subsequently, a de novo assembly was performed. In the RNA-seq data obtained, the sequence encoding the RNA-dependent RNA polymerase (RdRp) was detected with distant homology to members of the order Bunyavirales. Virus-specific primers were designed and used to detect the virus in the individual isolates by reverse transcription-polymerase chain reaction (RT-PCR). In this way, the presence of the virus was confirmed in an isolate of *Aphanomyces laevis*, a fish pathogen from the signal crayfish *Pacifastacus leniusculus* from Finland.
This is the first evidence of a viral sequence in freshwater oomycetes from the order Saprolegniales. Future studies should focus on deciphering the biological effects of the virus on the oomycete host, the mode of transmission and the host range. If the virus can reduce the virulence of the oomycete host, this research could open up the possibility for the development of alternatives to chemical treatment in aquaculture offering promising avenues for sustainable disease management.

PS1-17

Identification of citrus viruses and viroids in new weed hosts in Greece

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Citrus plants are infected by several viruses and viroid species, causing in some cases significant yield losses worldwide. A large-scale survey study of 3005 samples from five citrus-producing geographical areas was recently conducted in Greece about the presence of citrus viroids, and the results showed a widespread occurrence of five viroids in various citrus species. That study aimed to gain a deep knowledge on the presence of citrus viroids in order to control the CTV, as the only sustainable control measure is the use of resistance rootstocks which are very sensitive to viroids infections. The high incidence of the viroids (30-70%) raised important questions since their only know, yet, mode of transmission is by infected propagative material. Recent scientific works in different crops have shown the existence and the importance of reservoir hosts, and in citrus the information is limited apart from some viruses detected in other trees. In this study a total of 80 weed samples were randomly collected from 5 villages located at Chania prefecture (Crete island, Greece) from 21 different citrus orchards during autumn 2023 and spring 2024. The samples were identified as 39 different weed species and most of them were asymptomatic, whereas in some cases they showed symptoms such as yellowing in leaves. All samples were tested for the presence of CTV, CPsV, CLBV, CEVd, HSVd, CDVd, CBCVd and CBLVd by RT-PCR and direct sequencing of the PCR products. So far, the results have shown the presence of two viruses (CTV, CPsV) and four viroids (CEVd, HSVd, CDVd, CBCVd) in 9 different weed hosts (eg. Mediterranean asparagus, annual mercury, mallow, garland chrysanthemum, Chenopodium sp. etc). These isolates showed high nucleotide identities with other virus/viroids isolates from citrus. HTS analysis is under process to investigate also the whole virome in weed hosts. This study illustrates the first report of citrus viruses/viroids in weed hosts expanding our knowledge on their host-range.

«Acknowledgment: This research has been co-financed by the European Union (NextGenerationEU) and Greek national funds through the National Recovery and Resilience Plan (Greece 2.0), under the call Flagship actions in interdisciplinary scientific areas with a special interest in the connection with the productive sector (project code: TAEDR-0535675)»

PS1-18

Investigation of VSR coding capacity and possible synergistic effect of P0 and P4 protein of apple luteovirus 1

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RNA silencing is a homology-based gene inactivation mechanism. It plays a crucial role in plant immune responses during acute or chronic virus infections, which often pose serious threats to perennial fruit trees. Plant antiviral immunity is triggered by virus-derived double-stranded RNAs and suppresses virus activity via a sequence-specific degradation. Through plant-virus arms races, viruses evolved specific protein(s), known as viral suppressors of RNA silencing (VSRs) to combat plant antiviral responses by interacting with specific components of the plant RNA silencing machinery. Within the cell in which the virus is replicating VSRs can inhibit the production, accumulation of or effect locally-acting siRNAs (local suppression), or repress the spread of the silencing signal to distal tissues (systemic silencing suppression). Apple luteovirus 1 (ALV-1; genus Luteovirus) was described by high throughput sequencing (HTS) of apple trees affected by rapid apple decline disease. P4 protein of other Luteoviruses has been proven to act as VSR. ALV-1 has two ORFs (ORF0 and ORF5a) not present in other luteoviruses with possible protein-coding activity. ORF0 of ALV-1 is predicted to be present at the 5' end of its genome, in a similar position where P0, the VSR of Polemoviruses, Poleroviruses and Enamoviruses is encoded. In our work, we investigate the possible VSR activity of P0 and P4 expressed by ALV1 variants described in Hungary. For the VSR activity test, we used a transient assay. In this test possible VSR and GFP expressing Agrobacterium strains are co-expressed in Nicotiana benthamiana leaves. P0 and P4 coding regions of ALV-1 from different strains were amplified and inserted into HA-tagged binary plasmid by Takara In-Fusion kit. The constructs were tested for their ability to express P0 and P4 before testing them for their VSR ability. VSR activity was tested as a change in the intensity of the GFP fluorescence, GFP mRNA and protein level using qRT-PCR and Western blot, respectively. Our results revealed that both P4 and P0 proteins of ALV-1 displayed weak local VSR activity. Investigation of the systemic silencing effect of P4 and the effect of the possible synergistic action of P0 and P4 of ALV-1 are ongoing.

Our work was supported by the NKFIH K134895 and by the Flagship Research Group Programme of the MATE. A.J. is a PhD student at the Doctoral School of Plant Sciences of MATE with the Stipendium Hungaricum Scholarship of Tempus Foundation.

PS2-1

Novel SARS-CoV-2 E protein inhibitor identified in in vitro study using a surrogate murine hepatitis virus

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is constantly evolving and mutating over time into newer variants that threaten to disrupt established treatment strategies, making it imperative to find potential drug targets and effective therapeutics.
A high-throughput virtual screening (HTVS) campaign was performed using candidates from the ZINC15 database. Based on results, seven commercially available potential SARS-CoV-2 envelope protein inhibitor (EPRO) candidates were selected. EPRO, a structural protein and the smallest (8-12 kDa) of the major SARS-CoV-2 proteins is highly conserved and plays an important role in the pathogenicity of the virus and is, therefore, a promising target for drug development.
In this study, the L929 mouse cell line was used to propagate murine hepatitis virus (MHV, strain A59), a beta-coronavirus that served as a surrogate/model virus for SARS-CoV-2. It is evolutionarily and structurally very similar to SARS-CoV-2, while limiting the exposure of the environment and researchers to the dangerous pathogen.
Prior to antiviral testing, the cytotoxicity of the lead candidates on the L929 cell line was determined via CCK-8 assays. Hereafter, the lead candidates' feasibility was evaluated at two levels: (i) with plaque reduction assays determining the reduction of viral infectivity upon treatment with the inhibitor; (ii) with qPCR evaluating viral replication by assessing the presence and amount of the viral RNA. To gain insight into the mode of action of the candidates' different treatment conditions were applied.
One candidate was found to exhibit potent antiviral activity against MHV-A59 with an EC50 value of 12.1 uM, suggesting promising activity against SARS-CoV-2 EPRO. To confirm the observed antiviral activity of the candidate against SARS-CoV-2, further tests need to be performed. Molecular dynamics studies are planned to gain a more detailed insight into the binding within the EPRO ion channel and to determine the amino acid residues relevant for binding.
By expanding the known inhibitors, a step towards novel antiviral treatment options is made. The new findings can be used to investigate EPRO and its significance in coronaviruses in more detail.

PS2-2

Evaluation of antiviral activity of natural products on model bacteriophages and mammalian viruses

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Natural products are known sources of antiviral substances and have been used in many different applications to treat or prevent microbial infections, act anti-inflammatory, have antioxidative effect etc. Plant extracts (e.g. Helichrysum italicum) and animal byproducts (e.g. chitosan, propolis) have been shown also for their antiviral activity against different viruses. However, the characteristics of natural products present a challenge, due to variability in amount, availability, and solubility of the active substances, limiting the amount of reliable data. An antiviral activity testing platform was initially established on bacteriophages MS2 and phi6, model organisms for non-enveloped (e.g. enteric) viruses and enveloped (e.g. coronavirus) viruses, respectively. Chitosans, polysaccharides with high and low molecular weight, were shown to reduce bacteriophage phi6 concentration for 6 logarithms, while lower reduction was shown when applied to fabric. No such effect was observed on more robust MS2. However, evaluation of the antiviral activity on mammalian viruses can give insight into the mode of action of the substances in a more realistic environment, as model viruses are genetically and physiologically more related to human viruses. In vitro testing platform for evaluation of the antiviral activity of natural substances against respiratory viruses (Murine Hepatitis Virus, MHV, Coronaviridae; Adenovirus 5, AdV5, Adenoviridae) and enteric virus (Murine norovirus, Caliciviridae) was established. A commercially available aqueous dispersion of propolis was tested for its activity against model coronavirus. Different approaches of testing were applied to determine the best testing conditions and the post-incubation of the MHV infected cells with propolis, was shown to give the highest antiviral effect (SI ≈ 8). To optimise the search for active substances, in silico inverse docking approach using a natural compound library originating from H. italicum and targets from complete HMV proteome was performed. Moreover, hits were subjected to molecular dynamics simulations to evaluate binding pose stability. Selected hit compounds (e.g. phenolic acids, flavonoids) are being tested in parallel with *H. italicum* extracts, to confirm antiviral activity against MHV in vitro. Individual substances and extract will be further tested against AdV5 to widen the applicability of the *H. italicum* against respiratory viruses.

PS2-3

Viral landscapes of different crayfish species imported through pet trade

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Microbial and viral communities associated with hosts have been recognized as significant factors influencing the success of invasive species. These communities can either hinder or facilitate the establishment and spread of introduced species. Some members of these communities can be pathogenic, posing a severe threat to native hosts through a mechanism known as pathogen spillover. Conversely, pathogen spillback can occur when native pathogens infect introduced host species. Such transmissions can significantly alter local host-pathogen dynamics, increasing epidemiological risks to agriculture, aquaculture, wildlife ecosystems, and human health.
The risks and impacts of known and novel pathogens co-transported with their potentially invasive hosts are largely unexplored, unlegislated, and challenging to detect. To discover and detect such microbes sensitive and generic detection methods like high-throughput sequencing (HTS) based metagenomics are needed. This method offers unique opportunities for comprehensive detection of a broad range of microbes and viruses from different sample types.
The presence and role of viruses in association with non-native species have been understudied. However, in one of our previous studies the virome of the signal crayfish, one of the most successful invasive species of freshwater invertebrates in Europe, has been characterized by identifying novel and divergent RNA viruses associated with the species along its invasion range. Our current study focused on the characterization of virome of four popular exotic crayfish species imported to Europe through the pet trade, a major invasion pathway. Using an HTS-based metagenomic approach, multiple novel and divergent virus-like contigs were recovered in samples obtained from all four different crayfish species, spread through +ssRNA, -ssRNA, and dsRNA groups. Further examination placed these into a variety of orders and families within the Riboviria realm, including Picornavirales order and Permutotetraviridae, Hepeviridae, Totiviridae and Peribunyaviridae families. In addition, several known RNA and DNA viruses were detected in tested samples, including known and potential crayfish pathogens. This highlights the need for further research into the risks related to disease emergence and outbreaks associated with the pet trade.

PS2-4

Epidemiological and clinical characteristics of laboratory-confirmed MPOX cases in Greece - Central Public Health Laboratory (CPHL), June 2022-April 2023

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Introduction: In the context of the urgent response to the emergence of monkey pox disease/mpox, in non-endemic countries in 2022, CPHL was designated as one of the reference laboratories for the detection of virus. The epidemiological and clinical characteristics of the confirmed cases at CPHL are presented.
Materials and methods: Between June 2022 and April 2023, skin lesions from 79 suspected mpox cases originating from different hospitals were sent to CPHL. Nucleic acid extraction was performed using the Genesig Easy DNA/RNA kit, whereas, initially Real-time PCR was performed using the non-specific RealStar Orthopoxvirus PCR kit (Altona Diagnostics). Non-variola positive samples were confirmed as mpox positive by Sanger sequencing. Soon after, a special Monkeypox virus detection kit, (Real-time PCR Detection kit, Viasure /Spain) was used for the majority of samples.
Results: 50 confirmed cases of mpox were identified on the exact day the samples arrived at the laboratory, ensuring early diagnosis. The first case of mpox was detected on June 8, 2022, and the last on April 20, 2023. Sporadic cases were confirmed from the 38th week of the epidemic onwards. All cases were male. Their mean age was 38 years (range 23-61 years). 41 patients were Greek, while the foreigners were mainly European. All patients (100%) experienced rash, accompanied by fatigue (71.4%), fever (67.3%) and lymphadenopathy (65.3%). Six patients reported underlying immunodeficiency, 13 reported travel to a foreign country where cases of mpox had been reported, while 14 reported close contact with a known case.
Conclusions: The epidemiological and clinical characteristics of mpox cases in Greece were compatible with those of other EU countries. Imported cases were recorded early in the epidemic, while later, the epidemic was characterized locally by domestic cases and community transmission. Enhanced disease surveillance in Greece remains active to prevent a resurgence of the epidemic.

PS2-5

Echovirus 30 in Croatia during 2010-2019

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The enterovirus (EV) genus members from the Picornaviridae family are common etiological agents for a wide spectrum of infections, including neuroinvasive ones. Almost all Echoviruses, including Echovirus 30 (E30), belong to the species EV B. Here we report an analysis of epidemiology and molecular characteristics of E30 stains detected in Croatia, as a part of the EV surveillance program at the Croatian Institute of Public Health, Zagreb, for the period 2010 – 2019. Samples obtained from patients with suspected enteroviral disease were examined for EV presence by virus isolation in cell culture and/or RT-PCR. EV isolates were confirmed using serotyping following WHO guidelines. A phylogenetic analysis was conducted on the VP1 encoding genomic region sequences of the E30 isolates.
EV case positivity rate ranged from 2.53% in 2017 to 33.64% in 2012. Aseptic meningitis was the most common diagnosis. Enterovirus species B members dominated in the dataset (211, 75.89%), followed by A, D, and C species (17.99%, 1.44%, and 0,72%, respectively). E30 was the second most common type (24/278, 8,27%), after Echovirus 6. E30 first appeared in 2010 and 2011, reemerged in 2013-2015, prevailed in 2018, and declined in 2019.
Phylogeny data placed Croatian E30 strains within genogroups II, V, VIIa and VIIb. E30 strains from genogroup V were detected in the latest 2018 outbreak. Subgenogroup VIIa and VIIb circulated in preceding years. A single genogroup II member was detected in 2018.
E30 seems to occur every 3-5 years. The complex epidemiology of E30 and NPEV requires continued detection and surveillance of these underreported agents to increase clinical and epidemiological awareness.

PS2-6

PCR detection of herpes simplex virus 1 in patients with herpetic stromal keratitis

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Infection by the Herpes simplex virus 1 (HSV-1), of the Ortoherpesviridae family, is the leading cause of corneal damage and subsequent corneal blindness worldwide. A hallmark of HSV-1 is its ability to establish latency in sensory and autonomic ganglia. Reactivation of the virus by various stimuli can lead to recurrent disease, known as herpetic stromal keratitis (HSK). HSK is typically diagnosed solely based on clinical presentation, which can be problematic as other pathogens may present with similar symptoms. While laboratory tests such as viral culture, ELISA, and immunofluorescent antibody provide a more definite diagnosis, they are time-consuming and have limited sensitivity. Therefore, it is important to establish a simple but sensitive and reliable technique to set a correct diagnosis. We developed a PCR protocol using sets of primers specific for immediate early and late HSV-1 genes, previously described in the literature. After testing the sensitivity of each assay, using tenfold serial dilutions of bacterial artificial chromosome (BAC) carrying cloned HSV-1 genome as template DNA, two candidate primer sets for the amplification of VP16 and ICP4 were selected for further analysis. The sensitivity of selected assays was additionally tested using qPCR with the same template DNA. Results indicate that the VP16 assay can detect as few as a couple of hundred of viral genomes/µL, while the ICP4 assay has lower sensitivity, detecting thousands of genomes/µL. Potential Taq polymerase inhibition and sample quality were tested using 18S rRNA as a control. Furthermore, we show that adding genomic DNA to the BAC sample did not disrupt the sensitivity of the assays. In attempt to simplify the process, we tested multiplex assays on a clinical sample and BAC as a positive control. Another relevant question for clinical application is whether DNA isolation is needed prior to qPCR testing of samples. The ICP4 assay, tested on both isolated and non-isolated DNA from HSV-1 infected RPE cells, showed that DNA isolation is crucial for detection of viral genes. Based on these results, the two assays proved to be a potential upgrade from currently available diagnostic methods. However, the next step is to test the assays on a larger number of clinical samples in order to confirm their applicability. In summary, while current treatment options only control viral spread, providing an accurate and timely diagnosis via qPCR is vital to preventing corneal damage and blindness.

PS2-7

Extended HPV genotyping enables risk stratification for cervical cancer

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Human papillomavirus (HPV) infection is the main cause of cervical cancer and it can be proven in 99.7% of all cases of this cancer. The types that are most often associated with cervical cancer, called oncogenic or high-risk types (hrHPV), are: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. Genotypes HPV-16 and HPV-18 have the highest oncogenic potential and are the cause of about 70% of all cervical cancer cases worldwide. For this reason, PCR tests of the second generation, which provides individual genotyping for HPV-16 and HPV-18, and pooled detection of 12 other HPVs, are in everyday use for the diagnosis of HPV infection.
However, it is becoming clear that there are significant differences in the importance of individual genotypes that are included in the pool, and that certain HPV genotypes classified in the "12 other high-risk types" group have a significantly higher risk of developing ≥CIN3 than others in that group. New researches advocate the introduction of extended genotyping in additional risk stratification of HPV-positive women. According to the latest knowledge, the results of HPV genotyping can be classified into four groups according to the risk for the development of cervical cancer. Therefore, it is considered that extended genotyping provides clinically important information for further procedures, i.e. carrying out the appropriate treatment for the appropriate risk for the development of HSIL lesions that a certain HPV genotype has. These findings can help in better organization of cervical cancer screening.

PS2-8

SARS-CoV-2 Serological Diagnostic Development Using a Double Recognition Immunoassay Based on Plant-produced Nucleoprotein (N) and Receptor Binding Domain (RBD)

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Plants can be used as biofactories for recombinant protein expression, which is economically advantageous for producing large quantities of antigens or antibodies useful in serological diagnosis. During the SARS-CoV-2 pandemic, the rapid development of efficient and sensitive serological tests for monitoring the dynamics of the disease as well as the immune response after illness or vaccination was critical. The purpose of this study is to develop an enzyme immunoassay (EIA) for the detection of SARS-CoV-2 IgG in human serum, using plant-produced recombinant N and RBD Ag as coating proteins, and also to evaluate the sensitivity and specificity of this assay. A recombinant N protein and receptor-binding domain (RBD) bearing from hepatitis E virus capsid protein were transiently expressed in Nicotiana benthamiana plants, and were used to develop an in-house EIA. SDS-PAGE, Western blot, Maldi-TOF MS, and Mass photometry were used to analyze the expressed antigens. Western blot and Maldi-TOF analysis confirmed that N and HEV-RBD protein had been expressed well in plants. After purification through immobilized metal-anion chromatography (IMAC), the N (5µg/mL) and HEV-RBD (5µg/mL) were used to coat the plates. To validate the EIA tests, a panel of 84 sera from patients diagnosed with COVID-19 was used, and the results were compared to those obtained by another commercially available ELISA Kit (Dia.Pro D. B., Italy), which is already validated with clinical sensitivity 98 % and specificity 98%. The performance of a double recognition (N protein and HEV-RBD) EIA as a serologic test for SARS-CoV-2 antibody detection was shown to be highly satisfactory, with a calculated diagnostic sensitivity of 89.13% (95% Cl:76.43-96.38) and specificity of 94.7% (95% Cl:82.25-99.36) as compared to the reference ELISA, with a kappa (K) value of 0.832 and accuracy 91.67%. The assay cut-off value (A492 nm ≥ 0.10) was determined as the optimal value of sensitivity and specificity. The performance of an EIA based only on HEV-RBD (10 µg/mL) showed a sensitivity of 93.48% (95% Cl: 82.10 - 98.63), and a specificity of 92.11% (95% Cl: 78.62-98.34) with an assay cut-off value (A492 nm ≥ 0.12). Our study confirms that the transiently expressed in plants, N, and HEV-RBD proteins can be used to detect immune responses to SARS-CoV-2 in human sera reliably.

Acknowledgments: This research was funded by the Research Fund at the University of Plovdiv, MUPD23-BF-002.

PS2-9

Occurrence of highly pathogenic avian influenza in Croatia

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Highly pathogenic avian influenza (HPAI) is a highly contagious and often fatal viral disease in poultry, caused by highly pathogenic avian influenza viruses of subtypes H5 and H7. HPAI H5 viruses of A/goose/Guangdong/96-like (GD/96) lineage are of particular concern due to their presence in wild migratory birds and consequent global distribution including numerous outbreaks in poultry and occasional mammalian infections. GD/96 viruses of the H5N1 subtype were initially introduced into Croatia by wild birds in autumn 2005 with subsequent multiple introductions of H5N8 and H5N5 subtype in autumn/winter 2016/2017, H5N8 subtype in autumn/winter 2020/2021 and H5N1 subtype in all subsequent autumn/winters seasons. So far, the GD/96 viruses have been detected in mute swans, grey herons, mallards, great cormorants, black-headed gulls, common terns, gadwall, greylag goose, white-fronted goose, and common crane. Occasional outbreaks in poultry were also recorded annually from 2016 except in 2018 and 2019. All outbreaks were recorded in the area around the Drava, Sava, Danube, and Kupa rivers and on the coast of the Adriatic Sea. Seasonally, most occurred from October to March except a few outbreaks in May. Phylogenetically, the viruses detected in Croatia in the 2005/2006 season belonged to clade 2.2, which posed a serious threat to public health with a fatality rate of more than 50%. All other HPAI GD/96 viruses detected in Croatia belonged to different genotypes of clade 2.3.4.4b with typical avian makeup. However, recent events have shown the ability of these viruses to infect mammals, which could result in the virus adapting to new hosts increasing the risk of a new pandemic.

PS2-10

Characterization of infection and experimental evolution of Orsay virus in different species of the genus Caenorhabditis

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The nematode Caenorhabditis elegans has long been used as a model organism in the study of development, neurobiology, immunology, and aging, among other topics. With the discovery of the first natural viral pathogen of C. elegans, Orsay virus (OrV), the possibility of using the C. elegans-OrV pathosystem as a model for virus-host interactions and evolution opened up. Initial studies of the experimental evolution of OrV found few changes in the viral consensus sequence after multiple rounds of serial passages, which suggest that OrV is already well adapted to its host. The goal of this research is to study OrV adaptation to different host species and evaluate the cost of host-range expansion. Although in nature OrV is only known to infect C. elegans, a previous study has shown that several other species of the Caenorhabditis genus support OrV replication and horizontal transmission. To achieve the stated goal, experimental evolution of OrV in Caenorhabditis tropicalis, Caenorhabditis wallacei, Caenorhabditis macrosperma, and Caenorhabditis sulstoni has been started. An initial characterization of infection in the different species was performed which included (i) measuring viral load by RT-qPCR across several time points throughout the first 44 hours post-inoculation, (ii) visualization of viral RNA by FISH at different time points to characterize the progression of infection at a cellular resolution, and (iii) the impact of viral infection on the fitness of worms by measuring lifespan and fecundity of infected and healthy worm populations. At the end of the evolution experiments, the evolved viral lineages will be submitted to high-throughput sequencing to characterize new variants, and infection will be characterized as described earlier. The evolved lineages will be compared to the ancestral ones and this data will be used to establish links between sequence variation and infection outcome in order to gain a better understanding of the biology and evolution of OrV. The evolved lineages will be tested in C. elegans to evaluate the cost of host-range expansion, and used in subsequent evolution experiments in C. elegans to learn about the constraints imposed by adaptive radiation.

PS2-11

Viral population diversity changes after vertical and horizontal transmission

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Replication reliant on low-fidelity polymerases of RNA viruses causes a genetically diverse population of viral variants within an infected host. While this phenomenon has been extensively researched in animal and plant viruses, characterising intra-host diversity, infection rate and intra-host dynamics of mycoviruses has been scarce. Therefore, long-read sequencing by Pacific Biosciences (PacBio) was used on Cryphonectria parasitica/Cryphonectria hypovirus 1 model system to characterise the intra-host viral populations of the virus. Viral populations in naturally infected host, as well as the effect of vertical and horizontal transfer of the virus on its intra-host populations were studied. We analysed the intra-host virus diversity in 38 C. parasitica isolates naturally infected with CHV1, while the effect of vertical (via the conidia) and horizontal (via the hyphal anastomosis) transmission of the virus was studied in laboratory experiments on several hypovirulent C. parasitica isolates infected with French (F1) and Italian (I) CHV1 subtypes. Natural intra-host populations were diverse: we observed a mean of 40.2 intra-host mutations and up to 35 distinctive haplotypes per sample. Our analysis uncovered that new infections usually occur by only a few founder viruses: 1.99 ± 1.51. This was confirmed in laboratory experiments: in most cases, new populations established via vertical transmission were closely related to their parental populations. A bottleneck effect was observed as well, indicated by a significant drop in mutational diversity (π) after the transfer: for viral subtype F1, the π dropped from 0.0191054 to 0,000955 and for subtype I from 0,000394 to 0,000270. A significant bottleneck effect is also visible in the reduction of haplotype number for subtype I where the mean number of haplotypes dropped from 40 in the parental isolates to 25 in the progeny. Interestingly, for subtype F1 the mean number of haplotypes increased in the progeny from 211 to 230. In the horizontal transfer of the CHV1, the bottleneck effect was not as pronounced, as the mutational diversity and haplotype number remained approximately the same. Thus, it seems likely that naturally occurring viral populations usually arise by the spread of conidia, which causes a significant bottleneck effect and loss of mutational diversity. However, the haplotypic diversity of newly established viral populations recovers quickly, as we observed in our experiments.

PS2-12

Detection and phylogenetic characterization of porcine circovirus 2 and 3 strains in Hungary

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Porcine circoviruses (PCVs) are small DNA viruses endemic in swine populations globally. PCV2 systemic disease (PCV2-SD) was first identified in Canada in the early to mid-1990s, initially linked to nursery mortality, jaundice, diarrhea, respiratory disease, and wasting. Over time, the prevalence of different PCV2 genotypes has changed. Initially dominant, PCV2a was replaced by PCV2b in North America and Europe between 2003 and 2006, coinciding with more severe outbreaks. From 2010 to 2015, PCV2d gradually supplanted PCV2b globally, likely due to another genotype shift.
PCV3, identified in 2016, has been associated with porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, myocarditis, and multisystemic inflammation in piglets. Since its discovery, PCV3 has been reported across Europe, Asia, and America, predominantly linked to reproductive failures and multisystemic inflammation.
This study aimed to monitor the prevalence and genetic diversity of PCV2 and PCV3 in Hungarian herds, investigate within-herd infection dynamics, and assess pathogenicity. A total of 2505 serum samples (501 pools), 218 oral fluid samples, and 111 processing fluid samples were systematically collected from 27 large-scale swine herds and tested via real-time qPCR. Full-genome sequencing and phylogenetic analysis were also performed in some cases.
Results showed that at least one porcine circovirus was detected in 24 of the 27 examined farms (89%). PCV3 was the most widespread, identified in at least one sample type from all 24 farms, while PCV2 was detected in 11 farms (41%), all of which were also PCV3-positive. PCVs were present in all age groups, with the highest detection rate of PCV3 in processing fluid samples and PCV2 in 8–14-week-old pigs. Phylogenetic analysis revealed that Hungarian PCV2 strains belong to the PCV2d genotype, while PCV3 strains cluster into two clades, with Hungarian variants belonging to PCV3a. Our results indicate that PCV3, for which there is no vaccine available yet, and PCV2, despite regular vaccination, are widely spread in Hungary. Although the viruses appear to circulate subclinically, high levels of viral replication might be associated with wasting and multisystemic inflammation.

PS2-13

Establishment of whole genome sequencing-based Influenza A virus surveillance targeting avian and porcine cases in Austria

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The central geographic location of Austria poses an important and strategic hotspot for migratory bird routes and consequently, for introduction of seasonal avian influenza (AI) epidemics. Since avian Influenza viruses exhibit constant reassortments and viral mutations and recently H5N1 genotypes showed even adaptions to mammalian hosts, the implementation of NGS as a molecular method is deemed to be vital to keep track of the ongoing developments, especially in the context of domestic bird outbreak control. Therefore, an NGS-based workflow for whole-genome sequencing of Influenza A viruses was established in 2023 during the intensive AI season 2022/2023 in accordance with the SOP and guidelines of the EURL for avian Influenza.
The chosen approach entails a single primer pair PCR-based amplicon synthesis which is also suitable for all Influenza A viruses (including swine influenza,) and an additional quality control via automated electrophoresis of the amplified Influenza A segments. The obtained amplicons were processed by Illumina paired-end sequencing and analyzed by an in-house established Influenza bioinformatic pipeline.
This pipeline uses a mapping-based approach to determine the “optimal” reference for each of the eight viral segments. The optimal reference for each segment is chosen based on the percentage covered by the reference, the mean mapping quality of the reads, and the normalized number of reads mapping to the reference. These segment references are then used to perform a reference-based assembly of the cleaned-up reads. Variant calling is performed against a static reference using ivar. A subset of relevant and zoonotic awareness mutations reported to be associated with host specificity shift are automatically highlighted.
In summary, this presentation aims to outline the conducted efforts and encountered technical obstacles to set up a robust workflow for the generation of reliable whole-genome data of any diagnosed Influenza A virus, including avian, human, and swine strains. The established genotyping approach for Influenza A viruses and viral mutation screening, for both, avian and swine origins, as well as epidemiological outbreak analysis was implemented successfully with the help of the EURL for avian Influenza. Possible further analysis methods such as phylogenetic trees, as well as geographical mapping to set the generated information in context to metadata are also presented.

PS2-14

ADAR1 translocates from the nucleus to the cytoplasm during productive infection with herpes simplex virus 1

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Adenosine deaminase acting on RNA ("ADAR") is a protein family with the main enzymatic role of conversion of adenosine to inosine on dsRNAs (i.e. A-to-I editing), which is read as guanine by transcriptional and translational machineries. The ADAR protein has three members: ADAR1, ADAR2, and ADAR3. ADAR1 is expressed in two isoforms: p110, as constitutively expressed and localized in the nucleus, and p150, whose expression is inducible by type I interferons and localizes to the nucleus and cytoplasm. Additionally, ADAR1 has a non-editing function, it binds innate immunity proteins, which prevents immune hyperreactivity. Not much is known regarding the role of ADAR1 on infection with herpes simplex virus 1 (HSV-1). To investigate ADAR1 expression levels and its subcellular localization during productive HSV-1 infection, we used microscopy and cell fractionation techniques. We found that levels of ADAR1 protein decrease late in infection and that this phenotype is not dependent on cell type. These results indicate that the virus actively depletes ADAR1, suggesting an antiviral function of the ADAR1 protein. On the other hand, we observed increased levels of ADAR1 protein in the cytoplasm during infection, indicating translocation from the nucleus to the cytoplasm or aggregation of the cytoplasmic form (p150) of the protein. In addition, we observed perinuclear ADAR1 aggregates indicative of Golgi or ER structures. To determine whether ADAR1 translocates from the nucleus to the cytoplasm, we fractionated the cell compartments (cytoplasm and nucleus) and analyzed ADAR1 protein levels by Western blot. Our results show an even distribution of ADAR1 p150 in the nuclear and cytoplasmic fractions in uninfected and IFN-treated cells. Currently, we are analyzing the distribution of ADAR1 proteins after infection. Further studies are needed to determine the exact subcellular localization of ADAR1. Nevertheless, our study shows a massive redistribution of cellular ADAR1 in HSV-1 infection, which we are convinced plays an important biological role beyond viral infection.

PS2-15

Murine cytomegalovirus disrupts endosomal recycling by altering the ubiquitination status of EHD1 and MICALL1

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Murine cytomegalovirus (MCMV) infection leads to extensive rearrangements of host cell membranes to generate the viral assembly compartment (AC). The process is poorly understood, but it is known that it involves the endosomal system and results in strong disturbance in the physiology of numerous cellular proteins and their functions, either by affecting their expression or posttranslational modifications. MICALL1 and EHD1 are important regulators of the recycling processes on membranes of early endosomes (EE) and recycling endosomes (RE) and are known to be ubiquitinated by RFFL ubiquitin ligase. EHD1 is recruited to RE by MICALL1 and proper coordination of this process is crucial for maintaining the integrity of RE function. Therefore, this study aimed to investigate whether altering the ubiquitination of these proteins can be considered as one of the mechanisms used by MCMV for disruption of the structure and function of the endosomal system and formation of the AC. Using confocal imaging we have shown that both EHD1 and MICALL1 accumulate in the AC, however, western blot analysis showed that total expression of EHD1 stays unchanged during MCMV infection, and expression levels of MICALL1 decrease in infected cells. Since these proteins are known to be ubiquitinated by RFFL, we assessed the expression levels of RFFL in MCMV infected cells by Western blot, and we have shown that it was increased in MCMV infected cells. Therefore, we decided to test whether the ubiquitination of MICALL1 and EHD1 is altered in MCMV-infected cells. To assess the ubiquitination status of these proteins, we established an NIH3T3 HA-Ub wt cell line that can be induced to express HA-ubiquitin fusion proteins. After immunoprecipitation of Ub proteins and Western blot analysis, we found that MICALL1 was more ubiquitinated in MCMV infected cells than in uninfected cells, and ubiquitination of EHD1 was diminished in MCMV infected cells.
These results suggest that one of the mechanisms by which MCMV disrupts endosomal recycling could be mediated by impairing the function of EHD1 and MICALL1 by altering their ubiquitination status.

This work was supported by Croatian Science Foundation (IP-2020-02-2916, IP-2020-02-1323, IP-2019-04-3582), and by UNIRI (uniri-biomed-18-180, 18-88, 18-229).

PS2-16

Probiotics as antiviral agents for the treatment of rotavirus gastrointestinal infections in children

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Acute gastroenteritis is one of the most frequently reported infectious diseases in the world. The most common cause of acute gastroenteritis is various enteric viruses, including rotaviruses, noroviruses, astroviruses, adenoviruses, and other less presentable viruses. Rotaviruses are members of the *Sedoreoviridae* family and are characterized by their nonenveloped, segmented, double-stranded RNA genome (11 segments). The Rotavirus is classified into serogroups A to E based on antigenic properties. Only groups A to C have been shown to infect humans, and the most human Rotavirus disease is caused by group A Rotavirus. Group A rotavirus genotypes are classified by a nucleotide-sequence-based, complete genome classification system. Rotavirus gastroenteritis disease is especially prevalent among children, and studies over the past decade have revealed complex interactions between rotaviruses and gut microbiota. One way to treat and prevent dysbiosis is the use of probiotics as an antiviral agent. Probiotics are by definition ''live microorganisms that, when administered in adequate amounts, confer a health effect on the host''. The aim of this research was to focus on the latest scientific evidence on the antiviral properties of probiotics against rotavirus gastroenteric infections in children. 19 studies exhibited a statistically significant antiviral effect of probiotics. The main probiotics that were effective were Saccharomyces cerevisiae var. boulardii, Lacticaseibacillus rhamnosus GG, and various multi-strain probiotics. The underlying mechanism of the probiotics against rotavirus gastroenteric infections in children included immune enhancement and modulation of intestinal microbiota leading to the shortening of diarrhoea. Bacteriocins produced by probiotics, that prevent viral particle aggregation and inhibition of viral penetration into human cells, have also proven effective against viral infections. However, several clinical studies also found no significant difference in the probiotic group compared to the placebo group even though well-known strains were used, thus showing the importance of correct dosage, duration of treatment, quality of probiotics, and the possible influence of other factors, such as the production process of probiotics and the influence of immunisation on the effect of probiotics. Therefore, more robust, well-designed clinical studies addressing all factors are warranted.

PS2-17

Rubella in childbearing-aged women in Croatia: preliminary seroprevalence results

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Rubella is a viral infection caused by rubella virus (RUBV). It is a highly contagious disease but is typically mild and self-limiting. However, maternal infection during the first trimester of pregnancy can result in congenital rubella syndrome (CRS) which represents a global public health concern. Therefore, seronegative women are at risk of primary infection during pregnancy.
We analyzed the temporal trends of rubella seroprevalence in childbearing-aged women in Croatia.
The study included 1032 women aged 16-45 years tested consecutively for RUBV serology during ten years (2014-2023). Participants were from urban and rural areas of both continental and coastal Croatia. RUBV IgM and IgG antibodies were detected in serum samples using a commercial ELISA test (Novatec Immunodiagnostica, Dietzenbach, Germany). IgM/IgG-positive samples were further tested for IgG avidity (Euroimmun, Lübeck, Germany).
RUBV IgG antibodies were detected in 942 (91.3%; 95%CI=89.6-93.0%) participants. Significant differences in the seroprevalence were observed between years ranging from 84.5% in 2019 to 95.9% in 2014 (p=0.02). From 2014 to 2020, the seroprevalence curve was reverse U-shaped, while after 2020, the seropositivity remained stable (94.1-94.4%).
Seroprevalence was lowest in those under 20 years (81.3%) and highest in the 21-25 age group (95.4%), with no statistical significance (p=0.319). There was no difference in the seropositivity in urban (91.3%) vs. rural areas (91.2%) (p=0.956). A slightly higher seropositivity was observed in continental (91.7%) vs. coastal regions (90.3%) but without statistical significance (p=0.449).
Two participants were IgM positive (0.2%, 95%CI=0-0.7), however, a high IgG avidity index (77% and 83%) ruled out recent RUBV infection.
The presented results showed high and stable immunity to RUBV in childbearing-aged women in both urban and rural areas of continental and coastal Croatia. Continuous monitoring of immune status in this high-risk group and vaccination of seronegative women is needed to reduce the CRS burden.

PS2-18

Genetic stability of recombinant mumps viruses generated by reverse genetics technology

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Recombinant mumps viruses (MuVs) based on established vaccine strains represent attractive vector candidates as they have track records for high efficacy. We developed a rescue system based on the consensus sequence of L-Zagreb vaccine and generated seven different recombinant MuVs. In primary rescued stocks, low percentages of heterogeneous positions were found (maximum 0.12%) and substitutions were predominantly obtained in minor variants, with maximally four substitutions seen in consensus (relative to input plasmids). Still, during the virus stock generation, sub-consensus viral variability should be closely monitored. As we show for Pro408Leu mutation in the L gene, strongly positively selected variants can rise to frequencies over 85% in only few passages (1-4 in our experiments). Six substitutions were characteristic for recombinant viruses generated in our system; they repetitively occurred during the rescue processes, but their significance remains unknown.

PS2-19

Effect of short, non-viral sequence inserted in the 3' untranslated region of hemagglutinin-neuraminidase on mumps virus neurovirulence

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Due to neurotropic nature of mumps virus (MuV), mumps vaccine neurosafety testing and intense research on MuV neurovirulence markers are strongly required. We have designed and rescued two recombinant MuVs derived from the vaccine strain L-Zagreb; control virus containing green-fluorescent protein gene (MRV3), and experimental MRV3 with short, non-viral sequence added to the 3'untranslated region (UTR) of the hemagglutinin-neuraminidase (HN) gene (miscr-MRV3). A significant increase in in vivo neurovirulence, towards wild-type phenotype, was found for miscr-MRV3 compared to low level neurovirulence of MRV3. To elucidate the observed effect, we investigated specificities of virus growth in vitro and performed thorough genetic characterization of these viruses. Our data suggest that short, non-viral sequence inserted in the 3'UTR of HN contributes to observed neurovirulent behavior of miscr-MRV3.

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