



Spring 2023 Research Update

Compiled on May 23, 2023





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COVARR-NET PILLAR 1: IMMUNITY AND VACCINE PROTECTION

Years 1 and 2 FINAL Progress Report

5 May 2023

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ACRONYMS

AIM	activation-induced markers
BCR	B-cell receptor
BT	breakthrough infection
CD	cell differentiation
CMI	cell mediated immunity
COVID-19	coronavirus disease 2019
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunosorbent spot assay



IgA	Immunoglobulin type-A (a type of antibody enriched in mucosa)
IgG	Immunoglobulin type-G (a type of antibody found systemically)
INF- γ	interferon gamma
IRYIS	<u>I</u> mmune <u>R</u> esponse in <u>Y</u> oung <u>I</u> mmuno <u>S</u> uppressed children to COVID-19 vaccination
m-RNA	messenger ribonucleic acid
MTA	material transfer agreement
n	study population number
PBMC	peripheral blood mononuclear cells/white blood cells – includes B-cells and T-cells and their precursors and derivatives
PRNT	plaque-reduction neutralization test
RNAseq	ribonucleic acid sequencing
SARS	severe acute respiratory syndrome
scRNAseq	single cell ribonucleic acid sequencing
SPRING	<u>S</u> evere acute res <u>P</u> iratory syndrome- <u>R</u> elated coronavirus 2 prevalence <u>I</u> n children and you <u>NG</u>
TCR	T-cell receptor
VOC	variant of concern



VISION STATEMENT

Pillar 1 will contribute towards a better understanding of immune correlates of protection against pandemic pathogens and seek the best methods for measuring these correlates.

PILLAR 1 MANDATE AND EXECUTIVE SUMMARY

[Pillar 1](#) focuses on Vaccines and immune responses to VOC in pre-immune subjects who have had COVID-19 or been vaccinated against COVID-19. Our Pillar's mission is to *Measure de novo and recall immune responses to VOC in diverse populations and formulate an action plan for vaccination strategies*. Our stated objectives in 2020 were to:

1. Build capacity within CoVaRR-Net to answer key questions regarding VOCs to prepare us for emerging VOC's and future pandemics.
2. Bridge communication between ongoing COVID-19 vaccination clinical trials and research on VOC immunity, to ensure continuity in protocols, sample collection, and data collection/interpretation.
3. Create opportunities for discussion and potential collaboration with Canadian vaccine manufacturers.

In 2021, we reported evidence that the above objectives have been met. Briefly, we have (1) Collected samples from ~ n=135 adults across different vaccination platforms and n=100 children <12 years old. (2) Harmonized SOP generation for humoral (systemic and mucosal) and cellular immune assays and established MTAs between multiple sites for exchange of samples. (3) Engaged with Providence Therapeutics for further study of the mechanism of action of mRNA vaccines. Within this framework, we have formulated new Aims for Year 2 (2022).

YEAR 1 FUNDED AIMS/RESEARCH

YEAR 1, AIM 1: EVALUATE MUCOSAL IMMUNE RESPONSES

In adults who have received either 1 or 2 doses of a COVID-19 vaccine against Ancestral SARS-CoV-2 versus VOC, different prime-boost combinations will be compared by measuring antibody titres in the saliva by ELISA and flow cytometry.

- Completed study. Published [manuscript](#) at Mucosal Immunology; CoVaRR-Net acknowledged. Findings highlighted in the [New York Times](#).
- Collaborated on additional [manuscript](#) comparing saliva from Astra Zeneca vs J&J vs mRNA immunized subjects.

YEAR 1, AIM 2: CHARACTERIZE CELL MEDIATED IMMUNITY PROVOKED BY VACCINATION TO SPIKE IN ADULTS WHO HAVE RECEIVED COVID-19 VACCINE.

To correlate seroprevalence with cell-mediated immunity, the quality, breadth, magnitude, and durability of anti-SARS-CoV-2-specific CD4+ or CD8+ T cell responses from vaccinated individuals will be compared to that of previously infected and non-infected individuals. Using correlation, we will identify relationships between cell- and antibody-mediated responses to novel variants.

- With Pillar 6 (J. Fritz, J. Shapiro), CP used a computational framework to analyze SARS-CoV-2 humoral immune responses and mutational hot-spots in Spike and



Nucleocapsid B-cell epitopes which suggests that selective pressure for immune evasion mainly occurs upon transmission between hosts. A [study](#) preprint is available.

- Cell-mediated immunity was assessed through at the laboratory of HD (UdeM/CHUSJ). INF- γ positive T-cells were quantified in response to several VOCs (Alpha, Beta, Gamma, Delta, Omicron, and ancestral strain). Results show that individuals who contracted the Omicron variant after two doses of vaccine develop stronger cellular immunity against VOCs than individuals who have been vaccinated with two or three doses of vaccine. Infection prior to vaccination reduces the magnitude of this effect, suggesting the number and type of SARS-CoV-2 exposures is important for establishing cellular immunity.
- Post-dose 3 samples collected at ~1-2 mos, ~3-5 mos, and ~6-8 mos following vaccination or Omicron breakthrough (timing of breakthrough consistent with BA.1 wave). Includes participants who were boosted following breakthrough or experienced breakthrough following boost. Participant recruitment is complete.

YEAR 1, AIM 3: EVALUATE THE CLONOTYPIC DIVERSITY OF B CELL AND T CELL RESPONSES

In this aim we proposed to evaluate the clonotypic diversity of B-cell and T-cell responses to Spike in adults who have received either 1 or 2 doses of COVID-19 vaccine. Spike-specific B-cells and T-cells will be isolated by cell sorting. B-cell receptor (BCR) and T-cell receptor (TCR) genes will be determined by single-cell-RNAseq (scRNAseq).

- Implemented multi-plex ELISA assays to measure and compare Spike/RBD specific antibodies against ancestral and VOC strains in serum/plasma samples.
- Established cell stimulation, staining and sorting methods to identify and isolate VOC Spike/RBD-specific B-cells and T-cells.
- Established scRNAseq methods and bioinformatics analysis tools to examine BCR and TCR sequences in whole PBMC or isolated COVID-19-specific cell subsets. In collaboration with Pillars 6 (Jorg Fritz) and 5 (Giannis Ragoussis), we established a pipeline for the initial sequencing of the BCR before and after 3rd vaccine dose to interrogate the diversity of B cell clonotypes emerging. Using local participant specimens (Simon Fraser University), compared responses after 1, 2, and 3 vaccine doses.
- Extensive T-cell profiling was completed for Pillar 1 cohort who experienced Omicron Breakthrough and compared to those who received a booster dose of COVID-19 vaccine. Analysis was restricted to short duration AIM assay and unexpectedly, no significant differences in expansion of T-cell populations was observed between those experiencing breakthrough and those with 3rd doses.
- Data collection is ongoing for ten participants at SFU, of which five experienced breakthrough infection during the Omicron wave.

YEAR 1, AIM 4: COMPARE OUR DATA TO REPOSITORIES OF IMMUNOLOGICAL DATA FROM NATIONAL/INTERNATIONAL COLLABORATORS

Saliva: we will compare our results with laboratories of Irv Weissman and Michal Tal (Stanford University, MIT). **Cell mediated immunity:** we will compare with the National Microbiology Labs (collaborating with Pillar 1) as well as Tania Watts and Mario Ostrowski (University of Toronto)



who are actively involved in CMI studies in ongoing vaccine trials in Canada. **Clonotypic analysis:** as part of iReceptor (<https://gateway.ireceptor.org/login>) based at SFU, we will compare our results to a growing repository of BCR and TCR data from other COVID-19 studies.

- Prepared manuscript comparing saliva from subjects recruited at Stanford vs University of Toronto.
- Published manuscript at Mucosal Immunology; CoVaRR-Net acknowledged.
- New bioinformatics tools have been implemented at Simon Fraser University to compare BCR/TCR sequences to those reported by prior COVID-19 studies (in conjunction with the iReceptor project).
- Maintained ongoing communications with other CMI labs in Canada to share protocols, reagents, samples, and early results (including Watts, Ostrowski, Blake Ball).
- Establishment of an inter-Pillar collaboration for the characterization of immune cell receptors (BCR and TCR) pre/post vaccination or breakthrough infections
- Collaborated with Ostrowski lab examining effector and regulatory T cells responses in SARS-CoV-2 convalescent samples (natural infection versus vaccination-induced immunity). CoVaRR-Net to be acknowledged upon manuscript acceptance.

YEAR 2 FUNDED WORK/STATUS

Based on modification to vaccine series (3 doses versus 2), approval of the Pfizer vaccine in children aged 5-12, and the emergence of a highly divergent and continuously evolving variant (Omicron) we adapted in Year 2 (2022) to answer important Immunology questions related to vaccination:

1. Characterize the salivary antibody response to COVID-19 vaccines in children.
2. Compare the immune response of uninfected participants who have received 3 doses of COVID-19 vaccine versus 2 doses of COVID-19 vaccine followed by a breakthrough Omicron infection.
3. Assess the BCR repertoire of a mucosal vs systemic immune response to COVID-19 vaccination.

YEAR 2, AIM 1: CHARACTERIZE THE SALIVARY ANTIBODY RESPONSE TO COVID-19 VACCINES IN CHILDREN

(MS, JG, HD, and [Pillar 4: Functional Genomics and Structure Function of VOCs](#))

Studying Ab response to COVID-19 vaccination in children may help answer the question of why children disproportionately develop milder symptoms when infected with SARS-CoV-2. Saliva is an ideal fluid to measure antibody responses in children because it is a non-invasive procedure and can be performed in the home setting¹. In anticipation of vaccine roll-out in children aged 5-12 by early 2022, we collected:

- (1) A longitudinal cohort of n=100 kids aged 5-11. For each participant, saliva samples were collected at 5 timepoints: baseline, post-dose 1, and 1 month, 6-months and 12-months post-2. This timeline allows us to compare the results with adults in a previous study². A dried blood spot was taken at 2-weeks post-dose 2 to establish prior SARS-CoV-2 infection by serology (an exclusion factor).



- (2) A longitudinal cohort of n=20-40 kids with humoral immunodeficiencies who receive a 3-dose primary vaccination series. Saliva samples are collected by the [IRYIS](#) study at 2-weeks post dose 2 and 3, and 6- and 12-months post dose 3.
- (3) A cross-sectional cohort n=50 children (BC, [SPRING](#) Expansion study)

Using these samples, we will measure antibody levels for anti-Spike/RBD on all samples by ELISA^{1,2}. We will compare data with our adult data, ensuring that we match for vaccine type and will disaggregate data by sex. Because of their heightened mucosal immunity, compared to adults we expect children will mount a stronger and more durable mucosal response (IgA) but exhibit a similar systemic response (IgG) to COVID-19 vaccination. We expect that children with humoral immune deficiencies will exhibit an abrogated mucosal response compared to healthy children.

- Saliva measurements are being collected for 12-month timepoint. JG lab has begun analysing whether there are correlations with post-dose 2 antibody levels and confirmed subsequent breakthroughs.
- We are also comparing these results with those from the lab of HD to ascertain if immunocompromised children have abnormal mucosal antibody responses to vaccination. Most samples have already been collected and shipped. The 12-month timepoint is being collected now. Serum will be analyzed once all samples are shipped.
- Approximately 200 saliva and serum samples from SPRING study (run by MS) will be sent once samples for final timepoints are collected. These samples will be analyzed for antibody responses over 4 time points. We will monitor IgG and IgA levels in these children in response to vaccination / subsequent breakthroughs.

YEAR 2, AIM 2: COMPARE THE IMMUNE RESPONSE OF UNINFECTED PARTICIPANTS WHO HAVE RECEIVED 3 DOSES OF COVID-19 VACCINE VERSUS 2 DOSES OF COVID-19 VACCINE FOLLOWED BY A BREAKTHROUGH OMICRON INFECTION.

(JG, MB, CP, HD, HW and [Pillar 4: Functional Genomics and Structure Function of VOCs](#))

An important question for Canadians is how a 3rd dose of vaccine compares to a breakthrough infection. To answer this, we have recruited two groups of participants: 1) Participants who have had 2 doses of COVID-19 vaccine followed by an Omicron breakthrough infection (n=15 in Toronto and n=17 in Montreal), and 2) Participants who have had 3 doses of COVID-19 vaccine (n=17 in Toronto, n=24 in Montreal).

We have examined binding antibodies to SARS-CoV-2 Ancestral Spike and Omicron Spike in Saliva. We also measured antibodies to SARS-1 Spike to ascertain if a mucosal exposure results in epitope spreading at the mucosae itself. We analyzed serum samples for neutralizing antibodies against of SARS-CoV-2 variants of concern (VOC) by PRNT assay (HW) and Lentiviral assays (ACG). We analyzed PBMC samples using the ELISPOT readout (HD) for T-cell responses, and intracellular cytokine staining (CP).

Given the exposure to a new variant, we expect that the immune response to breakthrough infection will be more potent than that for those who received 3rd doses of vaccine.; Omicron infection will act as a mucosal “boost”. We are collaborating with Pillar 4: Functional Genomics and Structure Function of VOCs as they are employing the lentiviral panel of assays to these samples.



- We have completed most of our analysis for this study. Submission of a manuscript detailing these results is imminent.
- For the Toronto cohort we observed that the **serum** antibody response in 2-dose Omicron breakthrough (BT) subjects is superior to 3-dose non-BT subjects in terms of neutralizing antibody responses to ancestral SARS-CoV-2 and VOC (Pillar 4, ACG, and Pillar 1, HW). We found that serum antibody titres were likewise higher (Pillar 4, ACG). Interestingly, these results did not replicate for the Montreal cohort collected by HD. However, these subjects contracted SARS-CoV-2 prior to vaccination. and it is possible that infection prior to vaccination “evens out” the antibody response thus there is no further advantage conferred by Omicron infection.
- For both cohorts, BT infection did not augment the **T cell response** beyond what was observed with 3 doses of vaccine. Thus, the T cell response appears to have reached a threshold with 3 doses of vaccine and a BT or previous exposure.
- For the CoVaRR-Net cohort we observed that the **saliva** IgA antibody response in 2-dose Omicron breakthrough (BT) subjects is superior to 3-dose non-BT subjects in terms of antibody levels to omicron, ancestral SARS-CoV-2, and SARS-CoV-1. The same was not true for the IgG antibody response which was only augmented for Omicron.

YEAR 2, AIM 3: ASSESS THE BCR REPERTOIRE OF A MUCOSAL VS SYSTEMIC IMMUNE RESPONSE TO COVID-19 VACCINATION.

(CP, MB, JG, [Pillar 5: Viral Genomics & Sequencing](#), and [Pillar 6: Computational Biology and Modelling](#))

How do Spike-specific immune responses that are detected systemically compare with those induced in mucosal sites? How does this affect vaccine efficacy? Answering these questions requires strategies for B-cell isolation from systemic vs mucosal sites and their downstream functional and molecular characterization. Using the pipeline, we have established, we expect a more restricted Spike-specific B-cell repertoire (unique clonotypes) associated with vaccine protection.

- We have established cell stimulation, staining and cell-sorting methods (MB) to identify and isolate Spike/RBD-specific B-cells in PBMC (and that of 6 VOC-specific; can be expanded with evolution of variants). To evaluate the diversity of Spike-specific B-cell receptor clones that are acquired in adults who have received a COVID-19 vaccine, we have collaborated with Pillars 6 (Jorg Fritz) and 5 (Giannis Ragoussis) and established a scRNAseq method and bioinformatics analysis pipeline that examines BCR sequences in B-cell populations. This pipeline will be applied to mouse models for the study of virus and vaccine-induced B-cell clones. T-cells are identified by upregulation of activation-induced surface markers after stimulation using a pool of Spike peptides. T-cells are enriched as single-cell emulsions using cell sorting or magnetic bead methods and subsequently sequenced. Sequence data is processed using commercial software and custom tools being developed by the Brockman lab.
- We have initiated analyses BCR and TCR repertoires using PBMC specimens collected from ten individuals who had received 3 doses of vaccine from our



Vancouver-based cohort of vaccine recipients. Of these, five individuals experienced breakthrough SARS-CoV-2 infection during the Omicron wave. We are profiling Spike-specific BCR and TCR of all participants after their third vaccine dose to examine repertoire diversity. We are also profiling BCR and TCR of breakthrough cases to examine the impact of viral infection on vaccine-induced immune responses. Additional human studies are being planned using specimens collected by CoVaRR-Net investigators.

- To ascertain the clonotypic relationships between mucosal- vs systemic-derived anti-Spike B cells, we vaccinate mice with 1 dose of mRNA-based vaccine (in collaboration with Providence Therapeutics) and harvest mucosal versus peripheral tissues (spleen and draining-lymph node tissues) for BCR repertoire analysis, separating the analysis by isotype (IgG vs IgA). The nature, breadth, and diversity of Spike-specific CD4+ and CD8+ T cell responses will be monitored in parallel. Vaccine studies have been initiated by the Gommerman lab at UofT. Once sufficient immunogenicity data is available, samples will be identified and sent to SFU for BCR and TCR repertoire analyses.

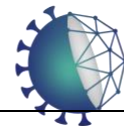
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- 1 Isho, B. *et al.* Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol* **5**, doi:10.1126/sciimmunol.abe5511 (2020).
- 2 Sheikh-Mohamed, S. *et al.* Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection. *Mucosal Immunol* **15**, 799-808, doi:10.1038/s41385-022-00511-0 (2022).



TABLE 1: PUBLICATIONS THAT ACKNOWLEDGE COVARR-NET FUNDING

Date	Corresponding Author	CoVaRR-Net Member	Title	Hyperlink	Type of Article	Acknowledgment
2021-08-30	Irving Weissman and Michal Tal	Jennifer Gommerman and Anne-Claude Gingras	Intramuscular SARS-CoV-2 vaccines elicit varying degrees of plasma and salivary antibody responses as compared to natural infection	https://doi.org/10.1101/2021.08.22.21262168	Pre-print	CoVaRR-Net will be acknowledged
2022-04-25	Sharon Strauss, Anne-Claude Gingras, and Jennifer Gommerman	Jennifer Gommerman and Anne-Claude Gingras	Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection	https://www.nature.com/articles/s41385-022-00511-0	Peer-reviewed	Funding
2022-05-11	Mark A. Brockman	Mark Brockman	Older adults mount less durable humoral responses to two doses of COVID-19 mRNA vaccine, but strong initial responses to a third dose	https://doi.org/10.1093/infdis/jiac199	Peer-reviewed	Funding
2022-06-01	J. Fritz and C. Piccirillo	Raphael Poujol, Guillaume Bourque, Julie G Hussin; Jesse Shapiro; Jörg Hermann Fritz; Ciriaco A Piccirillo	Selection for immune evasion in SARS CoV-2 revealed by high-resolution epitope mapping combined with genome sequence analysis	https://www.biorxiv.org/content/10.1101/2022.06.01.494373v1	Pre-print, in press with iScience (peer reviewed)	Funding
2022-06-07	Zabrina L. Brumme	Mark A. Brockman	People With Human Immunodeficiency Virus Receiving Suppressive Antiretroviral Therapy Show Typical Antibody Durability After Dual Coronavirus Disease 2019 Vaccination and Strong Third Dose Responses	https://doi.org/10.1093/infdis/jiac229	Peer-reviewed	Funding



2023-02-09	Mark A. Brockman	Mark A. Brockman	Impact of age and SARS-CoV-2 breakthrough infection on humoral immune responses after three doses of COVID-19 mRNA vaccine	https://doi.org/10.1093/ofid/ofad073	Peer-reviewed	Funding
2022-09-06	Zabrina L. Brumme	Mark A. Brockman	Serial infection with SARS-CoV-2 Omicron BA.1 and BA.2 following three-dose COVID-19 vaccination	https://doi.org/10.3389/fimmu.2022.947021	Peer-reviewed	Funding
2023-02-09	Zabrina L. Brumme	Mark A. Brockman	SARS-CoV-2 live virus neutralization after four COVID-19 vaccine doses in people with HIV receiving suppressive ART	https://doi.org/10.1097/QAD.0000000000003519	Peer-reviewed	Funding



TABLE 2: SUMMARY OF PROJECTS/PUBLICATIONS IN PIPELINE

Date	Corresponding Author	CoVaRR-Net Member	Title	Type of Article	Acknowledgment
2023-05	Patrick Budykowski	Arinjay Banerjee, Laurie Seifried, Anne-Claude Gingras, Jennifer Gommerman, and Samira Mubareka,	A Significant Contribution of Complement in Enhancing SARS-CoV-2 Neutralization of Convalescent and Vaccinee Sera	In submission	CoVaRR-Net will be acknowledged upon manuscript acceptance
2023-05	Helene Decaluwe, Jennifer Gommerman	Decaluwe, Gommerman, Gingras, Piccirillo, Salma Sheikh-Mohamad, Gary Chao, Sabryna Nantel	Omicron breakthrough Infection elicits superior antibody responses to SARS-CoV-2 variants in healthy adults	In submission	Funding, Membership
2023-05	Ciriaco A. Piccirillo, Jörg H. Fritz	Ciriaco A. Piccirillo, Jörg H. Fritz	SARS-CoV-2 Seroprevalence in an Orthodox Jewish Community in Montréal	In submission	CoVaRR-Net will be acknowledged upon manuscript acceptance
2023-05	Jorg Fritz*, Ciriaco Piccirillo*, Angela M. Crawley	Curtis Cooper, Marc-André Langlois, Jorg Fritz*, Ciriaco Piccirillo*, Angela M. Crawley	Investigation of the interplay between sex and age in antibody responses to SARS-CoV-2 infection and vaccination	In submission	Funding, Membership
2023-05	Mario Ostrowski and Ciriaco A. Piccirillo	Sebastian Grocott, Tho-Alfakar Al-Auboda, Zhiyang Liu, Ciriaco A. Piccirillo	High dimensional analysis of SARS-CoV-2-Spike-specific CD4 ⁺ T cell responses reveals heterogenous effector and regulatory branches at the level of the individual	In preparation	Funding, Membership



May 2022 Pillar 2 (Host-Pathogen Interactions) Research Update

Research Project Status Pillar 2

Co-Leads: Angela Rasmussen (VIDO, University of Saskatchewan) and Jason Kindrachuk (University of Manitoba)

Deputies: Arinjay Banerjee (VIDO, University of Saskatchewan), Samira Mubareka (University of Toronto, Sunnybrook), Bradley Pickering (Canadian Food Inspection Agency), Selena Sagan (McGill University)

Vision Statement

Exploring the interactions between emerging viruses, humans, wildlife, and the environment at the human-animal interface to understand infection and disease, both in the laboratory and the field.

Primary goal

We aim to advance a comprehensive understanding of SARS-CoV-2 variants in the context of One Health, with a particular understanding of how animal infections can influence human health. This includes the development of resources to further our overall pillar goals as well as foster fruitful cross-disciplinary collaborations across CoVaRR-Net, including reagents, access to virus stocks, susceptibility screening, a compendium of molecular profiling data on key host-virus interactions, and construction of a risk matrix to prioritize surveillance and future experiments. This project includes four research aims:

Aim 1. Development of reagents and access to virus stocks.

1.1 Isolation of novel emerging variants (Rasmussen and Banerjee).

Dr. Banerjee's laboratory led a collaboration with Drs. Falzarano and Mubareka to rapidly respond to the emergence of the omicron variant in Canada in December 2021. This involved isolation and characterization of all VOCs (Variants Of Concern), along with assessing their immune escape potential in naturally infected and vaccinated Canadians ([https://www.cell.com/med/fulltext/S2666-6340\(22\)00168-4](https://www.cell.com/med/fulltext/S2666-6340(22)00168-4)).

1.2 Development of ACE2 constructs and cell lines (Rasmussen).

Key points



- We assembled genomics, metagenomics, transcriptomics, and metatranscriptomics data publicly available on NCBI, to extract ACE2 sequences from 72 north american species.
- Using ACE2 protein sequences from 14 market species, we performed homology modelling, molecular docking, and binding affinity calculation and observed the interacting residues between spike RBD (Ribosomal Binding Domain) and host ACE2 PD (Peptidase Domain) interacting interface. The prediction indicates that ACE2 from market species can facilitate SARS-CoV-2 entry. However, the *in-silico* results need to be confirmed with wet-lab experimentation.
- We performed *de novo* transcriptomics assemblies of publicly available metagenomic data (collected from stall 6-29 at the Huanan Wholesale Seafood Market, Wuhan) on GISAID. Our analysis provides evidence of the presence of sequences from *Nyctereutes procyonoides* in cart Q61 environmental samples.

1.2.1 Extraction of ACE2 sequences from north American species.

Publicly available nucleotide and protein sequences of ACE2 were downloaded from GenBank nucleotide and protein databases, respectively. In the case of the north American animals with no ACE2 sequences, we have used genomes (if available), whole genome sequencing (WGS), metagenomics, RNA-seq and metatranscriptomics data available in the NCBI SRA and Genome databases, respectively. Depending on the data type, different datasets were *de novo* assembled using different programs such as Spades, Metaspades, Megahit, and Trinity. ACE2 sequence from humans was used as a query to find out the best hit regions in the assembled transcriptomes/genomes using blast search. The best hit regions (transcripts and/or scaffolds) were annotated using the “Augustus” program to extract the ACE2 protein and mRNA sequences. For species with no information available at Genbank, Taxonomy database searches were used to find ACE2 sequence the closest species.

1.2.2 Structure Prediction and Interaction analysis of ACE2 sequences from North American species with Wuhan-1 and Omicron spike protein:

Homology modelling analysis of the protein sequences was performed using Swiss-Model. Multiple homology models were generated of which the one with the best validation statistics was chosen for analysis. Structure quality assessment was performed using the structure assessment tool at the Swiss model and SAVES server. The best protein model was selected after manual examination of various statistics. An example of protein homology models and their evaluation has been shown in Figure 1 and Figure 2.

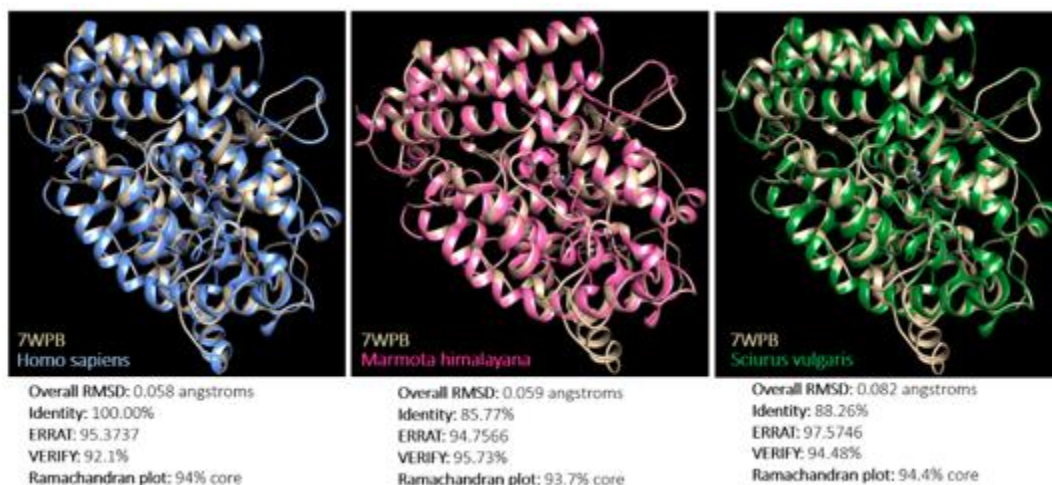


Figure 1: Homology models of ACE2 Peptidase Domain (with evaluations) from Human (*Homo sapiens*), Marmota (*Marmota himalayana*) and Red squirrel (*Sciurus vulgaris*)



For docking purposes, Spike protein structures from Wuhan (PDB:7CWU) and Omicron (PDB:7QO7) were downloaded from RCSB PDB. The best-modelled protein structures of ACE2 were docked with the spike glycoproteins from SARS-CoV2 Wuhan and Omicron B.1.1.529 using the Haddock 2.4 program. After the completion of the docking step, the top best cluster based on the best docking and z score was selected for further analysis. The top docking cluster (with four top best protein-protein complexes) from each host was selected based on an assigned docking score. Each complex was manually visualized in chimera and the best protein-protein complex was selected based on the highest number of key interacting residues. This final complex was used to calculate the binding affinities. In the case of pig and American mink, the docking analysis results indicated that the Wuhan-1 spike protein binds more strongly to ACE2 than the omicron spike (Figure 3; as indicated by the higher binding affinities). Whereas in the case of the red fox, the omicron spike binds strongly with the ACE2 as compared to the Wuhan-1 spike (Figure 3). All Species were ranked based on their binding affinities (Figure 4).

Figure 2: The evaluation (based on completeness) of homology models of ACE2 from *Neogale vison* (American mink), *Vulpes vulpes* (Red fox), *Sus scrofa* (Pig/wild boar), *Nyctereutes procyonoides* (Raccoon dog), and *Paguma larvata* (Masked palm civet). The predicted structures were compared with the crystal structure (6M17 Chain D) from *Homo sapiens*.

Higher binding affinities of Marmot (*Marmota himalayana*), Raccoon dog (*Nyctereutes procyonoides*), Coypu/nutria (*Myocastor coypus*), Masked palm civet (*Paguma larvata*), Malayan porcupine (*Hystrix brachyura*), and Red squirrel (*Sciurus vulgaris*) indicates that these animals can facilitate SARS-CoV-2 entry (Figure 4).

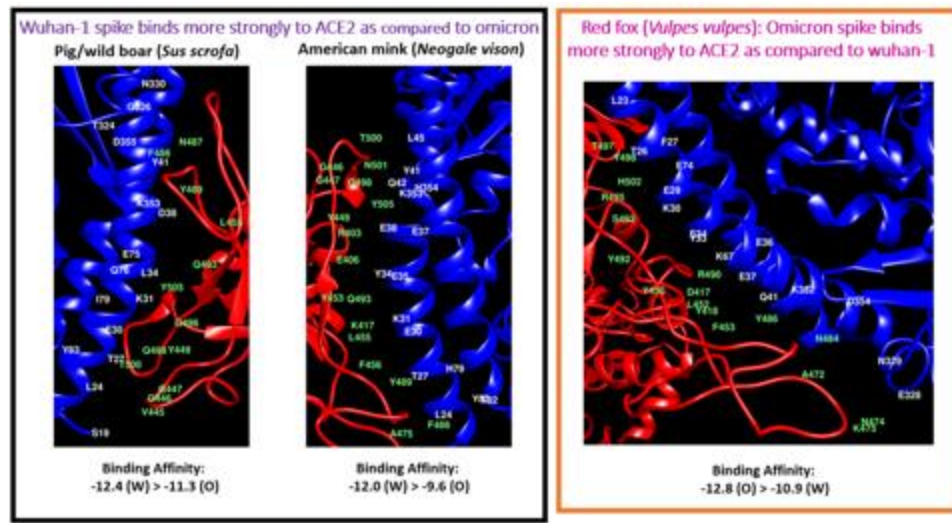


Figure 3: Wild (Wuhan-1) vs Mutant (Omicron B.1.1.529) spike-ACE2 binding interactions. Here the residue highlighted in white on the blue structure corresponds to the ACE2 chain and the residue highlighted in green on the red structure corresponds to the C-chain of the omicron spike protein.

Organisms	ACE2 Key Interacting Residues																												Binding Affinities	
Marmota himalayana	S19	L24	T27	F28	D30	K31	Q34	E35	E37	D38	Y41	Q42	L45	N53	L79	A82	Y83	N90	N322	N330	K353	G354	D355	R357	R393				-15.1	
Nyctereutes procyonoides	S19	L23	T26	F27	E29	K30	Y33	E34	E36	E37	Y40	Q41	L44	N52	L78	T81	Y82	D89	N321	N329	R352	G353	D354	R356	R392				-14.4	
Myocastor coypus	L19	Q24	A27	F28	D30	N31	Q34	K35	E37	D38	Y41	Q42	L45	N53	F79	A82	Y83	N90	H274	N282	K305	N306	D307	R309	R345				-13.7	
Paguma larvata	S19	L24	T27	F28	E30	T31	Y34	E35	Q37	E38	Y41	Q42	V45	N53	L79	T82	Y83	D90	N322	N330	K353	G354	D355	R357	R393				-13.6	
Hystrix brachyura		Q24	T27	F28	D30	K31	Q34	E35	E37	D38	Y41	Q42	L45	N53	F79	A82	Y83	N90	H322	N330	K353	N354	D355	R357						-13.4
Sciurus vulgaris	S19	S24	T27	F28	D30	K31	Q34	E35	E37	D38	Y41	Q42	L45	N53	L79	T82	Y83	N90	N322	N330	K353	G354	D355	R357	R393					-13.1
Erinaceus europaeus		V49	A52	F53	R55	L56	S59	T60	Q62	L63	N66	Q67	E70	N78	R104	L107	F108	D115	P336	K343	N367	G368	D370	R372	R408					-13
Meles meles		E80	R83	F84	E86	E87	R90	R91	L93	V94	N97	E98	E101	N109	W13	Q131	F131	N146	P367	K375	N398	G399	D401	R403	R439					-13
Arctonyx collaris		L24	T27	F28	E30	K31	R34	E35	E37	E38	Y41	Q42	L45	N53	H79	T82	Y83	D90	N322	N330	K353	H354	D355	R357	R393					-12.7
Odocoileus virginianus texanus	S19	Q24	T27	F28	E30	K31	H34	E35	E37	D38	Y41	Q42	L45	N53	M79	T82	Y83	N90	H321	N329	K352	G353	D354	R356	R392					-12.6
Mus musculus	S19	N24	T27	F28	N30	N31	Q34	E35	E37	D38	Y41	Q42	L45	N53	T79	S82	F83	T90	H322	N330	H353	G354	D355	R357	R393					-12.6
Felis catus	S19	L24	T27	F28	E30	K31	H34	E35	E37	E38	Y41	Q42	L45	N53	L79	T82	Y83	N90	N322	N330	K353	G354	D355	R357	R393					-12.4
Sus scrofa	S19	L24	T27	F28	E30	K31	L34	E35	E37	D38	Y41	Q42	L45	N53	L79	T82	Y83	T90	N322	N330	K353	G354	D355	R357	R393					-12.4
Oryctolagus cuniculus	S19	L24	T27	F28	E30	K31	Q34	E35	E37	D38	Y41	Q42	L45	N53	L79	T82	Y83	N90	S322	N330	K353	G354	D355	R357	R393					-12.2
Neogale vison	S19	L24	T27	F28	E30	K31	Y34	E35	E37	E38	Y41	Q42	L45	N53	H79	T82	Y83	D90	N322	N330	K353	H354	D355	R357	R393					-12
Puma concolor	S19	L24	T27	F28	E30	K31	H34	E35	E37	E38	Y41	Q42	L45	N53	L79	T82	Y83	N90	N322	N330	K353	G354	D355	R357	R393					-11.9
Vulpes lagopus	S19	L23	T26	F27	E29	K30	Y33	E34	E36	E37	Y40	Q41	L44	N52	L78	T81	Y82	D89	N321	N329	K352	G353	D354	R356	R392					-11.7
Homo sapien	S19	Q24	T27	F28	D30	K31	H34	E35	E37	D38	Y41	Q42	L45	N53	L79	M82	Y83	N90	N322	N330	K353	G354	D355	R357	R393					-11.5
Muntiacus reevesi															M17	T20	Y21	N28	H191	N199	K222	G223	D224	R226	R262					-11.5
Vulpes vulpes	S19	L23	T26	F27	E29	K30	Y33	E34	E36	E37	Y40	Q41	L44	N52	L78	T81	Y82	D89	N321	N329	K352	G353	D354	R356	R392					-10.9

Decrease

Figure 4. North American species ranked based on the ACE2-Wuhan spike binding interaction. The conserved Key interacting residues present in the protein-protein interactions are highlighted with green background.

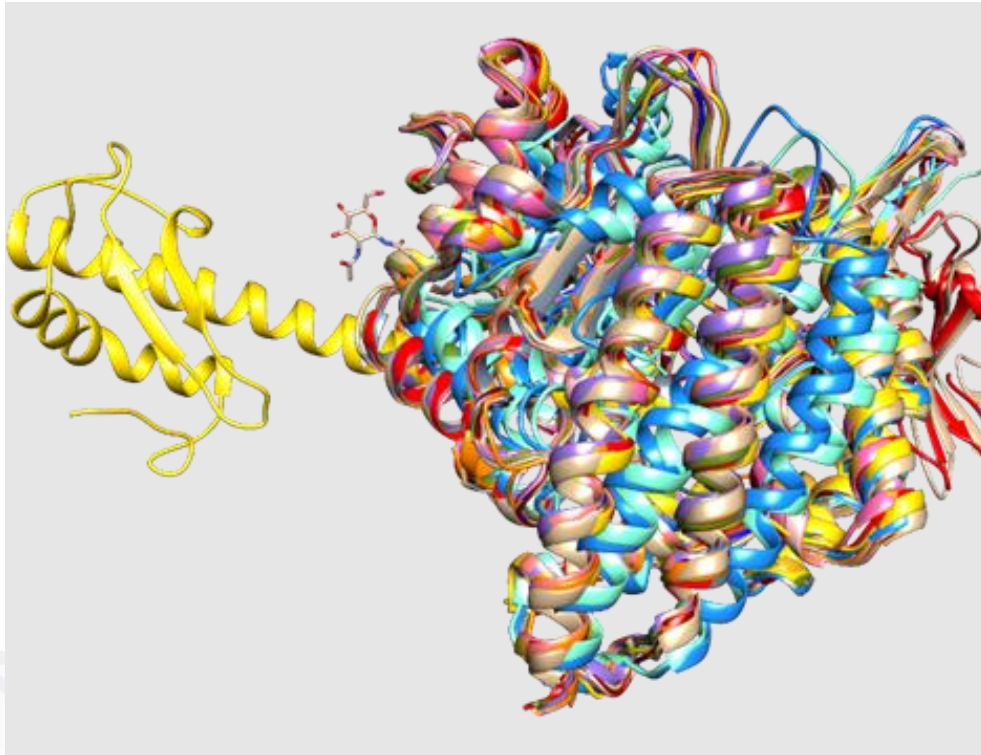
Summary

The ACE2 sequences used for the current analysis are downloaded from the NCBI protein database. In the case of the North American animals with no ACE2 sequences,



we have used either genomes/whole genome sequencing, metagenomics, transcriptomics, or meta-transcriptomics data available in the NCBI sequence read archive and genome databases, respectively, to extract the ACE2 sequences. For species with no information available at Genbank, taxonomy database searches were used to find the closest species and ACE2 sequences were extracted in fasta format.

We performed homology modelling of ACE2 protein sequences from *Homo sapiens*, *Neogale vison*, *Vulpes vulpes*, *Sus scrofa*, *Nyctereutes procyonoides*, *Paguma larvata*, *Marmota himalayana*, *Sciurus vulgaris*, *Myocastor coypus*, *Hystrix brachyura*, and *Muntiacus reevesi*. In the case of the *Meles leucurus*, *Arctonyx albogularis*, and *Erinaceus amurensis*, ACE2 sequences from a closely related species (i.e., *Meles meles*, *Arctonyx collaris* and *Erinaceus europaeus*) were used. Homology modelling analysis of the protein sequences was performed using Swiss-Model. A total of five homology models for each species were generated of which the model with the best statistics was chosen (superimposed structures shown in Figure 5) for the molecular docking analysis. The best modelled ACE2 structure of each species was docked with the trimeric spike protein of SARS-CoV-2 Wuhan-1 (PDB ID: 7CWU) and Omicron spike B.1.1.529 (PDB ID: 7QO7) using HADDOCK 2.4 webserver. The interacting residues of spike RBD and ACE2 PD were identified using Chimera and PDBePISA. Prodigy server was used to predict binding affinities. Our results indicated that most of the residues that are present in the human ACE2 PD and spike RBD interacting interface have been previously documented. The predicted binding affinities of *Muntiacus reevesi* (with truncated ACE2 PD binding site) are identical to humans, while *Marmota himalayana*, *Sciurus vulgaris*, *Myocastor coypus*, *Hystrix brachyura*, *Meles meles*, *Arctonyx collaris*, and *Erinaceus europaeus* have higher. Binding affinities and the presence of some conserved key interacting residues in the interacting interface of ACE2 PD and SARS-CoV-2 RBD indicate that the ACE2 from market species can facilitate SARS-CoV-2 entry.

**Figure 5. The architecture of the ACE2 peptide domain of chosen models:**

Homo sapiens in blue, *Neogale vison* in hot pink, *Vulpes vulpes* in red, *Sus scrofa* in olive drab, *Nyctereutes procyonoides* in goldenrod, and *Paguma larvata* in medium purple, *Marmota himalayana* in pink, *Sciurus vulgaris* in dark red, *Meles meles* in aquamarine, *Arctonyx collaris* in salmon, *Erinaceus europaeus* in dodger blue, *Myocastor coypus* in orange, *Hystrix brachyura* in gold, *Muntiacus reevesi* in slate gray, and reference human ACE2 (crystal structure PDB: 6M17 chain D) in tan. In the case of *Hystrix brachyura* the overall architecture of the model is different from others (structure in yellow) which is probably because of the incomplete sequence.

De novo transcriptomics assemblies of single-end short sequencing reads from the samples (i.e., Q61, Q64, Q68, Q69, and Q70) collected from stall 6-29 at the Huanan market was generated using Trinity. The resulting assembled transcripts were searched using blastn against the *in-house* blast database of genome sequences assemblies from market species and *Homo sapiens*, *Mus musculus*, *Canis lupus familiaris*, *Capra hircus*, *Bos taurus*, *Oryctolagus cuniculus*, *Rattus rattus*, *Anas platyrhynchos*, *Gallus gallus*, *Ovis aries*, and *Felis catus*. The unique best-hit transcript sequences were annotated using Transdecoder and the final annotated proteins were used for ortholog mapping against human proteome using OrthoFinder. Our results



confirmed the presence of 1,252 transcripts with 100% identity to the *Nyctereutes procyonoides* genome in sample Q61. 59.02% reads from sample Q61 mapped to the *Nyctereutes procyonoides* genome using bowtie.

1.2.3 Development of ACE2 ortholog expressing HEK293 cells.

The ACE2 ortholog constructs from North American animals were synthesized from Twist Biosciences in a CMV expression plasmid with a puromycin resistance gene. ACE2-expressing plasmids were transfected into human embryonic kidney (HEK293) cells and stably ACE2-expressing cells were selected with Puromycin (1 µg/ml). ACE2 expression was confirmed by immunoblot (Figure 6) and localization of the ACE2 protein was assessed using immunofluorescence (Figure 7). Up to date 58 ACE2 orthologs expressing plasmids have been transfected into HEK293 cells and ACE2 expression by immunoblot has been confirmed in 49 cell lines (Table 1). These cell lines were subsequently shared with Jason Kindrachuk at the University of Manitoba

Table 1. Development of ACE2 expressing HEK293 cell lines. Animal species in blue are from the Huanan wet market.

Number	Animal ACE2 orthologue	Species	Transfection completed	Confirmed expression
1	Alpaca	<i>Vicugna pacos</i>	Yes	Yes
2	American Mink	<i>Neogale vison</i>	Yes	Yes
3	Artic Fox	<i>Vulpes vulpes</i>	Yes	Yes
4	Artic ground squirrel	<i>Uroditellus parryii</i>	Yes	Yes
5	Big brown bat	<i>Eptesicus fuscus</i>	Yes	Yes
6	Black Rat	<i>Rattus rattus</i>	Yes	
7	Cat	<i>Felis catus</i>	Yes	Yes
8	Chicken	<i>Gallus gallus</i>		
9	Cougar	<i>Puma concolor</i>	Yes	Yes
10	Cow	<i>Bos taurus</i>	Yes	Yes



11	Creeping Vole	<i>Microtus oregoni</i>	Yes	Yes
12	Dog	<i>Canis lupus familiaris</i>	Yes	Yes
13	Elk	<i>Cervus canadensis</i>	Yes	Yes
14	Ermine	<i>Mustela ermina</i>	Yes	Yes
15	Eurasian badger	<i>Meles meles</i>	Yes	Yes
16	European hedgehog	<i>Erinaceus europaeus</i>	Yes	
17	Goat	<i>Capra hircus</i>	Yes	Yes
18	Grizzly Bear	<i>Ursus arctos horribilis</i>	Yes	Yes
19	Ground Squirrel	<i>Ictidomys tridecemlineatus</i>	Yes	Yes
20	Greater hog Badger	<i>Arctonyx collaris</i>	Yes	Yes
21	Harbour Seal	<i>Phoca vitulina</i>	Yes	Yes
22	Himalayan marmot	<i>Marmota himalayana</i>	Yes	Yes
23	Horse	<i>Equus caballus</i>	Yes	Yes
24	Human	<i>Homo sapiens</i>	Yes	Yes
25	Little brown Bat	<i>Myotis lucifugus</i>	Yes	Yes
26	Lynx	<i>Lynx canadiensis</i>	Yes	Yes
27	Malayan porcupine	<i>Hystrix brachyura</i>	Yes	Yes
28	Mallard	<i>Anas platyrhynchos</i>	Yes	Yes
29	Masked palm Civet	<i>Paguma larvata</i>	Yes	Yes
30	Mouse	<i>Mus musculus</i>	Yes	Yes
31	Mule Deer	<i>Odocoileus hemionus</i>	Yes	Yes
32	Muntjac	<i>Muntiacus reevesi</i>	Yes	
33	Muntjac_NTCE	<i>Muntiacus reevesi</i>	Yes	Yes
34	North American River Otter	<i>Lontra canadensis</i>	Yes	Yes
35	Northern Fur Seal	<i>Callorhinus ursinus</i>	Yes	Yes
36	Northern Sea otter	<i>Enhydra lutris kenyoni</i>	Yes	Yes



37	Norway Rat	<i>Rattus norvegicus</i>	Yes	Yes
38	Nutria	<i>Myocastor coypus</i>	Yes	Yes
39	Orca	<i>Orcinus orca</i>	Yes	Yes
40	Pig	<i>Sus scrofa</i>	Yes	Yes
41	Prairie Deer Mouse	<i>Peromyscus maniculatus bairdii</i>	Yes	Yes
42	Prairie Vole	<i>Microtus ochrogaster</i>	Yes	Yes
43	Pronghorn	<i>Antilocapra americanus</i>		
44	Rabbit	<i>Oryctolagus cuniculus</i>	Yes	Yes
45	Raccoon	<i>Procyon lotor</i>	Yes	Yes
46	Raccoon dog	<i>Nyctereutes procyonoides</i>	Yes	Yes
47	Red deer	<i>Cervus elaphus</i>	Yes	Yes
48	Red Fox	<i>Vulpes vulpes</i>	Yes	Yes
49	Red squirrel	<i>Sciurus vulgaris</i>		
50	Reindeer	<i>Rangifer tarandus caribou</i>	Yes	Yes
51	Sheep	<i>Ovis aries</i>	Yes	
52	Star Nosed Mole	<i>Condylura cristata</i>	Yes	Yes
53	Turkey	<i>Meleagris gallopavo</i>	Yes	
54	White Tailed Deer	<i>Odocoileus virginianus texanus</i>	Yes	Yes
55	Woodchuck	<i>Marmota monax</i>	Yes	
56	Yellow bellied marmot	<i>Marmota flaviventris</i>	Yes	Yes
57	Humpback whale	<i>Megaptera novaeangliae</i>	Yes	Yes
58	Virginia opossum	<i>Didelphis virginiana</i>	Yes	Yes
59	Moose	<i>Alces alces</i>		
60	Wolf	<i>Canis lupus</i>		
61	Black-footed ferret	<i>Mustela nigripes</i>		



		TOTAL	58	49
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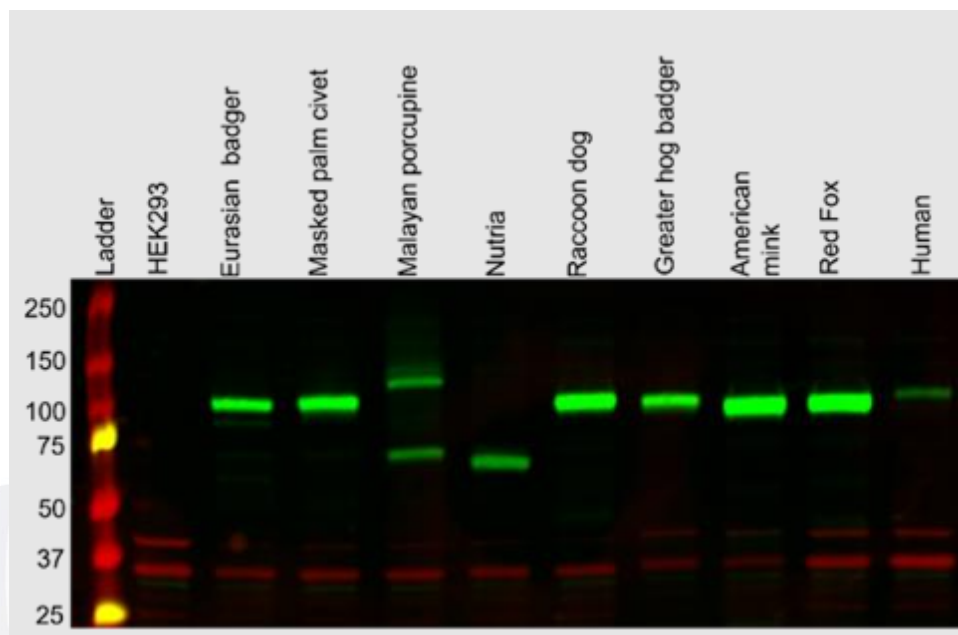


Figure 6. Representative Immunoblot of ACE2 expressing HEK293 cell lines.

HEK293 cells were transfected with the corresponding ACE2 ortholog expression plasmid and selected with Puromycin. Cells were lysed at 2 weeks post-selection and immunoblotting was performed using FLAG (green) and GAPDH (red) antibodies.

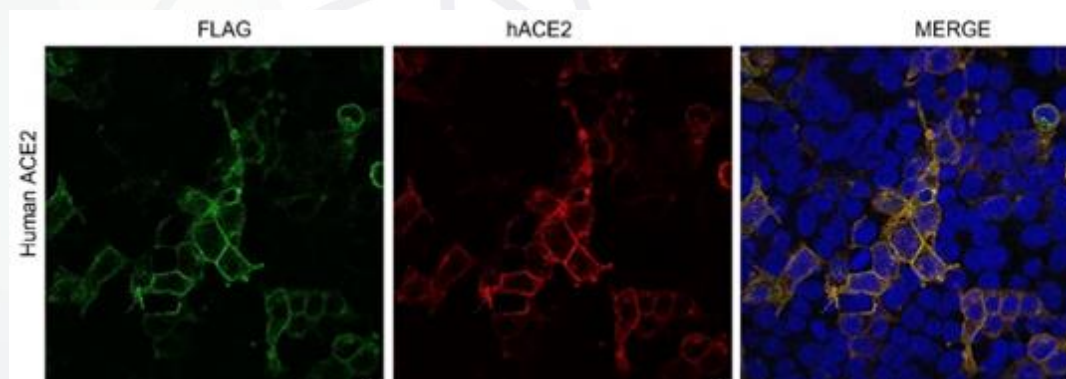


Figure 7. Representative Immunofluorescence of ACE2 expressing HEK293 cell lines. HEK293 cells were transfected with the human-ACE2 ortholog



expression plasmid and selected with Puromycin. Cells were fixed (methanol) after at least 2 weeks of selection and immunofluorescence was performed using FLAG (green) and human ACE2 (red) antibodies, the nucleus was stained using Hoescht (blue).

1.3 Development of pseudoviruses (Kindrachuk).

As a Risk Group 3 pathogen, SARS-CoV-2 requires the use of containment level 3 (CL3) infrastructure for work with fully infectious virus. Further, high-throughput assays that employ pseudotype virus systems can also increase screening capacity within laboratories and decrease laboratory time within CL3 spaces. The University of Manitoba is currently undertaking a \$1.7M renovation of existing laboratory space for upgrading to CL3; however, there is no publicly accessible CL3 laboratory in Manitoba for SARS-CoV-2 research. To meet this challenge, the Kindrachuk laboratory has employed SARS-CoV-2 pseudotype virus systems utilizing non-replicating vesicular stomatitis virus (VSV) for screening and down-selection of potential susceptible host species at CL2. SARS-CoV-2 pseudotypes have been constructed by the Kindrachuk lab as follows: VSVΔG containing the selected SARS-CoV-2 spike protein were prepared as described previously [4]. HDM plasmids containing the Delta spike protein gene (COVARR-NET, #HDM_Delta+_(B.1.617.2_K417N)_Spike), the Omicron spike protein gene (COVARR-NET, #HDM_Omicron_(B.1.1529)_Spike), and the BA.2 spike protein gene (COVARR-NET, #HDM_BA.2_Spike) were kindly provided by P4 lead Anne-Claude Gingras. Briefly, HEK293T cells were transfected with the desired plasmid using Lipofectamine with 10% fetal bovine serum. Cells were then infected with rVSVΔG-luciferase*G and incubated at 37°C with 5% CO₂ for 6 hours. The media was removed and replaced with fresh DMEM with 10% FBS, and cells were monitored for cytopathic effects (CPE) daily. Supernatants containing rVSVΔG-luciferase*S were harvested following observation of CPE (typically 24-48 hours postinfection). The presence of rVSV in the supernatant was determined by Britelite assay following the manufacturer's instructions. Incorporation of S into the pseudotypes was confirmed by western blot. The same approach is being used to generate pseudoviruses from newly emergent variants such as BA.4 and BA.5. The presence of rVSV replication was detected by Britelite assay and the presence of SARS-CoV-2 spike protein confirmed by western blot (Figure 8).

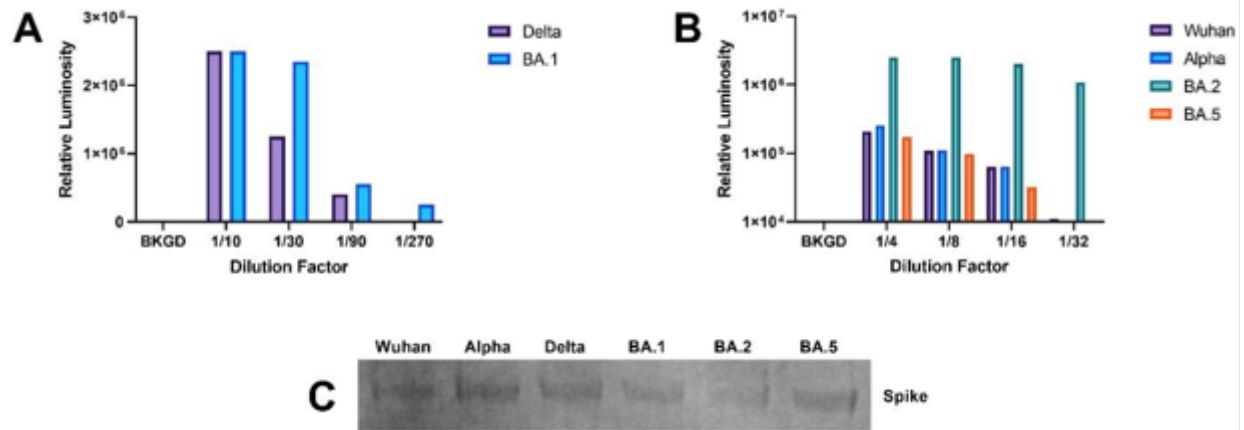


Figure 8. Construction of rVSV containing SARS-CoV-2 spike protein. HUH7 cells were infected with rVSV-Delta or rVSV-BA.1, with viral replication detected by BriteLite assay (A). HUH7 cells were infected with rVSV-Wuhan, rVSV-Alpha, rVSV-BA.2, or rVSV-BA.5, with viral replication detected by BriteLite assay (B). Spike protein was detected on all pseudotypes by western blot (C).

1.4 Assessment of SARS-CoV-2 binding and entry in Canadian wildlife species (Kindrachuk and Rasmussen).

Krist Loeb, MSc candidate in the Kindrachuk laboratory, identified 52 species of Canadian wildlife and livestock that may be of interest, consisting of 32 mammals and 20 birds. Forty-three of these species have previously described ACE2 protein sequences and the remaining 9 have a genome that may be used to extract the ACE2 sequence. We performed multiple sequence alignment on the previously described ACE2 sequences using Clustal Omega, which provided a phylogenetic organization of the mammals based on the similarity of ACE2 (Figure 9).

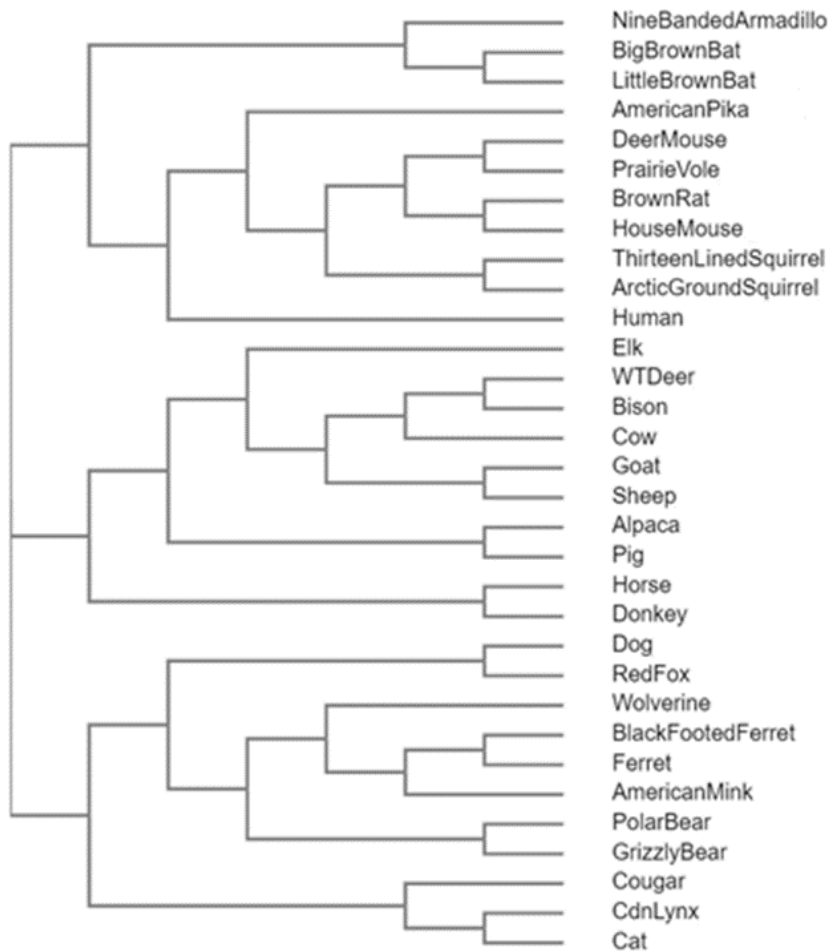


Figure 9. Phylogenetic tree of the ACE2 sequences of 32 mammals. Sequences were organized by multiple sequence alignment using Clustal Omega.

The Kindrachuk laboratory has acquired 28 lines of HEK293T cells stably expressing a given species' ACE2 and 38 plasmids containing a given species' ACE2. These are courtesy of the Rasmussen laboratory. We have tested susceptibility of six cell lines with various SARS-CoV-2 pseudotypes which has shown both species- and variant-specific effects (Figure 10).

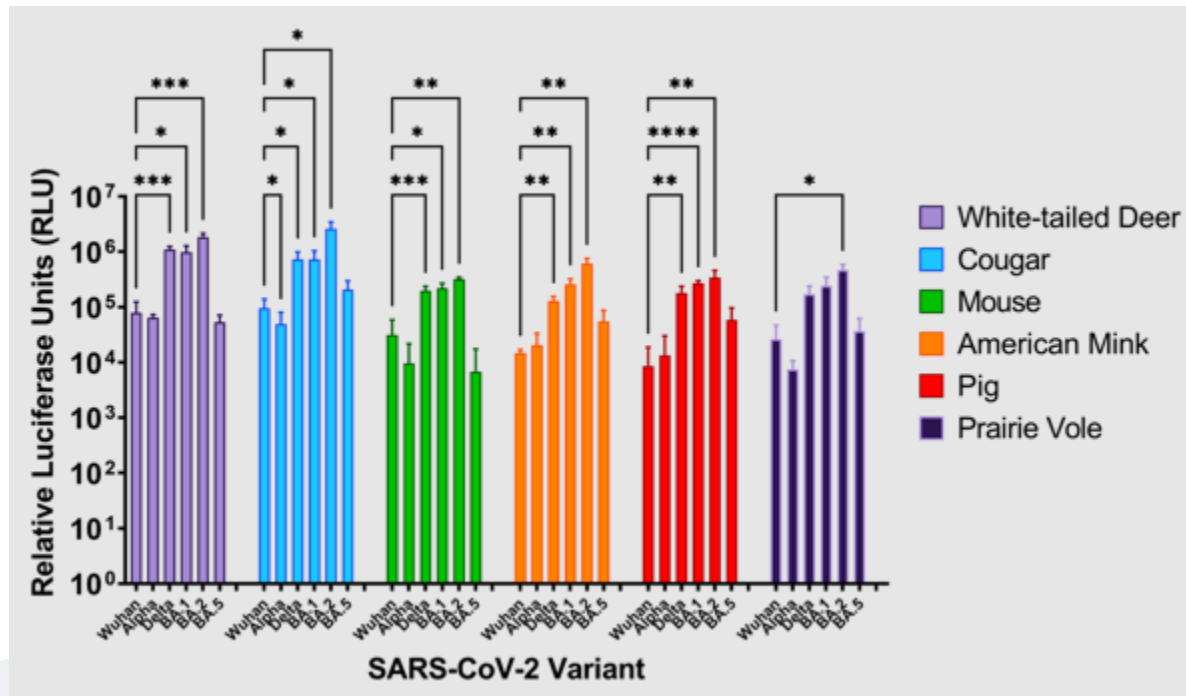


Figure 10. SARS-CoV-2 pseudotype infections of HEK293T expressing wildlife ACE2.

We are currently collaborating with Dr. Colin Garroway, a population ecologist at the University of Manitoba, for acquisition of primary tissues from various wildlife species. We are providing collaborative funding for a studentship for tissue acquisition in summer 2023 as well as for reagents and consumables.

1.5 Establishment of primary cell lines (Banerjee, Mubareka, and Pickering).

White-tailed deer (WTD) tissues were received from Pickering, Mubareka, and colleagues. We have generated primary lung and kidney cells and have generated immortalized lung cells (immortalization will be confirmed by colleagues who propagate the cells for a long time; we do not keep the cells in culture). Kidney cell immortalization/selection failed during the first attempt. Not repeating kidney cell immortalization at this moment. Cells propagated and metagenomics completed, analysis pending. Optimization of infection is underway.

1.6. Development of novel 3D organoids using primary animal cells to facilitate studies of virus-host interactions (Banerjee)

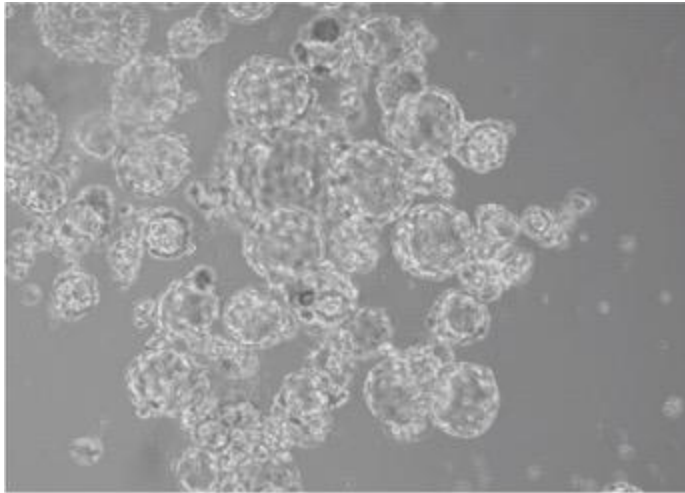


Figure 11. *Carollia perspicillata* (bat) primary kidney organoids developed in the Banerjee laboratory.

The Banerjee laboratory has developed primary 3D airway organoids to study emerging respiratory viruses. In this sub-aim, the Banerjee laboratory will attempt to develop 3D organoid cultures using animal cells for virus-host interaction studies. In cell line models, viruses such as MERS-CoV and SARS-CoV-2 typically induce complete cell death (cytopathic effect) of the entire cell population. However, real life infections do not lead to large-scale global infection, in part due to inherent differences in susceptibility and permissivity of different cells that make up our tissues. Thus, cell lines are not a feasible model to study long-term virus replication, viral persistence, and cellular regeneration due to the sheer lack of tissue complexity. The common alternative is to use animal models. However, *in vivo* imaging technology is still being refined and current strategies involve sacrificing animals at different times to assess infection and pathogenicity. Sacrificing animals is a terminal experiment and it does not allow researchers to capture the dynamic relationship of virus infection and tissue regeneration. Organoids are a newly developed model system. Self-organization of differentiating cells has enabled the development of various human epithelial and epithelial-mesenchymal organoids from human pluripotent stem cells (hPSCs), such as the optic cup, gut, liver, pancreas, brain, kidney, and retinal organoids. The main advantages of an organoid are the cellular diversity and structural sophistication of multiple lineages with a 3D architecture resembling that of the original tissue via self-organization. Organoids derived from animal tissues are rare and represent a significant lack of resources to facilitate studies on virus transmission and persistence in key wildlife and livestock species. Indeed, to address current gaps and needs, the Banerjee laboratory will develop organoids from priority animal species to facilitate studies on virus-host interactions (Figure 11).



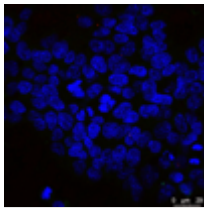
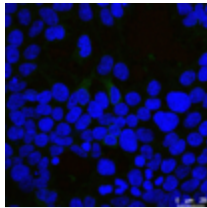
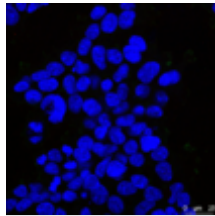
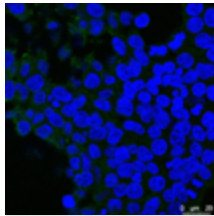
Aim 2. Susceptibility screening.

2.1 Variant screening (Rasmussen and Banerjee).

ACE-2 expressing HEK293 cells will be infected with ancestral SARS-CoV-2 and beta, delta, and omicron VOCs at an MOI of 1 for immunofluorescence analysis and or 0.1 for growth curves for 48 hours. ACE2 orthologs that support virus entry will be identified using immunofluorescence analysis to observe cells that express ACE2 and are infected with SARS-CoV-2. Production of infectious virus will be assessed at 0, 24, and 48 hours post infection (hpi) using median tissue culture infectious dose (TCID₅₀) assay, qRT-PCR, and immunoblot analyses. Data from these experiments will lead to the identification of mammalian ACE2 orthologs that are likely to facilitate SARS-CoV-2 infection. This will be the first step to systematically identify and robustly validate ACE2 orthologs from animal species that are likely to become infected with SARS-CoV-2. Once we have identified ACE2 orthologs that support SARS-CoV-2 entry, we will derive primary cells from tissues collected from these animals, which will help to identify the animals that are not only susceptible but also permissible to SARS-CoV-2 infection and study the specific host-virus interactions.

We will use primary cells as well as develop immortalized cell lines for long-term studies from animal species of interest. We will longitudinally assess primary and immortalized cells for SARS-CoV-2 infectivity at 6, 12, 24, and 48 hpi, followed by RNA extraction and bulk RNA sequencing (RNA-seq) to profile global host gene expression relative to time-matched, mock-infected controls. These transcriptomic studies will facilitate the discovery of cellular processes that are triggered or dampened during virus infection, along with an assessment of virus replication in these cells.

First, we have started assessing the susceptibility of animals that were reported to be at the Huanan Wholesale Seafood Market (Huanan market) to the ancestral SARS-CoV-2 variant. HEK293 cells expressing the ACE2 ortholog of the corresponding animals were Mock infected or infected with VIDO-1 SARS-CoV-2 at a MOI of 1 and at 24 hpi the cells were fixed using neutral buffered formalin 10% solution (NBF 10%) and processed for immunofluorescence analysis (Figure 12).

	MOCK	SARS-CoV-2 VIDO1		MOCK	SARS-CoV-2 VIDO1
HEK293			Malayan porcupine		

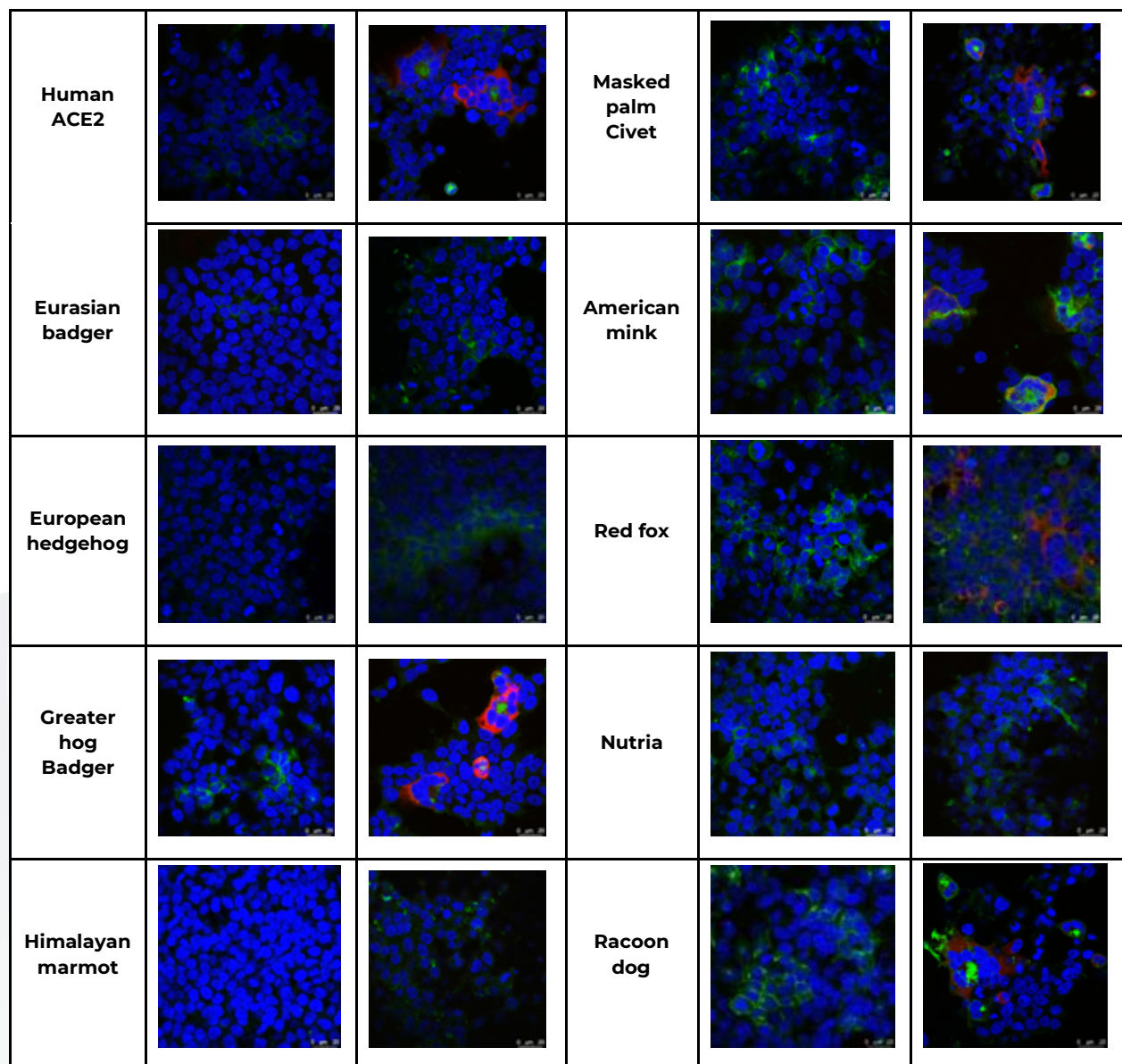


Figure 12. SARS-CoV-2 in HEK293 cells expressing the indicated ACE2 orthologs. HEK293 cells were Mock infected or infected with VIDO-1 SARS-CoV-2 at a MOI of 1 and 24 hpi the cells were fixed with NBF 10% and processed for immunofluorescence analysis using FLAG (green) and SARS-CoV-2 N protein (red) antibodies, the nucleus was stained using Hoescht (blue).

The ACE2 expressing HEK293 cells were also Mock infected or infected with the VIDO-1 SARS-CoV-2 at a MOI of 0.1 and the supernatant and whole cell lysate was



collected at 0, 24 and 48 hpi to assess virus replication by median tissue culture infectious dose (TCID₅₀) assay (Figure 13) and immunoblot analysis respectively (Figure 14). The human ACE2 HEK293 expressing cells were used as a positive control of susceptibility. The Eurasian badger, Greater hog badger, Masked palm civet, American mink, Red fox, and Raccoon dog ACE2 orthologs show a pattern of membrane localization, whereas Himalayan marmot, Malayan porcupine, and Nutria ACE2 orthologs show mainly cytoplasmic localization (Figure 12). Raccoon dog, Greater hog badger, Masked palm civet, American mink, and Red fox ACE2 expressing HEK293 cells have SARS-CoV-2 N-protein expression when the cells are infected at a MOI of 1 at 24 hpi (Figure 12) and at an MOI of 0.1 at 24 and 48hpi (Figure 9) indicating that these ACE2 orthologs support virus entry.

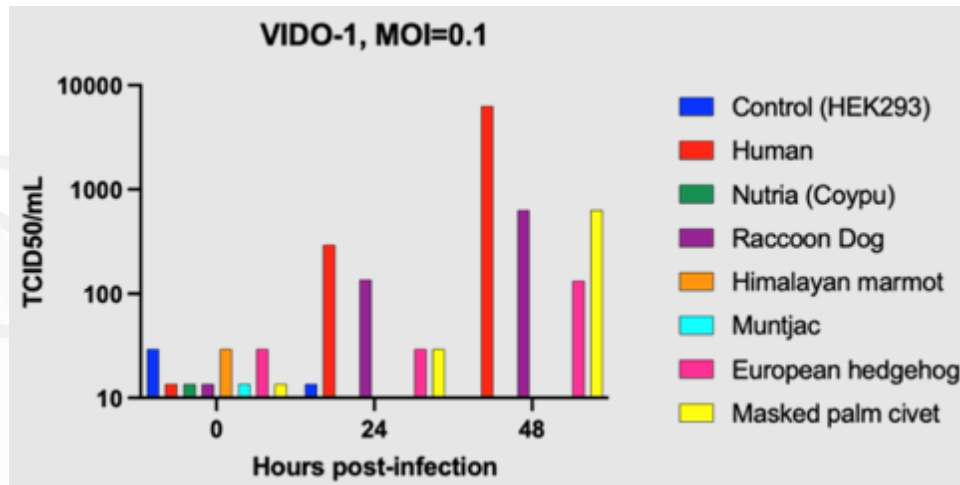
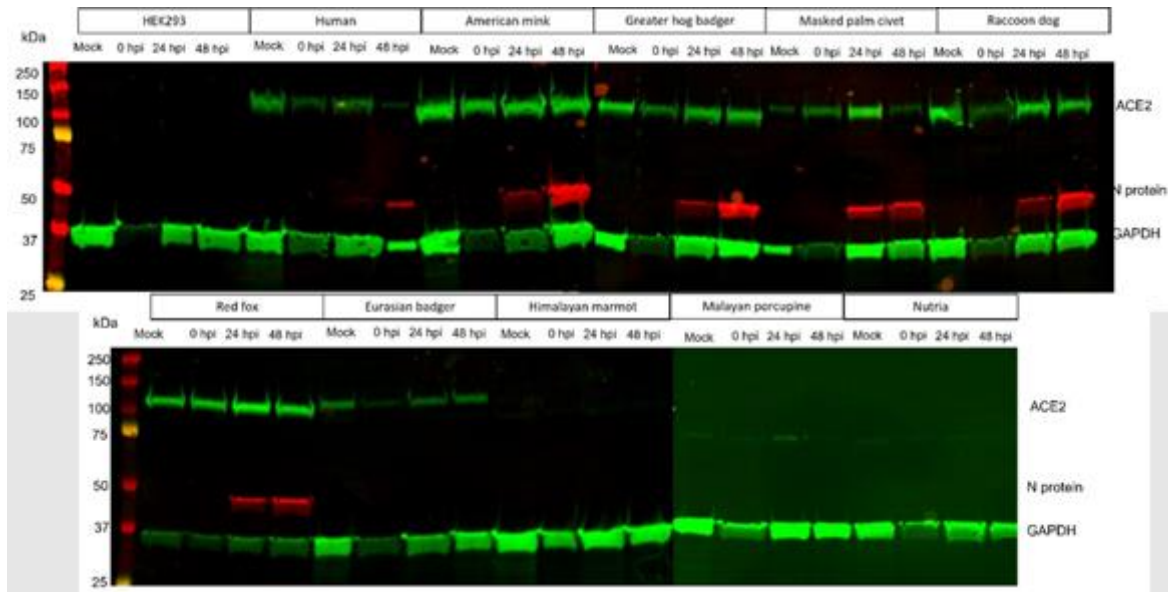


Figure 13. SARS-CoV-2 infection in HEK293 cells expressing the indicated ACE2 orthologs. HEK293 cells were infected with VIDO-1 SARS-CoV-2 at a MOI of 0.1 and supernatant was collected at 0, 24, and 48h to perform median tissue culture infectious dose (TCID₅₀) assay.

A



B

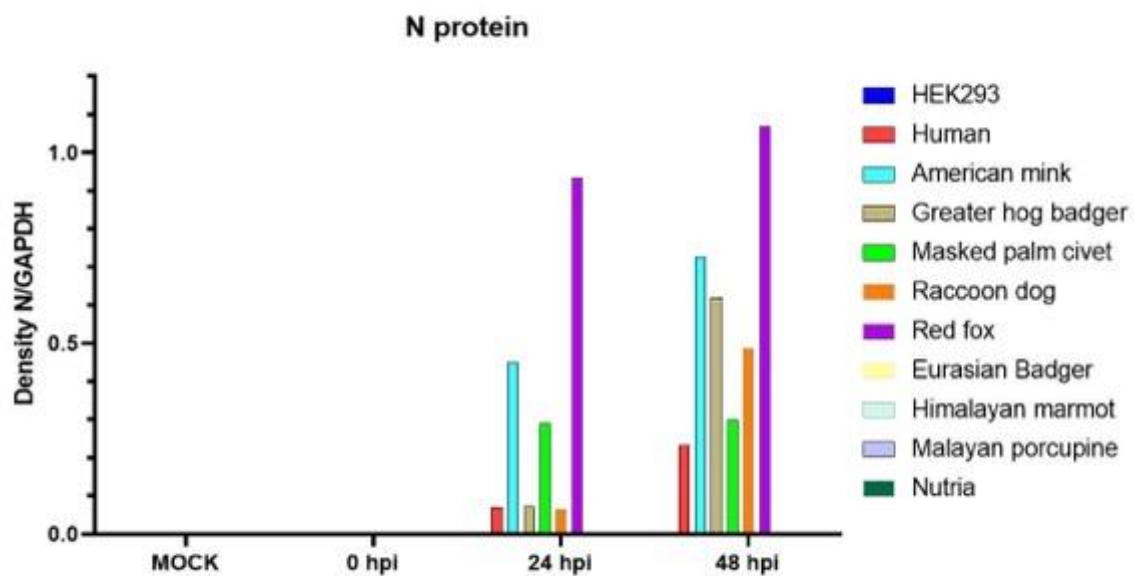


Figure 14. SARS-CoV-2 in HEK293 cells expressing the indicated ACE2 orthologues. HEK293 cells were infected with VIDO-1 SARS-CoV-2 at a MOI of 0.1 and whole cell lysates were collected at 0, 24, and 48h. (A) Immunoblotting was performed using N-SARS-Co-2 protein (red), FLAG and GAPDH (green) antibodies. (B) Densitometric analysis of N protein relative to GAPDH.



2.3 Characterization of genetically complex Collaborative Cross mouse strains that model divergent COVID-19 outcomes.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the coronavirus disease 2019 (COVID-19) pandemic, has highly variable levels of illness. Host inflammatory responses play a major role in the progression of serious outcomes after viral infection. The host contribution to COVID-19 pathogenesis, however, remains poorly understood. Elucidating key host regulatory networks and signaling pathways is essential to understanding how host gene expression contributes to COVID-19 severity. Understanding host-virus interactions require a robust animal model. Laboratory mice are less genetically diverse than the human population, which limits phenotypic variance in these models. We propose using the Collaborative Cross (CC), a panel of genetically diverse mice that vary significantly in their response to Ebola virus infection and manifest a variety of disease presentations and outcomes, from mild to severe. ***We hypothesize that CC mice will appropriately model the impact of genetic diversity and host responses on SARS-CoV-2 susceptibility, resistance, pathogenesis, and disease outcome seen in humans. We have 2 goals: 1) Developing critically needed mouse models that reproduce humanlike COVID-19 illness, ranging from no clinical disease to severe disease/death. 2) Identifying host genes/pathways driving COVID-19 pathogenesis.***

We are currently screening groups of female and male mice from 10 different CC strains backgrounds (CC011/Unc, CC041/Unc, CC074/Unc, CC019/Unc, CC023/Unc, CC032/Unc, CC033/Unc, CC041/Unc, CC043/Unc, and CC061/Unc) for COVID-19 severity after infection with rodent-adapted variants to understand how genetic variation contributes to differential COVID-19 outcomes. After intranasal infection with 10^4 TCID₅₀ of B.1.1.7/Alpha or BA.2/Omicron, animals (6-8 weeks) are monitored and scored daily for respiratory distress, weight loss, neurological deficits, and physical appearance. Oral swabs are collected on different days post-infection (p.i.) to assess viral load by quantitative RT-PCR (qPCR) and host gene expression in response to infection. Tissues and blood are collected from animals that survived until day 14 p.i. for virus titration, qPCR, microneutralization assays, and global transcriptomics by RNA-seq.

In both CC011 and CC041 lines infected with BA.2/Omicron, we observed 100% survival rates (Figure 15). Also we did not detect any virus replication in the oral swabs at any time p.i. across these lines. Furthermore, we observed differential weight loss in male



and female mice across these strains screened (Figure 16), suggesting that SARS-CoV-2 severity and host responses to infection are partly determined by sex which mimics the epidemiology of COVID-19 in humans. To assess the impact of viral background variables in these strains, we challenged additional groups CC011 and CC0.41 with B.1.1.7/Alphavirus (in process).

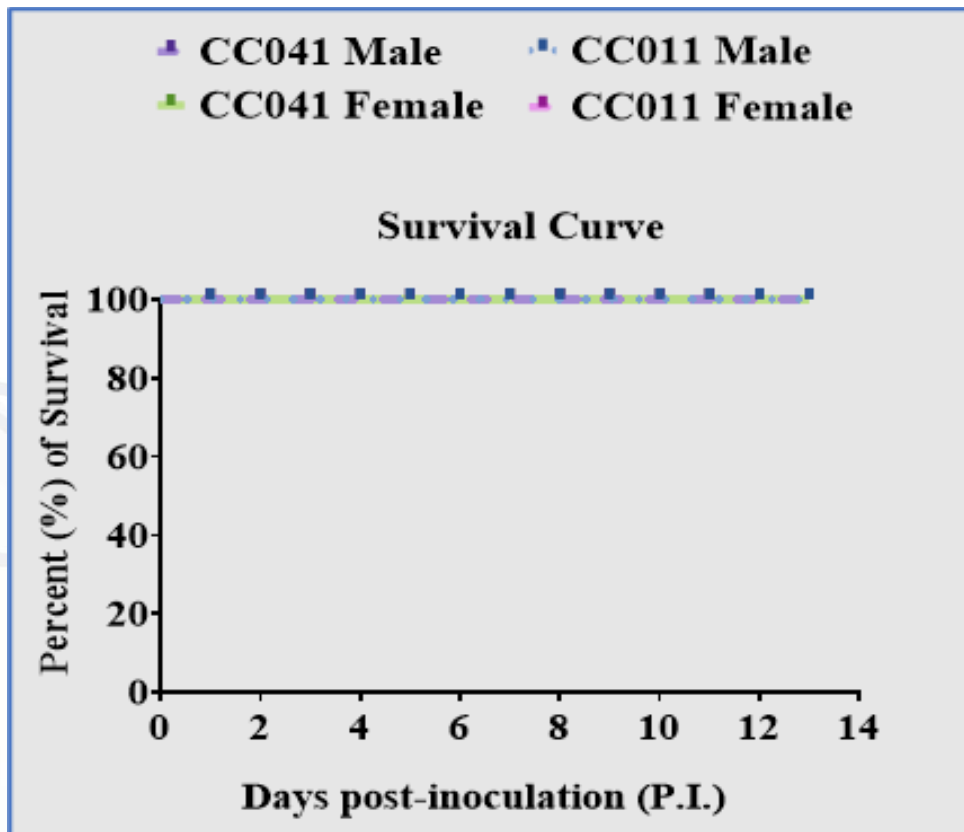


Figure 15. Survival Curve

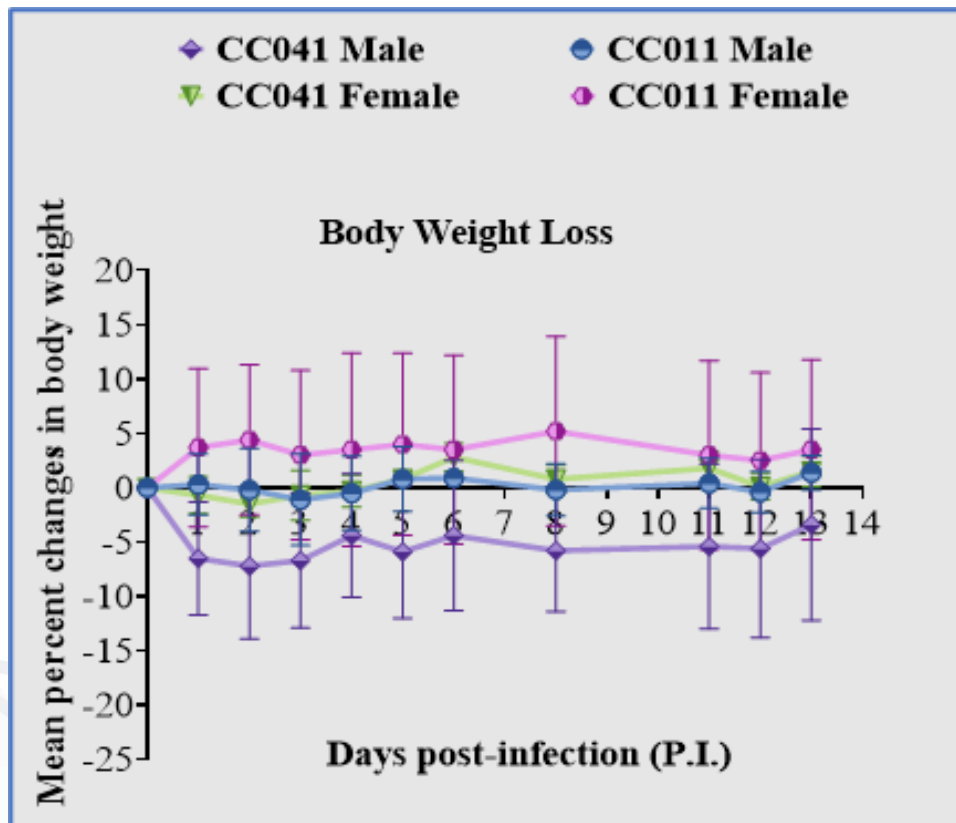


Figure 16. Body weight loss

The short-term plan is to continue phenotypic screening of the remaining 8 CC mouse strains (CC074/Unc, CC019/Unc, CC023/Unc, CC032/Unc, CC033/Unc, CC041/Unc, CC043/Unc, and CC061/Unc).

The phenotypic observations will lead us to select at least two lines of CC mice showing extreme SARS-CoV-2 responses, ranging from mild to severe symptoms. A detailed virological and histopathological analysis will be conducted on the CC lines showing the most phenotypic differences. Additional CC mice will be intranasally infected and serially sacrificed at days 2, 4, and 7 p.i. to collect tissues including nasal epithelium, trachea, lungs, heart, brain, spleen, liver, kidney, stomach, large intestine, small intestine, eyes, genital glands (ovaries and testis) and blood. The control CC mice will be euthanized at the same time point to harvest the same tissues. Samples were divided into three equally sized portions. One portion will be formalin-fixed for histopathology and immunohistochemistry. One portion will be placed in and frozen prior to homogenization and centrifugation for virus infectivity and quantification by TCID₅₀ and q-RT-PCR, respectively.



To define key host pathways regulating pathogenesis, RNA extraction from nasal, lung, and brain samples and RNA sequencing will be used to profile global gene expression in susceptible and resistant COVID-19 lines relative to time-matched, mock-infected controls with a view to identifying genes/pathways that affect its progression. Differential expression (DE) analysis will be first used to determine which genes are significantly expressed higher or lower relative to controls by comparing mice with varying disease phenotypes. DE genes will undergo functional analysis using Ingenuity Pathway Analysis to identify key host pathways that drive disease. This project will allow us to select CC strains with extreme respiratory and neurological symptoms. Further, the CC model will improve knowledge of how host responses drive COVID-19 pathogenesis and identify novel targets for the development of effective vaccines and antiviral therapies.

Aim 3. Molecular profiling data.

3.1 Transcriptomic profiling (Rasmussen).

3.1.1 To develop a compendium of global host response profiles associated with differential pathogenicity.

The National Center for Biotechnology Information (NCBI)/International Nucleotide Sequence Collaboration Database Collaboration (INSD) “Bioproject” database was searched using the term “SARS-Cov2 host response” to collect the publicly available transcriptomics data. All the available RNAseq datasets (as of 3rd June 2022) were sorted and curated in an excel list sheet based on different criteria such as the number of samples, viruses (i.e., SARS-CoV2 and other variants), experiment/study type (in-vivo/in-vitro/clinical), the tissue used for transcriptomics, disease outcome (mild/moderate/severe), the platform used for sequencing, read type (paired-end/single-end), etc. Then, RNAseq datasets from in-vivo/clinical/in-vivo and in-vitro studies from eight different organisms i.e., *Mustela putorius furo*, *Mesocricetus auratus*, *Mus musculus*, *Homo sapiens*, *Chlorocebus sabaeus*, *Rattus norvegicus*, *Macaca fascicularis* and *Macaca mulatta*, were selected for further analysis. The proteome of all eight species was downloaded from the NCBI genome database and the ortholog mapping was performed using the program “OrthoFinder (version 2.5.4)”. A total of 1,746 single-copy orthologues were found in all species. The purpose of orthologs mapping here is to find a common identifier so that gene expression datasets from different species can be integrated into a single matrix. Before integration, the RNAseq dataset from six species will be filtered further based on the number of samples, sequencing and read types, vaccination etc. To enable samples to be analyzed at the same scale, the datasets after integration will be



normalized using either the deseq-median ratio or trimmed mean of M values (TMM) including batch as a factor in the design.

In addition, the Rasmussen lab has received approval to begin infections of Collaborative Cross mice with the Alpha and Omicron (BA.2) variants to identify transcriptomic profiles associated with differential outcomes. These profiles will be analyzed independently, as well as incorporated into the compendium. These experiments are scheduled to begin on November 22nd.

3.2 Proteome/Serological/Kinomic profiling (Kindrachuk and Pickering).

Kinome profiling has been delayed due to availability of stably transfected HEK293T cells for screening and identification of species of interest as well as additional MTA requirements between VIDO and U.Manitoba for material transfers. However, as some cell lines have now been sent to the University of Manitoba, this is expected to begin in the near future.

3.3 Viral Genome-Wide Association Studies (Sagan).

We used publicly available viral genome sequences and phylogenetic analysis to systematically investigate transmission of SARS-CoV-2 between human and non-human species and to identify mutations associated with each species (Naderi et al. ELife 2023). We found the highest frequency of animal-to-human transmission from mink, compared with negligible transmission from other sampled species (cat, dog, and deer). Although inferred transmission events could be limited by sampling biases, our results provide a useful baseline for further studies. Using genome-wide association studies, no single nucleotide variants (SNVs) were significantly associated with cats and dogs, potentially due to small sample sizes. However, we identified three SNVs statistically associated with mink and 26 with deer. Of these SNVs, $\sim\frac{2}{3}$ were plausibly introduced into these animal species from local human populations, while the remaining $\sim\frac{1}{3}$ were more likely derived in animal populations and are thus top candidates for experimental studies of species-specific adaptation. Together, our results highlight the importance of studying animal-associated SARS-CoV-2 mutations to assess their potential impact on human and animal health. Moving forward, we are exploring within host diversity using a variety of statistical models.

Aim 4. Construct a risk matrix for susceptible animal species.

4.1 Data integration and risk matrix construction (Banerjee, Kindrachuk, Rasmussen, Mubareka, Pickering, and Sagan).



We collaborated with Andrea Osborn from the Community for Emerging and Zoonotic Diseases (CEZD) to consult with key stake holders regarding priority species for SARS-CoV-2 screening. In addition, the structural homology modeling and molecular docking simulations described in section 1.2.2. and results from susceptibility screening described in 2.1 will inform our initial efforts to rank species for surveillance. Gradually, surveillance and transcriptomic data will allow us to integrate risk of disease as well as infection and to better estimate the risk of variant pathogenicity for both humans and animals.

Table 2. Summary of publications and publications in the pipeline to date

Corresponding Author	CoVaRR-Net member	Title	Type of Article	Status
Michael Worobey, Kristian Andersen	Angela L. Rasmussen	The Huanan Seafood Wholesale Market in Wuhan was the early epicenter of the COVID-19 pandemic	Research paper	Published
Arinjay Banerjee and Darryl Falzarano	Arinjay Banerjee, Samira Mubareka	Immunogenicity of convalescent and vaccinated sera against clinical isolates of ancestral SARS-CoV-2, Beta, Delta, and Omicron variants	Research paper	Published
Jesse Shapiro, Selena M. Sagan	Brad Pickering, Jesse Shapiro, Selena Sagan	Zooanthroponotic transmission of SARS-CoV-2 and host-specific viral mutations revealed by genome-wide phylogenetic analysis	Research Paper	Published
Jason Kindrachuk	Jason Kindrachuk	SARS-COV-2 AND THE MISSING LINK OF INTERMEDIATE HOSTS IN VIRAL EMERGENCE - WHAT WE CAN LEARN FROM OTHER BETACORONAVIRUSES	Review Paper	Published



Arinjay Banerjee and Daniel Becker	Arinjay Banerjee	COUPLING FIELD AND LABORATORY STUDIES OF IMMUNITY AND INFECTION IN ZOO NOTIC HOSTS.	Comment article	Published
Michael Hiller	Arinjay Banerjee	REFERENCE-QUALITY BAT GENOMES ILLUMINATE ADAPTATIONS TO VIRAL TOLERANCE AND DISEASE RESISTANCE.	Research Paper	Under review
Karen Mossman	Samira Mubareka and Arinjay Banerjee	SARS-COV-2 R.1 LINEAGE VARIANTS ARE SENSITIVE TO NEUTRALIZING ANTIBODIES BUT RESISTANT TO TYPE I INTERFERONS.	Research Paper	Under review
T.D. Capellini	Arinjay Banerjee	REGULATORY DISSECTION OF THE SEVERE COVID-19 RISK LOCUS INTROGRESSED BY NEANDERTHALS.	Research Paper	Published
Arinjay Banerjee	Arinjay Banerjee	VERSATILE USE OF BAT ACE2 FOR CELLULAR ENTRY BY MERS-COV-LIKE VIRUSES.	Research Paper	Published
K. Chan	Samira Mubareka and Arinjay Banerjee	SURVIVAL-BASED CRISPR GENETIC SCREENS ACROSS A PANEL OF PERMISSIVE CELL LINES IDENTIFY COMMON AND CELL-SPECIFIC SARS-COV-2 HOST FACTORS.	Research Paper	Published



E. Jane Parmley	Samira Mubareka, Brad Pickering and Arinjay Banerjee	STRENGTHENING A ONE HEALTH APPROACH TO EMERGING ZONOSSES.	Research Paper	Published
Arinjay Banerjee and Karen Mossman	Arinjay Banerjee	LAYING THE FOUNDATION FOR SINGLE-CELL STUDIES IN BATS.	Research Paper	Published
Che Colpitts	Arinjay Banerjee	SARS-COV-2 MITOCHONDRIOPATHY IN COVID-19 PATIENTS EXACERBATES HYPOXEMIA.	Research Paper	Published
Arinjay Banerjee	Arinjay Banerjee	MOLECULAR, ECOLOGICAL, AND BEHAVIOURAL DRIVERS OF THE BAT-VIRUS RELATIONSHIP.	Research Paper	Published
J.A. Corcoran	Samira Mubareka and Arinjay Banerjee	HUMAN CORONAVIRUSES DISASSEMBLE PROCESSING BODIES.	Research Paper	Published
Arinjay Banerjee	Arinjay Banerjee	CYTOKINE CROSSTALK DURING MYCOBACTERIUM TUBERCULOSIS AND SARS-COV-2 INFECTIONS.	Research Paper	accepted
Mario Ostrowski	Samira Mubareka and Arinjay Banerjee	A SIGNIFICANT CONTRIBUTION OF COMPLEMENT IN ENHANCING SARS-COV-2 NEUTRALIZATION OF CONVALESCENT AND VACCINEE SERA.	Research Paper	Under review



Q. Wu	Arinjay Banerjee	HEART-ON-A-CHIP PLATFORM TO MODEL CARDIAC SARS-COV-2 PATHOGENESIS AND THERAPEUTIC SCREENING.	Research Paper	Under review
Selena M. Sagan and B. Jesse Shapiro	Selena M. Sagan, Bradley S. Pickering, Sana Naderi and B. Jesse Shapiro	ZOOANTHROPONOTIC TRANSMISSION OF SARS-COV-2 AND HOST-SPECIFIC VIRAL MUTATIONS REVEALED BY GENOME-WIDE PHYLOGENETIC ANALYSIS	Research paper	Published
Kristian G. Anderson, Michael Worobey and Florence Débarre	Reema Singh and Angela L. Rasmussen	GENETIC EVIDENCE OF SUSCEPTIBLE WILDLIFE IN SARS-COV-2 POSITIVE SAMPLES AT THE HUANAN WHOLESALE SEAFOOD MARKET, WUHAN ANALYSIS AND INTERPRETATION OF DATA RELEASED BY THE CHINESE CENTER FOR DISEASE CONTROL	Report	Published



Progress report Pillar 3: Virology. May 5, 2023

Lead: Louis Flamand (U Laval)

Deputies: Stephen Barr (Western U)
Jennifer Corcoran (U Calgary)
Darryl Falzarano (VIDO)
Denis Leclerc (U Laval)
Silvia Vidal (McGill)

Vision

Pandemic preparedness starts by having a dedicated network of scientists actively working in confinement level 3 facilities and developing experimental models enabling drug and vaccine testing and contributing to the training of highly qualified personnel.

Overview

Pillar 3 (P3) members are currently focused on the virological characterization of SARS-CoV-2 VOCs. As such, P3 members have engaged in a variety of diverse, yet complementary activities. Several milestones have been achieved. Members of P3 have successfully: 1) grown and produced stocks of most VOC isolates; 2) developed animal models (mice, hamsters, and ferrets) to study VOC pathogenesis, vaccine efficacy, and antiviral drug testing ; 3) developed key techniques such as microneutralization of live and pseudotyped SARS-CoV-2; and 4) a SARS-CoV-2 reverse genetic system has been developed, enabling the study of individual mutations in the context of the whole virus. P3 members collaborate closely with Pillar 5: Viral Genomics & Sequencing that is responsible for the sequencing and analysis of VOC. Similarly, Pillar 4: Functional Genomics and Structure-Function of VOCs supplies P3 with expression vectors encoding the Spike protein of VOC for generating pseudotyped lentiviruses.

Corcoran laboratory

Preamble: Since joining CoVaRR-Net, our work has focused largely on molecular virology of SARS-CoV-2, and we are keen to support the network with our expertise and tools in this area.



Goal: Despite a wealth of information, much remains to be learned regarding the pathogenesis of SARS-CoV-2 and its variants of concern (VOCs). In my lab, we are capitalizing on our experience with recombinant virology to study the genetic determinants of SARS-CoV-2 replication and pathogenesis. As part of Pillar 3, our contribution is to build SARS-CoV-2 recombinant viruses and perform *in vitro* characterization of these recombinants to better understand if the genetic changes confer a replication advantage. Other pillar members will perform *in vivo* characterization. As noted in our proposals, should 'gain of function' be observed for any of the mutants generated, we will restrain from disseminating the information and will inform CoVaRR-Net's executive committee.

Year 1 (2021-2022) Progress Report:

In Year 1, we proposed to study mutations within emerging VOCs that are not found in the spike protein using reverse genetics.

Two specific aims were proposed:

1.1 Use of reverse genetics to characterize VOC mutations not in Spike (focus on the VOC mutational hotspot in the nucleocapsid protein)

The nucleocapsid (N) protein is a structural protein that localizes to viral replication and transcription compartments, binds the viral genomic RNA and is essential for viral replication. The protein is comprised of a modular domain structure that includes an N-terminal RNA-binding domain, a C-terminal oligomerization domain, a central serine and arginine-rich linker, and three regions of intrinsic disorder. Several other functions have been attributed to the N protein, including antagonizing innate immune pathways and disruption cellular antiviral stress granules. However, it remains unclear how the various roles attributed to the N protein are regulated spatially and temporally during virus infection.

In our studies of the coronavirus N protein, **we made an exciting observation.** After ectopic expression of SARS-CoV-2 N protein, we observed several truncated N protein species by immunoblot that were not present when we overexpressed the N protein from several other human coronaviruses. Upon sequence inspection, we realized that the protein contained six in-frame methionine residues with the potential to produce these truncated products (N*) (Figure 1). After immunoblotting infected cell lysate, we observed N* products were present, and their relative amounts varied between VOCs. Closer inspection of the mutational hotspot in the N gene sequence (residues 203/204) revealed that three VOCs (Alpha, Gamma, and Omicron) contained a novel transcription regulatory sequence (TRS) predicted to produce a truncated N* sgRNA (Figure 2).

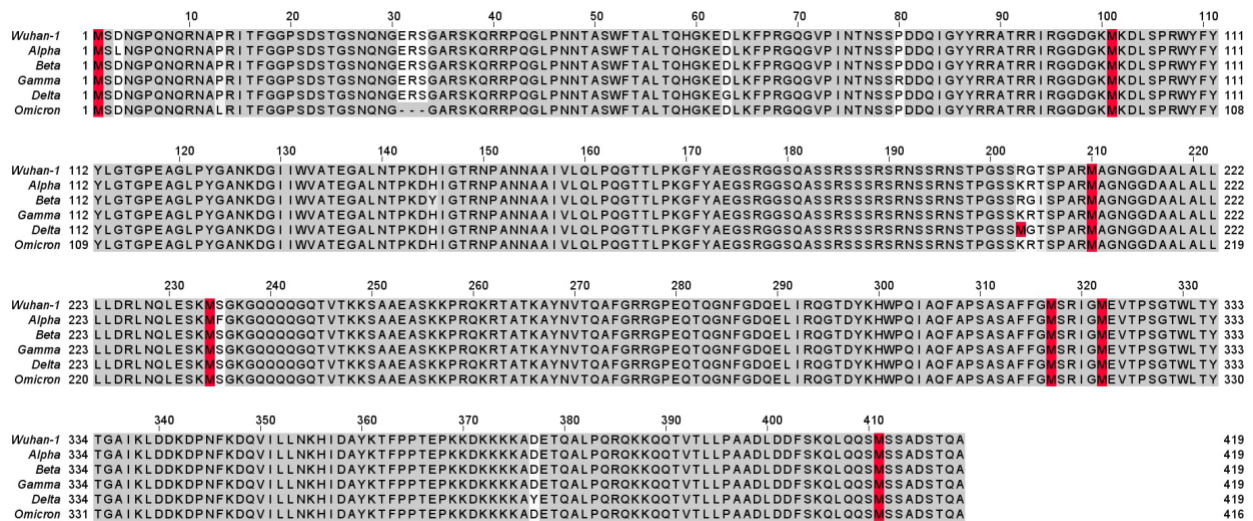


Figure 1. Alignment of SARS-CoV-2 nucleocapsid amino acid sequences. Non-synonymous mutations are white; in-frame methionine codons are red.

Variant	RNA sequence (canonical TRS: <u>ACGAAAC</u>)	Amino Acid 203-204
Ancestral	5' <u>GGCAGCAGUAGGGGAACUUCUCCUG</u> 3'	RG
Alpha	5' <u>GGCAGCAGUAA<u>ACGAAAC</u>UUCUCCUG</u> 3'	KR
Beta	5' <u>GGCAGCAGUAGGGGAUUCUUCUCCUG</u> 3'	RG
Gamma	5' <u>GGCAGCUCUAA<u>ACGAAAC</u>UUCUCCUG</u> 3'	KR
Delta	5' <u>GGCAGCAGUAGGGGAACUUCUCCUG</u> 3'	MG
Omicron	5' <u>GGCAGCAGUAA<u>ACGAAAC</u>UUCUCCUG</u> 3'	KR

Figure 2. SARS-CoV-2 nucleocapsid mutational hotspot. Amino acid substitutions to K203 and R204 in Alpha, Gamma and Omicron create a new TRS in the RNA sequence (underlined bold).

When produced, this N*sgRNA initiates upstream of the start codons for the N* truncated products initiating from Met210, the start codon immediately downstream of the mutational hotspot (R203K/G204R) where the novel TRS is found (Figure 3). We are actively pursuing several strategies to better appreciate the functional significance of the production of truncated N*. Using recombinant virology, we will either eliminate or enhance the production of the truncated N* product M210 to determine the impact of its production on viral replication and fitness.

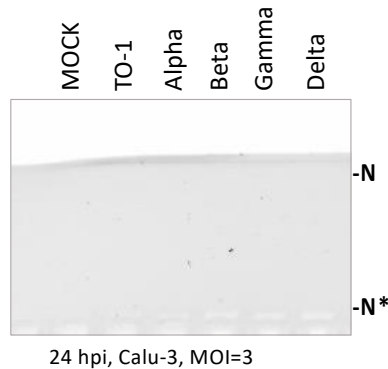


Figure 3. Alpha and Gamma SARS-CoV-2 nucleocapsid genes produce a truncated sgRNA for N*. qPCR amplification of nucleocapsid sequences reveal that Alpha and Gamma produce a truncated N* sgRNA.

We will build the following recombinant viruses to functionally uncouple the amino acid changes at positions 203/204 from the addition of the TRS sequences:

1. R203K/G204R + TRS: using the same codons as Alpha to add amino acid changes K203 and R204 with the new TRS
2. R203K/G204R – TRS: different codon usage to retain K203 and R204 amino acids but remove the TRS
3. M210opt – TRS: optimization of the M210 start with a Kozak consensus sequence
4. M210I – TRS: eliminate the start codon for the M210 truncation
5. M210I + TRS: eliminate the start codon for the M210 truncation in the presence of the K203/R204/TRS changes

1.2 Use of reverse genetics to generate error-prone SARS-CoV-2 mutants by eliminating proofreading activity

RNA viruses have high mutation rates because their polymerases typically lack proofreading ability. The *Coronaviridae* family is one fascinating exception because their polymerase complexes possess a unique RNA proofreading activity. In SARS-CoV-2, this activity depends on two viral gene products, non-structural protein 14 (Nsp14) and non-structural protein 10 (Nsp10). Nsp14 possesses 3'-to-5' exonuclease activity that repairs mismatches during replication. Nsp10 binds to Nsp14, stabilizing its association with the RNA polymerase and enhancing proofreading activity 30-fold.

The last two years of the SARS-CoV-2 pandemic have shown that we are limited in our ability to anticipate the emergence of new viral variants. **To help understand SARS-CoV-2 evolution, we propose to create a tool to study viral evolution safely, by rescuing attenuated, hypermutating viruses that lack RNA proofreading activity** (Gribble et al. 2020 The coronavirus proofreading exoribonuclease mediates extensive viral recombination. PLOSPath. 92:e01531–46). Proofreading-deficient coronavirus mutants are attenuated and do not persist in nature. These viruses accumulate



nucleotide substitutions at an elevated rate and could reveal mutational hotspots in a controlled CL3 lab environment. Using reverse genetics, we will create recombinant SARS-CoV-2 viruses with point mutations in the Nsp10 and Nsp14 genes that disrupt the proofreading function. We will serially passage these recombinant viruses, alongside wild-type controls, to track the accumulation of mutations in their genomes in different selective conditions. **We hypothesize that proofreading-deficient viruses will reveal mutational hotspots and offer a convenient and low-risk way to study viral evolution in laboratory, where specific questions can be asked.**

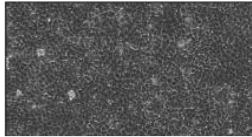
Towards these aims, we have made the following achievements:

Achievements:

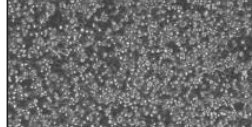
1. Our CL3 facility opened in April 2021. We established SOPs, cultured and titered SARS-CoV-2 (Toronto isolate), Alpha, Beta, Gamma, Delta and Omicron. We established several cell lines for growth of these viruses including ACE2-expressing HeLas, 293Ts and 293As, HUVECs, A-549s, VeroE6s, and Calu-3.
2. We obtained a bacterial artificial chromosome (BAC) that contains the entire ancestral SARS-CoV-2 sequence and reporter versions of this virus which express either fluorescent (Venus green or mCherry red) or bioluminescent (nano-luciferase) reporter genes (Ye C, Chiem K, Park JG, Oladunni F, Platt RN, 2nd, Anderson T, et al. Rescue of SARS-CoV-2 from a Single Bacterial Artificial Chromosome. *mBio*. 2020;11(5)).
3. The DNA for wild-type and reporter BACs was propagated in *E.coli* and then directly transfected into VeroE6 to create the initial virus passage (P0) eliminating difficult ligation and *in vitro* transcription steps required by other coronavirus reverse genetics systems. After transfection, we rescued these reporter SARS-CoV-2 infectious clones (mNeonGreen, nLuc, mCherry, Venus) (Figure 4).
4. We established numerous in-house and in-network collaborations of benefit to understanding SARS-CoV-2.



Untransfected

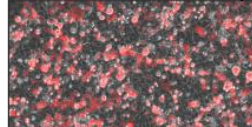


SARS-CoV-2



P1: 8×10^6 pfu/ml

SARS-CoV-2-mCherry



P1: 1×10^7 pfu/ml

Figure 4 Rescue of SARS-CoV-2 bacmid and SARS-CoV-2-mCherry reporter after bacmid transfection.

5. We successfully created several SARS-CoV-2 recombinant bacmids in *E. coli* for use in the above two aims. These were validated by sequencing and transfected into Vero E6 cells for rescue.
6. We successfully rescued, sequenced, passaged, and titered several recombinant viruses harbouring changes in the nucleocapsid gene. Growth curves and competition assays are in progress. These viruses will reveal whether the mutational hotspot in the N gene (R203K/G204R) provides a replication advantage because of the amino acid changes or because of TRS created within the gene that permits greater production of the N* truncated product that initiates from M210.
7. We have been unable to rescue any of the nsp14 or nsp10 mutant viruses that were successfully cloned (e.g., Nsp14D90A). This is disappointing but consistent with the results from one other study (Orgando et al. 2020 The enzymatic activity of the nsp14 exoribonuclease is critical for replication of MERS-CoV and SARS-CoV-2. JVI 94:e01246).

HQP Supported Year 1:

Dr. Danyel Evseev (0.5 FTE) Postdoctoral Fellow

Mr. Maxwell Bui-Marinos (0.25 FTE) Virology technician (help with CL3 SOP set up)

Year 2 (2022-2023) Progress Report:



In Year 2, our pillar proposed to study the genetic basis of VOC pathogenicity using open-ended approaches rather than the hypothesis-driven approaches of Year 1. Working with the Flamand lab, we will build new SARS-CoV-2 bacmids using sequences from different VOCs. Beta causes the most severe disease in animals while the Omicron causes relatively mild disease. These two viruses possess several signature mutations throughout the viral genome, yet the precise molecular basis for the different replication abilities of these VOCs is currently not known. Adopting the same strategy that was used to create the ancestral SARS-CoV-2 bacmid, our goal is to develop reverse genetic systems for Beta and Omicron. Using these recombinants as a starting point, we will determine the contribution of individual genes and mutations to viral replication and pathogenicity. Briefly, validated Beta/Omicron bacmids will be subjected to fragment/gene swaps. This research will also establish a platform for rapidly identifying key determinants of pathogenesis in new variants that are emerging or will emerge in the future.

Towards these aims, we have made the following achievements:

Achievements:

8. We have shared Beta and Omicron isolates with the Flamand lab to ensure we are working with the identical RNA sequences. While the Flamand lab will use yeast recombination to build the bacmid clones, our strategy will be to order gene blocks for Gibson assembly of large fragments into the pBeloBac backbone.
9. Dr. Danyel Evseev recently decided to leave my lab group in August 2022. Because he was the lead HQP for this CoVaRR-Network, our cloning progress has slowed. However, while searching for a new postdoctoral fellow to fill this role, Danyel is providing a current MSc student, Ms. Noga Sharlin, with guidance building the Beta and Omicron bacmids.

HQP Supported Year 2:

Dr. Danyel Evseev (0.5 FTE) Postdoctoral Fellow

Ms. Noga Sharlin (0.25 FTE) MSc student



Barr Laboratory

Use of a Clinically Relevant Hamster Model for Improved Detection of Pathogenicity

Preamble: In severe cases, COVID-19 is complicated by acute respiratory distress syndrome (ARDS), sepsis, endotheliitis, thromboinflammation (thrombosis associated with inflammation), and multi-organ failure, including the lungs, heart, liver and kidneys, often seen in patients with cardiovascular comorbidities such as hypertension. Notably, sepsis develops in all severe cases 1-2 days before ARDS and acute pulmonary embolus occurs in 30% of these patients, suggesting that sepsis and thromboinflammatory response are key contributors to the development of ARDS and multi-organ failure. To better simulate COVID-19 in humans with cardiovascular comorbidities, we are currently developing a novel clinically relevant model based on L-NAME-induced hypertension in Syrian hamsters to study the pathogenesis of key recombinant viruses developed in our Pillar or other Pillars for comparison to previous (e.g., Alpha, Beta, Gamma, and Delta) or currently relevant SARS-CoV-2 variants (e.g., Omicron).

Progress: We have received an approved animal use protocol to carry out development of our hypertensive hamster model for SARS-CoV-2 infection. We have purchased a Muromachi non-invasive blood pressure monitor with cuff-pulse and holders to allow us to restrain and detect changes in hamster blood pressure to indicate time of hypertension induction. Testing and adaptation of the monitor for the front limb of hamsters is complete, and we are successful in accurately monitoring blood pressure. We are currently planning with our vet team the first phase of hypertension induction in a small cohort of hamsters to optimize the dosing of L-NAME and monitoring of blood pressure and other clinical signs.

Pathogenesis in Syrian golden hamsters

Preamble: Hamsters will be used to study viruses showing altered pathogenesis in mice and to study virus competition *in vivo*. A high dose challenge model using 1×10^5 TCID₅₀ of the indicated virus delivered by the intranasal route of 5-7 week-old male hamsters will be used. Hamsters will be monitored daily for weight, and nasal washes will be collected every second day. Two groups will be euthanized for tissue collection on day 5 and day 10 post-infection for collection of nasal turbinate, lungs, and spleen for assessment of virus burden, cytokines, and pathology. Blood will be collected prior to challenge and necropsy to determine antibody levels. Weight loss, viral burdens, and pathology will be compared with the progenitor virus.

Progress: We have completed a test experiment on a small group of hamsters infected with Delta alone, Omicron alone, or equal amounts of SARS-CoV-2 Delta and



Omicron. Only hamsters infected with Delta (alone or in combination with Omicron) exhibited weight loss, albeit ~3%. Viral RNA collected from the lungs were sequenced and quantified. Analysis is currently in progress, but the sequencing data suggests that Delta almost completely outcompeted Omicron (BA.1). We are refining our sequencing and alignments to try and identify mutations in the viruses (if any) that emerged in either of the viruses for functional analyses. Plans are under way to assess competition between other variants and to correlate replication kinetics and fitness in vitro. These findings will provide key insight into the factors driving the dominance of certain variants and factors that may contribute to the emergence of one variant over another if people get infected with two variants at the same time.

Understanding the host interferon response towards SARS-CoV-2 infection

Preamble: Host survival during infection in vital organs, such as the lung, requires a delicate balance between host defense, which is essential for the detection and elimination of pathogens and disease tolerance, which is critical for minimizing collateral tissue damage rather than pathogen control. These defense/tolerance mechanisms are intertwined, and failure of one or the other may explain, at least in part, the wide range of clinical variability between individuals infected with SARS-CoV-2 ranging from asymptomatic to fatal infections. Studies of SARS-CoV-2 infected individuals have implicated a failure to launch a robust type I and type III interferon response while simultaneously inducing high levels of chemokines and pro-inflammatory cytokines in disease severity.

Progress: We and others have observed that exogenously administered Type I interferon inhibits SARS-CoV-2 replication in vitro. We have shown that the E3 ligase HERC5 is an essential mediator of this interferon response towards all SARS-CoV-2 variants tested. Interestingly, SARS-CoV-2 PLpro antagonizes HERC5 activity, and the level of this antagonism appears to differ among SARS-CoV-2 variants. We are currently investigating the molecular mechanisms by which HERC5 inhibits SARS-CoV-2 and PLpro antagonizes HERC5 and the amino acid determinants of PLpro antagonism. This study will help identify a critical SARS-CoV-2/host interface that could be exploited for small molecule inhibitor screening/development and to identify SARS-CoV-2 mutations that may confer better protection against the interferon response.



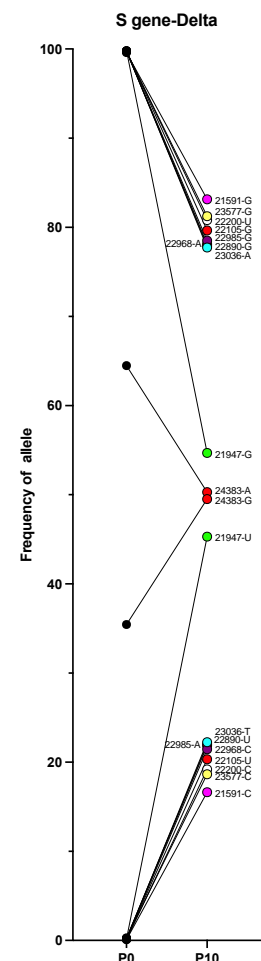
Flamand laboratory

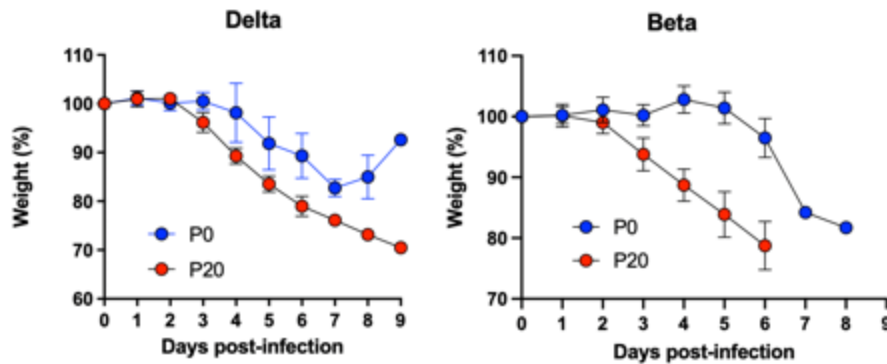
***In vivo* evolution and protection against VOCs.** Predicting the evolution and emergence of new VOC in humans is difficult. We proposed to study the evolution of the Beta and Delta VOC in mice by allowing this virus to evolve for a period of 60-80 days in mice (20 successive passages three to four days apart). Evolved Beta and Delta VOC (eVOC) will then be characterized for presence of mutations, virulence, neutralization (PRNT) and ability to cause infection/disease in immune animals.

Progress

We have passaged the Beta and Delta VOC 20 times in K18-ACE2 mice and sequenced the viruses present in lungs, in collaboration with Pillar 5: Viral Genomics & Sequencing. Results indicate the not all VOC mutate at similar rates, with Delta>Beta. An example of allele frequencies in the S gene of the Delta virus at passage 0 (P0) and at P10 is shown. Many of the mutations resulted in amino acid changes. Several mutations were present at very low frequencies in P0 (0-0,1%) but were selected by *in vivo* passaging. At P10, several mutations increased several folds and are detected at frequencies varying between 15-20% suggesting that these proved a growth advantage. Mice that developed immunity following Wuhan infection were protected from a challenge with a lethal dose of Delta and Beta P10 (not shown).

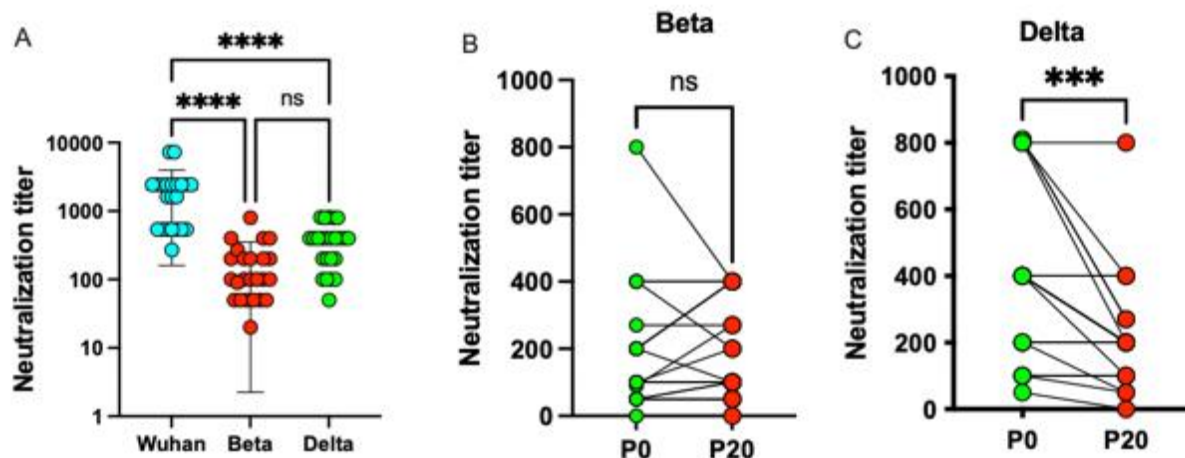
Based on these results, we decided to passage Delta P10 and Beta P10 an additional 10 times in three separate mice. RNA seq was performed at P13, P17 and P20 in various organs (lungs, heart, spleen, brain, kidney). There were 88 unique variants present in P13 across organs in Beta and Delta lineage virus, 100 in P17, and 93 in P20. Over all passages, there were 148 unique variants, 25 of which were present in Beta P0 virus and 27 being present in Delta P0 virus. Of these variants, there were 27 that changed in frequency across passages that had annotations with clinically-relevant traits, including mutations that may confer drug resistance, Omicron variants of concern in non-Omicron lineage virus, CD8 cell and antibody escape, mutations at vaccine targets, and at sites associated with recombination. These variants included C22674, or Spike S371F, that arose de-novo in P17 in Beta and Delta-infected animals. S371F is associated with antibody escape, affects a vaccine target, and is an Omicron variant of concern. As variants with clinically relevant traits arose and became fixed in the allele population, animals infected with P20 virus lost significantly more weight (left) than animals infected with P0 virus for both lineages with similar survival.





Pathogenesis of P0 and P20 viruses in K18-ACE2 mice. The weight of each mouse (n = 10/group) was monitored daily throughout the experiment and reported as mean percentage \pm SD of weight relative to day 0.

Results also suggest that sera from vaccinated donors neutralized Delta P20 less efficiently than Delta P0.



Neutralization of P0 and P20 viruses with sera from vaccinated subjects. (A) Neutralization assay of Wuhan-like, Beta, and Delta viruses using sera from 24 vaccinated subjects. Individual results and mean \pm SD neutralization titer against different SARS-CoV-2 isolates are presented. ****: $p < 0.0001$ determined using one way-ANOVA. ns: not statistically significant. (B) Pair-wise comparison of the sera used in A in neutralization assay against P0 and P20 Beta viruses. (C) Pair-wise comparison of the sera used in A in neutralization assay against P0 and P20 Delta viruses. ***: $p > 0.002$ determined using paired t-test.

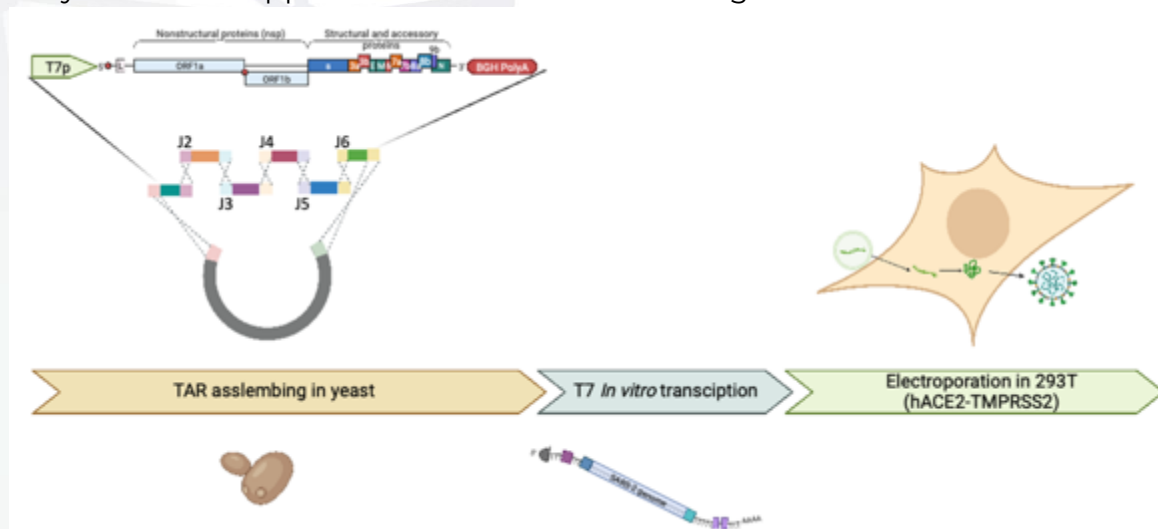


Understanding the pathogenesis of VOCs.

The emergence of VOCs that display variable pathogenicity in both humans and animal models provides a unique opportunity to investigate the genetic determinants of pathogenicity using recombinant virology. The Beta VOC (B.1.351) displays several hallmarks of increased pathogenicity in mouse and hamster models, with higher levels of pathology in the lungs, that take longer to resolve than with ancestral SARS-CoV-2 as well as the other VOCs. Also, the initial neutralizing antibody response appears to be delayed. In contrast, Omicron variants appear to be less pathogenic in hamster and mouse models (4, 5). The molecular basis for these differences in pathogenicity are currently not known. Our goal is to develop reverse genetic systems for Beta and Omicron VOCs. Using these recombinants as a starting point, we will determine the contribution of individual genes and mutations to viral replication, pathogenicity and immune evasion in cell culture and animal models (mice and hamsters). This research will also establish a platform for rapidly identifying key determinants of pathogenesis in new variants that are emerging or will emerge in the future (e.g., BA.4, BA.5, BA.2.12.1).

Two strategies are used. The Corcoran laboratory will attempt to clone Beta and Omicron (BA.5) genomes in bacterial artificial chromosome (BAC), whereas the Flamand laboratory will use a yeast-based approach, as described by Thou et al. (6).

The yeast-based approach is summarized in the figure below.

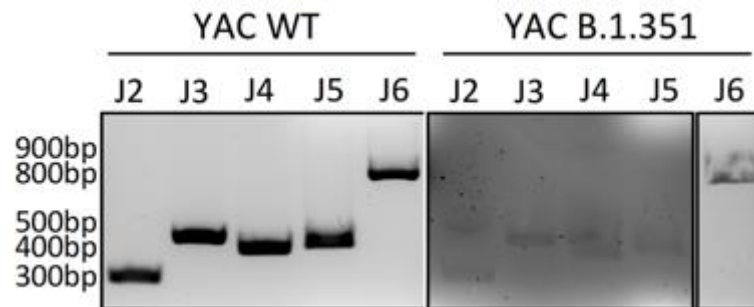


Using PCR, we successfully amplified the Wuhan and Beta B.1.351 viral genomes in 6 fragments with overlapping ends. These fragments were transformed, along with the BAC/YAC vector in yeast and following recombination, the viral genome was incorporated in a BAC/YAC vector. Using PCR primers spanning the junctions (J2-J6), we screened colonies and identified several clones containing the entire viral genome



(see figure on the left). We have performed T7 RNA transcription and electroporation of ACE2-293T to rescue the Wuhan virus. The virus was successfully rescued with high titers and sequence to confirm genome integrity. We will proceed with

the cloning of the Omicron BA.5 genome using a similar methodology. Once Bata and Omocron BA.5 viruses have been cloned and validated, we will proceed with the generation of hybrid viruses by swapping genomic fragments between viruses. Hybrid viruses will be studied for altered virulence to identify genomic region associated with pathogenic traits.



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Vidal laboratory

Host-pathogen interactions in experimental mouse models of VOC infection

1. Investigating mechanisms of resistance or disease tolerance to SARS-CoV-2 VOC infection

The precise molecular and cell mechanisms accounting for the wide range of clinical variability between individuals infected with SARS-CoV-2 remain largely unknown. Mouse models are essential for modeling the complex *in vivo* host-pathogen interactions that underlie SARS-CoV-2 pathogenesis. Moreover, mouse models can be developed that vary early immune responses and have divergent infection outcomes. We characterized a new human angiotensin converting enzyme 2 (hACE2) knock-in (KI) model generated in the CD-1 strain, hACE2KI mice. Following intranasal inoculation with SARS-CoV-2, the course of infection in CD-1 hACE2 KI mice was compared to the keratin 18 (K18)-hACE2 transgenic model in the C57BL/6J strain. CD-1 hACE2 KI mice showed no clinical signs despite substantial early virus replication and cytokine production in the lung in contrast to K18-hACE2 mice that showed severe clinical signs and high lethality. RNA sequencing of infected lung tissues demonstrated divergent early host response signatures (day 2). To control for strain-specific differences, transcriptomic analysis compared first the profiles of infected versus non-infected mice for each mouse strain. Gene ontology analysis of differentially expressed genes (CPM > 5; adjusted p-value < 0.05) between resistant and susceptible mouse strains identified shared transcriptional signatures of pathways involved in pathogen recognition and type I interferon responses. For further detail, gene set enrichment analysis identified numerous gene sets of which the 25 most significant (Fig. a, below) were used for leading-edge analysis. Despite these gene sets being common, many had higher normalized enrichment score (NES) in the susceptible mice (Fig. a) and leading-edge analysis on those identified, again shared and unique leading gene signatures (Fig. b). This analysis indicated that interferon stimulated genes (ISG) *Irf7*, *Trim25*, *Stat1* and *Isg15* are at the root of a SARS-CoV-2 response elicited in both strains (Fig. c). By contrast, pro-inflammatory interferon stimulated genes, including *Cxcl10*, *Ptpn6* and *Nfkb2* were found driving disease susceptibility in C57BL/6 K18-hACE2 (Fig. d). By contrast, a signal for activation of cell cycle genes (*Ccna2*, *Mcm4*) and GPCR signaling, which are associated with tissue regeneration in mucosal tissues, together with downregulation of clotting pathways were associated with survival in the resistant CD-1 KI hACE2 mice. Overall, the results revealed the combined effect of virus load, hACE2 expression, and mouse genetic backgrounds in the outcome of SARS-CoV-2 infection highlighting distinct



patterns of the innate response in a new mouse model of asymptomatic SARS-CoV-2 infection.

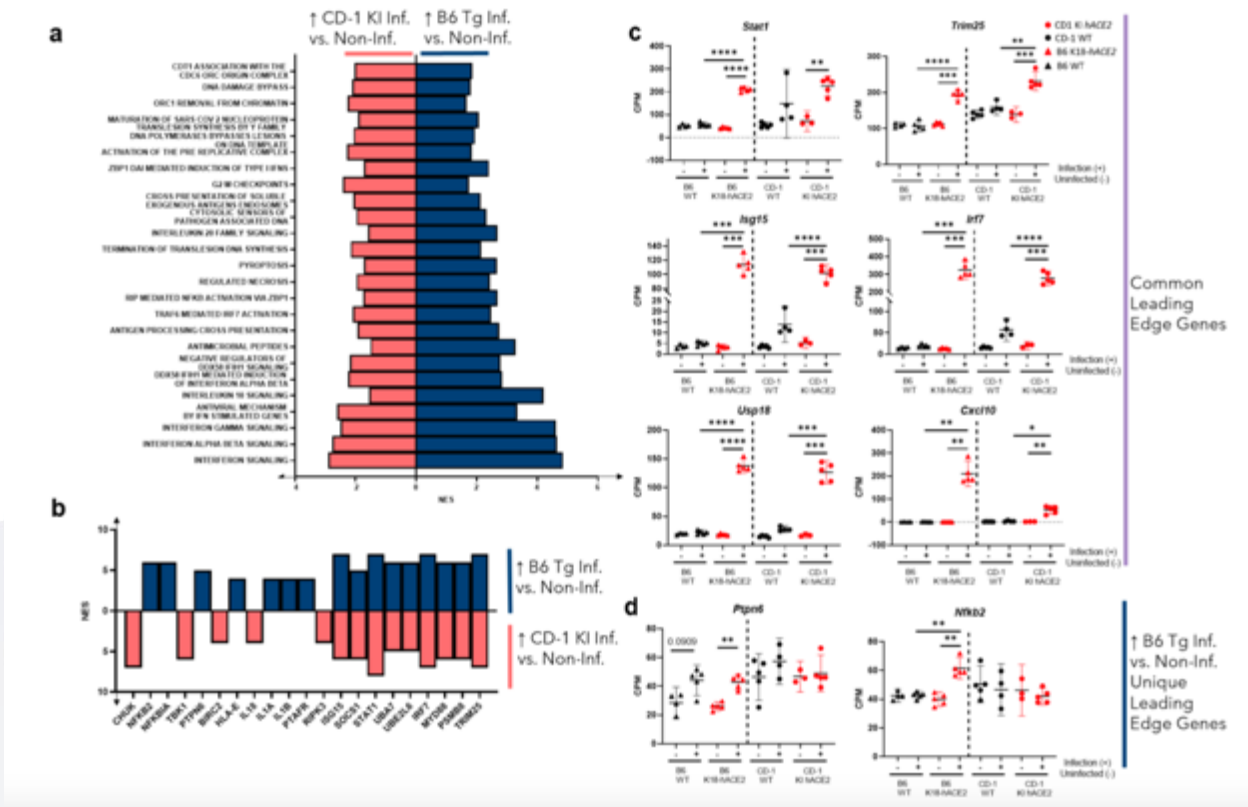


Figure legend. Shared and unique type I interferon gene response in C57BL/6 K18-hACE2 (susceptible) and CD1 KIACE2 (resistant) mice. Gene set enrichment analysis using GSEA_4.2.3 software from Broad Institute using the C2.CP.Reactome.v7.5.1 gene sets and all gene's CPM matrix. Common gene sets having an FDR < 0.25 and NES > 1.5 were ranked according to highest average (of both CD-1 KI and B6 Tg inf.) NES from which the top 25 were selected. **(a)** NES for gene sets upregulated in CD-1 KI inf. vs. non-inf. are shown in orange and upregulated gene sets in B6 Tg inf. vs. non-inf. are shown in dark blue. **(b)** Leading edge analysis was done for the genes appearing in the most sets for both CD-1 KI inf. and B6 Tg inf. mice, considering a threshold of either 4 or a difference between CD-1 KI inf. and B6 Tg inf. of 3. Y-axis displays number of gene sets each gene is found in. **c-d**, Notable CPM of leading edge genes are displayed for **(c)** common and distinct leading edge genes in shared datasets **(d)** unique up in B6 Tg inf. genes. Data are presented as mean +/- 95% CI, n=5 per group (except n=4 for B6 WT non-inf., n=4 for D-1 WT inf., and n=3 for CD-1 KI non-inf.). p* < 0.05, p** < 0.01, p*** < 0.001, p**** < 0.0001 using Brown-Forsythe's one-way ANOVA test with Dunnett's T3 multiple comparison test.



2. Investigating host response to PapMV-N vaccination in agammaglobulinemic mice

Bruton's Tyrosine (BTK) is a critical regulator of B cell development and a target for intervention in various B cell malignancies, including Chronic Lymphocytic Leukemia (CLL), which is the most frequent lymphoproliferative disease in the elderly of the Western world. Patients with CLL experience suboptimal vaccine efficacy, especially those receiving BTK-directed therapy. The antibody response rates are lower during active treatment and wane faster following vaccination. BTK-deficient mice present a similar deficit in humoral immunity. In this aim we will examine the level of protection afforded by PapMV-N vaccination using BTK-deficient C57BL/6 K18-hACE2 (and wild-type littermates). The cross is ongoing. **The vaccine has already demonstrated protection against ancestral SARS-CoV-2 (PMID:35850421) and should be tested for protection against Omicron variants in early 2023.**

3. Markers of Emerging SARS-CoV-2 Variants in Patients

Serial sequencing of the SARS-CoV-2 genome in immunosuppressed patients that have demonstrated chronic infection in these hosts allow for a rapid emergence of new variants that can escape anti-viral treatment or vaccination. For instance, all available evidence indicates that the Omicron variant emerged in an immunocompromised patient. To gain insights into the mutational signatures of secondary immunodeficiency (HIV-infection, antineoplastic drugs, immunosuppressive treatment) in SARS-CoV-2 genetic profiles, we queried the literature to review SARS-CoV-2 genome data from 44 patients with secondary immunodeficiency who underwent treatment against COVID-19. We retrieved 148 full genomes from 21 patients and partial genomes for 24 patients. By analyzing the viral genomes detected in these patients in comparison with circulating variants, we identified numerous new protein-coding mutations and inspected their predicted structural or functional impact at the protein level. We compared the frequency of the mutations in the patient population against the GSAID database, and only significant hits were analyzed. As expected, we identified several mutations in the Spike protein and the viral RNA-dependent RNA polymerase (RdRp) likely selected by the antiviral treatments. Surprisingly, among all the tracked mutations in the SARS-CoV-2 genome in the studied immunocompromised patients, recurrent mutations were found at a unique position in the Envelope (E) viroporin. In fact, out of 44 examined patients, 9 of them had the amino acid substitution T to I at position 30 (T30I). In addition to functioning as ion channel, the E protein plays critical roles during infection. Pre-entry, E binds to TLR2 in the cell surface and activation of NFkB-dependent pathways (PMID: 348666574). Post-entry, E blunts interferon signaling through binding to epigenetic reader proteins (BET) (PMID: 35839775). Plans include exploring the functional impact of the E T30I substitution (as well as several recurrent mutations identified in the shut-



off protein, NSP1, which principally abrogates interferon expression) during infection using pseudotyped viruses and site-directed mutagenesis experimental infections in cells and mice.





Leclerc laboratory

The Quest for a SARS-CoV-2 Vaccine Triggering a Broad Protection

The problem: After more than 3 years of the SARS-CoV-2 pandemic, the global population has heterogeneous immune histories due to various exposures, including vaccinations and infections by different variants. In Canada, mRNA vaccines were rapidly preferred over other types of SARS-CoV-2 vaccines and are still the main vaccines proposed to the population. Canadians have been offered more than 5 doses of vaccines in the last 2 years to maintain high levels of neutralizing antibodies directed towards the spike (S) protein. The first 4 doses used the S protein of the SARS-CoV-2 Ancestral strain as the antigen. However, recent studies suggest that the immune responses directed towards the specific regions of the BA.4/BA.5 S protein were deceiving compared to the immune response directed towards the common regions shared by the Ancestral and BA.4/BA.5 variants. These results suggest that the multiple priming of the population with the same vaccine containing the Ancestral S protein induced immune imprinting. Immune imprinting or 'original antigenic sin' refers to the immune system's propensity to repeatedly reuse the first cohort of B cells towards an antigen rather than recruiting new clones from the naïve repertoire. In November 2022, the CDC published data on the effectiveness of the BA.4 and BA.5 mRNA vaccines and reported that the extra protection associated with the bivalent booster is less than 30%.

Our solution is to broaden the immune response against SARS-CoV-2 and overcome immune imprinting against the S protein by developing vaccines based on the SARS-CoV-2 nucleocapsid (N) as the main vaccine antigen. We propose to use a nanoparticle-based vaccine platform that enhances the B and T cell responses to protein antigens attached to its surface, thereby maximizing the efficacy of this approach.

Status of the Year 1-funded research project (May 2021-May 2022)

a) Immunogenicity of the vaccine

We have already produced the vaccine candidate that is made of the papaya mosaic virus (PapMV) nanoparticles coupled to the N protein (Fig 1). The manufacturing of the vaccine is completed and the vaccine has already been used to perform several assays to confirm its immunogenicity in a mouse animal model (Fig.2). The results confirm that the vaccine is immunogenic and trigger the production of IgG2a and of CTLs directed to the SARS-CoV-2 N protein. The PapMV nanoparticle was compared with the adjuvants CpG and alum to confirm that the PapMV is a TH1 adjuvant (low IgG1 titers, high IgG2a titers), closer to CpG than alum that is a TH2 (high IgG1 titers, low IgG2a) adjuvant. Overall, the antibody response of the PapMV-N and the CpG-N show



a TH1 profile (IgG2c/IgG1 ratio > 1) and the alum shows a TH2 profile (IgG2c/IgG1 ratio < 1).

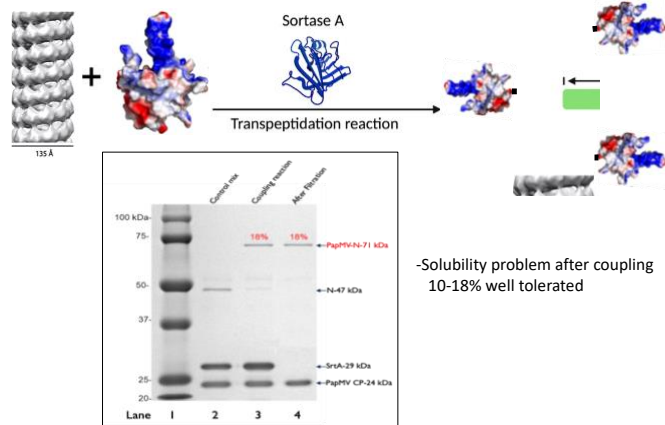


Figure 1. Coupling of the SARS-CoV-2 N protein (red-blue) to the surface of the PapMV nan using the sortase A (SrtA) transpeptidase.

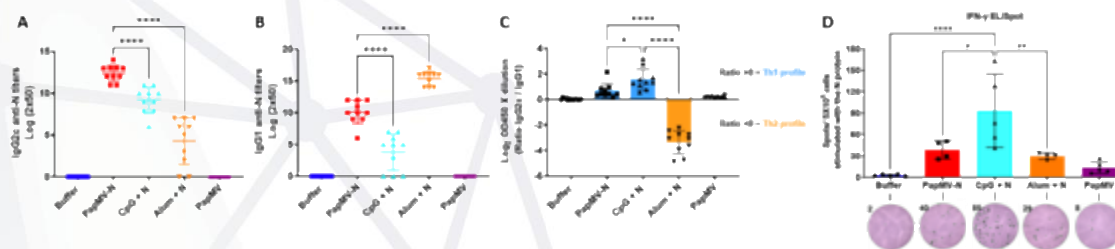


Figure 2. Immunogenicity of the different vaccine formulations after 2 immunizations i.m. at 21 days interval. **A-B:** IgG2c or IgG1 antibody titers against the N protein after two doses assessed by ELISA, **C:** Ratio of IgG2c/IgG1 antibody levels to assess the type of response (Th1 or Th2) induced by the immunizations, **D:** ELISpot measuring the frequency of IFN- γ -secreting N protein-specific T cells in the spleen of immunized mice. PapMV-N, PapMV alone, N protein alone, CpG+N, Alum+N and buffer (neg. control). * $P < 0,05$, ** $P < 0,01$, *** $P < 0,001$ and **** $P < 0.0001$.

b) Challenge of the vaccinated mice

The K12 mouse animal model where the animals express the human ACE2 receptor was used to perform the challenge with the SARS-CoV-2 ancestral strain. In brief, K12 mice were immunized i.m. twice at 21 days interval with either the buffer control or the 3 different vaccine formulations described earlier (Fig. 3).

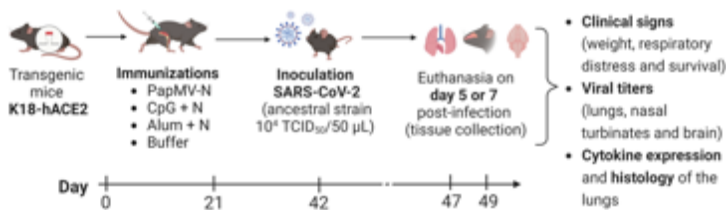


Figure 3. Schematic representation of the challenge stud

In brief, we have not seen significant differences in the viral load between the different groups in the lungs, in the nasal turbinates and in the brain (Fig. 4). However, it seems that the group receiving the N+alum vaccine tends to show higher virus titers in the brain. It appear obvious that the number of animal per group was too low to draw any clear conclusions at this point concerning the effect of the vaccine on the viral load.

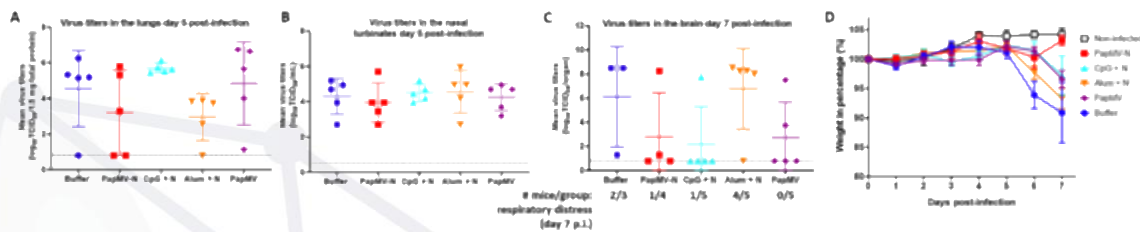


Figure 4. Challenge of vaccinated K12 mice with the ancestral variant. A-C: Viral titers in the lungs, nasal turbinates, and brain of challenged mice determined by end-point titration assay, D: Post-infection mice weight change measurement.

However, animals vaccinated with the PapMV-N and the Alum+N vaccines have showed a significant reduction in IL-6 secretion in the lung of the animals during the infection (Fig. 5A). This result was also supported by the histology score that revealed a significant decrease in the inflammation in the lungs of animals vaccinated with the PapMV-N and the alum+N vaccines. This result suggest that the PapMV-N and alum+N vaccines have contributed to significantly decrease the symptoms and the tissue damage caused by the viral infection.

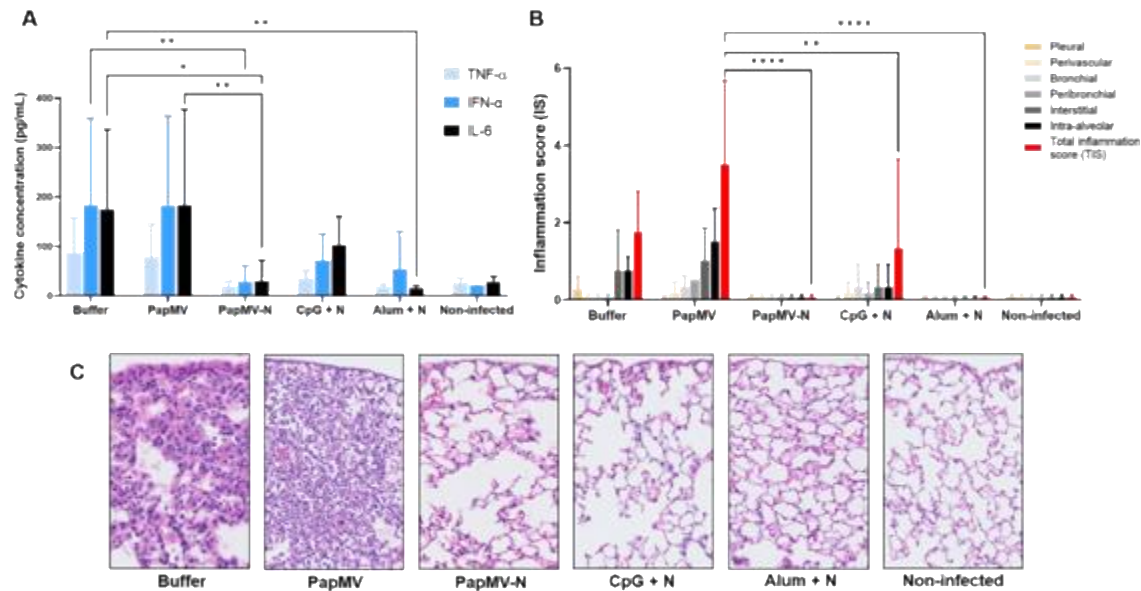


Figure 5. Measure of the inflammation in the lung of vaccinated animals. A: Cytokine concentrations in the lungs 5 days post-infection measured by MSD U-Plex, B: Semi-quantitative evaluation of inflammation in histological sections of the lungs 7 days post-infection by a pathologist, C: Histological sections of the lungs of challenged mice 7 days post-infection. * $P < 0,05$, ** $P < 0,01$, *** $P < 0,001$ and **** $P < 0.0001$.

These results are very encouraging and demonstrate that the vaccine is effective in controlling the level of inflammation and the tissue damage caused by the disease in the lung of the animals.

The Year 2-funded research project (May 2022-May 2023)

We will complete this study and perform several challenges on K12 animals using the PapMV-N and Alum+N vaccines to confirm two major points. For all the challenge protocols, we will use 10 animals per group to increase statistical power.

The first challenge experiment will clearly demonstrate that a vaccine triggering a TH2 response (Alum+N) significantly increases virus titers in the brain of animals compared to a TH1 vaccine (PapMV-N). We expect to show that the PapMV-N formulation is more effective and safer, with reduced virus titers in the lungs of animals 5 days post-infection. A similar protocol will be initiated to monitor weight loss in animals vaccinated with the PapMV-N vaccine compared to those vaccinated with buffer and the Alum+N vaccine over a period of 9 days after the challenge. We expect the PapMV-N vaccine to be more effective and prevent weight loss compared to the other two groups.



Second, we will perform a final challenge on PapMV-N vaccinated mice with the Omicron variant, using buffer-vaccinated animals as a control to confirm that the observed protection is effective and broad against any SARS CoV-2 variants. In this experiment, animals will be sacrificed on day 5, and virus titers will be assessed in the lungs, nasal turbinate, and lungs as previously presented.

In the first and second experiments, we will assess the level of inflammation by measuring the IL-6 and IFN- γ in the lungs of animals on day 5 post-challenge. We will also perform histology to assess tissue damage and the accumulation of immune cells (mostly neutrophils) in the infected lungs. The PapMV-N vaccine is expected to prevent inflammation in the lungs on day 5 after infection.

As previously stated, vaccination will be performed using the same vaccine preparations described earlier. These last experiments will complete the first chapter of this PapMV-N vaccine and its usefulness in preventing the development of SARS-CoV-2-induced severe disease.

Finally, we will provide the PapMV-N vaccine to the groups of Dr. Vidal and Dr. Falzarano to complete their investigation with the candidate vaccine.

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Falzarano laboratory

Heterologous challenge/re-challenge in hamsters

We demonstrated that previous infection with two divergent VOCs, Alpha and Beta, provided substantial protection against the other when re-challenge occurred at either one or three months. This contrasted with our initial hypothesis that re-challenge at one month would not be protective or would be less protective than challenge at three months due to a lack of antibody maturation at the earlier timepoint. Although infectious virus was still detected in nasal washes and turbinate tissues after re-challenge, indicating incomplete protection in the upper respiratory tract, both titers and duration of virus were reduced. Importantly, hamsters were protected from body weight loss (Fig.1 A, D) and lungs remained protected from the presence of virus (Fig.1 B, E), inflammation and pathology. Following the initial challenge, the neutralizing antibody response was biased towards the challenge virus (Fig.1 C, F). However, following re-challenge with a heterologous virus, neutralizing titers were boosted and were nearly comparable against all variants tested (Fig.1 C, F). Only titers against Omicron remained lower than the other variants (Fig.1 C, F). Titers were boosted to similar magnitudes in animals re-challenged at either one or three months and were sustained at sufficiently high enough levels to provide protection against re-challenge.

Given similar protection at both rechallenge timepoints and technical difficulties with the analysis of sequencing hamster antibodies due to a lack of pre-existing data we decided to pursue characterization of antibody binding and ACE2 blocking using the MesoScale Devices multiplex platform. This was used to assess whether the Alpha and Beta variants could induce cross-reactive IgG antibodies against full-length Spike and RBD derived from the Alpha and Beta variants, as well as against the ancestral, Delta and Omicron viruses. The initial challenge led to high IgG levels against both Spike (Fig. 1G, H) and RBD by day 20 post-challenge, regardless of the challenge virus, with the highest values being observed against wild-type, Delta and Omicron BA.2. Re-challenge of these animals at 21 days led to a small boost in titers against all variants by day 42 (Fig. 1G, H). In the 3-month cohorts, animals that were initially challenged with Alpha and then re-challenged with Beta received a small boost from the re-challenge. In contrast, in the animals that were initially challenged with Beta and then re-challenged with Alpha, IgG titers dropped slightly after re-challenge (Fig. 1G, H).

We also measured neutralizing responses before and after re-challenge using an ACE2 competitive ELISA assay. Both initial Alpha and Beta infections induced ACE2 competing antibodies against all viruses except Omicron BA.1 and BA.2. Neutralizing antibodies were observed by D20 against all viruses, including Omicron, although neutralization against Omicron was substantially reduced. Bias in the response towards the challenge virus was noted with Alpha challenge leading to higher ACE2 displacement and neutralizing responses against ancestral and Alpha, while Beta



challenge was biased toward Beta, with reduced responses to ancestral, Alpha and Delta. Heterologous re-challenge at 21 days led to a boost in ACE2 competing antibodies with Beta challenge boosted levels against Delta and BA.1 and BA.2. Similarly, Alpha challenge boosted ACE2 competing antibodies with ancestral, Alpha, Delta and BA.1 and BA.2. Following re-challenge levels were still lowest against BA.1 and BA.2 in both groups. Neutralizing titers remained highest against the original challenge virus following the initial infection (Fig. 1 C, F), but were observed against all the VOCs by D42, although Omicron titers remained lower than the other variants. At D76, animals from the Alpha challenged group had lower ACE2 competition than at D20 but retained their neutralizing titers (Fig. 1C); then after re-challenge with the Beta variant, both ACE2 competition and neutralizing titers were boosted. In contrast, animals from the Beta challenged group retained similar levels of both ACE2 competition (Fig. 1F) and neutralizing titers at D76. After re-challenge with the Alpha variant, neutralizing titers were boosted (Figure 1F) but ACE2 competition decreased (Fig. 1H) for all VOCs.

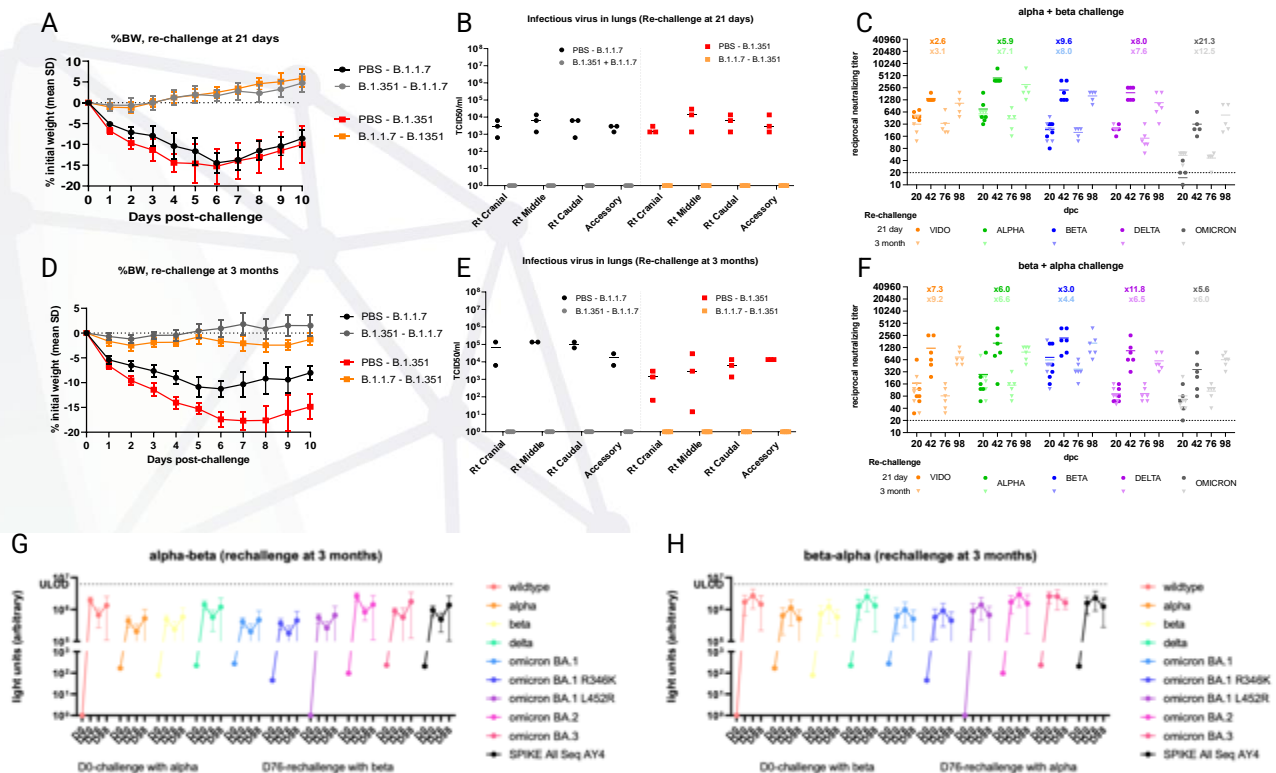


Figure 1. Challenge/rechallenge with heterologous SARS-CoV-2 variants of concern (VOC) Alpha and Beta in hamsters at 21 days or 3 months. (A) Weight loss following heterologous rechallenge at 21 days, (B) Virus titers in lungs following heterologous rechallenge at 21 days, (C) Microneutralization titers against the indicated VOC following heterologous rechallenge at 21 days, (D) Weight loss



following heterologous rechallenge at 3 months, (E) Virus titers in lungs following heterologous rechallenge at 3 months, (F) Microneutralization titers against the indicated VOC following heterologous rechallenge at 3 months, (G, H) Spike binding domain antibodies following the indicated heterologous rechallenge at 3 months.

Overall, this data demonstrates that immunity from a prior infection provides cross-neutralization against other SARS-CoV-2 VOCs, which marginally improves at the three-month mark in hamsters and can be boosted by a heterologous re-challenge. However, neutralizing titers against Omicron remained lower than against other variants even after re-infection.

Further characterization of the affinity of antibody binding to VOC RBDs using biolayer interferometry is ongoing (Octet BLI, Sartorius), but has been continuously delayed by difficulties obtaining both reagents and consumables for this instrument.

Improving the protection induced by the VIDO COVAC-2 vaccine with PapMV-N (Leclerc, Falzarano).

Hamster models of Alpha, Beta, Delta, Omicron BA.1 have been established and used in numerous vaccine studies outside of CoVaRR-Net activities. Omicron BA.2, BA.4, and BA.5 are available, but hamster challenge models have not yet been developed. Newer Omicron lineages are being sought from provincial public health labs for virus isolation for neutralization assay and hamster model development and for dissemination to other labs across Canada.

Separately (outside of CoVaRR-Net), we have broadened protection of COVAC-2 by generating a fusion protein that contains the original S1 antigen with a heterologous C-terminal receptor binding domain. These vaccines provide protection against VOCs, including Beta and BA.1, where S1 alone vaccines were sub-optimal against Beta and Omicron challenges.

Hamster studies in collaboration with Denis Leclerc are planned once proof-of-concept is demonstrated in mouse immunization experiments, data from which is expected in December 2022.

Outside of CoVaRR-Net, we have been assessing whether VOCs have similar or differential susceptibility to interferon (IFN)-Lambda (I). Currently, we have data that indicates that all VOC have at least a 10-fold increase in EC_{50} values to IFN-I. This had led to a cross-pillar collaboration with (the antiviral pillar), to pursue mechanisms. Interestingly, we have found that current antiviral agents against SARS-CoV-2 frequently have synergistic activity in combination with IFN-I, which given its ease of administration as a single subcutaneous injection is encouraging.



HQP and technicians presented 4 posters at the June 2022 Canadian Society for Virology meeting that were related to COVID-19 projects, most of which are at least in part associated with CoVaRR-Net.





CoVaRR-Net Pillar 4 – Functional Genomics and Structure Function of VOCs

Years 1 and 2 Progress Report

5 May 2023

Lead: Anne-Claude Gingras (ACG)

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Caron lab: David Hamelin, Loïze Maréchal

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Deputy: James Rini (JR)

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Prepared by: Laurie Seifried, Anne-Claude Gingras, Samuel King, Nozomu Yachie,
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ABBREVIATIONS

BLI	Bio-Layer Interferometry
HLA	Human Leukocyte Antigen
MS	Mass Spectrometry
Mut	Mutant
NSP	Non-Structural Protein
RBD	Receptor Binding Domain
SA-APC	Streptavidin-Allophycocyanin
SA-PE	Streptavidin-Phycoerythrin



SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2

VLP Viral-Like Particle

VOCs Variants of Concern

VOIs Variants of Interest

WT Wild Type (also, Ancestral)





PILLAR 4 VISION STATEMENT

[Pillar 4](#) will contribute to a better understanding of the structure-function of pathogen-associated proteins by using genetic engineering strategies and proteomics approaches to develop technologies for the study, monitoring and mitigation of pandemic-level threats.

EXECUTIVE SUMMARY

[Pillar 4](#) has successfully fulfilled its mandate by 1) creating and disseminating SARS-CoV-2 variants reagents, and 2) utilizing reagents in well-validated assays. We have created 26 SARS-CoV-2 spike variants, with three more in development. These spike variants, supported by CoVaRR-Net, are used to create lentiviral-based virus-like particles with variant spike proteins on their surfaces. This enables testing of antibody neutralization potential generated by vaccinations and infections.

Additionally, we have generated synthetic spike protein variants and receptor-binding domains in both membrane-bound and soluble forms. Soluble protein has been distributed to collaborators who use it to measure antibodies present in human biofluids, such as serum and saliva.

We have made significant progress in developing cutting-edge technologies for high- and medium-throughput neutralization assays, which measure antibody-mediated protection from both emerging and potential SARS-CoV-2 variants. Our goal is to create a technology that can simultaneously assess multiple variant-spike encoded virus-like particles for their ability to enter host cells. This will help identify variants and associated mutations that facilitate viral entry. By integrating this approach with our lentiviral neutralization assay, we will establish parallel neutralization assays capable of measuring multiple variants at once, effectively overcoming the current labour bottleneck of manual, single-variant assays.

We have introduced a second aim in year 2 that seeks to use proteomics-based techniques to understand how variant mutations affect cellular functions. We have begun to investigate the impact of SARS-CoV-2 mutations on the presentation and T-cell recognition of epitopes, identified through an immunopeptidomics approach. We have recently identified new immunogenic SARS-CoV-2 epitopes. We anticipate that variant mutations will disrupt recognition of these epitopes by T-cells.

A second proteomics project involves studying whether variant-associated mutations will affect the localization, protein interactions and function of non-spike proteins. We have begun to look at NSP3, NSP4, and NSP6 that are implicated in the formation of replication organelles. We created mutant and wild-type versions of these proteins that are tagged 1) to label proximally associated proteins and 2) to visualize their subcellular localization. With our tagged proximal proteins, we have observed colocalization with NSP6 and disruption of NSP6 localization. This work is being expanded to examine how variants affect these and other potential interactors.



Finally, we aim to characterize spike protein interactions using cell binding assays with human cells. We have generated large amounts of properly folded/assembled spike and RBD variants for our collaborators' studies. These studies have guided modifications and quality control efforts to ensure consistent results and in doing so enabled characterization of spike and RBD variants. By expanding our work on examining RBD interactions from endemic coronaviruses with human cells, we will observe which animal spike proteins readily interact with human cells.

PILLAR 4 MANDATE FOR YEARS 1 & 2

1: CREATE AND DISSEMINATE SARS-COV-2 VARIANTS REAGENTS

Construction of mammalian spike expression constructs for use in lentiviral assays: 26 variants fully validated, with 3 under construction (Gingras lab); distribution through <https://nbcc.lunenfeld.ca/resources/> (14 labs so far, including Gingras lab) is detailed in Table 1. Between May 2021 and May 2023, approximately 146 aliquots of plasmid for spike variants were distributed to collaborators. Since January 2022, 21 publications and 4 pre-print manuscripts have been enabled by these reagents.

The Rini lab has created four Omicron spike constructs for soluble and membrane-tethered recombinant protein expression (stable cell lines), and small-scale purification has been established. Protein preparations of Gamma, Omicron, and Delta/Delta+ receptor binding domains (RBDs) are currently being produced for collaborators.

2: USE REAGENTS IN WELL-VALIDATED ASSAYS

Pseudovirus neutralization assays with spike wild-type (WT), Alpha, Beta, Gamma, Delta, Omicron- and Omicron subvariants BA.2, BA.5, BQ.1.1, XBB.1 and XBB.1.5 have been used in ongoing and completed studies (**Tables 2, 3, and 4**). Spike variants Omicron BA.2.17.2 and BQ.1 are being tested for quality/virus titre before use with collaborator samples in ongoing viral-like particle (VLP) assays (**Table 3**). Collaborators have requested additional Omicron variants that are currently under construction (**Table 1**).

PILLAR 4 RESEARCH PROGRAMS INITIATED IN YEARS 1 & 2

PROGRAM 1: DEVELOPMENT INITIATIVES TO INCREASE THE THROUGHPUT OF VIRAL ENTRY AND NEUTRALIZATION ASSAYS.

In year 1, our research program aimed to replace current laborious spike-viral like particle neutralization and entry assays with higher throughput assays that maintain the same level of information and quality. This would enable larger scale investigation of multiple variants simultaneously and provide a generalizable strategy for pandemic preparedness. Two strategies were initiated in parallel for proactively mapping SARS-CoV-2 variants using tagged synthetic spike protein variants: a medium throughput



approach and a high throughput approach. This work was further developed in year 2 and is elaborated on below.

MEDIUM THROUGHPUT APPROACH

This approach involves individually tagging clones for each spike sequence, generating lentiviral particles, and pooling them for use in neutralization assays. Results of multiple neutralization assays can then be interpreted simultaneously. Once functional, this approach is expected to replace current manual assays for ongoing projects, best suited for profiling fewer than 100 variants simultaneously.

In a pilot experiment, lentiviruses have been pseudotyped with six variants of concern (VOCs), and each of these has been tagged with three unique tags. These tagged VOCs were pooled, and reference cell lines were infected. VOCs have been quantified, and data is currently being analyzed by [Pillar 4](#) labs. Once the proof-of-concept experiment analysis is completed, we will test the system using plasma/serum collected by Pillar 1 and larger pools of variants before expanding for use in ongoing projects.

HIGH THROUGHPUT APPROACH

GHOST: A genetic engineering method for highly scalable production of pseudotyped viruses

Advances in synthetic virology have enabled researchers to decorate virus surfaces with proteins of interest through viral pseudotyping. Viral pseudotyping of laboratory-regulated viral strains is helpful in medical therapies and evolutionary studies of viral variants since they are safe and easy to generate. However, creating and analyzing synthetic variants at a large scale remains difficult, as each variant must be produced individually (see above).

We are establishing a scalable viral pseudotyping technology to produce and screen synthetic viral variant libraries in single pooled pseudotyping and infection steps (Yachie lab). The pseudotyped viruses can be harvested and used in large-scale assays of choice, such as serum neutralization or drug screening. We have designed the technology to be compatible with common methods such as microscopy, flow cytometry, and sequencing. We envision that the technology will create new possibilities in synthetic virology, including enhanced pandemic preparation procedures, drug testing, and therapeutic development.

Applications of the technology include:

- ❖ **Big data for predicting viral evolution:** While computational models are rapidly advancing, an experimental testbed such as our platform is required to determine whether predicted sequences are biologically viable and to provide the big data needed to train powerful predictive AI tools.



- ❖ **Preventative pandemic measures and vaccine design:** Large-scale screening of observed and unobserved viral variants through our technology could allow for pre-emptive vaccine design in the early stages of a pandemic.
- ❖ **Large-scale drug screening and therapeutic development:** Our platform is highly modular in the viral and host cell proteins that can be tested. During the screening process, the synthetic viruses can be treated with drugs/compounds or patient sera, which is useful for therapeutic development and understanding of patients' responses to treatment.
- ❖ **Direct generation of targetable viral vectors:** The successfully infectious variants produced from screening assays using our technology can be directly implemented for genome engineering or delivery of genetic agents to specific cell types tested.

PROGRAM 2: USING PROTEOMICS APPROACHES TO UNDERSTAND EFFECTS OF VARIANTS ON SARS-COV-2 INDUCED CELLULAR CHANGES

Understanding CD8+ T cell escape using advanced immunopeptidomics to guide vaccine design. Based on recent work (Caron lab)¹, our goal is to determine the impact of SARS-CoV-2 mutations on the processing, presentation and T-cell recognition of new SARS-CoV-2 epitopes that can only be identified using advanced mass spectrometry (MS)-based immunopeptidomics approaches. We are using SARS-CoV-2-infected HLA mono-allelic cell lines and cells expressing different Spike variants. HLA peptide complexes are isolated, and peptides are identified¹⁻⁴ and quantified^{5,6} using in-house immunopeptidomics techniques. Recently, we have discovered new immunogenic SARS-CoV-2 T-cell epitopes and anticipate that mutations found in Omicron sub-lineages will interfere with the processing, presentation, and recognition of those novel T cell epitopes. This work is being performed with [Pillar 1: Immunology & Vaccine Protection](#) and [Pillar 6: Computational Biology and Modelling](#). The Caron lab has hired a research associate who has initiated work on identifying SARS-CoV-2 HLA-associated peptides by MS-based immunopeptidomics. This work will guide T-cell epitope vaccine design.

Understanding the effect of variant-associated mutations on non-spike protein-protein networks using proximity labelling and fluorescence-tagging. Several mutations in non-spike proteins associated with SARS-CoV-2 variants have been recurrently identified in variants of concern, suggesting a functional advantage and/or severe outcomes following infection^{7,8}. We previously defined the proximal interactome of each ancestral SARS-CoV-2 protein in model cell lines, revealing multiple host-viral protein associations⁹⁻¹¹. We have generated proteins with variant-specific mutations and compared their proximal proteomes to the ancestral strain. Subcellular localization and protein-protein networks are being explored using microscopy and biochemistry techniques.

We have focused on SARS-CoV-2 NSP3, NSP4 and NSP6 proteins that are implicated in the formation of replication organelles¹². NSP6, NSP4, and NSP3 proteins are



recurrently mutated in SARS-CoV-2 VOCs and Variants of Interest (VOIs). Mutant NSP proteomes and subcellular localization of these proteins are being compared to those of the ancestral virus proteins. Proximity labelling of NSP3/4 complexes and NSP6 proteins has helped us to identify changes in proximally associated proteins that result from variants, and fluorescent tags enable us to track location of NSP6 and NSP3/4 complexes that may result from variants. To date, we have identified 20 potential NSP6 interactors and have tagged 10/20 of these potential interactors with fluorescent protein/epitope tag and the proximity tagging enzyme miniTurbo to determine how they affect NSP6 localization and function. Localization of three proximal proteins has been examined, with two of these proteins colocalizing with NSP6 at punctate spots and one disrupting NSP6 localization to the punctae. Our next step will be to expand the proteomes of network interactors and other non-spike proteins. Mechanisms of action identified from this work may correlate with clinical features and could be followed up in models of infection set-up by [Pillar 2: Host-Pathogen Interactions](#) and [Pillar 3: Virology](#).

PROGRAM 3: CHARACTERIZATION OF SPIKE: IDENTIFICATION OF ANIMAL (BAT) SPIKE PROTEIN AND HOST (HUMAN) RECEPTOR INTERACTIONS

The Rini lab has generated and characterized forms of SARS-CoV-2 spike protein (ancestral and variants). This includes soluble forms of the RBD and spike trimers. The proteins have been used by us and distributed to others, primarily as reagents to study receptor binding and immune recognition¹³⁻¹⁶.

The yield of properly folded/assembled protein varies considerably from one variant to another, so we have developed procedures to ensure consistently high-quality protein can be reproduced. Quality control measures have contributed to the quality of the data generated by our collaborators in [Pillar 1: Immunology & Vaccine Protection](#) who required large amounts of spike and RBD variants to determine the levels of anti-spike/RBD antibodies in saliva from various human populations¹³. The results have been correlated with that of serum antibody levels determined in work done by the Gingras and Gommerman labs.

We have also developed protocols, including size exclusion chromatography and negative stain electron microscopy, for generating well-defined streptavidin-APC (SA-APC) and streptavidin-PE (SA-PE) conjugates with both the RBD and spike. These reagents are being characterized in cell binding experiments by both the Watts and Gommerman labs. The RBD of the SARS-CoV-2 VOCs Omicron, Delta, and Gamma, have been produced and distributed in large quantities (> 65 mg) to partners within CoVaRR-Net ([Langlois](#), [Gommerman](#)) and external partners (Ostrowski, Hu, Watts). Production of these RBD reagents for our collaborators will continue as needed.

We have begun to analyze animal coronaviruses with the potential to infect humans. One aspect of this work is to assess binding of SARS-CoV-2 proteins and peptides to human cells/receptors. The SA-PE and SA-APC conjugates (RBD and spike) described



are being used in Bio-Layer Interferometry (BLI)-based binding studies. We have evaluated the binding of various SA-PE complexes (RBDs and carbohydrate binding domains from endemic human coronaviruses) to human airway epithelial cells and established human cell lines. This method will be extended to selected variants and animal viruses.

]





TABLE 1: SARS-COV-2 VARIANT PLASMID REAGENTS

See [here](#) to request validated expression plasmid reagents used in VLP-assays.

No	WHO name	Pango Lineage	Complete/C Ongoing/O	Plasmid Fate:
1	Ancestral (Wuhan)		C	Distributed, Validated Use
2	Ancestral + D614G		C	Distributed, Validated Use
3	Alpha	B.1.1.7	C	Distributed, Validated Use
4	Beta	B.1.351	C	Distributed, Validated Use
5	Delta	B.1.617.2	C	Distributed, Validated Use
6	Delta +	B.1.617.2_K417N (AY.1)	C	Distributed
7	Delta_Q613H	B.1.617.2_Q613H	C	
8	Delta_Y145H	B.1.617.2_Y145H	C	
9	Delta_Y145H/A222V	AY.27	C	
10	Delta_A222V/Q613H	AY.4.2	C	
11	Gamma	P.1	C	Distributed, Validated Use
12	Lambda	C.37	C	Distributed
13	Mu	B.1.621	C	Distributed
14		C.1.2	O	Cloning
15		B.1.1.318	C	
16	Omicron	B.1.1.529/BA.1	C	Distributed, Validated Use
17	Wuhan-1/Omicron-RBD		C	
18	Omicron-	BA.2	C	Distributed, Validated Use
19	Omicron-	BA.2.12.1	C	Distributed
20	Omicron-	BA.5 (BA.4/5)	C	Distributed, Validated Use
21	Omicron-	BA.2.75	C	Distributed
22	Omicron-	BA.2.75.2	C	testing in VLP assay
23	Omicron-	BQ.1.1	C	Distributed
24	Omicron-	BF.7	C	Validated Use
25	Omicron-	CK.2.1.1.	O	Cloning
26	Omicron-	BQ.1	C	testing in VLP assay
27	Omicron-	XBB.1	C	Distributed
28	Omicron-	XBB.1.5	C	Distributed
29	Omicron-	XBB.1.16	O	Cloning



TABLE 2: STUDIES USING COVARR-NET LENTIVIRUS REAGENTS IN YEARS 1 AND 2

Principal Investigator	Inst.	Topic	Status	Source
Eric Marcusson, Mario Ostrowski	Providence Ther.; UofT	Preclinical vaccine development	Published	PMID: 35044832
Atul Humar, Deepali Kumar	UHN	Solid transplant recipients	Published + ongoing	PMID: 34807716 PMID: 36975097 PMID: 35266606 PMID: 35927279
Steven Drews	CBS	Blood donors	Published + ongoing	PMID: 34662434 , PMID: 35171006 , PMID: 32870820 PMID: 36125288
T Watts/ AC Gingras/ V Chandran / M Silverberg / V Piguet	UofT, UHN, Sinai	Autoimmune disease patients	Published + ongoing	PMID: 35471956
AC Gingras / A McGeer/ H Wood	Sinai, NML	LTCH residents	Preprint + ongoing	https://doi.org/10.1101/2021.08.06.21261721
Eric Marcusson, Natalia Orozco	Providence Ther.	Phase I vaccine trials	Preprint + ongoing	https://doi.org/10.1101/2022.05.06.22274690
A McGeer / AC Gingras	Sinai	Cross-variant neutralization	Published + Ongoing	PMID: 32870820
Michelle Hladunewich, AC Gingras	Sunnybrook	Dialysis patients clinical trial (BOOST)	Ongoing	
Darren Yuen, Michelle Hladunewich, AC Gingras	Unity Health, Sunnybrook	Kidney transplant and dialysis recipients	Published + Ongoing	PMID: 36518793 PMID: 36950028
J Gommerman /AC Gingras/H Decaluwe	Sinai, UofT, UdeM	Mucosal Immunity in healthy Adults and Children	Published + Ongoing	PMID: 35468942
Christopher Kandel	Michel Garron	Household contacts correlates of protection	Ongoing	
Mario Ostrowski	UofT	Complement contribution to neutralization	Ongoing	
Sharon Walmsley	UHN	Hybrid immunity study in STOPCov participants (older adults)	Published + Ongoing	PMID: 37073374 DOI: 10.3138/jammi-2022-0011



TABLE 3. PUBLICATION SUMMARY – COVARR-NET PILLAR 4

Peer-reviewed publications using CoVaRR-Net-funded reagents and/or citing CoVaRR-Net for Pillar 4 from 2022-2023

Date	First Author(s)	CoVaRR-Net Member	Title	Hyperlink	Journal
19 Jan 2022	Liu J	Samson R, Hu Q, Banerjee A, Hardy WR, Rathod B, Colwill K, Mubareka S, Gingras AC	Preclinical evaluation of a SARS-CoV-2 mRNA vaccine PTX-COVID19-B	https://www.science.org/doi/10.1126/sciadv.abj9815	Sci Adv
03 Feb 2022	Yau, K., Abe, K.T.	Abe KT, Colwill K, Gingras AC	Differences in mRNA-1273 (Moderna) and BNT162b2 (Pfizer-BioNTech) SARS-CoV-2 vaccine immunogenicity among patients undergoing dialysis	https://www.cmaj.ca/	CMAJ
15 Feb 2022	Kumar, D, Ferreira FH	Hu Q, Samson R, Gingras AC	Neutralization of SARS-CoV-2 Variants in Transplant Recipients After Two and Three Doses of mRNA-1273 Vaccine : Secondary Analysis of a Randomized Trial	https://doi.org/10.7326/M21-3480	Ann Intern Med
16 Feb 2022	Drews S	Hu Q, Samson R, Abe KT, Rathod B, Colwill K, Gingras AC	SARS-CoV-2 Virus-Like Particle Neutralizing Capacity in Blood Donors Depends on Serological Profile and Donor-Declared SARS-CoV-2 Vaccination History.	https://journals.asm.org/doi/10.1128/spectrum.02262-21	Microbiol Spectr
21 Feb 2022	Mostefai, F	Hamelin D, Caron EC, Hussin J, Shapiro J, Poujol R, Murall CL	Population Genomics Approaches for Genetic Characterization of SARS-CoV-2 Lineages	https://doi.org/10.3389/fmed.2022.826746	Front Med (Lausanne)
23 Feb 2022	Tuite A	Abe KT, Rathod B, Colwill K, Gingras AC	Estimating SARS-CoV-2 Seroprevalence in Canadian Blood Donors, April 2020 to March 2021: Improving Accuracy with Multiple Assays.	https://journals.asm.org/doi/10.1128/spectrum.02563-21	Microbiol Spectr
10 Mar 2022	Kumar D	Hu Q, Samson R, Hardy W, Gingras AC	Neutralization against Omicron variant in transplant recipients after three doses of mRNA vaccine	https://doi.org/10.1111/ajt.17020	Am J Transplant



25 Apr 2022	Sheikh-Mohamed S	Sheikh-Mohamed S, Isho B, Chao GYC, Rathod B, Colwill K, Mubareka S, Rini JM, Gingras AC, Gommerman JL	Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection	https://www.nature.com/articles/s41385-022-00511-0	Mucosal Immunol
26 Apr 2022	Dayam, RM, Law, JC	Dayam RM, Chao GY, Abe KT, Hu Q, Hardy WR, Samson R, Rathod B, Gingras AC	Accelerated waning of immunity to SARS-CoV-2 mRNA vaccines in patients with immune-mediated inflammatory diseases	https://insight.jci.org/articles/view/159721	JCI
04 Aug 2022-	Ferreira VH, Solera JT	Hardy WR, Hu Q, Samson R, Gingras AC	Homotypic and heterotypic immune responses to Omicron variant in immunocompromised patients in diverse clinical settings	https://www.nature.com/articles/s41467-022-32235-x#Ack1	Nat Comms
09 Sept 2022	Cheung MW	Dayam R, Chao GYC, Gingras AC	Third dose corrects waning immunity to SARS-CoV-2 mRNA vaccines in immunocompromised patients with immune-mediated inflammatory diseases	http://dx.doi.org/10.1136/rmdopen-2022-002622	RMD Open
20 Sept 2022	Lin, YCJ	Hu Q, Samson R, Abe KT, Rathod B, Colwill K, Gingras AC	Utilization of the Abbott SARS-CoV-2 IgG II Quant Assay To Identify High-Titer Anti-SARS-CoV-2 Neutralizing Plasma against Wild-Type and Variant SARS-CoV-2 Viruses	https://doi.org/10.1128/spectrum.02811-22	Microbiol Spectr
11 Oct 2022	Wamsley S	Gingras AC	COVID-19 breakthrough infections in vaccinated participants of the Safety and Efficacy of Preventative COVID Vaccines sub-study	https://doi.org/10.3138/jammi-2022-0011	JAMMI
5 Dec 2022	Ferreira VH, Hu Q	Hu Q, Kurtesi A, Gingras AC	Impact of Omicron BA.1 infection on BA.4/5 immunity in transplant recipients	https://doi.org/10.1016/j.jt.2022.10.004	Am J Transplant
07 Dec 2022	McEvoy C	Hu Q, Abe KT, Gingras AC	Humoral Responses in the Omicron Era following Three-Dose SARS-CoV-2 Vaccine Series in Kidney Transplant Recipients	https://doi.org/10.1097/txd.0000000000001401	Transplant Direct
20 Mar 2023	Yau K, Enilama O	Abe KT, Hu Q, Colwill K, Gingras AC	Determining the Longitudinal Serologic Response to COVID-19 Vaccination in the Chronic Kidney Disease Population: A Clinical Research Protocol	https://doi.org/10.1177/20543581231160511	Can J Kidney Health Dis
28 Mar 2023	Ferreira VH	Kurtesi A, Hu Q, Hardy WR, Gingras AC	Omicron BA.4/5 Neutralization and T-Cell Responses in Organ Transplant Recipients	https://doi.org/10.1093/cid/ciad175	Clin Infect Dis



			After Booster Messenger RNA Vaccine: A Multicenter Cohort Study		
28 Mar 2023	Breznik JA	Colwill K, Dayam R, Gingras AC	Protection from Omicron infection in residents of nursing and retirement homes in Ontario, Canada	https://doi.org/10.1016/j.jamda.2023.02.105	JAMDA
28 Mar 2023	Walmsley SL	Colwill K, Dayam R, Gingras AC	COVID-19 vaccine antibody responses in community-dwelling adults to 48 weeks post primary vaccine series	https://doi.org/10.1016/j.isci.2023.106506	iScience
26 Apr 2023	Abubakar H	Colwill K, Dayam RM, Gingras AC	Feasibility and Acceptability of Self-Collected Dried Blood Spots for SARS-CoV-2 Vaccine Response in Community-Dwelling Elderly: A Large Decentralized Prospective Study	https://doi.org/10.29011/2577-2228.100309	J Community Med Public Health
05 Apr 2023	Solera JT	Kurtesi A, Hu Q, Hardy WR, Gingras AC	Differential serum neutralisation of omicron sublineages in patients receiving prophylaxis with tixagevimab–cilgavimab	https://doi.org/10.1016/S1473-3099(23)00208-6	Lancet Infect Dis

TABLE 4. MANUSCRIPTS IN THE PIPELINE

Pre-prints/projects in the in progress (not yet peer-reviewed/published) citing CoVaRR-Net for Pillar 4.

Date	First Author(s)	CoVaRR-Net Member	Title	Hyperlink	Preprint server
27-Aug-21	Abe KT, Hu Q, Mozafarihashjin M	Abe K, Hu Q, Samson R, Gommerman J, Colwill K, Wood H, Gingras AC	Neutralizing antibody responses to SARS-CoV-2 variants in vaccinated Ontario long-term care home residents and workers	https://www.medrxiv.org/	medRxiv
10-Feb-22	Walmsley S	Colwill K, Dayam R, Gingras AC.	Safety and Efficacy of Preventative COVID Vaccines: The StopCoV Study	https://www.medrxiv.org/content/10.1101/2022.02.09.22270734v1	medRxiv
10 May-22	Martin Orozco N	Samson R, Hu Q, Gingras AC	Phase I study of a SARS-CoV-2 mRNA vaccine PTX-COVID19-B	https://doi.org/10.1101/2022.05.06.22274690	medRxiv
05 Mar-23	Cheung MW	Dayam R, Gingras AC	Third and fourth vaccine doses broaden and prolong immunity to SARS-CoV-2 in immunocompromised adult patients	https://doi.org/10.1101/2023.03.01.23286513	medRxiv



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- 2 Kovalchik, K. A. *et al.* MhcVizPipe: A Quality Control Software for Rapid Assessment of Small- to Large-Scale Immunopeptidome Datasets. *Mol Cell Proteomics* **21**, 100178, doi:10.1016/j.mcpro.2021.100178 (2022).
- 3 Kubiniok, P. *et al.* Understanding the constitutive presentation of MHC class I immunopeptidomes in primary tissues. *iScience* **25**, 103768, doi:10.1016/j.isci.2022.103768 (2022).
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Pillar 5: Viral Genomics and Sequencing — May 2023 Research Update

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Pillar 5 is collaborating with Pillars 1, 3 and 6 in partnership with Illumina Inc.

Pillar 5: Viral Genomics and Sequencing Team

Lead	Ioannis (Giannis) Ragoussis	McGill Genome Center/ McGill University
Deputy	Robert Delatolla	University of Ottawa
Deputy	Caroline Quach Thanh	Sainte-Justine Hospital
Deputy	Terry Snutch	University of British Columbia
Deputy	Jeff Wrana	LTRI in Mount Sinai Hospital
Member	Marc Desforges	Sainte-Justine Hospital
Member	Ryan Ziels	University of British Columbia

Pillar 1: Immunology & Vaccine Protection

Lead	Jen Gommerman	University of Toronto
Lead	Ciriaco Piccirillo	McGill University

Pillar 3: Virology

Lead	Louis Flamand	Université Laval
Deputy	Darryl Falzarano	University of Saskatchewan
Deputy	Silvia Vidal	McGill University

Pillar 6: Computational Biology and Modelling

Lead	Jesse Shapiro	McGill Genome Center/ McGill University
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Collaborators:

Dominique Frigon	McGill University
Tony Mazzuli	Mount Sinai Hospital
Laurence Pelletier	LTRI in Mount Sinai Hospital
Brad Wouters	University Health Network
Michael Parkins	University of Calgary
Casey Hubert	University of Calgary
Guillaume Bourque	McGill University

Illumina Inc.



Pillar Vision Statement:

In order to prepare and prevent for the next health emergency, it is critical to have genomic surveillance to detect and monitor emerging pathogens. This work will enable us to rapidly identify and share the genomic information of detected pathogens to aid public health surveillance effort by providing feedback on the emergence of dangerous pathogens/ threats, identification of threatened regions/ municipalities, disease prevalence and the impact of public health measures. The immediate sharing of genetic sequences by monitoring pathogens such as viruses, bacteria, fungi and parasites as well as antimicrobial resistance alleles (AMR) will help us to understand their genetic similarities and differences as genomic surveillance. It will also establish a proactive program that can be implemented to accelerate the rapid response for effective development of medical interventions such as diagnostic testing, vaccines and therapeutics as what we learnt from SARS-CoV-2 pandemic.

Pillar Overview:

Pillar 5 was established in March 2021 to provide expert genomics support including high-throughput virus genome sequencing to the other pillars, establish strong links with Public Health Laboratories in support of public health SARS-CoV-2 surveillance efforts, contribute to national pandemic preparedness networks, establish links with international efforts and lead in the application of genomics in wastewater-based surveillance. As a result, Pillar 5 (Ragoussis) chaired the CanCOGeN virus sequencing committee and the Steering committee (Snutch) until the end of the program, played a key role in genomic surveillance of SARS-CoV-2 in the Greater Toronto area (Wrana) and was instrumental in establishing wastewater genomic surveillance networks. Pillar members lead or co-lead efforts in Ontario (DelaTolla), Quebec (Ragoussis), Alberta (Hubert, Ragoussis) and British Columbia (Ziels). They are also part of the CoVaRR-Net's Wastewater Surveillance Research Group (WWSRG).

Importantly, Pillar 5 (Ragoussis) together with Pillar 6 (Shapiro) and members of the CoVaRR-Net WWSRG (Vanrolleghem) are members of the technology transfer program CentreEau, which is performing genomic sequencing-based SARS-CoV-2 wastewater surveillance in Quebec and transfers the technology and methodologies to Quebec Public Health (INSPQ), funded by the Ministry of Health and Social Security (MSSS). It is important to emphasize that this effort was seed funded by CoVaRR-Net and the results produced, in conjunction with dissemination to the news media played a key role in convincing MSSS to provide funding. The role of CoVaRR-Net was also highlighted when Minister Duclos visited McGill University to inaugurate the CentreEau program.

In addition, Pillar 5, (with Pillar 6), participates in the European Wastewater surveillance COST action proposal, representing Canada, which has been submitted in 2022. For the 2022-23 half of the award period, Pillar 5 will play a key role also in the



proposed Immuno-Genomics platform and provide expertise in single cell genomics across pillars.

Pillar Project Deliverables:

1. Comparison of wastewater results with corresponding school/workplace/population efforts **(achieved)**
2. Early identification and tracking of VOCs and feeding this information to whole Network and public health labs **(achieved)**
3. Further methodologies developments for highly sensitive viral genome sequencing to detect emerging SARS-CoV-2 variants in wastewater and clinical samples and expand the detection to other pathogens as well as AMR **(in progress)**
4. Genomics and sequencing assay support to other Pillar members in a variety of technologies, including direct RNA sequencing, single cell RNA-seq and a wide range of short and long read sequencing methods **(achieved)**
5. Collaboration with Pillar 6 and the Wastewater Surveillance Research Group on the VirusSeq database structure and deposit the information in the portal **(achieved)**
6. Collaboration with Pillar 6 and the Wastewater Surveillance Research Group on the new COVID-19 in the Urban Built Environment (CUBE) pilot project. **(in progress)**

Aim 1: Development of high sensitivity sequencing assays for the genomic variation detection of SARS-CoV-2 VOCs which enables us to expand our detection to other pathogens as well as AMR

Introduction and Summary:

With the emergence of new variants, to ensure the early identification of them, we need further technological developments to increase sensitivity of viral genome sequencing for rapid response. Therefore, Ioannis Ragoussis lab proactively established partnerships with Illumina, Roche as well as Swift Biosciences to develop highly sensitive virus detection methodologies in wastewater as well as clinical samples. Further partnerships have been established with the Boucherville NRC lab as part of the NRC/CNRC Collaborative Initiative Pandemic Response Challenge Program and with industry (Miroculus Inc.).



For this part of work, Ioannis Ragoussis, Terry Snutch and Jeff Wrana labs will perform virus whole genome sequencing methodology optimization to adapt to meet the challenges of low virus titers, low quality and wastewater samples as well as new variations in the virus genome sequence affecting the performance of whole genome capture/enrichment and amplification assays. In summary, we tested different upstream sample preparations to understand which methods can increase RNA concentration, maintain RNA integrity, and generate more cDNA input in order to compare different sequencing assays and methods.

Research Update:

1. In order to develop rapid and novel NGS assays for the identification of known and emergent SARS-CoV-2 variants, we take advantage of the Epocrates project [2]. The school screening samples collected from January 25th to June 10th, 2021 were obtained from the Caroline Quach-Thanh laboratory as leftovers of gargle samples and/or remaining solutions from rapid or PCR tests. All samples were organized and used to extract RNA. 2230 samples were analyzed by LamPore, a method that detects SARS-CoV-2 by generating sequences and results which were analyzed in real-time in a purpose build Nanopore GridION device. Evaluation of the LamPore as a novel screening tool for SARS-CoV-2 (or other viruses and/or their combination) has been carried out in collaboration with Oxford Nanopore. Further development of the assay included establishing a full automation process (manuscript and protocol in preparation). In addition to LamPore assay, we also performed whole virus genome sequencing on the positive samples of the Epocrates project. 7 lineages were revealed and B.1.1.7 represents 50% of the positive lineage assignments. The data will be compared to geographically matched clinical surveillance and wastewater data. Lab work on this project is completed. **(Work is completed and a manuscript and protocol is in preparation.)**
2. For sensitive assay development, we performed a comprehensive testing with different viral loads and lineages to compare 6 assays. Lineages are tested including B.1 (Wild-type), B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), BA.1 (Omicron) and BA.2 (Omicron). Cultured variants were obtained in collaboration with L. Flamand and LSPQ and digital droplet PCR was performed in L. Flamand lab to get concentration at viral copy number level. Cultured variants are tested at three different dilution factors representing different viral loads. All these samples, in combination with synthetic standards from Twist and Accu Genomics, were used to generate a comprehensive series of 28 standard samples for assay benchmarking purposes. Six tested assays are tiled PCR- based WGS approaches including short amplicon PCR (Swift Biosciences, Qiaseq and a modified version of ARTIC plus rolling cycle amplification as well as ARTIC V3/V4/V4.1 as control) and long amplicon PCR by



using primer sets of Midnight [3] and Entebbe [4]. Further methodological developments target a further sensitivity increase through the modification of PCR-based assays to allow for further post-PCR and pre-library prep amplification. The results of this assay development will be implemented into population screening efforts and wastewater surveillance in collaboration with Public Health leading to an improvement in whole virus genome coverage and overall data quality. **(Work is completed and a manuscript is in preparation.)**

In parallel, we are also testing capture-based protocols through the partnerships with Roche and Illumina for clinical and wastewater samples. As RNA/cDNA input requirement is higher for capture-based assays, currently we are testing different reverse transcription kits to increase the cDNA generation for low input fragmented RNA such as wastewater samples. This testing also enables us to expand our detection to other pathogens (e.g., influenza, RSV, 40 different viruses, bacteria, fungi as well as antimicrobial resistance alleles) with different targeting probes including SARS-CoV-2. Our preliminary results suggest that we are able to detect different pathogens in addition to SARS-CoV-2 in wastewater samples including influenza, RSV, mastadenovirus, respirovirus and etc. by using Illumina capture panel. **(Work is in progress)**

3. Terry Snutch lab collaborates with Ryan Ziels to pursue untargeted, primer-free enrichment of SARS-CoV-2 genomic fragments in wastewater samples for accurate reference-free detection of emerging variants. Magnetic beads (obtained from Ceres Nanosciences company) that have a high specificity for enveloped viruses will be tested for its specificity towards SARS-CoV-2 and those 'untargeted' RNA resulted libraries will be used for long-read sequencing. This project will focus on detecting emerging SARS-CoV-2 variants in wastewater without relying on their previous detection in clinical genomic sequencing. As current population-based clinical testing and sequencing is waning across Canada, this work will provide us an opportunity to detect the emergence of future variants without depending on known mutations from consensus viral genomic references. Currently, Ryan Ziels lab finished the sample preparation from 3 different wastewater concentration methods, including previously mentioned magnetic beads, filtration and PEG. Those RNA extracts were sequenced with tiled PCR-based WGS approaches including artic v4.1 and midnight primer sets with nanopore long-read sequencing. For data comparison, the same RNA samples will be sent to IR lab for Illumina sequencing with capture-based methods, and purified amplicon samples will be also provided for short-read sequencing. This work will help us to understand which viral enrichment methods can maintain the integrity of viral RNA. **(Work is in progress)**



4. To further understand the degradation of viral RNA in wastewater, Robert Delatolla lab collaborates with Ragoussis lab for degradation project on sludge/stool samples tracking over 60 days to understand the viral genome degradation during sewer transit and whether the viral genome degradation is uniform. Collected sludge/stool samples will be tested under 3 different conditions for sewer transport. RNA extracts will be provided to Ragoussis lab for sequencing with capture-based methods, and RNA profile will be evaluated to track the degradation status over different timepoints. We finished the initial work planning for this project by sequencing the timepoint 0 of a SARS-CoV-2 positive stool sample (5 bio-replicates). Two different sequencing methods including ARTIC v4.1 and Illumina capture panel were performed, and we found that capture-based method gave us better genome coverage than ARTIC v4.1 for the sequencing results. **(Work is in progress)**
5. As part of our SARS-CoV-2 virus genomic sequencing work, we have developed methodology and data analysis pipelines to identify and sequence expressed SARS-CoV-2 RNAs by native RNA sequencing using nanopore technology. In collaboration with the Pelletier and Hobnan groups, Pillar 5 produced and analysed direct virus RNA sequencing data that led to the identification of defective virus genomes (DVGs) (Girgis et al. 2022). Specifically Direct RNA sequencing (DRS) of total RNA isolated from P1-, P14, P15- and P30-infected VERO cells in two independent experiments, revealed that by P30, a significant proportion of DVGs that arose during serial passaging retained genomic segments spanning Nsp10-Nsp12 (~13.3 – 16.8 kb). Transcript models constructed from the DRS information and retaining 5' and 3' end sequences (as these regions harbor essential replication signals), allowed the study of the evolution of these RNAs over passages and identified RNAs that dominated the DVG population at the different passage stages. Ongoing efforts focus on the epitranscriptomic analysis of the DVG. Overall, the work, leading to the identification and characterization of DVGs, has major implications for the development of Defective Interfering (DI) particle-based antiviral therapies. **(Work is completed and published.)**
6. Methodological developments and method dissemination is coordinated with the European SARS-CoV-2 Wastewater Surveillance Working Group.

Aim 2: Unique Canadian monitoring program by combining “above ground” screening of COVID-19 positive population with “below ground” wastewater surveillance of the entire population

Quebec Effort



Introduction and background:

In collaboration with Dominic Frigon, around 1,000 wastewater samples collected between March 2020 and July 2021 from Montreal, Quebec City, and Laval were sequenced using a combination of technologies and the data analyzed in order to evaluate the possibility of not only detecting the presence of SARS-CoV-2, but also identifying lineages, their relative proportions, and variants from whole virus genome data. The data were compared to data generated in-house, in collaboration with LSPQ, from geographically matched clinical and outbreak samples collected at the same time period. In this work [1], different sequencing depths were applied to establish the calling thresholds for single nucleotide variants from sequence reads. The key findings indicate the feasibility of wastewater surveillance as a complementary approach in providing a detailed picture of the prevalence of variants in a population, especially at places or times when sufficient clinical sampling is infrequent or infeasible.

Research Update:

1. Ioannis Ragoussis lab continues to produce SARS-CoV-2 genome sequencing data from wastewater samples and geographically matched clinical samples for Quebec province.

For wastewater samples, we completed the sequencing work for the retrospective samples collected between November 2021 to April 2022 for 4 cities including Montreal, Gatineau, Laval and Quebec City. Since June 2022 to March 2023, we started to sequence 70-80 wastewater samples per week under CentrEau program and those samples were collected from 6 cities including Montreal, Gatineau, Laval, Quebec City, Sept-Îles and Rimouski. This dataset will enable us to assess an additional 3,000 samples from April 2022 to March 2023 and will also increase the datapoints in terms of timepoints. Please see below the link for SARS-CoV-2 monitoring data in Quebec wastewater. <https://www.inspq.qc.ca/covid-19/donnees/eaux-usees>. All these efforts will be supported by the Quebec MSSS from June 2022, and Pillar 5 will provide a link between these efforts and CoVaRR-Net. No funding was requested for this effort, but it is relevant for providing a link between CoVaRR-Net and the Quebec and Ontario public health surveillance efforts.

For clinical samples, we continue to sequence weekly around 180 clinical samples in collaboration with the Quebec Public Health Laboratory (LSPQ). LSPQ clinical samples are randomly selected from across the whole province and a small, separated batch of samples is target sequencing for travelers and hospitalized patients. Samples received from LSPQ represent the variants circulated around the whole Quebec provincial area.



In addition to the collaboration with LSPQ, we also sequence positive clinical samples from Sainte-Justine Hospital, and those samples will increase the representation of variants present in Montreal Island to match the wastewater samples collected from Montreal Island stations. So far, we have been sequencing 92 clinical samples from August 23 to September 29, 2022 for 5 consecutive weeks. Sequencing data were shared with Pillar 6 for data analysis. We will continue to sequence positive clinical samples provided from Caroline Quach-Thanh laboratory for another 5-6 consecutive weeks. This part of our work is funded by CoVaRR-Net. **(Work is in progress)**

Moreover, to support the provincial biobanking for COVID-19 study, BQC19 (Biobanque Québécoise de la COVID-19), a partner of CoVaRR-Net biobank network, 1223 positive clinical samples collected from the whole Quebec provincial area across 5 separate waves for the time frame of July 2020 to March 2022 will be sequenced in Jiannis lab. This effort is funded by MSSS and PHAC.

2. Gabrielle Jayme from Terry Snutch lab will perform data analysis focusing on comparison and integration of mutations between Illumina (short read) and Nanopore (long read) sequencing in order to identify variants in these two different SARS-CoV-2 wastewater datasets and to determine haplotypes, specifically those without a known lineage. 248 wastewater samples from Montreal, Quebec City and Ottawa collected on early 2021 were sequenced with both Illumina and Nanopore, as well as positive and negative control samples with AccuGenomics reference to minimize background error. An initial comparison between the two datasets showed a large number of extra mutations from Nanopore sequencing, determined to be background noise. After filtering, Nanopore sequencing results show a more comparable dataset to the Illumina analysis. Using the tool Freyja, as well as the detection of signature and marker mutations, it was determined that the samples are dominated by the B.1.1.7 (Alpha) variant with other VOCs /VOIs in low abundance. The detection of certain variants within a sample increases when taking into account both Illumina and Nanopore datasets. The increased accuracy and depth that Illumina provides over Nanopore allowed for the improved detection of variants that are less abundant within a mixed population sample. Illumina sequencing preferentially detects mutations with a predominant lineage that is in low abundance across the population or unknown for that time period, while Nanopore preferentially detects mutations predominantly found in the high abundant Alpha lineage. The co-occurrence of signature mutations within the same amplicon was also compared as it is a more robust indicator of variants within a sample. Due to longer reads that are able to span the entire amplicon, Nanopore sequencing was determined to



detect these co-occurrences more often and can be used to detect unknown lineages where they are present. **(Work is in progress)**

Ontario Effort

Introduction and background:

In Year 1 of CoVaRR-Net funding, Jeff Wrana with Laurence Pelletier continued in collaboration with Dr. Tony Mazzulli and Brad Wouters to profile the dynamics and emergence of variants within the greater Toronto area (GTA) using automated SPAR-Seq, which is a high throughput NGS-based method to identify variant signatures in key functional regions including the receptor binding motif (RBM) and polybasic region of the Furin cleavage site of the S gene, as well as a region of RdRP. So far, the team has profiled over 40,000 samples from the GTA region. To define how variants spread within the GTA, we also mapped basic patient demographics (age and gender) and geographic (postal code forward sorting addresses; FSAs) information. Interestingly, analysis of the FSAs that showed the highest levels of each of the three major variants that spread within the GTA (Alpha, Delta, and Omicron) showed that FSAs were rarely disproportionately affected by more than one variant relative to the entire population. We also tracked the emergence of novel variants and subvariants within the population, which were frequent in the beginning of 2021, but by the end of 2021 were rare or non-existent. These variants displayed broad targeting of the RBM and PBS regions, often in residues strongly associated with immune evasion, such as E484 and F490. These studies can thus identify novel variants and subvariants at an early stage, and when coupled to high throughput variant fingerprint characterization, provide insight into impact on ACE2 binding, Furin cleavage efficiency and possible immune evasive properties. To understand how the major variants, Alpha, Beta/Gamma, Delta and Omicron were dispersed by FSA in the GTA, we plotted the timing of peak prevalence in each FSA relative to the peak in the entire population. The preliminary evidence suggests that most variants display geographic spread patterns through the GTA that resemble wavelets, except for Omicron, that spread simultaneously. These studies will be extended to explore whether network-based strategies can be implemented to predict future spread, and the findings could provide important implications for public health interventions and disease trajectory for precision-based containment strategies.

The “above ground” COVID-19 positive population screening can also support the wastewater monitoring effort by identifying new variants when they are still rare. For the Ontario wastewater surveillance effort, Ontario Genomics collaborates with Genome Canada and Illumina to introduce large-scale viral genome sequencing for wastewater samples by using whole genome tiled amplicon based methodology. In order to integrate the data between Toronto above-ground population screening and their below-ground wastewater screening to determine how and when, in terms of prevalence, geographically-defined variants can be detected in matched wastewater



samples, Jeff Wrana and Robert Delatolla will continue to exchange their datasets for analysis.

Research Update:

1. Jeff Wrana and Robert Delatolla labs will continue to produce SARS-CoV-2 genome sequencing data from wastewater samples and 4,000 clinical samples in Ontario regions including greater Toronto area (GTA) and Ottawa. Please see below the link for SARS-CoV-2 monitoring data in Ottawa wastewater. <https://613covid.ca/wastewater/> **(Work is in progress)**
2. Jeff Wrana is collaborating with Robert Delatolla and Jiannis Ragoussis to combine above- ground patient-based surveillance with surveillance data of wastewater to determine how prevalence and dynamics of variant spread is manifested in wastewater collection. These studies would provide valuable information about the sensitivity of wastewater for variant detection and how above-ground distributions translate to viral distribution in the sewershed. **(Work is in progress)**
3. Explore how variants spread within a large urban environment. Our preliminary results suggest that variants display distinct modes of transmission that are reflected in perdurance of the population-wide wave, which would thus have predictive value. We are also applying network graph concepts to explore whether geographic expansion in distinct forward sorting addresses can be predicted based on early correlations in variant dynamics, thus providing a second predictive tool for mapping variant spread. These tools could have value if public health transitions to a “precision intervention” model for pandemic management. **(Work is in progress)**
4. SPAR-Seq provides massive sequencing depth of viral sequences in individual patients allowing us to explore emergence of putative quasi-species. Our preliminary data suggest that there is tremendous intrapatient variability in the RBD of individual patients when compared to the Furin site and the RdRP regions. We will continue to pursue these studies to define what residues are most frequently targeted during quasi-species emergence and determine if putative quasi-species anticipate the emergence of subvariants in the population. Development of assays that anticipate what potential variants might emerge at the population level would provide a useful tool for understanding the trajectory of this pandemic that could be applied in future pandemics. **(Work is in progress)**



5. Ioannis Ragoussis lab will continue working with Wrana and Delatolla labs to provide support for methodological development of sensitive virus whole genome sequencing assay. **(Work is in progress)**

Calgary Effort

Background and Research Update:

Ioannis Ragoussis lab established a collaboration through Caroline Quach Thanh with Michael Parkins and Casey Hubert (CH) at the University of Calgary. The Calgary effort (Genome Canada funded, (CH and IR)), will be coordinated with CoVaRR-Net efforts through Pillar 5 and the CoVaRR-Net Wastewater Surveillance Research Group. Please see below the link for SARS-CoV-2 monitoring data in Alberta wastewater. <https://covid-tracker.chi-csm.ca/>

Eighty Calgary wastewater samples obtained from Casey Hubert and Michael Parkins were re-sequenced for 10M reads of each sample to get deeper sequencing data for high-confidence variant calling. Data were shared with Casey and Michael Groups for them to do the data analysis to compare different wastewater concentration methods. **(Lab work on this project is completed. Data analysis is in final stage.)**

Vancouver Effort

Background and Research Update:

Our Pillar 5 member, Ryan Ziels, will sequence SARS-CoV-2 genomes from three wastewater samples collected each week from five wastewater treatment plants serving 2.5 million people in Metro Vancouver, BC, with geographically matched clinical samples. The clinical samples are sequenced by collaborators at the BC Center for Disease Control. In addition, previously-collected data from this two-year time-series will be shared with CoVaRR-Net to establish a broader view of genomic variants in Canadian wastewater. Please see below the link for SARS-CoV-2 monitoring data in British Columbia wastewater. <http://www.metrovancouver.org/services/liquid-waste/environmental-management/covid-19-wastewater/Pages/default.aspx>

Aim 3: Collaboration with other Pillar Members towards Cross-Pillar Projects

Background and Research Update:

1. **Predictive immune responses (Pillars 1 (Immunology & Vaccine Protection), 3 (Virology), 5 (Viral Genomics & Sequencing) and 6 (Computational Biology and Modelling):** Pillar 5 will provide support for the characterization of antigen-specific B cell and T cell receptor diversity and VOC cross-recognition profiles



as it has established single-cell RNA sequencing methods to assess the diversity of SARS-CoV-2 antigen-specific B cell receptors (BCR) and T cell receptors (TCR) following infection or vaccination at high resolution and sensitivity. In collaboration with the [iReceptor](#) consortium, which hosts nearly 1 billion BCR/TCR sequences from COVID-19 patients and vaccine recipients, SARS-CoV-2-specific BCR/TCR repertoire diversity metrics will be defined and comparative sequence analyses of these repertoires will be performed to identify genetic features of epitope-specific B cell and T cell responses that can be used to infer the specificity (and VOC cross-reactivity) of any new sequence. This work will allow us to develop a genomics-based approach to evaluate host immune responses to SARS-CoV-2 that complements current diagnostic approaches and informs future vaccine development and testing strategies. This project will be part of the proposed Immuno-Genomics Platform to build high-content models of virus-host immune interactions and to prioritize computational approaches to assess both the quality of an individual's immune response and to predict the consequences of viral mutations on those immune responses, thus providing novel tools to rapidly examine the evolution and adaptation of SARS-CoV-2 variants that can be readily deployed against localized outbreaks as well as future epidemics. We have established the bioinformatic pipelines required for the analysis of single cell TCR/BCR and RNA-seq data. Samples have been collected by Pillar 1 members and we are in the process of carrying out the single cell data production part. **(Work is in progress)**

2. **Virus evolution studies (P3 and P5):** In collaboration with Louis Flamand, we supported the studies by sequencing 7 viral samples derived from mice infected with several different variants including B.1, B.1.617.2, B.1.351 and P.1 at different passages (0, and 10x) and 95 samples from multiple tissues (lungs, spleen, kidney, heart and brain) of mice infected with B.1.351 (Beta) and B.1.617.2 (Delta) with higher passage cycles (13x, 17x and 20x). To further confirm our findings, we also sequenced an additional 14 viral samples from lung tissue of mice infected with B.1.351 (Beta) and B.1.617.2 (Delta) with passage cycles (18x and 19x). This project is to investigate if passaging unmodified B.1.351 (Beta) and B.1.617.2 (Delta) in K18-ACE2 mice, which express human ACE2 receptor, in a BSL-3 laboratory without selective pressures would drive evolution, if any evolution was lineage-dependent, and whether results could inform human health. We found that after 20 passages, late-passage virus caused more severe disease, at organism and lung tissue scales, with only late-passage Delta virus demonstrating antibody resistance and interferon suppression. This resistance co-occurred with a *de novo* spike S371F mutation, linked with both traits. S371F, an Omicron-characteristic mutation, was observed to be co-inherited at times with spike E1182G per Nanopore sequencing, existing on different genomes as quasi-species at others. Both variant alleles have been linked to interactions with mammalian GOLGA7 and ZDHHC5, which mediate SARS-CoV-2-cell entry



and play an essential role in viral protein palmitoylation, that contributes to antiviral response. This study demonstrates SARS-CoV-2's tendency to evolve, highlights how its evolution varies by lineage, experimentally links SARS-CoV-2 genetic changes with phenotypes at several scales, and suggests non-dominant quasi-species could play a role in these traits. Overall, this work illustrates the importance of combined viral-mammalian studies when characterizing high-morbidity disease in humans. **(Work is completed and published on bioRxiv. A revised manuscript for peer-reviewed journal is in final stage of preparation.)**

This dataset will be compared with GTA population-based SPAR-Seq data to detect quasi-species. These studies will help us determine mutational hotspots of several SARS-CoV-2 genes in different VOCs associated with humans and/or animals and better understand the consequence of these mutations. **(Work is in progress)**

3. **Virus evolution studies in immunocompromised mice (P3 and P5):** In collaboration with Silvia Vidal, we supported this studies by sequencing 13 viral samples derived from immunocompromised mice infected with SARS-CoV-2 with different passage cycles (0x, 4x and 10x). All viral RNA samples were extracted from lung tissues. For this study, we would like to understand the serially infected immunocompromised mice in a BSL-3 laboratory if these sequential passages of unmodified virus yielded changing disease metrics, and if genetic changes coincided with any shifts observed at the organism level. While we are still collecting data for this study, our initial data suggest some variation in disease severity by mouse immuno-status, with genetic correlates in progress. **(Work is in progress)**
4. **COVID-19 in the Urban Built Environment (CUBE):** This pilot project is being led by Rees Kassen (uOttawa). Pillar 5 will support the effort by generating whole virus genome sequences using methodologies adapted from the wastewater efforts from its central budget allocation. It will be a combined effort made by Pillar 5, Pillar 6 and the Wastewater Surveillance Research Group. This project will allow us to evaluate the prevalence of SARS-CoV-2 and its variant distributions using environmental sampling of the built environment, especially floors from a diversity of community sites and from vulnerable populations, preceding or parallel to wastewater surveillance efforts over time. Complementing the wastewater sampling, built environment sampling can provide more spatially refined detection, and it is easier for expanding the geographic range. We can also test these samples for detecting other respiratory pathogens (e.g., influenza, RSV) and non-respiratory pathogens (including those not shed in the gut). Rees Kassen lab has been



testing and implementing multiplex qPCR assays for several pathogens such as SARS-CoV-2 and RSV. The qPCR dataset will be matched with a wastewater dataset. Rees Kassen lab is planning to collect samples from long-term care facilities, libraries, emergency rooms and hospitals over a 4–6-month period. Each location should cover a population of 10 to 100 people. To start the sequencing work, 24 samples with different Ct values from different collection sites on different collection dates were sent to the Ragoussis lab to test. 11 of them were collected from hospitals and 12 from non-hospital sites plus 1 processing negative control. The SARS-CoV-2 qPCR Ct value is 30-36 for samples collected from hospitals and 35-40 from non-hospital sites. **(Work is in progress)**

5. **Viral Sequencing Support for other Pillars:** Pillar 5 continued supporting other Pillars for their sequencing needs. For example, we have been providing virus whole genome sequencing to Pillar 3 Deputy, Darryl Falzarano lab (43 viral samples) for his functional experiments to isolate approximately 4 atypical SARS-CoV-2 isolates per month. Those samples are originally from human clinical samples with varied numbers of passages cycles up to 3 in cell culture.

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Table for Publications in the pipeline

Date	Pillar	Corresponding Author	CoVaRR-Net Member	Title	Hyperlink	Type of Article	Acknowledgment	Lay Summary			Lay Summary + publication posted to website	French Translation completed & posted Date
								Requested	Received	Sent to Comms		
	5 and 3	Jiannis Ragoussis	Ragoussis and Flamand	A Benchmark of methods for SARS-CoV-2 whole genome sequencing and development of a more sensitive method		Research	CoVaRR-Net					



Table for Publications related to research funded by CoVaRR-Net

Date	Pillar	Corresponding Author	CoVaRR-Net Member	Title	Hyperlink	Type of Article	Acknowledgment	Lay Summary			Lay Summary + publication posted to website	French Translation completed & posted Date
								Requested	Received	Sent to Comms		
2023, Mar 10	5 and 3	Giannis Ragoussis and Louis Flamand	J. Ragoussis and L. Flamand	SARS-CoV-2 variant natural evolution in K18-ACE2 mice increases virulence and produces treatment resistance-linked variant alleles	https://www.biorxiv.org/content/10.1101/2023.01.16.523994v3.full	Research	CoVaRR-Net	Yes	Yes	Yes	Yes	Yes (Mar 17, 2023)
2022, Oct 27	5	T. Hobman and J. Pelletier	J. Ragoussis	Evolution of naturally arising SARS-CoV-2 defective interfering particles	https://pubmed.ncbi.nlm.nih.gov/36302891/	Research	CoVaRR-Net	Yes	Yes	Yes	Yes	Yes (Nov 11, 2022)
2022, Jun 10	2, 4, 5 and CCABL3	Darryl Falzarano	A. Banerjee, V. Gerdt, A.C. Gingras, J.L. Wrana, S. Mubareka and D. Falzarano	Immunogenicity of convalescent and vaccinated sera against clinical isolates of ancestral SARS-CoV-2, Beta, Delta, and Omicron variants	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9008123/	Research	CoVaRR-Net	Yes	Yes	Yes	Yes	Yes (Nov 7, 2022)
2022, Feb 01	5 and 6	J. Ragoussis, S.D., D. Frigon and J. Shapiro	J. Ragoussis and J. Shapiro	Detection of prevalent SARS-CoV-2 variant lineages in wastewater and clinical sequences from cities in Québec, Canada	https://doi.org/10.1101/2022.02.01.22270170	Research	CoVaRR-Net	Yes	Yes	Yes	Yes	Yes (Feb 14, 2022)
2022, Jan 23	5	Caroline Quach	C. Quach, M. Desforges and J. Ragoussis	Evaluation of real-life use of Point-Of-Care Rapid Antigen TEsting for SARS-CoV-2 in schools (EPOCRATES)	https://doi.org/10.1101/2021.10.13.21264960	Research	Québec Ministry of Health and Social Services					
2021, Oct 28	5, 6 and Data Platform	Jesse Shapiro	C.L. Murall, G. Bourque, J. Ragoussis and J. Shapiro	A small number of early introductions seeded widespread transmission of SARS-CoV-2 in Québec, Canada	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8550813/	Research	CoVaRR-Net					
2021, Oct 25	2, 4 and 5	Marc-André Langlois, Anne-Claude Gingras and Yves Durocher	J.L. Wrana, S. Mubareka, M.A. Langlois and A.C. Gingras	A scalable serology solution for profiling humoral immune responses to SARS-CoV-2 infection and vaccination	https://onlinelibrary.wiley.com/doi/10.1002/cti2.1380	Research	CoVaRR-Net					



CAMEO: Computational Analysis, Modelling and Evolutionary Outcomes (Pillar 6) Year 1 & 2 Progress Report

Vision Statement

CAMEO aims to achieve a unified Canadian network for the detection, molecular characterization, and epidemiological modelling of emerging pathogens within our borders. Our vision is to make genomic epidemiology actionable in real time, for the benefit of the research community and public health practitioners. Our team strives to build relationships, computational tools, and data standards, as well as train highly qualified personnel to realize this vision.

[List of publications](#)

Projects and Progress

Year 1 projects = highlighted in blue

Year 2 projects = highlighted in yellow

Year 1 & 2 projects = highlighted in green

1) Developing and applying strategies for identifying and monitoring new variants internationally and nationally

a) Monitoring for potential variants of concern to alert CoVaRR-Net and public health officials

Summary: All CAMEO members share information on a weekly basis about potential variants of concern that are being found nationally and internationally, providing data analyses (e.g., phylogenetic analyses, estimating unusual mutation signatures, predicting functional impact of mutations, highlighting mutations of interest to further study by other pillars, and inferring selection coefficients). Analyses are shared in our modelling resources webpage ([duotang](#)) on the CoVaRR-Net website. Results of importance are shared with the broader CoVaRR-Net community and have been shared externally, including with the Council of Chief Medical Officers of Health (CCMOH), Canadian Public Health Lab Network (CPHLN), Canadian COVID-19 Genomics Network (CanCOGeN), and Variants of Concern Leadership Group with the Deputy Minister of Health (Canada), and the National Advisory Council on Immunization (NACI). Near future work will investigate the expansion of [duotang](#) features to genomic data on influenza from Canada.

Status: ongoing (to continue for as long as funding is available)

CAMEO Personnel: All members

b) Mathematical and phylogenetic approaches to characterize variants

Summary: We are continuously analyzing SARS-CoV-2 genomic sequence data to identify and characterize the epidemiology of new and existing variants. These activities allow us to predict the variants' rate of spread and their



potential impact on health care systems across the country (Otto et al. 2021). We communicate this information across CoVaRR-Net pillars and to PHAC to assist in the formation of public policies.

Status: ongoing. As CoVaRR-Net begins to investigate additional emerging pathogens, they will be analyzed in a similar fashion. Publication in *Current Biology* on the shifting selection pressures on SARS-CoV-2 with increasing vaccination rates (Otto et al. 2022)

CAMEO lead: Otto, Murall, Colijn

CAMEO trainees: Jia, Poujol

a) Monitoring the evolution and spread of Delta sublineages AY.25 and AY.27 in Canada

Summary: A notable achievement was our [Virological report](#) on Delta sublineages AY.25 and AY.27 (which includes the novel Spike Q613H mutation), in close collaboration with at the Public Health Agency of Canada (PHAC) / National Microbiology Laboratory (NML), three provincial public health labs, and several universities. We showed that these variants, which became dominant in Western Canada over summer 2021, had a modest selective advantage compared to other circulating variants. These results are largely reassuring and show that we are poised to catch more concerning variants should they arise – pending timely sharing of sequence data.

Status: complete

CAMEO leads: Shapiro, Otto, Colijn, Brinkman, Fritz, Hussin

CAMEO trainees: Murall, Poujol, Kraemer, N'Guessan, Naderi, Gill

b) Model projections for the spread of Omicron and the potential impact on hospital occupancy in Canada

Summary: Following early international estimates for the rate of spread of Omicron, modelling was conducted and shared on the CoVaRR-Net website to [predict the potential impact on hospital occupancy in Canada](#). The model examined different potential values of vaccine effectiveness against infection and against severe disease, as well as different potential values for the transmissibility and severity of Omicron.

Status: complete

CAMEO leads: Otto, Brinkman, Colijn, Shapiro, Gordon, Joy

CAMEO trainees: Murall, Kraemer

Collaborators involved: Pillar 4: Functional Genomics & Structure-Function of VOCs (Gingras)

c) Data-driven approaches for genetic characterization of SARS-CoV-2 lineages



Summary: We present a set of computational tools that span phylogenomics, population genetics and machine learning approaches. To illustrate the utility of this toolbox, we detail an in-depth analysis of the genetic diversity of SARS-CoV-2, using 329,854 high-quality consensus sequences published in the [GISAID database](#) during the pre-vaccination phase. Among other tools, we discuss how time series analysis of Tajima's D provides a powerful metric of population expansion and how unsupervised learning can be used to detect variants. The computational framework presented here is useful for real-time genomic surveillance of SARS-CoV-2 and could be applied to any pathogen that threatens the health of worldwide populations of humans and other organisms.

Status: complete. Published in [Frontiers in Microbiology](#). Many of these methods were also implemented in a study of the first pandemic wave in Québec, resulting in a publication in [Genome Medicine](#).

CAMEO leads: Hussin, Shapiro

CAMEO trainees: N'Guessan, Murall, Naderi, Poujol

Collaborators involved: Pillar 1: Immunology & Vaccine Protection (Hamelin), Pillar 5: Viral Genomics & Sequencing (Ragoussis)

2) Inferring mutations and variants from wastewater samples across Canada

a) Detection of prevalent SARS-CoV-2 variant lineages in wastewater and clinical sequences from cities in Québec, Canada

Summary: Here we report sequencing and inference of SARS-CoV-2 mutations and variant lineages in 936 wastewater samples and thousands of matched clinical sequences collected between March 2020 and July 2021 in the cities of Montreal, Québec City, and Laval. We found that variant frequency estimates in wastewater and clinical samples are correlated over time in each city, with similar dates of first detection. Most variants were first observed in clinical and outbreak data due to higher sequencing rate. However, wastewater sequencing is highly efficient, detecting more variants for a given sampling effort. This shows the potential for wastewater sequencing to provide useful public health data, especially at places or times when sufficient clinical sampling is infrequent or infeasible.

Status: Preprint posted in [medRxiv](#); journal submission in preparation.

CAMEO lead: Shapiro, Poon

CAMEO trainees: N'Guessan, Kraemer

Collaborators involved: Pillar 5: Viral Genomics & Sequencing (Ragoussis)

b) Environmental monitoring and sequencing

Summary: In collaboration with the [CUBE](#) project and the CoVaRR-Net Wastewater Surveillance Research Group (WSRG), we are working to develop,



benchmark and apply methods to track SARS-CoV-2 mutations and variants in the built environment and in wastewater (WW).

Status: In collaboration with Québec public health (INSPQ), we are developing a [bioinformatic pipeline](#) for long-term surveillance of SARS-CoV-2 variant-lineages from WW.

CAMEO lead: Shapiro, Poon

CAMEO trainees: Sutcliffe

Collaborators involved: Wastewater Surveillance Research Group, Pillar 5: Viral Genomics & Sequencing (Ragoussis)

c) Benchmarking wastewater SARS-CoV-2 variant-lineage calling

Summary: Following the development of our analysis of wastewater [data](#) in Québec, there was a surge in novel bioinformatic approaches to tackle SARS-CoV-2 [1-4]. We are collaborating with software developers to test multiple tools against a synthetic WW benchmark dataset.

Status: The benchmark dataset has been [created](#), and tool developers have submitted their analyses. We are writing the results up for publication.

CAMEO lead: Shapiro, Poon

CAMEO trainees: Sutcliffe, Kraemer, Jia

3) Identifying and monitoring mutations associated with immune escape

a) Selection for immune evasion in SARS-CoV-2 revealed by high-resolution epitope mapping combined with genome sequence analysis

Summary: In this study, we studied the humoral immune responses of asymptomatic or recovered COVID-19-positive patients relative to COVID-19-negative patients. We used a novel high-density peptide array (HDPa) spanning the entire proteomes of SARS-CoV-2 and endemic human coronaviruses to identify B cell epitopes recognized by distinct antibody isotypes in patients' blood sera. While some epitopes are common across all CoVs, others are private to specific hCoVs. We also highlight the existence of hotspots of pre-existing immunity and identify a subset of cross-reactive epitopes that contributes to increasing the overall humoral immune response to SARS-CoV-2. Using a public dataset of ~40,000 SARS-CoV-2 genome sequences from the early phase of the pandemic, we studied the mutations that occurred in or outside of predicted epitopes. We showed that mutations in S and N epitopes emerge more rapidly upon transmission rather than within patients suggesting that most of the selective pressure for immune evasion following SARS-CoV-2 infection occurs upon transmission between hosts.



Status: Preprint posted in [bioRxiv](https://www.biorxiv.org/). Manuscript in second round of review at iScience. We are currently working to expand this work to more recent mutations and VOCs, and to epitopes beyond the spike protein.

CAMEO lead: Fritz, Shapiro

CAMEO trainees: N'Guessan, Kraemer

4) Tracking evolution in animal reservoirs and identify mutations associated with humans or animals

a) Estimation of animal-to-human transmission events

Summary: We are in the process of analyzing ~2000 SARS-CoV-2 genome sequences of animal origin and inferring their phylogenetic relationships with ~2000 matched human sequences. The number of animal-to-human transmission events that have happened during the pandemic will then be estimated, and a genome-wide association study will be conducted to identify any animal-associated mutations. We will then develop methods to track these mutations in WW.

Status: Preprint posted in [bioRxiv](https://www.biorxiv.org/). Manuscript under review at eLife. Methods to track mutations in WW in development.

CAMEO lead: Shapiro

CAMEO trainees: Naderi, Murall, Pujol, Kraemer

Collaborators involved: Pillar 2: Host-Pathogen Interactions (Sagan & Pickering)

5) Expanding efforts to integrate sequence data from across Canada, from both clinical and wastewater data

a) Data sharing

Summary: CAMEO is committed to working with the [Canadian VirusSeq Data Portal](https://canadianvirusseq.ca/) to ensure that Canadian SARS-CoV-2 genome sequences generated by the CPHLN and regional health authorities are publicly available without restriction to encourage data re-use for scientific discovery and innovation. Building on the [Canadian COVID Genomics Network](https://canadacovidgenomics.ca/) (CanCOGeN) and CHARGES (Canadian public Health Alliance for Research in Genomic Epidemiology and Surveillance - a CPHLN-led consortium to implement genomic based infectious disease surveillance) efforts to standardize and harmonize contextual information critical for genomics analysis, we will work with the VirusSeq Data Portal to explore more flexible and comprehensive ways of sharing contextual data in a trusted environment. Our collaboration is focused on ensuring maximum user-friendliness of the Data Portal, while working to add features that are useful to researchers seeking to analyze the data in the Portal. We are also working towards supporting the analyses and



visualization of WW data from across the country and national priority pathogens.

Status: ongoing. Adding WW data to the Data Portal is a priority. Publication in *CCDR* on the need for and benefits of linking genomic data to four key data sources: (Colijn et al. 2022)

CAMEO lead: Hsiao, Brinkman, Shapiro, Murall

CAMEO trainees: Anwar, Gill, Jia, Pujol

Collaborators involved: Wastewater Surveillance Research Group

b) COVID-MVP

Summary: [COVID-MVP](#) is an interactive heatmap-based visualization tool for SARS-CoV-2. It tracks the prevalence of mutations of SARS-CoV-2 along with annotations of the functional impact of these mutations in Variants of Concern (VOCs), Variants of Interest (VOIs), and user-defined subpopulations in near-real time. COVID-MVP visualization is powered through an independent, scalable and reproducible genomics workflow ([nf-cov-voc](#)) using functional annotations manually curated in [Pokay](#). Pokay is also maintained by our team and is regularly updated by curating new functions from the literature. Near future work includes estimating selection by the change in frequency of each mutation within different lineages to disentangle selection on lineages (as reported in [duotang](#)) and selection on individual mutations, connecting the insights provided by COVID-MVP and [duotang](#).

Status: ongoing. Preprint published in [bioRxiv](#).

CAMEO lead: Hsiao, Gordon, Brinkman

CAMEO trainees: Anwar

New Findings for Comms Amplification

- CAMEO is interested in spreading the word about [duotang](#) (described in section 1a.), our public modelling resources webpage. The page is updated on a weekly basis and provides diverse information on topics such as SARS-CoV-2 lineages that are showing the most growth in Canada, mutations that are prevalent in circulating lineages, how the variant landscape has evolved over time and more. Please contact Erin Gill (erin.gill@gmail.com) for more information.

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CIEDAR vision statement

CIEDAR (Pillar 7, Indigenous Engagement, Development and Research, of the CoVaRR-Net Network) supports Indigenous communities and organizations to address issues relevant to Indigenous wellbeing. CIEDAR consists of academic researchers from Canada, New Zealand, and the USA. CIEDAR research supports and works to ensure that Indigenous voices are heard before, during, and after pandemic challenges. CIEDAR has partnerships with First Nation, Inuit, and Métis communities in Canada and Māori communities in New Zealand for Indigenous land-based healing. CIEDAR collects survey data from an Indigenous data panel from Canada and the USA to understand the life experiences of Indigenous peoples and to elucidate the attitudes and opinions of Indigenous people. CIEDAR also serves a virtual community of Indigenous peoples across the earth to share stories of Indigenous thriving and disseminate science-based information to promote and support Indigenous wellbeing. CIEDAR connects Indigenous communities, organizations, and partners to academic researchers to support the needs of communities as identified by themselves and to amplify Indigenous voices and stories to effect change.

Pillar 7: CoVaRR-Net's Indigenous Engagement, Development, and Research (CIEDAR) Research Update

CIEDAR (P7) Year 1 Report

Milestone 1: Building of Infrastructure

- CIEDAR's Advisory Council (AC) has been formed (Felix Lockhart (Elder), Maureen Dobbins, Renee Masching, and Shelia Blackstock).
- Successful first meeting of advisory council. AC reviewed the BeadAndThrive social media campaign and survey. They were in support of the effort. AC members will disseminate BeadAndThrive social media campaign recruitment flyers in their communities.
- Hired 7 part-time research assistants, 1 part-time research manager, and in the process of hiring 1 full-time project coordinator.
- International Collaborations with Dr. Karina Walters, University of Washington and Dr. Aggie Yellow Horse, Arizona State University.

Milestone 2: Community Engagement, Building of Research Collaborations & Assessments

Project: Wastewater Needs Assessment

Timeline: It was conducted and completed in June, 2021 and results were shared with Pillar 8: Public Health, Health Systems and Pillar 9: Knowledge, Implementation and Training Team (KITTT) in Sept. 2021.



Dissemination Plan: Due to low response rate the wastewater results will not be widely shared.

- *Status:* Completed project

Project: Association between COVID-19 and Social Determinants of Health

Timeline: From June 2021 to September 2021, data was collected for the project from the Census, COVID-19 dashboards, and location of First Nation communities. The analysis and mapping occurred in Year 2.

Project: Evaluation of Indigenous community-led interventions, initiatives, and responses to the COVID-19 pandemic

We have been evaluating the success of a land-based healing initiative being carried out with community members of Łutsël K'é Dene First Nation in the Taché area, Northwest Territories. This will be one of the first land-based healing camps that seeks to focus on thriving after the COVID-19 pandemic through returning to culture and the land. The aim of the current study is to identify facilitators and barriers to the successful implementation of the Land-based healing camp pilot.

Timeline: CIEDAR began engagement with First Nation Elder Felix Lockhart about community research with Łutsël K'é Dene First Nation in March 2021.

- *Status:* In July 2022, CIEDAR evaluated the initial pilot project with Elder Felix Lockhart. CIEDAR attended and evaluated the Ya'a de Land-based healing camp pilot and, as part of the evaluation, interviews with key informants were conducted. Our work with the community will continue.

Project: Building CIEDAR's National Presence in Indigenous Communities - #BeadAndThrive

Timeline: Design of social media campaign and methodology to connect with Indigenous communities beyond in-person meetings, #BeadAndThrive, from December 2021 to April 2022. #BeadAndThrive was launched in May 2022 using our CIEDAR_7 social media accounts on Instagram, Twitter, and Facebook.

- *Engagement activities:*
 - Virtual On-line engagement: CIEDAR has made relationships with 27 Indigenous artists, and 6 have partnered with CIEDAR for the #BeadAndThrive campaign. CIEDAR has made relationships with 34 Indigenous beading influencers, and 5 have partnered with CIEDAR for the #BeadAndThrive campaign.
 - Outreach to Indigenous organizations and communities: CIEDAR has made relationships with 21 community organizations, and 12 have partnered with CIEDAR for the #BeadAndThrive campaign.



- *Status:* Ongoing.

Other Community Engagement Milestones

- CIEDAR developed partnerships with the Global Alliance for Genomics and Health, CanCOGeN, UBC Vaccine Literacy Club, and some CIHR grantees with relevant projects.
- CIEDAR implemented educational initiatives on issues related to COVID-19 and Indigenous peoples, including webinars and panels on vaccine awareness, ethical considerations for biobanks, and a panel on genomics and Indigenous rights. In total, our presentations have reached over 500 people.
- CIEDAR has developed partnerships with Tache Healing Waters Society. Tache Healing Waters Society has invited CIEDAR to partner and co-develop a land-based healing research design and program evaluation protocol into the Ya'a de Healing Camp.

CIEDAR (P7) Year 2 progress Report

Infrastructure

- Additional CIEDAR's Advisory Council member from Metis Nation of Saskatchewan knowledge keeper, Calvin Racette
- Hired a full-time project coordinator
- Hired a full-time communications and social media assistant
 - Created a communications team.
- All team members have completed The First Nations principles of ownership, control, access, and possession (OCAP) training.
- CIEDAR leadership, manager and graduate research assistant attended the annual Community-Based Participatory Research (CBPR) Institute in Indigenous and Critical Methodologies summer Institute at the University of New Mexico.

AIM 1: Social Determinants of Health During the COVID-19 Pandemic

Aims: (1) To investigate the SDOH factors that may protect or increase the vulnerability of Indigenous Peoples to COVID-19 and its VOCs. (2) To understand the responses of Indigenous communities that mobilized to protect Indigenous communities and individuals from COVID-19 and its VOCs. (3) To learn the tools needed by Indigenous communities to monitor and respond to health concerns, particularly for VOCs.

Project: Association between COVID-19 and Social Determinants of Health

Timeline: Literature review and construction of a database of SDOH factors and COVID-19 case numbers has been ongoing since June, 2021. Data analysis and



mapping to identify particularly vulnerable regions to COVID-19 and VOCs began in Oct, 2021. Final report was submitted for publication in Summer, 2022 and published in September 2022.

- *Status:* Manuscript “Understanding the associations among social vulnerabilities, Indigenous Peoples, and COVID-19 cases within Canadian health regions” is published. Lay summary published on CoVaRR-Net website. Knowledge translation posts on our social media platforms instagram and twitter.
- Academic dissemination: presented findings at The American Sociological Association (ASA) Annual Meeting in Los Angeles, August 2022.
- Community dissemination: Congress of Aboriginal Peoples: Youth and Elder Dinner, Native Women's Association of Canada, Gatineau, Québec. September 21, 2022
- Publication: Huyser KR, Yellow Horse AJ, Collins KA, Fischer J, Jessome MG, Ronayne ET, Lin JC, Derkson J, Johnson-Jennings M. Understanding the Associations among Social Vulnerabilities, Indigenous Peoples, and COVID-19 Cases within Canadian Health Regions. *Int. J. Environ. Res. Public Health* 2022, 19(19), 12409; <https://doi.org/10.3390/ijerph191912409>.

Project: Web-based Survey of Indigenous Peoples

We have collaborated with Dr. Karina Walters of the University of Washington to develop a large-scale survey that will allow us to assess Indigenous persons' experiences in the pandemic, knowledge of the pandemic and its changes, vaccine hesitancy, resources used for mental health concerns, and willingness to engage with research.

Timeline: Data collection began fall 2022. Dependent upon response rate, a final report will be prepared by Spring 2023. Additional assessments will be carried out based on the findings of the large-scale survey.

Dissemination Plan: Dissemination outlets have not yet been identified.

- *Status:* The survey received ethics approval, and data collection began in October 2022. The analysis began in the Winter of 2023. Will present preliminary finding in June 2023. Manuscript is expected to be ready for publication in Winter 2023.

Aim 2: Community-Engaged Research with Indigenous Communities

Aims: (1) To learn the tools needed by Indigenous communities to monitor and respond to health concerns, particularly for VOCs. (2) To educate on systemic and individual (implicit and explicit) bias in science and provide an easy-to-understand framework for ethical community-engaged research for both CoVaRR-Net



researchers and the wider scientific community. (3) To share knowledge of successful solutions, and build Indigenous community capacity. The community-based collaborations will ensure that CIEDAR will make a significant contribution to the health of Indigenous communities across Canada and position CIEDAR and the Network to be a global leader in Indigenous health.

Project: Wastewater Feasibility

Timeline: In March 2022, CIEDAR began gathering data that will inform CoVaRR-Net's wastewater efforts in Indigenous communities. Next steps will be determined in collaboration with Doug Manuel: Director of the Wastewater Surveillance Research Group (WWSRG).

Dissemination Plan: Results will be reported to CoVaRR-Net's WWSRG.

- *Status:* Next steps are to be determined.

Project: Conceptual Framework for Community Engaged Research with Indigenous Communities

Timeline: Literature review has been ongoing. CIEDAR leadership met in Fall 2022 to complete the manuscript.

Dissemination Plan: We are planning on publishing the article in *The Lancet Public Health*.

- *Status:* Preliminary framework developed; Status: Manuscript is expected to be ready for publication in Summer 2023.

Project: Crowdsourcing and Mapping Stories of Indigenous Thrivance During the Pandemic

CIEDAR launched a social media campaign to solicit stories of Indigenous thrivance, educate community members on various health behaviours, and encourage family, community, and cultural connection or reconnection as a means of coping.

Timeline: Project was designed and submitted for ethics approval. The campaign was launched in May 2022. Dependent on the response rate, the final report will be prepared in Fall of 2024.

Dissemination plan: The map will be posted on the CoVaRR-Net website. Dissemination outlets have not yet been identified.

- *Status:* The study has been submitted for ethics approval and the campaign launched June 2022. Manuscript is expected to be ready for publication in Fall 2024.

Project: Evaluation of Indigenous community-led interventions, initiatives, and responses to the COVID-19 pandemic



We have been evaluating the success of a land-based healing initiative being carried out with community members of Łutsël K'é Dene First Nation in Taché area, Northwest Territories. This will be one of the first land-based healing camps that seeks to focus on thriving after the COVID-19 pandemic through returning to culture and the land. The aim of the current study is to identify facilitators and barriers to the successful implementation of the Land-based healing camp pilot.

Timeline: CIEDAR attended and evaluated the Ya'a de Land-based healing camp pilot and, as part of the evaluation, interviews with key informants were conducted. Our work with the community will continue. A Ya'a de Land-based healing camp pilot with Łutsël K'é Dene First Nation families will be evaluated in Summer 2024.

Dissemination Plan: The pilot camp facilitator evaluation has been analyzed. We expect to develop knowledge translation resources to share with Indigenous and other community members via CIEDAR social media, to other researchers on the CoVaRR-Net website, and in an academic article in winter 2023. We expect to share the findings of the 2024 pilot community in the winter of 2025.

Status: An evaluation of camp facilitators took place in July 2022. The analysis began in the Winter of 2023. Presented preliminary finding in New Zealand in February 2023. Manuscript is expected to be ready for publication in Winter 2023. A community engagement visit with Łutsël K'é Dene First Nation community members will take place in the Summer of 2023. A pilot evaluation with Łutsël K'é Dene First Nation community members will take place in the Summer of 2024. Manuscript is expected to be ready for publication in Winter 2025.

Project: Podcast

Timeline: Series has been designed, and dissemination outlets have been identified. We will be connecting with members of the community through fall 2022, and the interviews will be held in winter 2023. Dissemination will likely occur in Spring 2023.

Dissemination plan: We plan to publish the podcast on Spotify, the CoVaRR-Net website, and on radio stations with primarily Indigenous listeners.

- *Status:* Series designed and Dissemination Outlets identified. Production began Winter 2023. Interviewed Indigenous researchers, elders, artist and community members across North America and New Zealand. First episode will be release in June 2023.

Aim 3: Community Outreach

Project: Evaluation of Social Media as an Alternative Method for Conducting Community-Engaged Research When In-Person Meetings Are Not Feasible



Timeline: Project was designed and submitted for ethics approval. The campaign was launched in May 2022. Dependent on the response rate, the final report will be prepared in Winter 2023.

Dissemination plan: The map will be posted on the CoVaRR-Net website. Dissemination outlets have not yet been identified.

- *Status:* Evaluation will be ongoing through Summer 2023, the manuscript is expected to be prepared in Fall 2023.

Other Community Engagement Milestones:

- In partnership with Tache Waters Healing Society, CIEDAR has been invited to participate in a community engagement visit to Łutsël K'é Dene First Nation.
- Awarded SSHRC Partnership Development Grant to continue our work with Tache Waters Healing Society in the Northwest Territories
- CIEDAR has connected with the Métis Nation of Saskatchewan. They have referred our newest AC member and have begun communication about potential research collaboration.
- CIEDAR built relationships with researchers at the University of New Mexico: Drs. Tassy Parker, Nina Wallerstein, Lisa Cacari Stone, Lorenda Belone, Vincent Werito, and Shannon Sanchez-Youngman about their Indigenous community research on COVID-19.
- CIEDAR has partnered with the Congress of Aboriginal Peoples (CAP). CIEDAR presented on "The COVID-19 pandemic and its effect on off-reserve Indigenous communities in Canada." at the Congress of Aboriginal Peoples: Youth and Elder Dinner, Native Women's Association of Canada, Gatineau, Québec. September 21, 2022. CAP has partnered with CIEDAR for their youth holiday gift bundles. 550 Indigenous youth across Canada will be gifted a beading kit, and CAP will promote the #BeadAndThrive campaign to 1,100 Indigenous youth across Canada. CIEDAR will partner with CAP for future survey dissemination and project pilots.
- CIEDAR has partnerships with Māori communities, organizations and researchers in New Zealand (Massey University, University of Waikato, the Ngāti Apa research group, Te Atawhai o Te Ao, Te Runanga o Nga Wairiki and the Kakano Rangatahi). CIEDAR Leadership presented at the Community Kōrero at Tū Tama Wahine o Taranaki and the Ngāti Tāwhirikura at the Land and Sea symposium in November 2022. CIEDAR team presented at Tangata Whenua Tangata Ora project team gathering and Te Ruunanga o Ngaa Wairiki Ngaati Apa, Marton. Rising Before Dawn Indigenous Learnings from the Pandemic
- Hosting a dialogue session in June 2023 highlighting Indigenous peoples' experiences and triumphs on Turtle Island (Hearing Indigenous Voices during the COVID-19 Pandemic). The session will be an opportunity for Canadian and



US researchers to discuss the lessons learned about research and Indigenous resilience during the COVID-19 pandemic.

- 3 UBC awards to support our activities: UBC Health HIFI award (\$25k) to support our work in the NWT, and for our upcoming international symposium: UBC Health after 2020 award (\$10k), Indigenous cluster pilot award (\$10k).
- Awarded two NSERC Undergraduate research awards to fund a summer research placement for two Indigenous students.
- CIEDAR developed a partnership with the UBC Indigenous Land-Based Health Wellness Research & Education Cluster. CIEDAR was a part of the panel discussion at the UBC Land-based symposium, "Pathways to Regenerative Indigenous Land-Based Research & Education."
- CIEDAR developed partnership with UBC Centre for Teaching, Learning and Technology (CTLT) Indigenous Initiatives.
- CIEDAR will host a webinar Summer 2023. It will be carried out in partnership with the Global Alliance for Genomics and Health, Genome Canada, and CanCOGeN and will cover topics related to health equity.
- In July 2022 CIEDAR hosted a community beading event, Bead in the Park. In partnership with Indigenous beader Breanna Deis we facilitated a beading workshop in Central Park, Burnaby.
- CIEDAR facilitated a workshop at the Gathering Our Voices Indigenous Youth Leadership Training Conference in Vancouver March 2023.

Summary of Publications

Title	Hyperlink	Type of Article	Lay Summary		
			Request ed	Receiv ed	Sent to Comms
Understanding the associations among social vulnerabilities, Indigenous Peoples, and COVID-19 cases within Canadian health regions	https://www.mdpi.com/1660-4601/19/19/12409/htm	Manuscript/Sub mitted	YES	YES	YES



Wastewater testing in Indigenous communities - a scoping review to provide insight on the feasibility in Indigenous communities		Manuscript/In preparation	N/A	N/A	N/A
Community engagement - Designing and developing Indigenous land-based healing camp		Manuscript/In preparation	N/A	N/A	N/A
A conceptual framework on how to engage with Indigenous communities.		Manuscript/In preparation	N/A	N/A	N/A
Attitudes toward COVID-19 public health mandates: A Canada & US comparison		Data being collected	N/A	N/A	N/A
Social Media as Community Engagement for Indigenous Populations		Data being collected	N/A	N/A	N/A



Pillar 8- Public Health, Health Systems and Social Policy Impacts

Progress Report

May 2023

Nazeem Muhajarine, Co-Lead

Cory Neudorf, Co-Lead

Cheryl A. Camillo, Deputy

Doug Manuel, Deputy

Andrew Morris, Deputy



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VISION STATEMENT

Our vision is to create a healthier and more resilient Canada through the integration of cutting-edge research and innovative approaches to public health, health systems, and social policy impacts. We strive to empower public health and health systems actors with the most up-to-date and contextually relevant information on variants of concerns (VOC) in order to enhance preparedness, prevention, and response to emerging threats. By bridging the gap between CoVaRR-Net and the diverse public health systems in Canada, we aim to facilitate collaboration, knowledge-sharing, and evidence-based decision-making for the benefit of all Canadians.

PILLAR 8 TEAM

Co-Lead	Muhajarine Nazeem	Mehdiyeva Khatira	Coordinator
		Singh Sarbjeet	Public Health Researcher
Co-Lead	Neudorf Cory	Bandara Thilina	PhD
		Ali Bukhari	Research Analyst
		Sakshi Goyal	Research Assistant
Deputy	Camillo Cheryl	Allin Sara	PhD
		Fitzpatrick Tiffany	PhD
		Roerig Monika	Research Associate
Deputy	Manuel Doug	Bennett Carol	Public Health Researcher
		Thomson Mathew	Public Health Researcher
Deputy	Morris Andrew	Barrett Lisa	PhD
		Bonnar Paul	PhD
		Ramsey Tasha	PhD



PROJECT 1. CREATING THE STANDARDS FOR A NATIONAL VACCINE EFFECTIVENESS SENTINEL SURVEILLANCE SYSTEM

Introduction

Sentinel surveillance is a form of surveillance that provides early information to advise actors on issues of specific interest. Written records date carefully established sentinel surveillance systems to at least as early as the late ancient Roman Empires,[1] and the method is used in a variety of fields including politics, military, and health. In public health, sentinel surveillance typically selects a method to collect health information that allows quicker insight regarding a public health event, a condition, or an intervention, that would afford stakeholders the opportunity to respond to initial information before universal, general surveillance becomes available.

Today's sentinel surveillance systems in public health can take many forms. For example, one of the most common methods of performing sentinel surveillance is gathering surveillance data from a selected group of healthcare workers who have agreed to make timely reports on a very specific issue. Other methods to perform sentinel surveillance can potentially take a variety of forms including automated electronic medical record analysis,[2] self-reporting on web or mobile applications,[3] wastewater detection,[4] or even implantable monitoring devices with wireless communication capabilities,[5] and more.

With regards to SARS-COV-2, understanding the real-world effectiveness of the various vaccines and their regimens is a pressing priority to help assess risk of individual and population-level infections. At this stage of the pandemic, and transitioning into a possible endemic period, this is now an imperative. We know of several individual attempts to assess vaccine effectiveness across Canada. With the help of public health data custodians and researchers from across Canada, our intention is to learn from these isolated studies to help develop standards and protocols towards a routinized vaccine effectiveness sentinel surveillance system.

Research questions

1. With what level of coherence do different jurisdictions across Canada link COVID-19 vaccine registries to COVID-19 outcome data?
2. What are common protocols used in attempts to understand COVID-19 vaccine effectiveness?
3. What level of infrastructure is required to develop a routine sentinel surveillance system for COVID-19 vaccine effectiveness?



Activities

- Assess the COVID-19 vaccine effectiveness landscape to understand the scope of the work already done.
- Engage experts in the area to engage in a Delphi process to reach consensus on standards for vaccine effectiveness.
- Assess feasibility of a COVID-19 vaccine effectiveness sentinel surveillance system.

Completed Activities

- Engaged several parties already engaged in the work to assess metrics used in COVID-19 vaccine effectiveness.
- Consulted with other pillars in CoVaRR-Net working on the feasibility of doing meta-analysis of vaccine efficacy data from across Canada. Determined that further public health input to this topic would be better placed after that work is complete or further along so as to reduce duplication.
- Conducted mathematical and epidemiological analysis on sub-provincial COVID-19 outcome trend data to characterize 120 distinct COVID-19 wave patterns from across the country.
- Through consultation with UPHN membership, have decided that priority around this topic has waned, and larger issues around *immunization equity* are of more interest as immunization program planning requires more equity-based evidence.
- Engaged with National Collaborating Centre for the Determinants of Health to collaboratively leverage their access to knowledge translation and public health workforce expertise.

Upcoming Activities

- Developing a primary data collection strategy to survey Canadians regarding immunization status; knowledge, attitudes and beliefs regarding immunizations, and sociodemographic variables. Through representative sampling, we will conduct a stratified equity-based analysis of self-reported immunization status across Canada to detect inequities.
- Conduct further analysis on COVID-19 wave patterns to assess clusters and determinants of waves across the country.

Manuscripts in Progress:

Bukhari A, Muhajarine N, Plante C, Bandara T, Neudorf C. (2023) Spatiotemporal Variation in The Experience of the COVID-19 Pandemic in Canada: A Cross-Regional Comparison of Local Wave Patterns.



PROJECT 2. LESSONS LEARNED FROM PROVINCIAL/TERRITORIAL VACCINATION CAMPAIGNS

Introduction

Vaccination with primary and booster doses is key to ending the COVID-19 pandemic. In fact, with Canadian jurisdictions having rescinded public health orders requiring proof of vaccination, masking, and social distancing, vaccination is now the primary tool to protect the public. The uptake of first and second doses of COVID vaccines and boosters has slowed across Canada. Vaccination rates are significantly lower in certain provinces/territories and amongst certain vulnerable groups. Behavioural public administration research shows that the ways in which health services, such as immunizations, are administered impacts patient access. Yet, research examining vaccine administration across Canada is lacking. Throughout the pandemic, each province and territory (PT) has administered vaccinations differently. Gathering policy data on these approaches while considering the epidemiological outcomes and effectiveness through jurisdictional comparison will facilitate evidenced-based decision making for future pandemics.

Our Social Policy team is building on our first year of collaborative research performed with the North American Observatory on Health Systems and Policies (NAO) at the University of Toronto to analyze in depth how each PT administered vaccinations, including how the provincial health systems made decisions, allocated workforce, partnered with the non-profit and private sectors, and notified the public of appointment availability. Our research entails: collecting comprehensive baseline data describing components of each PT's vaccine administration; producing reports summarizing each PT's vaccine administration; and, performing analyses of the determinants of PT vaccine administration approaches, including the extent to which factors like the organization of the health system, governance of public health, scientific advice, population distribution, and other variables influenced the effectiveness of vaccine administration. In Year 1, we completed data collection for four diverse provinces (British Columbia, Nova Scotia, Ontario, and Saskatchewan), have released two reports (Ontario and Saskatchewan), and have prepared the remaining two reports for publication. We also performed, presented, and published a comparative descriptive analysis that shows significant differences in provincial rollout campaigns that may have impacted outcomes, such as vaccination rates. Our ongoing research will identify lessons and promising practices for administering COVID-19 vaccinations to Canadians, and sub-groups thereof, and will also provide insights into planning for future pandemics.



Activities

- Vaccine administration reports for each PT. Updated reports for the first 4 provinces and the remaining nine PT reports will be published on the CoVaRR-Net and NAO websites.
- Multiple peer-reviewed journals accessible to public health researchers and decision-makers. Revise and resubmit a promising second manuscript employing an event study to understand the effects of provincial proof of vaccination policies on vaccination uptake; Additional manuscripts will examine the effects of provincial health system governance on vaccination campaign approaches and/or health policy statutes on access to vaccines.
- Presentations of findings to additional audiences, including at scientific research conferences. These may include the Canadian Association for Health Services and Policy Research (abstract submitted), the Canadian Public Health Association's (CPHA) Public Health Conference, European Public Health Conference, and a public lecture (e.g., as part of the NAO Lecture series).

Completed Activities

Published manuscript:

Fitzpatrick, T., Camillo, C.A., Hillis, S., Habbick, M., Roerig, M., Muhajarine, N., & Allin, S. (2023). Comparison of COVID-19 vaccination rollout approaches across Canada: Case studies of four diverse provinces. *Health Reform Observer – Observatoire des Réformes de Santé* 11(1): 1-27. DOI: 10.13162/hro-ors.v11i1.5118

Completed presentations since 2022:

Fitzpatrick, T., Camillo, C.A., Hillis, S., Habbick, M., Roerig, M., Muhajarine N., & Allin, S. (2023). The Impact of Provincial Proof of Vaccination Policies on Age-specific Uptake of First Doses of COVID-19 Vaccines in Canada (poster presentation by T. Fitzpatrick). Canadian Immunization Conference (CIC), Ottawa, Ontario, April 25-27, 2023.

Camillo, C.A. (2022). Differences in Provincial/Territorial Roll-out of COVID-19 Vaccination Campaign Programs: Findings from Pilot Case Studies, Canadian Association for Immunization Research, Evaluation and Education (CAIRE) Research Sponsor Advisory Board Meeting, Toronto, Ontario (and virtually), November 30, 2022.

Fitzpatrick, T., & Camillo, C.A. (2022). The Impact of Provincial Proof of Vaccination (POV) Policies on Age-Specific First Dose Uptake Of Covid-19 Vaccines In Canada, Urban Public Health Network (UPHN)/Public Health Physicians of Canada (PPHC) Biannual Meeting, Toronto, Ontario (and virtually), October 14, 2022.



Camillo, C.A., Allin, S., Fitzpatrick, T., Habbick, M., Mauer-Vakil, D., Muhajarine N., Roerig, M., & Rowein, S. (2022). Comparing the Goals, Governance, Policies, and Communication Strategies of Provincial COVID-19 Vaccination Campaigns (oral presentation by C.A. Camillo). Public Health 2022 Virtual Conference, June 15, 2022.

Rowein, S., Camillo, C.A., Allin, S., Fitzpatrick, T., Habbick, M., Mauer-Vakil, D., Muhajarine N., & Roerig, M. (2022). Comparison of COVID-19 Vaccination Rollout Approaches across Canada: Case Studies from Four Diverse Provinces (oral presentation by S. Rowein). Virtually CAHSPR 2022, June 1, 2022.

Camillo, C.A. (2022). COVID-19 Lessons Learned from Provincial/Territorial Vaccination Campaigns, VOC (COVID-19 Variants of Concern) Expert-User Panel, Regina, Saskatchewan, March 3, 2022.

Upcoming Activities

Camillo, C.A. (2023). The Impact of Provincial Proof of Vaccination (POV) Policies on Age-Specific First Dose Uptake Of Covid-19 Vaccines In Canada, CoVaRR-Net Spring 2023 Meeting, Ottawa, Ontario (and virtually), May 17, 2023.

Advance of case study reports through internal and external peer review and copyediting.

Manuscripts in Progress:

Fitzpatrick, T., Camillo, C.A., Hillis, S., Habbick, M., Roerig, M., Muhajarine, N., & Allin, S. (under second round of revision for journal). "The impact of provincial proof of vaccination policies on age-specific first doses uptake of COVID-19 vaccines in Canada."



PROJECT 3. EQUITY-BASED ANALYSIS ON THE EFFECTS OF THE COVID-19 RESPONSE IN CANADA¹

*(formerly CoVaRR-Net and MIICOVAC project)

Pillar 8 analysis of public health policies² has highlighted the significant diversity in responses across the country, revealing that the borders of policy initiatives often differ from our traditional jurisdictional understanding of our federal system. Similarly, our epidemiological research has demonstrated that COVID-19 outcomes varied depending on the jurisdictional levels and the timing of the pandemic. Our comparative analyses of vaccination campaigns have identified, similarly, a variety of approaches to encouraging vaccine uptake, and balancing vaccination and reopening schemes.

This leads us to an important question about which populations have benefited the most, and the least, from exposure to policy-based interventions. To answer this question and taking advantage of the work Pillar 8 have been engaged in, we will conduct a retrospective population study that focuses on equity and engages Canadians themselves. Our approach consists of three steps.

Step 1. Using our provincial level COVID-19 vaccine policy analysis we will develop a survey sampling frame that identifies jurisdictions that represent a spectrum of policy regimes related to COVID-19. Having a sampling frame that is informed by the spectrum of policy regimes is important as it enhances our ability to learn what worked or didn't work, and at the same time conduct research efficiently and use appropriate resources.

Step 2. The sampling frame will help us draw adequate samples from within-province/territory for a population survey. We will engage a national polling firm with online and telephone survey capacity to survey Canadians. The survey will take into account previous national population surveys (e.g., Canadian COVID-19 Antibody and Health Survey, CCAHS) to enable comparability, and include factors such as:

- Socio-demographic information
- Vaccination status
- Knowledge, beliefs and attitudes with regards to vaccinations
- Trust in government measurements
- General public health knowledge
- Collectivist vs. individualistic world views

¹ Previously, in Y2, we proposed this project, in part, as 'CoVaRR-Net and MIICOVAC' project. We made some progress in implementing the project, and due to key personnel changes, we had to transition the project in Y3 that is broader and much more relevant to this stage of the pandemic (in 2023) in Canada.

² Used here to mean broad policies to prevent, mitigate and contain spread of SARS-CoV-2 virus and uptake of COVID-19 vaccinations, at sub-national levels including regional (e.g., Canadian Atlantic provinces), provincial/territorial and within-province regions.



Step 3. Survey data will be complemented by a series of focus group conversations at local public health unit levels (UPHN and NCCDH will be key partners) to add more depth of understanding.

Partners This work will be cross-pillar and involve external partners. We will follow up with CIEDER (P7) and EDI within CoVaRR-Net and external partners: Urban Public Health Network and National Collaborative Centre for Determinants of Health.

By combining policy, epidemiological and vaccination data with a representative survey sample, and complement this with qualitative data, we can assess the equity-based impact of COVID-19 responses across the country. Understanding these differential responses to interventions across populations is essential for effective local public health preparedness. With this knowledge, public health agencies can proactively engage communities and relevant partners to promote resilience in the future.





KITT Vision Statement

- KITT (Pillar 9, Knowledge, Implementation, and Training Team) supports the development and application of research evidence to address the challenges of pandemics.
- Brings together expertise from a range of disciplines, including implementation science, behavioural and social sciences, knowledge synthesis, and health policy.
- We coordinate, facilitate, support, and accelerate the design and execution of applied health research projects to contribute to ending the current pandemic and to create a stronger and more nimble system in Canada to be able to respond to future pandemics and public health challenges.
- Our aim is to maintain our existing knowledge assets and create implementable evidence-informed resources that will support decision making among basic scientists, clinicians, public health leaders, and decision-makers.

Pillar 9: CoVaRR-Net's Knowledge, Implementation, and Training Team (KITT) Research Update

KITT (P9) Year 1 Report

Infrastructure

- 1 full-time research manager.
- 1 full-time research assistant.

Project: Developing an Asset Map of COVID-19 Research Funded Across Canada

Aim: To systematically review the allocation of Canadian research investments targeting COVID-19, and to produce a readily accessible inventory of various research resources available in Canada that can be leveraged to ensure informational needs among various stakeholders can be quickly addressed.

Status:

- Data extraction and interpretation completed.
- Manuscript currently in preparation

Anticipated completion:

- On-going

Project: Mapping the Evidence Regarding SARS-CoV-2 VOCs

Aim: To synthesize high-quality information on SARS-CoV-2 VOCs available from reviews in existing COVID-19 inventories.



Status:

- Complete review of COVID-END database up to August 1, 2021
- Preliminary report written describing findings

Anticipated completion:

- Complete as of August 2021, with potential to update as needed.

Project: Establishing a SARS-CoV-2 VOC Knowledge Intelligence Service

Aim: To adapt an existing multipronged search engine to retrieve daily sound evidence from the published literature, pre-prints, and various websites regarding VOCs, and to generate a weekly/bi-weekly digest of relevant literature regarding the VOCs that can be circulated to CoVaRR-Net members and external stakeholders as necessary.

Status:

- Infrastructure adaption completed
- Collaboration with colleagues from basic science research disciplines is underway to refine searching algorithm and quality appraisal of retrieved studies.
- Next steps: pilot testing and generation of weekly/bi-weekly reports

Anticipated completion:

- On-going

Project: Conducting and Maintaining Living Evidence Syntheses Regarding SARS-CoV-2 VOCs

Aim: To support the maintenance of three living systematic reviews that are relevant to VOCs: (1) public health measures, (2) health system arrangements, (3) vaccine efficacy and effectiveness.

Status:

- Several updates to each review completed between June and December 2021
- Reports are available on the COVID-END website

Anticipated completion:

- Complete as of December 2021
 - Ongoing updates to vaccine efficacy and effectiveness review, but not directly funded by CoVaRR-Net



Project: Digital Health Communication Tool for COVID-19

Aim: To adapt an existing web application as a means for communicating evidence about VOCs to the Canadian public.

Status:

- Infrastructure has been developed to support the addition of new data regarding VOCs.
- Post-doctoral student hired to parameterize the application
- Protocol for incorporating new data from modelling studies in progress.

Anticipated completion:

- September 2022 for the pilot launch of web app

KITT (P9) Year 2 Report

Infrastructure

- Hired 1 full-time research manager (maternity leave replacement), 1 full-time research associate, and 1 full-time research coordinator.
- Partners with many high quality personnel.

Carry Over Projects Year 1

Project: Establishing a SARS-CoV-2 VOC Knowledge Intelligence Service

Aim: To adapt an existing multipronged search engine to retrieve daily sound evidence from the published literature, pre-prints, and various websites regarding VOCs, and to generate a weekly/bi-weekly digest of relevant literature regarding the VOCs that can be circulated to CoVaRR-Net members and external stakeholders as necessary.

Status:

- Infrastructure adaption completed
- Collaboration with colleagues from basic science research disciplines is underway to refine searching algorithm and quality appraisal of retrieved studies.
- Next steps: pilot testing and generation of weekly/bi-weekly reports

Anticipated completion:

- On-going
- Discuss continuation with Executive Team for 2023-2024 season



Project: Conducting and Maintaining Living Evidence Syntheses Regarding SARS-CoV-2 VOCs

Aim: To support the maintenance of three living systematic reviews that are relevant to VOCs: (1) public health measures, (2) health system arrangements, (3) vaccine efficacy and effectiveness. Partners: uOttawa, Dalhousie University, McMaster University.

Status:

- Several updates to each review completed between June and December 2021
- Reports are available on the COVID-END website

Anticipated completion:

- Pivot to long-term invested projects (see below).

New Projects Year 2

Project: Monkeypox Living Evidence Profile

Aim: This living evidence profile was requested by the Public Health Agency of Canada to evaluate accumulating evidence related to the animal and zoonotic characteristics of the monkeypox virus, understand their relevance to the Canadian context, and to identify important gaps in literature. Partners: Public Health Agency of Canada, Knowledge Synthesis and Application Unit

Status:

- We updated the LEP every two weeks from July 2022 to October 2022.
- Completed the final iteration of the LEP in December 2022
 - All eight iterations are published on the CoVaRR-Net website.
- Review registered on PROSPERO (registration number: CRD42022349554)
- Abstract submitted to the *Journal of Infectious Diseases*.
 - Manuscript expected to be completed by June 2023.
- Findings will be presented at the CoVaRR-Net Spring Meeting in Ottawa, May 2023

Anticipated completion:

- Summer 2023 for publication submitted.
- Project Complete.

Project: CoVaRR-Net Webinar Series

Aim: To provide CoVaRR-Net pillars the opportunity to showcase their work and achievements, and to increase understanding of the importance of knowledge synthesis and implementation science in the context of a pandemic.



Status:

- We facilitated monthly webinars from September 2022 to March 2023.
 - Presentations from six pillars (Pillar 3, 5, 7, 9, BioBank Team, and EDI&I).
- All webinar recordings posted on the CoVaRR-Net website.

Anticipated completion:

- Completed year 1 as of March 2023
- Discuss continuation with Executive Team for 2023-2024 season

Project: A Living Evidence Approach to Variants of Concern (VOC) and COVID-19 Vaccine Effectiveness

Aim: The rapid development of safe and effective COVID-19 vaccines is an outstanding scientific achievement. As an increasing proportion of the world's population is vaccinated, societies have begun to regain some form of normalcy. However, it is evident that COVID-19 will remain a serious public health concern in the coming years, largely driven by variants of concern (VOC - virus mutants with increased spread and/or severity of disease and/or decreased vaccine effectiveness). In many regions the majority of new cases are due to VOC. It is critical to continuously monitor vaccine effectiveness against VOC (e.g., breakthrough infection rates, transmission rates). Systematic surveillance of the rapidly evolving scientific evidence base is necessary to inform public health action and research aimed at improving the use of current vaccines and development of vaccines and/or boosters. Living evidence syntheses use rigorous scientific methods to identify, appraise and summarize evidence, proving their usefulness when the evidence base is rapidly developing and bears significant policy and practice importance. Since April 2021 we have deployed 15 editions of a living review to determine the effects of VOC on vaccine efficacy, regularly communicated to PHAC and HC among others. We will extend this series, also considering additional outcomes such as immunity duration, new emerging VOC and new vaccines. We will search the global peer-reviewed and grey literature daily, update our synopsis weekly, and our full synthesis on at least a monthly basis. We will maintain an up-to-date summary of our findings on our website (including plain language summaries in French and English) and disseminate at least monthly briefings through our networks of decision makers. Our living evidence summaries will ensure that citizens, healthcare and public health professionals and policy makers continue to have access to timely trustworthy evidence to inform their decisions in response to VOCs.

Status:

- On-going project.



- CoVaRR-Net funding amplified by CIHR grant (PIs Little, Iorio, CIs Brouwers, Grimshaw, et al, \$475,940)

Anticipated completion:

- On-going into Year 3

Project: Innovations in Behavioural Sciences Related to Public Health Measures

Aim: To understand factors contributing to adherence and acceptance towards public health measures and vaccination, and to use behavioural perspectives to create public-facing materials that will maximize adherence and acceptance towards COVID-19 measures and future pandemics. Partner Ottawa Hospital Research Unit (OHRI), Public Health Agency of Canada.

Status:

- Preparation and publication of rapid reviews
- Currently maintaining the currency of the living systematic reviews
- Explore expansion to (a) interventions to increase perceptions of trustworthiness and (b) interventions to mitigate misinformation
- CIHR grant to be submitted

Anticipated completion:

- On-going into Year 3

Project: Establishing a SARS-CoV-2 VOC Knowledge Intelligence Service

Aim: To adapt an existing multipronged search engine to retrieve daily sound evidence from the published literature, pre-prints, and various websites regarding VOCs, and to generate a weekly/bi-weekly digest of relevant literature regarding the VOCs that can be circulated to CoVaRR-Net members and external stakeholders as necessary.

Status:

- Infrastructure adaption completed
- Collaboration with colleagues from basic science research disciplines is underway to refine searching algorithm and quality appraisal of retrieved studies.
- Next steps: pilot testing and generation of weekly/bi-weekly reports

Anticipated completion:

- On-going
- Discuss continuation with Executive Team for Year 3.



Project: Vaccine Effectiveness Risk of Bias Tool

Aim: Living evidence synthesis work of Pillar 9 revealed significant variation in the quality of vaccine effectiveness trials. Poor quality trials can lead to poor quality decisions. However, there is currently no international standard to evaluate the risk of bias of vaccine effectiveness trials. This project aims to fill this gap by using international methods comprised of three stages to develop a RoB tool for these studies. This will then lead to a vaccine effectiveness trials toolkit and study reporting guideline.

Status:

- Protocol developed
- Partners identified
- Research staff hired and student recruited
- Plans for a research grant submission to augment and accelerate this project have been sorted to support stages 2 and 3

Anticipated completion:

- On-going to year 3 and beyond.

Project: Repository of Training Modules for Pandemic Preparedness and Management & Clinical Practice Guidelines and Behavioural-Action Guidance

- Pivot to other priorities in response to existing coverage (training) and disbandment of science tables and receptors for guidance.

Summary of Publications

Title	Hyperlink	Type of article	Lay Summary + publication posted to website
Zoonotic Characteristics of Monkeypox: Living Evidence Profile	https://covarnet.ca/zoonotic-characteristics-of-monkeypox-living-evidence-profile/	Living evidence profile (type of systematic review); manuscript in progress	CoVaRR-Net website
Public Health Implications of SARS-CoV-2 Variants of Concern	https://sporevidencealliance.ca/wp-content/uploads/2021/12/Deliverable-7-Public-Health_Report-Updated_2021.12.03.pdf	Living synthesis (type of systematic review)	COVID-END website



Understanding the role of personal risk perceptions during the COVID-19 pandemic: a rapid behavioural science evidence synthesis	https://www.mcmasterforum.org/docs/default-source/product-documents/rapid-responses/riskperceptionsarossacovid-19pandemic.pdf?sfvrsn=7da9dd7f_5	Rapid evidence synthesis	COVID-END website
COVID-19 Living Evidence Synthesis 6.41 - What is the efficacy and effectiveness of available COVID-19 vaccines in general and specifically for variants of concern	https://www.mcmasterforum.org/docs/default-source/product-documents/living-evidence-syntheses/covid-19-living-evidence-synthesis-6.41---what-is-the-efficacy-and-effectiveness-of-available-covid-19-vaccines-in-general-and-specifically-for-variants-of-concern.pdf?sfvrsn=6a3ce34c_5	Living evidence synthesis (type of systematic review)	COVID-END website
Factors affecting COVID-19 vaccination acceptance and uptake among the general public: a living behavioural science evidence synthesis	https://www.researchgate.net/publication/353045219_Gen_Pub_Vaccination_Living_Behavioural_Science_Evidence_Synthesis_v2_May_31	Living Evidence Review	Research Gate.



Behavioural determinants of COVID-19 vaccination acceptance among healthcare workers: a rapid review. Public Health. 2022 Jun 14.	https://www.sciencedirect.com/science/article/pii/S0033350622001603	Rapid Review	
Health system impacts of SARS-CoV-2 variants of concern: a rapid review.	https://doi.org/10.1186/s12913-022-07847-0	Living Systematic Review	
Public health implications of SARS-CoV-2 variants of concern: a rapid scoping review	https://bmjopen.bmj.com/content/11/12/e055781.abstract	Rapid Review	



PILLAR 10: ANTIVIRAL STRATEGIES & ANTIVIRAL THERAPEUTICS

RESEARCH REPORT: MARCH 31, 2023

Y2 REPORTING PERIOD: APRIL 1, 2022 TO MARCH 31, 2023

VISION:

As SARS-CoV-2 Omicron variants continue to dominate globally and accumulate novel mutations leading to immune evasion, antiviral treatments are becoming increasingly important to limit COVID-19 hospitalizations and deaths. The drug resistance against the few available antivirals against SARS-CoV-2 has not been well studied, however the potential for drug resistance to develop is high for a rapidly mutating virus, such as SARS-CoV-2. Moreover, studies are still lacking to understand which combination therapy approaches will have the greatest potency and synergy against SARS-CoV-2 Omicron sub-variants.

In Year 2, we identified priority projects directed at performing pre-clinical studies of novel pan-coronavirus lead antivirals to counteract drug resistance and to study promising combination antiviral therapies with antivirals approved by Health Canada—remdesivir and paxlovid.

- Identify and profile leading antivirals effectiveness alone or in combination with direct-acting antivirals (DAA) approved by Health Canada (e.g., remdesivir and Paxlovid, against emerging SARS-CoV-2 Omicron variants
- Use cell-based assays to understand how current antiviral approaches may be improved, when combined with leading clinical candidates for novel antivirals targeted at host proteins (host-directed antivirals; HDA) needed for the viruses to enter cells, or replicate, and regulate the host immune response.
- Study and test how viral mutations impact the viral fitness, susceptibility to antivirals, and how the mutations affect binding of antiviral drugs to their targets at the atomic level, and potentially impact innate immune signalling.

This work is providing molecular insights into promising pan-coronavirus lead drugs; and unraveling potential combination antiviral therapies—based on HDAs and DAAs acting synergistically—as alternatives to monotherapy thereby improving COVID-19 treatment and reducing the risk of antiviral drug resistance.



COVARR-NET PILLAR 10 TEAM		
LEAD	François Jean, PhD	University of British Columbia
DEPUTY	Nathalie Grandvaux, PhD	Université de Montréal
DEPUTY	Natalie Strynadka, PhD	University of British Columbia
DEPUTY	Deanna Santer, PhD	University of Manitoba
MEMBER	Rob Kozak, PhD	SUNNYBROOK HEALTH SCIENCE CENTRE
MEMBER	Prameet Sheth, PhD	QUEEN'S UNIVERSITY
MEMBER	Joyce Wilson, PhD	UNIVERSITY OF SASKATCHEWAN
COLLABORATOR	MEL KRAJDEN, MD	BCCDC
COLLABORATOR	IOANNIS RAGOUSIS, PHD	PILLAR 5 LEAD; MCGILL UNIVERSITY
COORDINATOR	CARL PEREZ, PHD	University of British Columbia

Year 2 Publications:

Title: A TMPRSS2 inhibitor acts as a pan-SARS-CoV-2 prophylactic and therapeutic.

- **Shapira, T.**, Abrey, I. M., Dion, S. P., Buchholz, D. W., Imbiakha, B., **Olmstead, A. D.**, Jager, M., Désilets, A., **Gao, G.**, Martins, M., Vandal, T., **Thompson, C. A. H.**, **Chin, A.**, Rees, W. D., Steiner, T., Nabi, I. R., Marsault, E., Sahler, J., Diel, D. G., Van de Walle, G. R., August, A., Whittaker, G. R., Boudreault, P. L., Leduc, R., Aguilar, H. C., and **Jean, F.**
- **Nature** 2022 May; 605(7909):340-348. doi: 10.1038/s41586-022-04661-w.
- Impact Factor: 69.5; **F. Jean:** Corresponding author
- <https://news.ubc.ca/2022/03/28/new-nasal-spray-treats-delta-variant-infection-in-mice-indicating-broad-spectrum-results/>
 - We report on N-0385, a NOVEL potent small-molecule protease inhibitor of human TMPRSS2 and the first SARS-CoV-2 pan-variant host-directed antiviral (HAD) effective in vivo against the Delta B.1.617.2 VOC.
 - The nanomolar potency of N-0385 against SARS-CoV-2 infection in human Calu-3 cells and patient-derived colonoids without detectable toxicity yields a striking selectivity index of $>10^6$.
 - Furthermore, in the K18-hACE2 mouse model, treating with N-0385 resulted in complete protection against SARS-CoV-2 induced mortality and significantly protected against weight loss, lung pathology, and viral infection when treatment occurred at the time of, or 12 hours after, infection with the SARS-CoV-2 B.1.617.2, suggesting that N-0385 may provide a novel effective early treatment option against emerging SARS-CoV-2 VOCs.



Title: Discovery of lead natural products for developing pan-SARS-CoV-2 therapeutics.

- **Pérez-Vargas J, Shapira T, Olmstead AD, Villanueva I, Thompson CAH**, Ennis S, **Gao G, De Guzman J**, Williams DE, Wang M, **Chin A, Bautista-Sánchez D**, Agafitei O, Levett P, Xie X, Nuzzo G, Freire VF, Quintana-Bulla JI, Bernardi DI, Gubiani JR, Suthiphasilp V, Raksat A, Meesakul P, Polbuppha I, Cheenpracha S, Jaidee W, Kanokmedhakul K, Yenjai C, Chaayosang B, Teles HL, Manzo E, Fontana A, Leduc R, Boudreault PL, Berlinck RGS, Laphookhieo S, Kanokmedhakul S, Tietjen I, Cherkasov A, Krajden M, Nabi IR, Niikura M, Shi PY, Andersen RJ, **Jean F**.
- **Antiviral Res.** 2023 Jan; 209:105484. doi: 10.1016/j.antiviral.2022.105484. Epub 2022 Dec 8.
- Impact Factor: 10.018; **F. Jean**: Corresponding author
- <https://news.ubc.ca/2023/01/09/b-c-sea-sponge-has-covid-blocking-powers/>
 - Applied cell-based high-content screening of a natural product (NP) library of 373 compounds to identify new antivirals for SARS-CoV-2 infection.
 - Identified > 70 NPs with anti-SARS-CoV-2 activity (hit rate ~20%) including three NPs (holyrine A, alotaketol C, & bafilomycin D) with EC₅₀ values in the nanomolar range.
 - Demonstrated the pan-SARS-CoV-2 activity of holyrine A, alotaketol C, and bafilomycin D against SARS-CoV-2 highly transmissible Omicron subvariants (BA.5, BA.2 and BA.1) and highly pathogenic Delta VOCs in human Calu-3 lung cells.
 - Demonstrated the potential of NPs with highly diverse chemical structures for discovering pan-SARS-CoV-2 therapeutics including host-directed antivirals.

Title: Berbamine suppresses intestinal SARS-CoV-2 infection via a BNIP3-dependent autophagy blockade.

- Cloherty AP, Rader AG, Patel KS, **Perez-Vargas J, Thompson CA**, Ennis S, Niikura M, Wildenberg ME, Muncan V, Schreurs RR, **Jean F**, Ribeiro CM.
- **Emerg Microbes Infect.** 2023 Mar 23:2195020. doi: 10.1080/22221751.2023.2195020. Online ahead of print.
- Impact Factor: 19.568; **F. Jean**: Corresponding author
- <https://covarnet.ca/cross-continental-collaboration-identifies-new-covid-blocking-natural-product/>
 - Identified berbamine dihydrochloride as a potent inhibitor of SARS-CoV-2 entry and replication in human intestinal epithelial cells
 - Demonstrated that berbamine, a molecule derived from traditional Chinese medicinal herbs, is a promising option for a cost-effective, over-the-counter antiviral that could be fast-tracked to reach COVID-19 patients.



Title: DEEMD: Drug Efficacy Estimation Against SARS-CoV-2 Based on Cell Morphology With Deep Multiple Instance Learning.

- Saberian MS, Moriarty KP, **Olmstead AD**, Hallgrimson C, **Jean F**, Nabi IR, Libbrecht MW, Hamarneh G.
- **IEEE Trans Med Imaging**. 2022 Nov;41(11):3128-3145. doi: 10.1109/TMI.2022.3178523. Epub 2022 Oct 27.
- Impact factor: 11.037.
 - DEEMD identified known SARS-CoV-2 inhibitors, such as remdesivir and aloxistatin, supporting the validity of our approach.
 - DEEMD can be explored for use on other emerging viruses and datasets to rapidly identify candidate antiviral treatments in the future.

Y3 Manuscripts in Preparation

Title: Targeted 3C-like protease inhibitors as broad-spectrum antivirals for SARS-CoV-2 variants of concern.

- **Jimena Perez-Vargas**, Liam J. Worrall, **Andrea D. Olmstead**, Anh-Tien Ton, Jaeyong Lee, **Ivan Villanueva**, **Connor A. H. Thompson**, **Svenja Dudek**, Siobhan Ennis, Jason R. Smith, **Tirosh Shapira**, **Joshua De Guzman**, **Ellie Gang**, Fuqiang Ban, Marija Vuckovic, Michael Bielecki, Suzana Kovacic, Calem Kenward, Paul N. Levett, **Mel Krajden**, Richard Leduc, Pierre-Luc Boudreault, Masahiro Niikura, Mark Paetzel, Robert N. Young, Artem Cherkasov, **Natalie C. J. Strynadka**, **François Jean**.
- **In preparation: *Emerg Microbes Infect.***
- Impact Factor: 19.56; **F. Jean**: Corresponding author
- Complete ED50 determination of XBB.1.5. and emerging XBB.1.6 in human airway air-liquid interface (ALI cultures) and studies of synergistic combinations.
 - Developed and validated a novel class of 3CLpro inhibitors needed to address the urgent need for improved therapeutics against current and future SARS-CoV-2 variants
 - The co-crystal structures of SARS-CoV-2 3CLpro complexed with our new inhibitors provide useful insight to optimize inhibitory and pharmacokinetic profiles for further development.

Title: N-0385, a highly potent TMPRSS2 inhibitor, shows high antiviral activity against SARS-CoV-2 omicron subvariants and acts synergistically in combination with Paxlovid and Remdesivir.

- **Jimena Perez-Vargas**, **Ivan Villanueva**, **Connor A. H. Thompson**, Siobhan Ennis, Richard Leduc, Pierre-Luc Boudreault, Masahiro Niikura, **François Jean**.
- **In preparation: *Emerg. Microbes Infect.***
- Impact Factor: 19.56; **F Jean**: Corresponding author



- Complete ED50 determination of XBB.1.5 and emerging XBB.1.6 in human airway ALI cultures and studies of synergistic combinations.
 - Demonstrated the pan-SARS-CoV-2 antiviral activities of N-0385 against Omicron subvariants in human lung cells.
 - Demonstrated the synergistic inhibition of SARS-CoV-2 Omicron variants infection by combined use of paxlovid or remdesivir with N-0385 in human Calu-3 cells.

Title: Discovery of a novel broad-spectrum natural product therapeutic for human pathogenic enveloped viruses.

- **Pérez-Vargas J., Olmstead AD, Bautista-Sánchez D., Schulz AL, Villanueva I, Thompson CAH,** Vogl, W., Nabi, I. R., Brown, C., Wilkens S., Andersen RJ., **Jean F.**
- **In preparation: *Nature***
- Impact Factor: 69.5; **F. Jean:** Corresponding author
- Complete ED50 determination of XBB.1.5 and emerging XBB.1.6 in human airway ALI cultures and efficacy in small animal of human viral diseases.
 - Identified and patented a very potent, broad-spectrum natural product antiviral, cladoniamide A (CA), that inhibits viral entry of SARS-CoV-2, influenza A, and Zika in human cells .
 - Discovered that CA acts as a V-ATPase inhibitor and triggers the alkalisation of the host endo-lysosomal network (acidification inhibitor), thus preventing viral entry.
 - Demonstrated synergistic antiviral effects of CA combined with clinically relevant drugs against human pathogenic viruses.

Title: Synthetic Analogs of the Sponge Sesterterpenoid Alotaketol C are Potent Inhibitors of Infection by Covid Variants of Concern In Vitro.

- Polina Blagojevic, **Jimena Perez-Vargas,** Kunzhong Jian, David Williams, **Ivan Villanueva,** **Connor** Thompson, Siobhan Ennis, Masahiro Niikura, Ian Tietjen, **François Jean** and Raymond J. Andersen.
- **In Preparation: *Organic Letters***
- Impact Factor 6.072; R. Andersen and **F. Jean:** Corresponding authors
- Complete ED50 determination of XBB.1.5 and emerging XBB.1.6 in human airway ALI cultures and studies of synergistic combinations.
 - Synthesized analogs of the sponge sesterterpenoid alotaketol C.
 - Two of the analogs are more potent and have better selectivity indices than alotaketol C.
 - Our alotaketol C analogs represent readily accessible tool compounds to explore our proposed host-directed ACE2 shedding antiviral mode of action of alotaketol C—which has no precedent, and thereby are potential SARS-CoV-2 drug candidates.



Title: Identifying low-frequency mutations in patients treated with remdesivir using whole genome sequencing. (Ongoing compilation.)

- **Robert Kozak**, Samira Mubareka
- **Manuscript in revision: *JAMA Open Network***
- Impact Factor: 13.353

Title: Identifying low-frequency of intra-host variants in the nsp5 gene of SARS-CoV-2 in viral isolates circulating in Ontario. (Ongoing compilation.)

- **Robert Kozak** and **Prameet Sheth**
- **Manuscript in submission: *Influenza and Other Respiratory Viruses***
- Impact Factor: 5.663

Title: TBD

- **Natalie Strynadka**
- **Manuscript in preparation.**
- Developing a novel fluorescence-based assay, where linkers between the two TIM barrel fluorescent proteins (donor & acceptor pair) are systematically substituted with the 11 cuts sites of SARS-CoV-2 3CLpro. The aim is to provide a consistent and thorough kinetic analysis of all of the cut sites to coordinate our 3CLpro 3D structural analyses (Lee et al., *Nature Communications*, 2022). This will be the first complete analysis of all cut sites of any coronaviral protease—including SARS-CoV-2.

Title: Combination Therapy Enhances the Antiviral Activity of IFN- λ Against SARS-CoV-2 and MERS-CoV In Vitro.

- **Deanna Santer**, and Dr. Darryl Falzarano (VIDO)
- **Manuscript in preparation** with (VIDO)

Title: Combination of repurposed autophagy inducing drug (AID) with direct antiviral compounds (Paxlovid, Molnupiravir and Remdesivir) against SARS-CoV-2.

- **Nathalie Grandvaux**
- **Manuscript in preparation**
 - AID displayed efficient antiviral activity against ancestral (D614G) and BA.5 SARS-CoV-2 has been identified.
 - Combination experiments have been finalized in Vero E6 cells.
 - Analysis of the synergistic action is currently being finalized.
 - Hits will be confirmed in Calu-3 cells using ancestral (D614G), Delta, Omicron BA.1 and BA.5 variants for which all conditions have been set up.
 - Further evaluations of the combinations will be performed in collaboration with Deanna Santer.



Equity Diversity Inclusion and Indigeneity (EDI&I) Progress report

Vision Statement

We are committed to a national network that embodies health equity for **all** those engaged with CoVaRR-Net (patients, researchers, students, partners, and community members) to ensure research conducted is grounded in foundations of belonging and justice. Our research and actions are based on the principle of justice for all and we strive to remove any differences in power that can be used in discriminatory ways. First Nations, Inuit and Métis people have a place in health equity considerations due to the historic and ongoing impacts of colonialism and systemic racism.

Progress

In adding Indigenous inclusion into our Equity Diversity and Inclusion portfolio, a major priority was to identify and recruit a manager to round out the EDI&I team. A careful, consultative process was successful in the recruitment of Michelle Zinck, an Indigenous student with experience in land-based healing, community engagement and health outcomes. Together with Associate Director Claire Betker, this provides the EDI&I team with breadth in expertise in Indigenous health justice issues, health equity and employment equity policy and practice.

Our first initiative was to work with [CIEDAR](#) (CoVaRR-Net's Indigenous Engagement, Development, and Research Pillar 7) to elevate awareness of data sovereignty needs in under-served populations, primarily Indigenous health data principles of OCAP (Ownership, Control, Access and Possession). We supported CIEDAR on their Indigenous Genomics webinar. We next identified the First Nations Information Governance Centre's (FNIGC) OCAP online course as ideal for training. We impressed on leadership that this course be strongly recommended for all Pillar Leads and co-Leads. Work on this is ongoing with help from the CoVaRR-Net secretariat and is a foundational principle we are working to elevate across the network.

Before planning and embarking on an EDI&I training program, Michelle has undertaken a listening tour to: learn about each pillars' research activities and the potential areas involving inclusion; strengthen the notion of reciprocal respect in partnership building and pandemic preparedness research; introduce OCAP principles to elevate health data collection through partnership building. EDI&I principles are being codified in preparation for the next stage of dissemination of wise practices.



CoVaRR-Net's Wastewater Surveillance Research Group (WWSRG)

Vision statement

Our vision is to harness the power of wastewater testing and monitoring as a crucial tool in addressing the challenges posed by the COVID-19 pandemic and future health threats. Wastewater testing provides surveillance capacity that detects infection at all stages including asymptomatic cases, costs less than clinical testing, enables quick identification of outbreaks and waves, and is equitable in its ability to both include everyone and focus on vulnerable populations. CoVaRR-Net has established the [Wastewater Surveillance Research Group](#) to ensure the effective use of wastewater testing not only during the COVID-19 pandemic but also as a crucial component of a preparedness toolkit for future pandemics and health threats. This tool will play a vital role in detecting emerging infections, endemic diseases, drugs, toxins, and pesticides, serving as a key means of protecting public health and providing early warnings.

Year 2-funded work status

1. Wastewater infrastructure: development of data and data standards - The Public Health and Environmental Surveillance Open Data Model (PHES-ODM) is a dictionary and set of tools/templates to support the collection and use of wastewater and environmental surveillance data, including data on the SARS-CoV-2 virus and variants. It provides a singular and unified way to record and store data; so that wastewater-based surveillance data and metadata can be compared and aggregated across different research teams and public health departments.

- PHES-ODM version 2.0 and beta version 2.1—which includes provisions for more robust location data storage and other surveillance targets (polio, monkeypox, anti-microbial resistance)—released
- Release of robust written and video documentation to support uptake of ODM among users
- Release of tools for automatic data validation and transformation for the ODM
- Fostering data sharing at the local, provincial, and national level (ODM compliant data)

2. Methods for performing wastewater surveillance - This project is focused on establishing best measurement practices for wastewater testing and surveillance using a multidisciplinary, consensus approach. The project focuses on reporting wastewater metrics, including smoothing and outlier detection, growth parameters, and short-term projections.



- Ongoing fostering of collaborative studies within the Canadian community of over 250 testing sites and 200 researchers and public health staff.
- International collaboration for improved modelling of wastewater recovery and clinical cases, hospitalization, and death along with environmental parameters of SARS-CoV-2 viral load.

3. Evaluation and Public Health: evaluation of wastewater-based surveillance programs - We are facilitating public health analysis of wastewater surveillance methods by developing an evaluation framework to ensure that wastewater surveillance efforts moving forward are guided by the best evidence-informed practices. The process, which includes highly rigorous methodological standards and an open-science approach to establish best practices for wastewater testing and surveillance, is informed by our multidisciplinary, multinational executive group. The executive group includes members from the largest international wastewater groups, low- and middle-income countries, and world-leading methodologists.

4. Wastewater infrastructure: development of international partnerships - Support national and international (focus on low- and middle-income countries in Latin America, the Caribbean, and South Asia) research projects for the development and evaluation of wastewater testing and surveillance.

- The Open Data Model for wastewater, developed by CoVaRR-Net, has been adopted by 27 countries. It has been adopted, internationally, as the standard for airport surveillance. The goal is to make it the de facto international standard for sharing detailed wastewater and environmental data between researchers and surveillance programs.
- International collaborating bodies include, among others, the World Bank in Latin America and the Caribbean, the United States Center for Disease Control, the European Union Joint Research Centre, the Wastewater SPHERE (SARS Public Health Environmental REsponse), the UK Health Security Agency, the South African National Institute for Communicable diseases, and the Tata Institute for Genomic and Society (India).



Publications

Title	Status	Format
Data Models. Environmental Surveillance. A Cookbook for Wastewater Surveillance.	European Union Joint Research Centre. In Print 2023	Report
Documentation and Use. A Cookbook for Wastewater Surveillance.	European Union Joint Research Centre. In Print 2023	Report
Data Visualization. A Cookbook for Wastewater Surveillance.	European Union Joint Research Centre. In Print 2023	Report
A comprehensive, open-source data model for wastewater-based epidemiology	Submitted to Water Science and Technology	Peer review manuscript
Bayesian smoothing and predictive modeling of SARS-CoV-2 in wastewater in Ontario, Canada	Submission pending	Peer review manuscript
Validation and standardization of wastewater surveillance data for SARS-CoV-2 using serology data in Ontario, Canada	Submission pending	Peer review manuscript
Protocol for the development of an evaluation framework for environmental surveillance: a multinational, Delphi consensus approach	Submission pending	Peer review manuscript
Current evaluation practices for public health and environmental surveillance systems: a scoping review protocol	Submission pending	Peer review manuscript
Lessons learned from wastewater surveillance during the COVID-19 pandemic: a scoping review protocol	Submission pending	Peer review manuscript
Health care worker absenteeism as a surveillance tool for COVID-19: modelling and trends from a hospital in Ottawa, Canada	Draft	Peer review manuscript



Presentations

Date	Title/Headline	Audience	Format
November 28, 2022	Wastewater-based surveillance: update on progress and viewpoint from Canada	Variants of Concern Leadership Group Meeting	Invited talk
November 14, 2022	Development of a wastewater-based surveillance evaluation framework	EU Townhall Meeting	Invited talk
May 12, 2022	Evaluating wastewater surveillance and the performance of smoothing, outlier detection and projection	Eastern Ontario AGM	Invited talk
April 3, 2022	SARS-CoV-2 in wastewater: Canadian overview	Canadian Immunity Task Force	Invited talk

Media interviews

Date	Headline	Source(s)	Reach
January 6, 2023	'Big uptick' in Ottawa's coronavirus wastewater levels in recent days	CBC.CA News; Yahoo! News Canada; CBC News Ottawa	7 254 539
January 6, 2023	Wastewater levels suggest Ottawa is in another COVID wave: expert	newsexplorer.net; Vietnam Explorer News Channel; CBC News Ottawa; Yahoo! News	7 325 698



		Canada; CBC.CA News	
July 20, 2022	Ottawa's coronavirus wastewater nears record levels	Canada Today, CBC.CA News; Yahoo Movies (CA); Head Topics	7 159 622
July 20, 2022	Ottawa's wastewater levels projected to reach record high	Heromag; CBC.CA News; Yahoo! News Canada	6 315 224
April 30, 2022	COVID in the (waste) water; How testing sewage for coronavirus variants can be 'life-saving'	Regina Leader Post; Edmonton Journal; Calgary Herald; Montreal Gazette; London Free Press; Windsor Star; National Post; Ottawa Citizen	15 219 214



CUBE: Coronavirus in the Urban Built Environment

Research Progress Report - May 2023

VISION STATEMENT

CUBE develops tools to detect and analyze SARS-CoV-2 and other microbial pathogens in the places where people regularly meet, live, and work. Our aim is to make the invisible visible and, in the process, strengthen Canada's public health response to emerging pathogens. We aim to create scalable (high throughput end-to-end processing), translational (targeted predictive modeling), and transferable (peer-reviewed open access protocols) products to bolster environmental surveillance in the built environment. Our current work focuses on optimizing detection from low biomass surfaces samples; developing and validating tools for simultaneously monitoring multiple pathogens of concern (e.g., SARS-CoV-2, RSV, Influenza A and B, bacterial pathogens), including through 'omics approaches; documenting trends and prediction modelling; expanding our research collaborations across Canada; training highly qualified personnel to work effectively at the interface between research and society; and optimizing our communications pipeline with key public health decision-makers.

RESEARCH PROGRESS

Prior work: The CUBE team has validated a method of environmental sampling for SARS-CoV-2 in which swab samples are taken from surfaces in the built environment—specifically floors—and processed using polymerase chain reaction (PCR). The approach is similar to wastewater surveillance, but with greater spatial resolution and lower financial and technical requirements. Since 2020, we have processed and analyzed over 13,500 swabs collected from long-term care homes (LTCs), daycares, schools, libraries, university buildings, hospitals, and (most recently) an airport. Our largest study to-date (now complete) was a 14-month, multicentre prospective study at 10 LTCs in Ontario with three main findings: First, the percentage of floor swabs detecting SARS-CoV-2 can be used to effectively rule out an active outbreak. Second, the percentage of floor swabs positive for SARS-CoV-2 rises days, and sometimes weeks, before an outbreak is identified. Third, floor swabs provide spatial resolution to identify where in the LTC the COVID-19 cases are occurring (e.g., specific floor, staff-only areas, etc.). Taken together, our data show that floor swabs can be used as a proactive surveillance method and have geographic localization. (Full details of our prior work can be found in the publications linked below.)

CVN YEAR 2 PROJECT: Coronavirus in the Urban Built Environment (CUBE): Community-wide built environmental surveillance for SARS-CoV-2 and Variants



Project aim: To evaluate whether: (1) prevalence of SARS-CoV-2 from floors at a diversity of community sites follows a temporal pattern that precedes or parallels that of wastewater; (2) variant distributions from floor samples precede or parallel those in wastewater over time; and (3) built environment surveillance of key populations precedes or parallels wastewater detection for cases and variants (e.g., from schools and emergency department waiting rooms).

Deliverables: Building upon our prior work (i.e., in hospital settings, LTCs, and schools), this project aims to validate the use of built environment screening for population-level SARS-CoV-2 variant surveillance; demonstrate the ability to detect SARS-CoV-2 variants from low-biomass built environment samples; and determine the value of built environment screening for surveillance of COVID-19 in key populations.

Status:

We collected 1,866 swabs over a 26-week period (Oct 2022 - Mar 2023). Swabs were collected weekly, across a total of 16 locations (5 hospitals, 5 schools, and 6 libraries). Swabbing locations were selected with the aim of identifying geographically distinct regions, with considerations to feasibility and access, as well as matching to possible wastewater data (catchment areas with ongoing monitoring). Swab variables recorded included date the swab was taken, site, specific location within the site, and name of swabber. All swabs underwent quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) processing for detection of SARS-CoV-2; positivity and quantification cycle were recorded, and number of viral copies was extrapolated thereafter.

Selected samples (n=12) identified in discussion with Pillar 5 collaborators (led by Dr Ioannis Ragoussis) are currently being used to optimize sequencing techniques from low-biomass samples. Based on these results, we will query samples in our collection for SARS-CoV-2 sequence variation in collaboration with CAMEO (Pillar 6; Drs Sally Otto and Jesse Shapiro, co-leads). The broader aim of this work is to provide environmental surveillance data to complement and extend the data available from individual clinical sampling (i.e., human-based testing) and wastewater sampling.

Findings:

At present, analysis for our Year 2 project is in progress. The leading result to date is a strong difference in the percent of swabs positive for SARS-CoV-2 among the different sites, with hospitals having a substantially higher percent positivity than schools or libraries. Further analyses are on-going. At present, none of these findings require CoVaRR-Net Communications support; once we have completed the analysis, we can provide a further update.



PUBLICATIONS TO DATE

Title	Authors	Journal	Publication date	DOI / link
PEER-REVIEWED ARTICLES				
The spatial and temporal distribution of SARS-CoV-2 from the built environment of COVID-19 patient rooms: A multicentre prospective study*	M. Fralick, M. Burella, A. Hinz, H.S. Mejbél, D.S. Guttman, L. Xing, J. Moggridge, J. Lapp, A. Wong, C. Nott, N. Harris-Linton, R. Kassen, D.R. MacFadden	PLOS One	13 Mar 2023	doi.org/10.1371/journal.pone.0282489
Detection of Covid-19 Outbreaks Using Built Environment Testing for SARS-CoV-2*	M. Fralick, C. Nott, J. Moggridge, L. Castellani, A. Raudanskis, D.S. Guttman, A. Hinz, N. Thampi, A. Wong, D. Manuel, A. McGeer, E. Doukhanine, H. Mejbél, V. Zanichelli, M. Burella, S.L. Donaldson, P.W. Wang, R. Kassen, D.R. MacFadden	NEJM Evidence	17 Feb 2023	10.1056/EVIDo2200203
SARS-CoV-2 detection from the built environment and wastewater and its use for hospital surveillance	A. Hinz, L. Xing, E. Doukhanine, L.A. Hug, R. Kassen, B. Ormeci, R.J. Kibbee, A. Wong, D. MacFadden, C. Nott	FACETS	20 Jan 2022	doi.org/10.1139/facets-2021-0139
PRE-PRINTS				
Detection of SARS-CoV-2 in Schools Using Built Environment Testing in Ottawa, Canada: A Multi-Facility	N. Thampi, T. Burhunduli, J. Strain, A. Raudanskis, J.A. Moggridge, A. Hinz, E. Doukhanine, L. Castellani, R. Kassen, J.	Posted to pre-print server MedRxiv on 10 Mar 2023. Submitted to a peer-reviewed journal; currently in		doi.org/10.1101/2023.03.03.23286750



Prospective Surveillance Study*	McCready, C. Nott, A. Wong, M. Fralick, D.R. MacFadden	review.	
NON-PEER-REVIEWED ARTICLES			
Beyond wastewater surveillance: refining environmental pathogen detection in the built environment*	S. Abeygunawardena, R. Kassen and M. Fralick	CMAJ	28 Nov 2022 doi.org/10.1503/cmaj.147480-l

*partially supported by CoVaRR-Net

**wholly supported by CoVaRR-Net

UPCOMING PUBLICATIONS

Working Title	Authors	Status	Brief description
Community-wide built environmental surveillance for SARS-CoV-2 and Variants: A prospective study**	TBC	Analysis and manuscript drafting in progress.	This manuscript will share our findings from the CUBE Y2 CoVaRR-Net project, which is aimed at assessing built environment screening for surveillance of SARS-CoV-2 in key populations across various community locations (i.e., hospitals, schools, and libraries).

*partially supported by CoVaRR-Net

**wholly supported by CoVaRR-Net

PRESENTATIONS

Date	Organizer	Title	Type	Presenters
International				
21 Feb 2023	Institute for Advancing Health Through Agriculture, Texas A&M	Surface sampling for the detection and surveillance of disease outbreaks	Invited Speaker	A. Wong



10 Nov 2022	Yale Institute for Biodiversity Studies, Yale University	Islands of infection: adaptation and diversification in pathogenic microbes	Invited Speaker	R. Kassen
21 Jul 2021	US Library of Congress	SARS-CoV-2 Detection from the Built Environment in Hospital Surveillance	Invited Speaker	C. Nott, R. Kassen
Provincial (ON)				
23 Mar 2023	Prosserman Centre for Population Health Research	Using environmental swabs to detect SARS-CoV2 in the built environment: Multicentre prospective cohort study	Invited Speaker	M. Fralick, J. Moggridge
23 Aug 2022	Public Health Ontario	Coronavirus in the Urban Built Environment (CUBE): Evaluating the use of Environmental Swabs for the Detection and Surveillance of SARS-CoV-2 in School and Congregate Settings	Invited Speaker	C. Nott, M. Fralick
19 Aug 2022	ESA-CSEE Annual Meeting	The Covid-19 in the Urban Built Environment (CUBE) initiative	Invited Speaker	R. Kassen
13 May 2022	Toronto Public Health	CUBE: COVID-19 in the Urban Built Environment	Invited Speaker	M. Fralick, D. MacFadden, C. Nott, R. Kassen
12 May 2022	Toronto IPAC Hub	CUBE: COVID-19 in the Urban Built Environment	Invited Speaker	M. Fralick, D. MacFadden, C. Nott, R. Kassen
11 May 2022	Ottawa Public Health	CUBE: COVID-19 in the Urban Built Environment	Invited Speaker	N. Thampi, R. Kassen, D. MacFadden, C. Nott, M. Fralick



6 May 2022	Algoma Public Health	CUBE: COVID-19 in the Urban Built Environment	Invited Speaker	L. Castellani, M. Fralick, R. Kassen
1 Apr 2022	TEDxGuelphU	CUBE: COVID-19 in the Urban Built Environment	Invited Speaker	M. Fralick

MEDIA APPEARANCES

Date	Outlet	Participants	Description
16 Mar 2023	1310News (CIWW)	R. Kassen	Radio interview with 1310 News Ottawa.
15 Mar 2023	CTV News	M. Fralick	Print interview with CTV News Northern Ontario. "Sault-area nursing homes involved in COVID-19 study." Interviewer: Mike McDonald. Available from: https://northernontario.ctvnews.ca/sault-area-nursing-homes-involved-in-covid-19-study-1.6315272
15 Mar 2023	CBC	R. Kassen	Radio interview on CBC Ottawa Morning with Robyn Bresnahan. "New study shows it's possible to detect a COVID-19 outbreak before it happens." Available from: https://www.cbc.ca/listen/live-radio/1-100-ottawa-morning/clip/15972240-new-study-shows-its-possible-detect-covid-19-outbreak
15 Mar 2023	CBC	R. Kassen, A. Hinz, M. Fralick	Video/print interview with CBC News Ottawa. "Floor swabs at LTC homes early predictor of COVID outbreaks, study finds." Interviewer: Priscilla Ki Sun Hwang. Available from: https://www.cbc.ca/news/canada/ottawa/long-term-care-home-floor-swab-predict-outbreak-1.6769836
21 Feb 2022	CBC	R. Kassen	Radio interview with CBC. "Looking for COVID? Try the floor." Interviewer: Blair Sanderson. Available from: https://www.cbc.ca/listen/live-radio/1-90-columnists-from-cbc-radio/clip/15896240-looking-covid-try-floor



15 Feb 2022	The Conversation	A. Hinz, R. Kassen	Print article. "Swabbing floors to detect COVID-19 could be a useful indicator of the disease's spread." Available from: https://theconversation.com/swabbing-floors-to-detect-covid-19-could-be-a-useful-indicator-of-the-diseases-spread-175832
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OTHER KNOWLEDGE DISSEMINATION

Date	Activity	Description
20 Apr 2023	The Rounds Table Podcast	Podcast. The Rounds Table. Episode 59: "The Floor is COVID-19." Available from: https://healthydebate.ca/podcast/episode-59-the-floor-is-covid-19/
Apr 2023	CUBE LTC study infographic	Infographic depicting NEJM Evidence study. Available from: https://cube-ontario.github.io/about.html#:~:text=Long%2DTerm%20Care%20Homes%20Study
Jul 2022	Lay-facing infovideo	4-minute video, designed for lay audience. Available from: https://www.youtube.com/watch?v=oVdM8T9QVmo
Mar 2022	CUBE website	https://cube-ontario.github.io/

HQP & TRAINEES

TRAINEES OVERVIEW (Mar 2020 - present)				
	Undergraduate	Master's	Doctoral	Post-Doctoral Fellow
Current	2	2	0	0
Past	1	1	0	1
TOTAL	3	3	0	1



CURRENT HQP / TRAINEES			
Name	Role	Career Stage	Dates
Aaron Hinz	Senior Lab Manager	Research Associate	Apr 2020 - present
Jason Moggridge	Data Analyst	MBinf	Sep 2021 - present
Alex Hicks	Junior Lab Technician	MSc candidate	Sep 2022 - present
Tamara Van Bakel	Project Manager	BA	Sep 2022 - present
Hanna Ke	Research Assistant	BMSc & HBA candidate (dual degree)	Mar 2022 - present
Tasha Burhunduli	Research Assistant	BSc candidate	Mar 2022 - present
Engluy Khov	Research Assistant	BSc candidate	Nov 2022 - present
Makenna Wiebe	Research Assistant	BSc	Nov 2022 - present
Bryant Lim	Research Assistant	MD & MEng candidate (dual degree)	Jan 2023 - present

PAST HQP / TRAINEES				
Name	Role	Career stage (when with CUBE)	Dates	Position after CUBE
Veronica Zanichelli	Research Coordinator	MD	Aug 2021 - Mar 2022	Infectious Diseases Physician (Italy)
Hebah Mejbil	Junior Lab Technician	Post Doctoral Fellow (PhD)	Mar - Aug 2022	Mitacs Science Policy Fellow
Ashley Raudanskis	Project Manager	BSc	Aug 2021 - Sep 2022	Undergraduate MD student at Queen's University
Jamie Strain	Research Assistant	MSc candidate	Mar - Oct 2022	Research Assistant, CHEO Research Institute



Madison Burella	Research Assistant	Undergraduate MD candidate	Sep 2021 - Jul 2022	General Internal Medicine residency at University of Western Ontario
Sawith Abeygunawardena	Research Assistant	BSc	May 2022 - Jan 2023	Research Administrator at Humber College
Sana Ahsan	Research Assistant	BSc	Mar - Aug 2022	MPH student at Queen's University

INDUSTRY PARTNERS

DNA Genotek Inc.

DNA Genotek, an Ottawa-based company with 20+ years in microbial sample collection and processing, has provided (in-kind) the collection devices for the CUBE initiative to date. The P-208 Environmental Surface Collection Prototype kit consists of a flocced swab and a semi-lytic nucleic acid stabilization solution for post-collection swab immersion.



CoVaRR-Net Biobank Team: Biobank, Data Platform and Bioethics

Progress Report May 2023

CoVaRR-Net Biobank Team Vision Statement: Supporting rapid, accessible, and responsible sharing of materials and data in response to, and in anticipation of, emerging health threats to Canadians addressing this gap for Canada in tandem with an international movement.

The CoVaRR-Net Biobank Team facilitates rapid research through the sharing of data, biological materials, and pathogens among CoVaRR-Net members, external researchers and industry. The Team is fundamental and foundational to CoVaRR-Net activities, particularly as it transitions towards a state of pandemic preparedness. The Team is currently **a)** maintaining ongoing operations of its supported COVID-19 research; **b)** conducting prioritized post-pandemic activities; and **c)** establishing pandemic preparedness operations and innovations. The Biobank Team is focused on identifying and supporting ongoing studies of key cohorts relevant to CoVaRR-Net researchers such as the *Stop the Spread Ottawa* cohort. It is working with the CoVaRR-Net Wastewater Surveillance Research Group to preserve their collected material and data resources and integrate the effort into the preparedness biobanking model. Building capacity in Environmental and Animal Pathogens with experts will aid in reaching an overall ONE HEALTH approach in this effort.

A major initiative of the CoVaRR-Net Biobank is the establishment of the Canadian COVID-19 Biobank and Data Alliance which is essential to the national preparedness biobank activity. The Alliance boasts pan-Canadian membership with large “nodes” in BC, AB, ON and QC and small “hubs” in SK, MB, NS, NB, and NL. Alliance members exchange resources, materials, data and expertise and strive to harmonize practices, integrate catalogs and share common data models. The CoVaRR-Net Biobank operates in a federated model that allows each partner biobank to retain custody over the materials and data they oversee.

The CoVaRR-Net Biobank acquired a state-of-the-art, customized, biobank inventory management system known as SLIMS from Agilent Technologies to catalogue its inventory, SLIMS is available to all Alliance partners who require assistance with inventory management.

The Biobank team continue to work alongside the CoVaRR-Net Data Platform to support storage and management of data associated with the samples stored at the Biobank. The Data Platform team has created a novel, searchable, national biobank catalogue (Metabase) that integrates query-relevant data from multiple biobank inventory management systems across Canada. The data platform dashboard allows researchers to query the sample repository comprised of resources across the Alliance biobanks and subsequently apply for access to these data and/or materials, facilitated by the Biobank procedures. All requests are subject to review by a Scientific Review



Panel for approval, followed by approval by the principle investigator responsible for the data/materials requested. The Data Platform team are currently in the beta-testing phase of importing data from Alliance partners into the Metabase. The Biobank Team is conducting several pilot projects to test the strength and identify challenges with the sharing and resources in the Alliance, to be leveraged in future funding opportunities.

Exchange of samples, data and reagents between institutions is made possible with the implementation of the *Universal Data and Biological Materials Transfer Agreement* (UDBMTA), a contractual agreement pre-established in partnership with the University of Ottawa and Ottawa Hospital Research Institute and vetted by 31 Canadian research institutions. The CoVaRR-Net Biobank facilitates the exchange of samples and data between academics by ensuring the proper use of the UDBMTA and other contractual documents. This pre-established, ready to use contractual tool is integral for the conduct of efficient research exchanges and vital for a rapid response in case of emergency, avoiding cumbersome delays associated with *de novo* contracts.

The Biobank is registered with the Canadian Tissue Repository Network (CTRNet), a leader in biobank training and practices. The Biobank is in the process of receiving certification with the program, through training of Biobank staff and researchers to operate according to national standards (completion expected end of August).

The CoVaRR-Net Biobank continues to support and build strong partnerships with various partners.

- The University of Ottawa High Throughput Serology and Diagnostic Facility
- CoVaRR-Net Indigenous Engagement, Development, and Research (CIEDAR) – encourage collaboration with Indigenous and vulnerable populations, demonstrating knowledge and consideration of the Ownership, Control, Access and Possession (OCAP®) Principles developed by First Nations. The CoVaRR-Net Bioethics group will collaborate with CIEDAR to engage with Indigenous communities and other underserved communities.
- CoVaRR-Net Canadian Consortium of Academic Biosafety Level 3 (CCABL3) Laboratories – support ongoing biobanking and sharing of level 3 pathogens between researchers
- CoVaRR-Net Wastewater Surveillance Research Group (WWSRG)
- Long COVID Web
- Canadian Training Platform for Trials Leveraging Existing Networks (CAN-TAP-TALENT)
- POPCORN (**P**ediatric **O**utcome im**P**rovement through **C**Oordination of **R**esearch **N**etworks)



The Biobank Team has been engaged in multiple knowledge translation activities:

- **Publication about pandemic biobanking:**
 - Saginur, R, Robblee, JA, Vranjkovic, A, Tamblyn, L, Hsu, A, Cooper, CL, Vinh, DC, Langlois, MA, Crawley, AM. *No time for complacency: The CoVaRR-Net Biobank is an essential element of laboratory preparedness for infectious disease outbreaks*. JAMMI, Dec 2022.
- **2023 Association of Medical Microbiology and Infectious Disease Canada – Canadian Association for Clinical Microbiology and Infectious Disease (AMMI-CACMID) Annual Conference**
 - Poster title: “No time for complacency: The CoVaRR-Net Biobank is an essential element of laboratory preparedness for infectious disease outbreaks”. Presented by Nikita Rayne (Biobank Manager) March 29-31, 2023. Toronto, ON.
 - Promotional booth hosted by Aliisa Heiskanen (Biobank Coordinator)
- **COVID-19 Immunity Task Force Scientific Meeting 2023**
 - Oral presentation title: “CoVaRR-Net Biobank support of CITF projects establishes framework for pandemic preparedness biobanking.” Presented by Dr. Angela M. Crawley (Biobank Director) March 8-10, 2023, Vancouver, BC.
- **CoVaRR-Net Webinar**
 - Webinar title: “Collaborative Biobanking: Trailblazing for Pandemic Preparedness”. Presented by Drs. Angela M. Crawley, Amy T. Hsu (Data Platform Director) and Bioethics Co-Directors Raphael Saginur and James Robblee. February 24, 2023.