What can sewage tell us about disease? Norovirus in English sewage samples

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Norovirus concentrations were measured in sewage from across England. Norovirus concentrations increased rapidly as the country come out of COVID-19 lockdown measures. Data also showed a potential outbreak of norovirus genogroup I in the north of England in 2022. Norovirus concentrations in wastewater are likely to be related to the number of cases within a community. Work is ongoing to sequence the norovirus in these samples.

INTRODUCTION

The COVID-19 pandemic accelerated the adoption of wastewater-based epidemiological (WBE) tools worldwide, including England's extensive monitoring program. WBE, which has an 80-year history, employs modern techniques like PCR to analyse wastewater for disease surveillance. Besides COVID-19, WBE's potential extends to tracking other pathogens like antimicrobial resistance, respiratory viruses, and norovirus (NoV), particularly due to NoV's high shedding in faeces. NoV outbreaks exhibit seasonality but saw reduced prevalence during the pandemic due to non-pharmaceutical interventions (NPIs). However, when NPIs eased, NoV cases surged, influenced by various factors.

In this study, we quantified norovirus in a representative subset of samples from the English SARS-CoV-2 sewage monitoring programme to determine how norovirus levels changed across the country as NPIs were decreased.

METHODS

Viral nucleic acids were concentrated from a total of 3,452 sewage samples from 163 sites across England between May 2021 and March 2022 as part of the Environmental Monitoring for Health Protection programme[1]. Norovirus genogroups I (GI) and II (GII) were quantified by duplex RT-qPCR[2]. The data were normalised against nutrient concentrations and the norovirus copies per capita were calculated using 2019 population data for the sewage catchment provided by ONS. Relative strength index (RSI) were used to show upward or downward trends in norovirus concentrations in sewage over the time course of the study.

Spatial autocorrelation of the norovirus concentrations across sies was carried out using Global and Local Moran's I analyses on monthly average data for each site. This was to determine whether there was any clustering of high or low results between sites within a 90 km proximity of each other.

Norovirus case numbers were obtained from UKHSA surveillance reports[3] and compared with norovirus concentrations in wastewater by linear regression.

RESULTS & DISCUSSION

Norovirus concentration trends over time

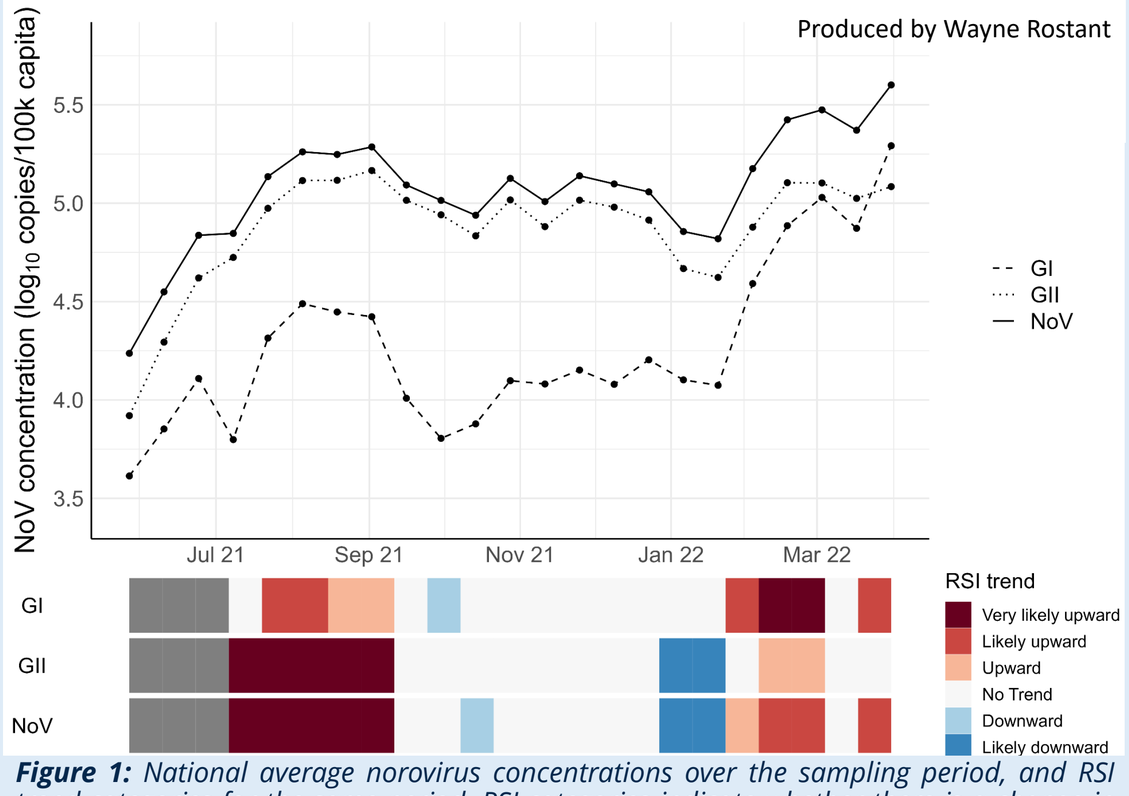

Figure 1: *National average norovirus concentrations over the sampling period, and RSI trend categories for the same period. RSI categories indicate whether there is a change in the concentration of NoV in wastewater over a rolling 6-week period.*

Clinical cases vs wastewater norovirus concentration

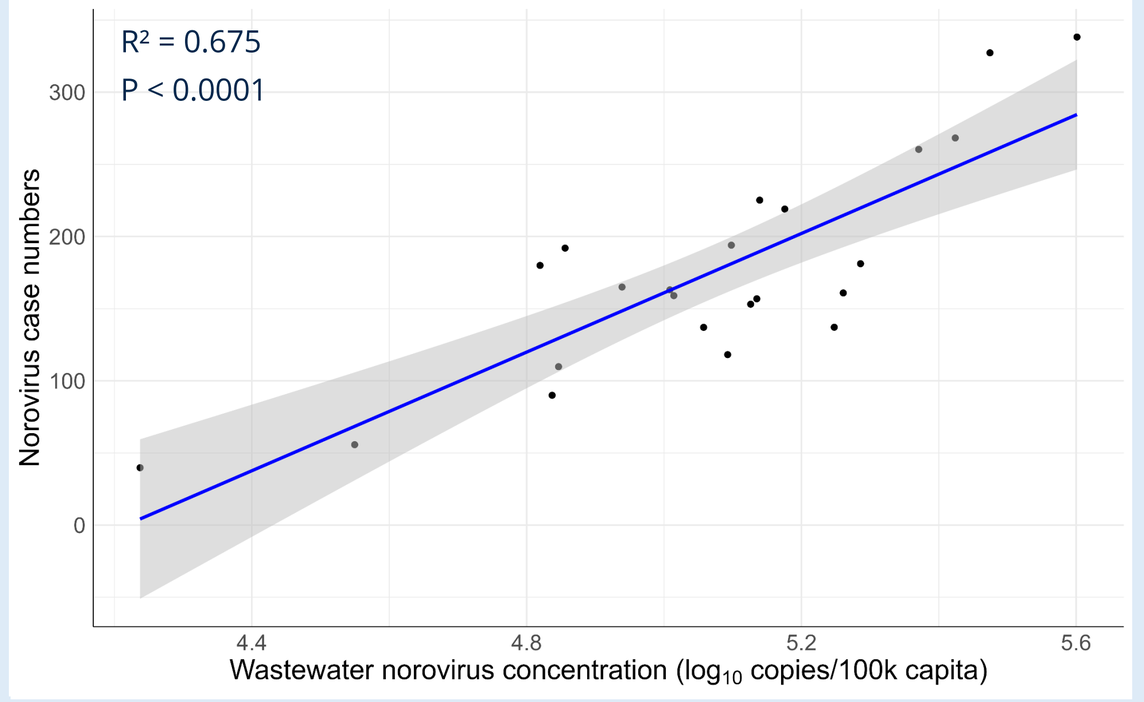

Figure 3: *Comparison of 2-weekly national average wastewater norovirus concentrations vs. the number of clinical cases reported for the same period. Grey areas represent 95% confidence intervals for the linear model (blue line).*

The national average trends in norovirus concentrations in wastewater between May 2021 and March 2022 are shown in Figure 1. Norovirus GII accounted for the majority of the norovirus measured in wastewater for much of the sampling period. However, in 2022, GI levels increased relative to GII until it became the dominant genogroup. These changes were reflected by the RSI measures with GI concentrations increasing at a higher rate than GII in 2022. RSI measures also showed that norovirus concentrations increased during the spring of 2021, a time that coincided with the easing of NPIs (national lockdowns) in England.

Spatial autocorrelations

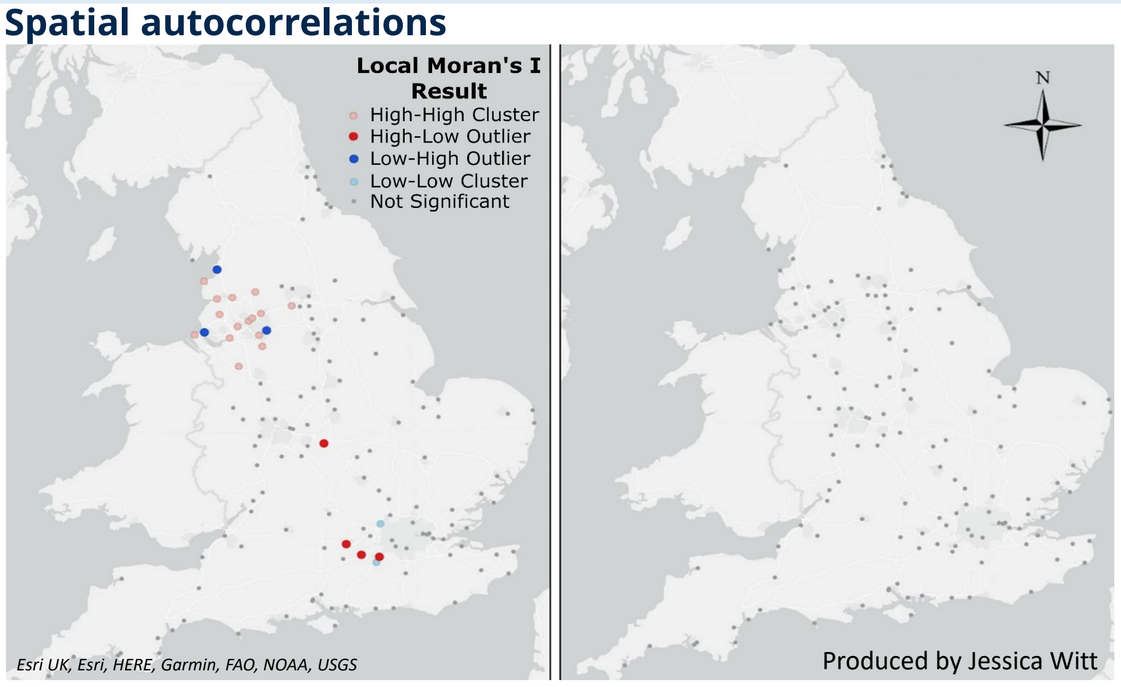

Figure 2: *Spatial autocorrelations between sites within 90 km of each other in March 2022 showed a high degree of high-high clustering of GI concentrations in wastewater in the North-West.*

Spatial autocorrelation of the norovirus concentrations across sites indicated possible outbreaks of GI and GII throughout the sampling period. Figure 2 shows these results for March 2022. At this time, there was an indication that there were higher levels of GI in the Northwest of England relative to the rest of the country. This indicates the possibility of a norovirus outbreak caused by GI in the Northwest at that time. This reflects the increase in GI seen relative to GII in 2022. Unfortunately, no regional norovirus case data were available to investigate this further.

Interestingly, at the same time, there were three sites within the Northwest that did not follow the same trend of high GI concentrations. Conversely, some sites in the Southeast and the midlands had high GI concentrations relative to nearby sites. The cause of these outliers is not clear. It could indicate a genuine disconnect between communities served by the different sewage catchments, or they may indicate methodological shortcomings, which should be addressed in future research and surveillance programmes.

Linear regression was used to compare the national average norovirus concentrations in wastewater with the numbers of reported clinical cases (Figure 3). This showed a significant relationship between the two measures, with a R^2 of 0.675. This indicates that norovirus concentrations in wastewater are somewhat predictive of the number of cases in the community, at least at a national level.

FURTHER WORK

In addition to collecting quantitative data for norovirus in wastewater, we are currently collecting genomics data for the norovirus populations within the wastewater samples. This work is being carried out at the Cefas Genomics Facility in Exeter. It follows on from the development of new methods developed by Cefas for sequencing norovirus from complex samples. These data will help us to understand the diversity of norovirus across England and to investigate how different genotypes move across the country.

ACKNOWLEDGEMENTS

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REFERENCES

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