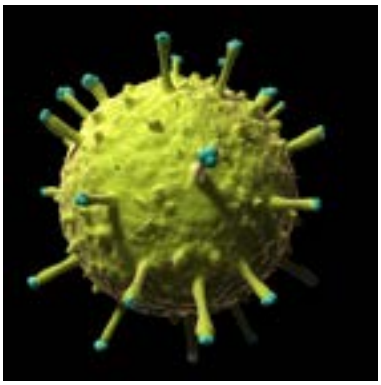
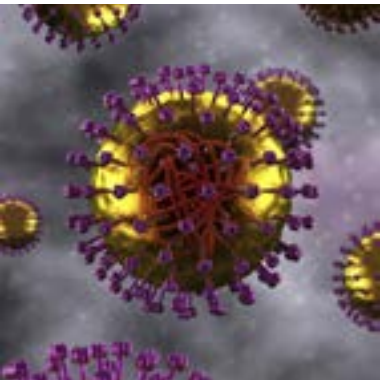
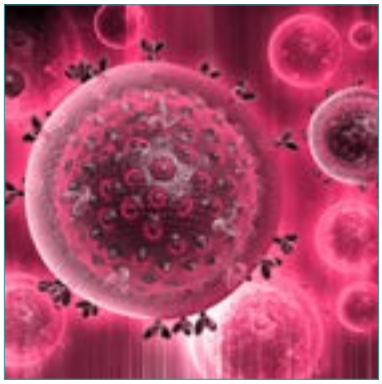
Poster Presentation

Virology Conference 2017







International Virology Conference

October 30-31, 2017 | Toronto, Canada

A new look at an old virus: Phylogenetic relationship between an Aleutian mink disease virus from Nova Scotia and global strains

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Infection with Aleutian mink disease virus (AMDV) causes economic losses to the multi-million-dollar mink industry in Nova Scotia (NS). There is no cure or vaccine for the disease and culling seropositive animals has not been effective in permanent eradication of the virus from many farms in NS. It is important to identify the sources of persistent infection or re-contamination of mink farms to develop strategies for controlling the virus. Sequencing of viruses on a farm, and comparing the sequences with existing sequence databases, is the only way to identify the source of contamination. The objective of this study was to find the phylogenetic relationship between one AMDV isolate from Cape Breton Island, NS (NS-CB), which has not been sequenced before, and the global strains. The NS-CB isolate originated from a farm which has been infected by AMDV for over 40 years. DNA was extracted from the spleen of one randomly selected mink from this farm. The entire coding region of the virus, from nucleotides 206 to 4349, was amplified by polymerase chain reaction (PCR) and sequenced by the Sanger sequencing method. NS-CB was compared with 14 global AMDV strains from North America, Europe and Asia, available on Genbank, which had the same sizes as the NS-CB isolate. Pairwise sequence identities were calculated by the Sequence Demarcating Tool (SDT) software, multiple sequence alignment was performed using Muscle program and phylogenetic analysis was performed by Mega7. The NS-CB isolate was the closest to the non-pathogenic AMDV-G, moderately pathogenic SL-3 from Germany and highly pathogenic Utah strain from USA. The four Chinese and four Newfoundland isolates were classified into different branches. It was concluded that the NS-CB isolate is different from the Newfoundland isolates, although they are the closest geographically, and that its pathogenicity could not be predicted from the nucleotide sequence of its entire coding region.

Speaker Biography

P P Rupasinghe has completed her BSc at the University of Peradeniya, Sri Lanka majoring Biology and Chemistry. After moving to Canada, she worked as a Research Assistant at the University of Guelph. During that time she has completed Certificate in Food Science Program of the University of Guelph. She has co-authored four peers-reviewed publications. Currently, she is a part-time Master's student and Research Assistant at Molecular Microbiology laboratory at Dalhousie University.

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Enteroviral infection leads to cytoplasmic mislocalization of TDP-43 in mouse brain

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that targets the motor neurons in the brain and spinal cord, which control the motor movements of the body. The disease is present in similar proportions in the majority of ethnic groups around the world, with the male being the more likely gender to contract the disease. Currently, without any effective therapies, the destruction of the motor neurons will first lead to paralysis, and eventually death. Even though 5% of all ALS cases have been associated with inherited genetic mutations that have been categorized as familial ALS, the majority of all ALS cases are actually sporadic (95%). In other words, these cases occur in the absence of prior ALS history in the family. Enterovirus (EV), a family of positive-stranded RNA viruses including poliovirus and coxsackievirus, is suspected to influence ALS pathogenesis due to the viruses' ability to target motor neurons. In addition, it has also been shown that patients with prior poliomyelitis, paralysis caused by poliovirus, are at a higher risk of ALS than those without. Our lab recently found that *in vitro* EV infection results in protein aggregation, RNA-processing defects and disruption of autophagy via EV-encoded proteases. Of particular interest was the finding that EV infection is able to impair nucleocytoplasmic trafficking, and initiate cytoplasmic aggregation and cleavage of transactive response DNA binding protein-43 (TDP-43), one of the hallmarks of ALS. Together with these findings, we hypothesize that EV infection is a causative and/or risk factor in the development of sporadic amyotrophic lateral sclerosis.

Methods & Results: Neonatal BALB/C mice were infected intracranially with eGFP-coxsackievirus or mock (DMEM) infected. Brain tissues were then collected at 2, 5, 10, 30 and 90 days post-infection for performing H&E and immunohistochemical staining. Based on our preliminary data, we were able to show brain lesions and inflammation, identified using IBA1 (microglia), pSTAT3 (astrogliosis) and GFAP (reactive astrocytes) in the cortical and hippocampus regions in parallel with viral protein detection through GFP staining as early as 2 days post-infection. Even though the viral protein was significantly decreased to only 10% of the original intensity at 90 days post-infection, there was sustained inflammatory and immune responses at the later time points. Most notably, our pilot data demonstrated clear ALS-like pathologies, such as cytoplasmic mislocalization and nuclear down-regulation of TDP43 at the areas of infection/tissue damages starting at 5 days post-infection and maintained until 90 days post-infection. Moreover, localization of markers such as p62 and ubiquitin has also been strongly detected within the infected regions.

Conclusion: Our preliminary results reveal that enterovirus infection, such as coxsackievirus, is able to cause ALS-like pathology, especially in the case of localization in abnormal TDP-43, p62 and ubiquitin within the virus infected regions of the mouse brains.

Speaker Biography

Yuan Chao Xue is a PhD student from University of British Columbia, Canada, Centre for Heart Lung Innovation, St. Paul's Hospital 2 Department of Pathology and Laboratory Medicine, University of British Columbia, Canada.

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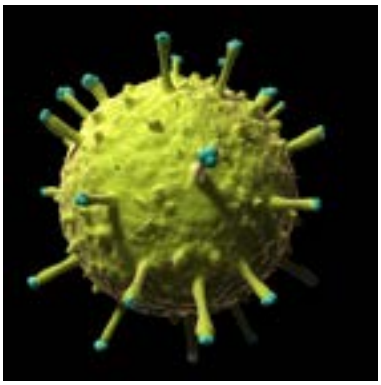

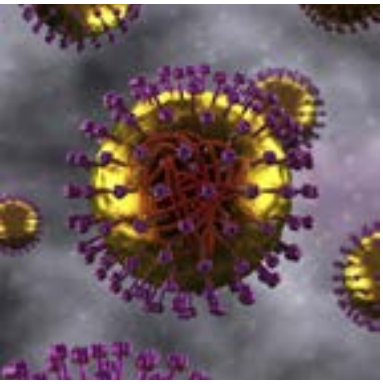

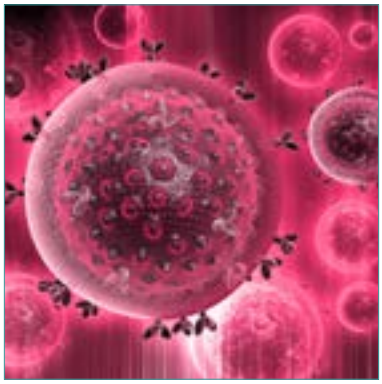

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Modification of grapevine virus A genome for vector development

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The development of new vectors based on plant viruses does not discontinue for the goal of creation a more efficient vector with high expression of heterologous proteins. We attempted to develop such vector based on genome of grapevine virus A (GVA). For creation of this viral vector we inserted gene encoding coat protein of Apple chlorotic leafspot virus (ACLSV) before ORF4 of GVA. PCASS vector carrying the complete genome of the grapevine virus A (pCASSgva) was used for creation a viral vector based on GVA. The viral genome was modified by introducing a CP gene of ACLSV before ORF 4 within restriction sites XmaI and XbaI. The overlapping region of 3'- terminus ORF3 and 5'- terminus of ORF4 was intact. The CP gene of ACLSV was placed under control of CP subgenomic promoter of GVA. The modified viral genome was subcloned into a pCambia 2300 binary vector. The expression of the CP of ACLSV in agroinfiltrated *N.benthamiana* leaves after 3-4 days of infection was confirmed by using western blotting. Agroinfiltration of transgenic plants carrying CP of GVA was not successful, we assume due RNA-silencing. It will be investigated the expression level of the viral vector and its usefulness as a vector for the expression of avian influenza hemagglutinin.

Speaker Biography

Gritsenko D A is a PhD- student at Kazakh National University named after al- Farabi. She performs her diploma work at Institute of Plant Biology and Biotechnology. The title of diploma is "Development of Viral Vector for Heterologouos Protein Expression in Plants". She developed 2 vectors based on genome of Grapevine virus A by using main strategies for vector engineering such as "deconstructed virus" and "full virus". Currently, these vectors were investigated for successful expression of eGFP and coat protein of Apple chlorotic leafspot virus. Moreover, she developed transgenic plants carrying coat protein of GVA for increasing of target protein yield since GVA cannot move between cells in non-encapsidated form.

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HTLV antisense proteins role in the nf-κb modulation

Stefania Fochi, Simona Mutascio, Francesca Parolini, Donato Zipeto and **Maria Grazia Romanelli**
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The retrovirus HTLV-1 is the causative agent of adult T-cell leukemia, whereas the genetically related sierotype HTLV-2 is sporadically associated with neurological diseases. The HTLV-1 genome encodes regulatory proteins, such as the oncoprotein Tax and the antisense proteins HBZ, involved into T-cells proliferation and transformation. Tax-1, HBZ, and the HTLV-2 homologs, Tax-2 and APH-2 interact with many host cell factors imparing cell signaling pathways involved in the mechanisms of survival, and proliferation, including the NF-κB pathway. The aim of this study is to investigate the involvement of the regulatory proteins HBZ and APH-2 in the constitutively Tax-mediated NF-κB activation. We demonstrated that HBZ and APH-2 differ in the NF-κB promoter suppression. The APH-2 protein, differently from HBZ, localizes into the cytoplasm in presence of Tax, where it prevents the degradation of the inhibitor IκB, hindering the nuclear translocation of p65. Unlike HBZ, we found that APH-2 interacts with the E3 ubiquitin ligase TRAF3, an upstream inhibitor of the alternative NF-κB pathway. By generating a TRAF3-KO cell line applying the CRISPR/Cas9 technique, we are investigating the HBZ and APH-2 activity on the alternative NF-κB cell signaling. This study may provide insight into the effect of host-viral interactions in human viral oncogenesis.

Speaker Biography

Stefania Fochi is a PhD student from Departement of Neurosciences, Biomedicine and Movement Sciences, University in Verona, Italy.

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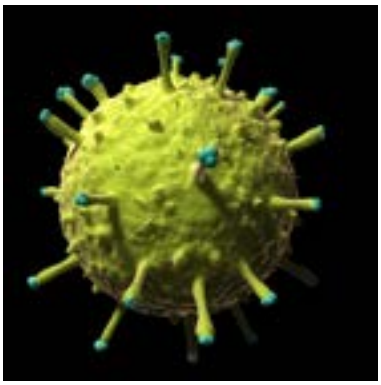

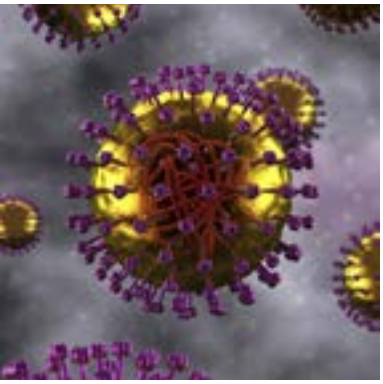

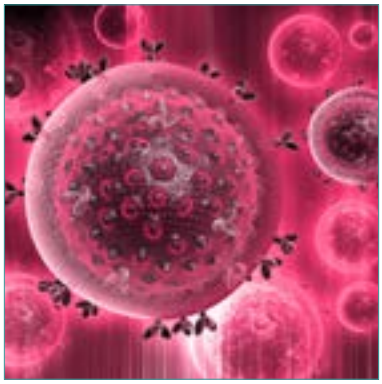

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LSDV100 and LSDV101 lumpy skin disease virus-specific PCR and real-time PCR for rapid diagnosis and vaccine quality control

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Capripoxviruses are genetically and antigenically similar. Sheeppox virus (SPPV) and goatpox virus (GPV) cause diseases in ovines and caprines, respectively. Lumpy skin disease virus (LSDV) causes lumpy skin disease (LSD) in cattle. LSD is endemic in Africa and the Middle East, and was recently introduced into Europe and Russia. Live attenuated SPPV is used as a vaccine in endemic areas. Cattle vaccinated using SPPV can develop LSD due to induction of partial protection, or as a result of vaccine seed contamination with non-highly-attenuated LSDV. LSD control and vaccine production can be enhanced by differentiation between LSDV and SPPV using a highly specific, simple, rapid, and inexpensive PCR assay. In this study, primers were designed to specifically amplify conserved LSDV sequences spanning parts of LSDV100, and LSDV101 genes. The design allowed the amplification of a 503 bp PCR product that was used for diagnosis. An alternative reverse primer allowed the amplification of a LSDV-specific 1583 bp PCR product for sequencing. The diagnostic assay detection limit was 585 genome-copy-equivalents of LSDV/5 ul of extract. A real-time assay was 10 times more sensitive. LSDV DNA was detected in skin samples collected from 1988 to 2015. Amplification of LSDV sequences was not affected by lesion size and distribution (localized or generalized) on infected animals. Application of the developed assay for the quality control of local LSD vaccines resulted in the detection of LSDV contamination of a local SPPV vaccine. The incorporation of the developed assay in LSD control programs was recommended.

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Aspects in tobamovirus management

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In the recent decade, a new outbreak of old and new tobamovirus occurred worldwide. The disease caused by the *Cucumber green mottle mosaic virus* (CGMMV) in cucurbits melon watermelon and cucumber reported in Israel, North-Europe, Canada, USA, Australia and the Far-east. Recently, the *tobamovirus Tomato mottle mosaic virus* (ToMMV) was discovered in tomato grown in Central America. In the Middle East; in Jordan and Israel, a new Tobamovirus isolate infects tomato plants harboring *Tm-2*^{2} resistance genes putatively named tomato brown rugose fruit virus (TBRFV). The epidemiology and strategies for the tobamoviruses management were studied and developed in our national initiative project for CGMMV coordinated by our lab. Growers in large-scale fields adopted the outcome of this extensive study. The experience with CGMMV management was rapidly applied also for the new tobamovirus disease management in tomatoes grown trellised in protected structures (greenhouses, walk-in tunnels etc.).

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A viroid structural compendium

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The subviral pathogens known as viroids are composed of a single stranded and circular RNA genome in the size range of 246-401 nt. The 30 species known are causing a wide array of symptoms to many plants. An important feature is their non-coding genome. This has for consequence that they depend on their sequence and structure to infect a host. The classification of viroids based only on their sequence was previously shown to be insufficient. To strengthen that classification, we believe that the use of the secondary structure of the viroids is useful. Generally, their structures were predicted with thermodynamics-based RNA folding programs, which were shown to lack precision for RNA longer than 200 nt. Thus the predicted structure of a viroid needs to have more information on its folding to produce accurate models for interpreting any systematic studies. Following the adaptation of SHAPE probing and computer assisted structure prediction to the viroid, we have elucidated the structure of all the known species. In fact, the structures in solution for one variant of all *Avsunviroidae* members as well as 30 *Pospiviroidae* species have been elucidated. There were many significant differences compared to predicted structure in absence of probing data, confirming the importance of this study. In addition to providing a complete compendium of viroid structure, this analysis permitted to ascertain structural motifs that could be important for their biology and classification.

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Identification of miRNA for expression in rice to provide resistance to RYMV

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The worldwide importance of rice, Oryza *sativa*, as food crop is well-understood. In order to maintain a consistent supply of rice globally, control of pathogens affecting crop production is matter of due concern. Rice yellow mottle virus (RYMV) is known to cause a variety of symptoms in *Oryza sativa* plants and certain symptoms account for death and hence reduce yield. Four ORFs can be identified in the genome of RYMV encoding for P1 (ORF1), Polyprotein (processed to produce VPg, protease, helicase, RdRp4); (ORF2), putative RdRp; (ORF3) and capsid/coat protein (ORF4). This research was executed to identify genome encoded miRNAs of *O. sativa* that are targeted to the genome of Rice Yellow Mottle Virus (RYMV). A consensus of four prediction algorithms (RNA22, miRanda, TargetFinder and psRNATarget was considered, thus allowing a multitude of miRNA target prediction parameters to be implemented including minimum free energy of binding, folding energy, seed pairing, target site accessibility and multiple target sites. A phylogenetic tree was constructed to portray the evolutionary relationships between RYMV strains isolated to date. Finally, target site conservation was also evaluated which revealed a varying degree of miRNA target site conservation in the genome of RYMV. Results of this research are expected to act as precursor for the development of RYMV resistant rice varieties around the world by using recombinant expression of selected miRNAs in *O. sativa*.

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Inhibition of respiratory virus infection by cholesterol reducing agents

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University of Rochester Medical Center, USA

Many enveloped viruses utilize cholesterol-rich lipid rafts at the plasma membrane for virus assembly and production. However, the functional role of cholesterol in virus formation and infectivity is unclear. In this study, we investigated the effects of FDA approved cholesterol-reducing agents on raft formation and the production of infectious parainfluenza virus (PIV), influenza A virus (IAV) and respiratory syncytial virus (RSV) in human airway cells. Depletion of cholesterol with the agents, especially when combined, significantly decreased production of all infectious viruses. Depletion of cellular cholesterol reduced cell surface accumulation of PIV glycoproteins and inhibited virus assembly and release. In contrast, depletion of cellular cholesterol did not decrease IAV and RSV surface glycoproteins accumulation, and virus particles were efficiently released from the cells. However, the released virus particles were less stable due to abnormal virion density and decreased cholesterol content in the viral membrane. Replenishing the virus released from the treated cells with cholesterol rescued virus stability and infectivity. Collectively, our findings suggest that cholesterol is critical for PIV assembly, and maintaining the stability of infectious IAV and RSV particles. Our data suggests that cholesterol is an attractive target for antiviral agents against various clinically important respiratory viruses.

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Modeling the spread of Ebola

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This study aims to create a mathematical model to better understand the spread of Ebola, the mathematical dynamics of the disease, and preventative behaviors. An epidemiological model is created with a system of nonlinear differential equations, and the model examines the disease transmission dynamics with isolation through stability analysis. All parameters are approximated and results are also exploited by simulations. Sensitivity analysis is used to discuss the effect of intervention strategies. The system has only one equilibrium point, which is the disease-free state. If traditional burials of Ebola victims are allowed, the possible end state is never stable. Provided safe burial practices with no traditional rituals, the endemic-free state is stable if the basic reproductive number is less than one. Model behaviors correspond with empirical facts. The model can predict the total number of infected, number of deaths and duration of outbreaks among others, and it can be used to educate about prophylactic behaviors, and develop strategies that alter environment to achieve the disease-free state. A future work of this research is to incorporate vaccination in the model when the vaccines are developed and the effects of vaccines are known better.

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