Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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**Methods & Materials:** We conducted a retrospective cohort investigation amongst 2,253 attendees. A cluster was defined as the identification/presence of ≥ two laboratory confirmed COVID-19 cases amongst individuals who attended the Rage between 27 November to 4 December 2020. COVID-19 confirmed cases were identified using the organizers ticket purchaser and crew record list and the national COVID-19 laboratory confirmed cases line-list. A standardized questionnaire was circulated to 1814 attendees using Google Forms. A case was defined as any person within the cohort with SARS-CoV-2 RT-PCR positive results.

**Results:** Of the 2,253 attendees, 848 (37.6%) cases were identified, of which 846 (99.8%) were revelers and two were crew members (0.2%). Age ranged from 16 to 58 years (Median: 18, IQR: 18-18). The 15-19-year-old age group accounted for 802 (94.6%) of the cases, while 53.0% (425/802) were males. Most cases were from Gauteng (66.2%, 561/848), followed by KwaZulu-Natal (30.0%, 254/848). Two of the attendees had positive SARS-CoV-2 results approximately 7-10 days before the festival. The questionnaire response rate was 1.0% (19/1814). Revelers attended other large events and private parties and mask wearing and social distancing was not always practiced.

**Conclusion:** Although organizers observed COVID-19 precautions and protocols; social distancing and mask wearing were compromised. Factors such as mass gathering without using appropriate personal protective equipment, crowded spaces, poor hygiene and ventilation may have produced a conducive environment for SARS-CoV-2 transmission. The study limitations included delayed questionnaire circulation, lack of contact tracing data to determine secondary attack rate, and lack of clinical information amongst cases. Non-pharmaceutical interventions are effective recommended prevention and control measures.

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**OP04.02 (797)**

**Excess Deaths during COVID-19 pandemic in Alberta, Canada**

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**Purpose:** To determine if there was excess mortality in Alberta, Canada during the pandemic. We sought to confirm if excess mortality affected all age groups equally and determine what proportion of excess deaths is directly related to COVID-19.

**Methods & Materials:** Excess mortality was calculated by comparing observed to expected number of deaths. Monthly Crude death rates (CDR) for 2015 to 2019 was calculated by dividing monthly deaths by the mid-year population. Expected deaths was calculated by multiplying mean monthly CDR by the mid-year population in 2020 and the projected mid-year population in 2021 to calculate expected deaths. Age-adjusted monthly mortality rates for January 2020 to March 2021 was compared to the previous 5 years.

**Results:** From January 2020 to May 2021 there was a 11% excess mortality corresponding to an average of 248 monthly excess deaths with a minimum of 49 deaths in January 2020 (no COVID-19-related deaths) and a maximum of 781 excess deaths in December 2020. COVID-19 related deaths (n=2266) account for 53.8% of the total excess deaths (n=4214) that occurred in the 17 months.

Increase in all cause -excess deaths was proportionately higher, and in significantly greater numbers, in the younger age groups. Deaths directly linked to COVID-19 were: 8 (20-29 years), 12 (30-39 years), 32 (40-49 years), 74 (50-59 years), 225 (60-69 years), 409 (70-79 years), and 1274 (>80 years)

Statistically significant increases in monthly drug poisoning deaths from March 2020 to April 2021 with a total of 1819 deaths. Excess 731 drug poisoning deaths representing 18.2% of total all cause excess mortality affected mostly those age 25-60. 53.9% of all excess deaths is directly related to COVID-19 and 18.2% are drug poisoning related excess deaths. The remaining 27.9% of excess deaths are likely due other factors such as limited access to urgent medical care.

**Conclusion:** There was statistically significant increase in all-cause mortality. Although older adults are more likely to die of COVID-19, there was massive increase in non-COVID-19 related mortality among the youth. These should be factored in public policy decisions on epidemic/pandemic management.

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**OP04.03 (153)**

**Implementing a SARS-CoV-2 Early Warning System in Valencia (Spain) and its Correlation with Epidemiological Indicators**

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**Purpose:** To implement a SARS-CoV-2 wastewater surveillance system in Valencia and study the correlation between SARS-CoV-2 concentration levels and epidemiological indicators.

**Methods & Materials:** The working strategy developed in the municipality of Valencia consisted of monitoring wastewater effluents collected by 790,000 people. The city was divided into 24 different hydraulic sectors. 3,023 samples of untreated wastewater were collected and analysed between May 2020 and May 2021. RNA extraction from sewage material was carried out using the NucleoSpin RNA virus Kit. SARS-CoV-2 RNA detection was performed by RT-qPCR using One-Step PrimeScript™ RT-PCR Kit (Perfect Real Time), targeting the nucleoprotein (N), N1 and N2 fragments, and envelope protein (E) gene. Mengovirus RNA recovery rates were used as quality assurance parameters according to ISO 15216-1:2017.

**Results:** A total of 2,169 samples were positive for SARS-CoV-2 RNA material. SARS-CoV-2 variations were detected throughout the entire study evidencing trends during the first, second and third wave. As the National State of Emergency ended (June 2020), SARS-CoV-2 values began to increase reaching the first and most significant concentration spike of the study (20th-26th of July 2020), with a weekly average aggregate concentration of 34.5 MGC/L (an increase of 2 units in the log scale). This translated in the worsening of epidemiological indicators (number of cases, hospitalizations, deaths and cumulative incidence (CI)), which maintained a stable increase until a second spike was detected during an important bank holiday in October (9th-12th), where the concentration changed from 28 MGC/L to 345 MGC/L in a 3-day period while the city was registering 1,100 new weekly cases and a CI of 200 cases/100,000 residents. Finally, a third wave placed Valencia with the worst historical epidemiological data (6.545 new cases; 1,000 new hospitalizations; CI= 1,318,04 cases/100,000 in-
Estimating SARS-CoV-2 prevalence from large-scale wastewater surveillance: insights from combined analysis of 44 sites in England

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Purpose: Accurate surveillance of the COVID-19 pandemic can be weakened by under-reporting of cases, particularly due to asymptomatic or pre-symptomatic infections, resulting in bias. Quantification of SARS-CoV-2 RNA in wastewater (WW) can be used to infer infection prevalence, but uncertainty in sensitivity and considerable variability has meant that accurate measurement remains elusive.

Methods & Materials: Data from 44 sewage sites in England, covering 31% of the population, are used in this analysis where samples are available from July 2020 to present day. Samples include the raw SARS-CoV-2 gene copy number and associated metadata. To establish the sensitivity and specificity of the WW data, we compare to population representative prevalence surveys available across England (the ONS Covid Infection Survey - CIS). The WW data were mapped to sub-regional data of the CIS and fitted using mathematical modelling. First, a phenomenological model was developed to model how infected individuals shed SARS-CoV-2 into WW and how the markers may degrade in time and compare this to the data. Second, we develop a model to estimate SARS-CoV-2 prevalence directly from WW data which is trained on the CIS data.

Results: Data from 44 sewage sites in England, shows that SARS-CoV-2 prevalence is estimated to within 11% of estimates from representative prevalence surveys (with 95% confidence). Using machine learning and phenomenological models, differences between sampled sites, particularly the WW flow rate, influence prevalence estimation and require careful interpretation. SARS-CoV-2 signals in WW appear 4-5 days earlier in comparison to clinical testing data but are coincident with prevalence surveys suggesting that WW surveillance can be a leading indicator for asymptomatic viral infections.

Conclusion: Wastewater-based epidemiology complements and strengthens traditional surveillance, with significant implications for public health. Using WW to quantify infection prevalence requires knowledge of additional meta-data and outbreak detection needs to account for unexplained aberrations in WW data to improve reliability.

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Sub-genomic RNA Expression in SARS-CoV-2 B.1.411 and B.1.1.7 Infections in Sri Lanka

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Purpose: As experienced by many countries, Sri Lanka is currently experiencing a large COVID-19 outbreak, with over 90 cases/one million population. The previous outbreak, which was due to the B.1.411 virus (Sri Lankan lineage) resulted in a significantly fewer number of cases and deaths compared to the current outbreak caused by B.1.1.7. Therefore, we sought to explore if the differences in the transmission rates and higher mortality rates with the introduction of B.1.1.7 is due to an increased expression of sub-genomic RNA, which is an essential step in the virus life cycle.

Methods & Materials: Sputum or nasopharyngeal samples of 472 patients with SARS-CoV-2 infection were included in the analysis. Samples with the cycle threshold <30, were sequenced using 247 amplicons targeting the SARS-CoV-2 genome (MN908947v3). Library preparation was done using AmpliSeq prep kit and sequenced either on illumina iSeq100 and Nextseq550 platforms. Basecalling and demultiplexing were done using the default bcl2fastq (v2.20) pipeline. Raw index-trimmed fastqs were analyzed for sub-genomic RNA using Periscope (https://github.com/sheffield-bioinformatics-core/periscope). Raw reads were aligned and checked for the leader sequence at the start of each open reading frame (ORF). The sgRNA detected reads were counted, classified into ORFs and normalized using the genomic RNA counts at each position. Groups were compared with an unpaired Wilcoxon test using Rrstatix package. Figures were generated in R ggpubr.

Results: Out of the remaining 434 datasets after the quality control step, 164 were of B.1.1.7 lineage while 237 were B.1.411. Means of the normalized sgRNA counts between B.1.411 and B.1.1.7 viruses were significantly different in six ORFs. Viruses of the B.1.411 lineage expressed significantly higher sgRNA for Spike protein \( p = 0.014 \), ORF3a \( p = 0.0001 \), Membrane protein \( p = 2.62E-10 \), ORF8 \( p = 1.81E-05 \), and ORF7a \( p = 0.0004 \) than those in B.1.1.7 samples. Contrastingly, Nucleocapsid (N) protein had significantly higher sgRNA expression in B.1.1.7 samples \( p = 0.0001 \).

Conclusion: Our results suggest that increased expression of sgRNA for a particular virus lineage does not necessarily associate with higher transmissibility as higher expression of sgRNA of B.1.1.7 compared to the B.1.411 lineage virus was only seen for the N protein.

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