Variance in the variants; a comparison of the symptomatology of SARS-CoV-2 variants in Wales, February 2020 – July 2022

Felicity Simkin[1]‡, Mark Postans[1], Nicole Pacchiarini[1], Jiao Song[1], Simon Cottrell[1], Catherine Moore[3], Thomas R Connor[2,4], and Christopher Williams[1]

Author affiliations:[1]Communicable Disease Surveillance Centre (CDSC), Public Health Wales, Cardiff, Wales, UK [2]Pathogen Genomics Unit, Public Health Wales, Cardiff, Wales, UK; [3]Wales Specialist Virology Centre, Microbiology, Public Health Wales, Cardiff, Wales, UK; [4]Cardiff University School of Biosciences, Cardiff University, Cardiff, Wales, UK.

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Running title: Variance in symptomatology of the SARS-CoV-2 variants

‡ Corresponding author: Felicity Simkin, Felicity.Simkin@wales.nhs.uk, Communicable Disease Surveillance Centre (CDSC), Public Health Wales, Cardiff, Wales, UK

Abstract

Background: Comparisons of SARS-CoV-2 variants' severity in terms of outcomes such as hospitalisation are commonly reported. By contrast, despite the vital importance of understanding evolution of the SARS-CoV-2 symptom profile for public health surveillance, few comparative studies have investigated differences in variants' symptomatology. The aim of this comprehensive analysis was to investigate whether successive variants were indeed associated with distinct symptom patterns in a large Welsh sample covering a broad time period and multiple variant waves.

Methods: Symptom and variant typing data were derived from contact tracing data and sequencing of positive SARS-CoV-2 PCR tests collected in Wales, respectively. Descriptive epidemiology and binary logistic regression modelling were then used to investigate differences in the symptom profile of N = 226,002 cases infected with Wild-Type, Alpha, Delta, or Omicron variants of SARS-CoV-2.

Results: The median number of symptoms and the proportion of cases reporting as symptomatic were lower for those infected with Omicron (BA.4) and Omicron (BA.5) compared to those infected with Wild-Type (B.1.612), Alpha (B.1.1.7), Delta (B.1.617.2 and descendent lineages, excluding AY.4.2), Delta (AY.4.2), Omicron (BA.1) and Omicron (BA.2). Indeed, the odds of being symptomatic when infected with the Omicron (BA.4) or Omicron (BA.5) variant were 83% and 84% lower, compared to those infected with Wild-Type. Anosmia in particular, was reported in non-Omicron variants at approximately three-to-five times the proportion of cases (range 20.23% - 26.41%) compared to Omicron variants (range 4.18% - 7.65%). Indeed, the odds of having anosmia when infected with an Omicron variant were 85% – 91% lower than when infected with Wild-Type, versus 32% – 43% lower for non-Omicron variants, relative to Wild-Type.

Conclusion: We suggest, given these changes in symptomatology and the vast reduction in testing and sequencing, symptom surveillance should continue, to approximate burden of

infection and facilitate rapid updating of clinical case definitions following any further evolution in the symptom profile. While disentangling the contribution of genomic, immunological and sampling changes to our findings remains challenging, symptom surveillance could also potentially aid emerging variant detection when used in combination with other surveillance approaches.

Introduction

The first case of SARS-CoV-2 was confirmed in Wales on 28th February 2020 (GOV.UK, 2020c). Following rapid global spread, a pandemic was declared on 11th March 2020. That same month the United Kingdom released the official list of COVID-19 symptoms which included the presence of a new continuous cough and fever (GOV.UK, 2020a). On 18th May 2020, following monitoring of emerging data and evidence, anosmia was added to the list (GOV.UK, 2020b). On 1st April 2022 the list was substantially expanded to include upper-respiratory, constitutional and gastrointestinal symptoms (GOV.UK, 2022b). Despite the initial small number of symptoms included on the list and "flu-like symptoms" occurring most commonly, the symptoms of SARS-CoV-2 are diverse and several others, not included in the current version of the list, have been reported (Whitaker et al., 2022).

Whilst SARS-CoV-2 can have a diverse clinical profile, a meta-analysis found that 40.5% of confirmed cases are asymptomatic, however, this excludes the untested asymptomatic cases distorting the likely higher, true proportion of cases which are asymptomatic (Ma et al., 2021). This asymptomatic proportion presents a potential driver in transmission, cases being unaware of their positive infection status and therefore not engaging in social isolation allows rapid spread in a short time frame (Vermund & Pitzer, 2021). As such, the proportion of asymptomatic cases per SARS-CoV-2 variant could be a marker for which variants will establish themselves successfully and indicate potential to become dominant.

Whilst many publications discuss the severity of SARS-CoV-2 variants in terms of outcomes such as hospitalisation, to date, few discuss differences in their symptomatology. We aim to make this comparison and ascertain if a significant difference exists in Wales. Indeed, Wales' robust testing and sequencing efforts - averaging over 25% genomic sequencing coverage over the study period - provided a unique opportunity to undertake this analysis. The present study period also afforded inclusion of the comparatively understudied Omicron variants BA.4 and BA.5 in our analyses. Informed by similar reports from other countries (e.g.,(Whitaker et al., 2022)), the primary hypotheses addressed by the current study were that a) overall symptom expression, and b) expression of specific symptoms would differ between SARS-CoV-2 variants. In the context of the end of free mass testing and reduced genomic surveillance, symptom surveillance could then prove a useful complementary epidemiological strategy for emerging variant surveillance (GOV.UK, 2022a).

Method

Data

From February 2020 until 31 March, 2022, in Wales, polymerase chain reaction (PCR) tests for COVID-19 were free for those with symptoms (BBC, 2020; Welsh Parliament, 2023). A PCR test could be booked through the UK government portal by individuals with any of the following symptoms: a high temperature, a new continuous cough, or a loss of/change in the ability to smell/taste. PCR tests were also available at walk-in testing sites, regardless of symptom status of those attending. A proportion of these community samples were tested within the NHS laboratory network in Wales whilst the majority were tested in the lighthouse laboratory network. All SARS-CoV-2 testing of hospitalised patients occurred within the NHS Wales laboratory network. In addition to PCR tests, antigen tests (lateral flow tests [LFTs]) were available in the UK via pharmacies or the UK Government portal at no cost. All individuals with a positive antigen LFT were encouraged to have a confirmatory PCR test, regardless of symptom status, until 7 January 2022. After this date ~94% of positive LFT test results reported up to the end of the study period were not associated with a confirmatory

PCR test (466,816 / 496,955 non-deduplicated tests), and could not, therefore contribute to the sequencing analyses reported below.

In Wales, whole genome sequencing (WGS) of positive PCR samples identified Variants of Concern (VOC) and Variants (V) of SARS-CoV-2. Positive samples from residents of Wales were mostly tested in the UKHSA lighthouse laboratory based at Newport Imperial Park 5 (IP5) in Wales. Positive samples tested at Welsh NHS labs or IP5 were sent to the Pathogen Genomics Unit (PenGU) for sequencing and analysis. The small proportion of Welsh tests that were sent to other parts of the lighthouse lab network, depending on site, were either tested at that laboratory (by reflex variant or PCR) or referred for WGS at the Wellcome Sanger Institute, and results reported back to the Welsh NHS system.

Contact tracing for all PCR positive COVID-19 cases within Wales was performed by the Test, Trace and Protect (TTP) service. Patients were asked about their symptoms (if they were symptomatic and if they had any of the following symptoms: cough, fever, anosmia, diarrhoea, vomiting, nausea, loss of appetite, runny nose or sneezing) and information was entered into the TTP contact tracing system. Enhanced contact tracing was undertaken by the TTP team in response to outbreaks. The present analysis of SARS-CoV-2 variant symptom profiles leveraged this TTP symptom data and is therefore constrained to the above nine consistently queried symptoms.

The COVID-19 vaccination register in Wales (the Welsh Immunisation System; WIS), which contains vaccination details for all NHS-registered individuals resident in Wales, was utilised to extract vaccination status of confirmed cases.

All variant-typed records derived from PCR sample sequencing between 28th February 2020 (first Welsh SARS-CoV-2 Wild-Type case) and 21st July 2022 (data cut-off), were therefore linked to the symptom data available via TTP (linkage performed by individual-specific reference codes), and the WIS vaccination status data (by Episode number), to facilitate the present analyses. Individual-specific reference codes were created, utilising a combination of

date of birth and a set number of characters from the individuals first name and surname. To ensure that symptom data for an individual was linked to the relevant episode within the sequencing data, date fields within the symptom data were utilised in order to retain symptom data that was within 42 days of the date of the PCR test for a given episode within the sequencing data. Finally, a binary Reinfection Status variable (Yes versus No) was derived for each record by grouping cases by individual-specific reference code, to denote if the current period of illness with an associated sequenced test was their first infection (No) or if they have had a previous period(s) of infection with an associated sequenced test (Yes).

Analysis

Descriptive statistics were calculated by SARS-CoV-2 variant (Wild-Type, Alpha, Delta, Delta [AY.4.2], Omicron [BA.1], Omicron [BA.2], Omicron [BA.4] or Omicron [BA.5]), overall symptomatic status (symptomatic versus asymptomatic), specific symptoms (cough, fever, anosmia, diarrhoea, vomiting, nausea, loss of appetite, runny nose and sneezing), age group (<5, 5-14, 15-29, 30-59 or 60+), sex (Male or Female), vaccination status (unvaccinated, one dose, two doses, three doses or booster), keyworker status (keyworker or not keyworker), and keyworker sector (1. Education and Childcare, 2. Food and other Necessary Goods, 3. Health and Social Care, 4. Key Public Service, 5. Local and National Government, 6. Public Safety, Emergency and National Security, 7. Transport, 8. Utilities, Communication and Financial Services).

Binary logistic regression was used to model the relationship between overall symptom status (symptomatic versus asymptomatic) and variant adjusted for: cases' age group, sex, vaccination status , keyworker status and reinfection status. Note that for the purpose of all regression analyses the vaccination status was collapsed into three levels: unvaccinated, vaccinated (individuals who has received one, two or three doses or a booster dose) and unknown vaccination status. Results are reported as adjusted odds ratios with 95% confidence intervals, along with the model AIC. Cases whose age, sex, symptom status or

keyworker status were unknown were excluded from the regression analyses (11,206 / 226,002 excluded, yielding a total of 214,796 complete cases available for analysis). Note that interactions were not included in this regression model or those described below even though, for example, an interaction between variant and vaccination status may be expected due to immune modulation. This interaction was not included here because vaccination was not available for all 'variants' considered (e.g., the natural reference: Wild-Type), precluding estimation of the corresponding odds ratios via maximum likelihood estimation. A complementary overall symptomatic status model that includes this variant-by-vaccination status interaction, is however, reported in Supplementary Information for reader interest (Supporting Information S1: Table 5).

Separate binary logistic regression models were also used to explore the association between each specific symptom and variant, adjusted for vaccination status, age group, sex, keyworker status and reinfection status. Each of these symptom-specific models were therefore considered planned comparisons addressing a separate primary hypothesis.

All data analysis was undertaken in R Studio (R v4.1.3), with logistic regressions models fitted using the base glm() function in the stats package (v4.1.3, part of base R). P values of <0.05 were considered statistically significant. The reported regression models were checked for multicollinearity issues using the vif() function in the car package (v3.1-1), which confirmed VIFs < 5 across all models (*R Companion 3E,* 2019).

Results

Sample demographics/characteristics

In total, 226,002 sequenced cases were included in the study. Samples came from two sources. One set of samples (N = 60,146, 26.61%) came from the NHS Wales laboratory network. These samples were predominantly hospital and staff samples, as well as some community cases. The second set (N = 163,980, 72.56%) of samples came from the lighthouse laboratory network, most frequently from Alderley Park lighthouse laboratory, and predominantly reflect community cases, tested as part of the national TTP programme. The origin of a further 1,876 (0.83%) of samples was not coded but these were also available for analysis. The characteristics of the study sample are summarised in Table 1; percentages within the table utilise the total number of cases for a given variant, reported within the column headers, as the denominator. Of these, 3.4% overall were Wild-Type cases, 5.5% were Alpha cases, 44.9% were Delta cases, 6.7% were Delta (AY.4.2) cases, 25.3% were Omicron (BA.1) cases, 12.9% were Omicron (BA.2) cases, 0.4% were Omicron (BA.4) cases and 1.0% were Omicron (BA.5) cases. Symptom data was unavailable for 4.49% of cases overall; across variants this ranged from 2.5% to 16.2% (see Figure 1).

By variant, the proportion of cases reported as keyworkers ranged from 12.51% - 27.82% (the greatest proportions were observed in the Omicron variant cases), the proportion of cases for which keyworker status was unknown ranged from 2.62% - 16.22%. The most common keyworker sector was the Health and Social Care sector where the proportion of cases working in this sector ranged from 4.23% - 26.25% (the greatest proportions were observed in the Omicron variants).

The proportion of cases which self-reported as male ranged from 37.73% - 48.62% across the variant groups (the proportion was lowest in Omicron (BA.4) and Omicron (BA.5) cases).

Across all variants the greatest proportion of cases were reported as aged 30-59, 40.85% - 47.47%, with the exception of Omicron (BA.5) in which the greatest proportion of cases were reported as aged over 60 years of age.

The proportion of cases within each level of vaccination status generally reflected the UK vaccine rollout schedule (e.g. the general decline in proportion of unvaccinated individuals with successive variants).

2,504 cases (1.1%) were reinfection cases. The proportion of cases which were reinfection cases was very low across all variants (range 0.08% - 5.28%), with a general pattern of increasing proportion of cases with successive variants. 2,482 individuals had two infections and just 11 individuals had three infections.

Table 1. Characteristics (age, sex, vaccination status, keyworker status, keyworker sector and reinfection) of SARS-CoV-2 variant cases. Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus

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Figure 1. Overall symptomatic status of the study population. A) Overall symptomatic status of non-Omicron variants. B) Overall symptomatic status of Omicron variants.

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The median number of symptoms reported for each of the variants was one symptom, with the exception of Omicron (BA.4) and Omicron (BA.5) for which it was no symptoms (See Supporting Information S1: Figure 1).

Overall symptomatic status analysis

A summary of the proportion of cases with symptoms reported, by variant, is described within Table 2; percentages within the table utilise the total number of cases for a given variant, reported within the column headers, as the denominator. Overall, Alpha had the greatest proportion of cases reported as symptomatic with any of the nine symptoms of interest (66.6%), whilst Omicron (BA.4) and Omicron (BA.5) had the lowest proportion of cases reported as symptomatic, 31.9% and 32.7% respectively (see Figure 2. and Table 2).

Table 2. Summary of reported symptoms in SARS-CoV-2 variant cases reported as symptomatic. Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus

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Figure 2. Proportion of cases which were reported as symptomatic/asymptomatic by SARS-CoV-2 variant. Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2. Wild-Type n = 7,676, Alpha n = 12,518, Delta n = 101,504, Delta (AY.4.2) n = 15,040, Omicron (BA.1) n = 57,115, Omicron (BA.2) n = 29,128, Omicron (BA.4) n = 861 and Omicron (BA.5) n = 7,676.

INSERT FIGURE 2 AROUND HERE

The odds ratios (ORs) derived from the fitted symptom status model are reported in Table 3. The odds of being symptomatic were significantly lower for all variants compared to Wild-Type when holding all other model variables fixed. In particular, the odds of being symptomatic when infected with Omicron (BA.4) and Omicron (BA.5) were 84% and 83%, (OR = 0.16, 95% CI = [0.14, 0.19], $p < 0.001$; OR = 0.17, 95% CI = [0.15, 0.19], $p < 0.001$, respectively) lower, respectively, compared to Wild-Type infections.

Table 3. Odds ratios, 95% confidence intervals and p-values for the fitted overall symptomatic status model. 134,031/214,796 cases included in the model reported being symptomatic. AIC = 270,486.

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More commonly reported symptoms status analysis

The most commonly reported symptom was cough, in all eight SARS-CoV-2 variants (Figure 3 and Table 2). The other most commonly reported symptoms across the variants included fever, anosmia, runny nose and sneezing.

Fever and cough were reported at the lowest proportion in Omicron (BA.4) and Omicron (BA.5) cases, (16.49% and 18.29%; 24.51% and 23.38%, respectively) and at a greater proportion in the remaining variants (range 25.13% - 28.94% and 34.38% - 43.18%, respectively). Anosmia was reported at the greatest proportion in non-Omicron variant cases (range 20.23% - 26.41%) and at a lower proportion in Omicron variant cases (range 4.18% - 7.65% respectively). Runny nose and sneezing were reported at the greatest proportion in Delta, Delta (AY.4.2), Omicron (BA.1) and Omicron (BA.2) cases (range 27.99% - 32.69% and 21.15% - 24.94%, respectively) and at a lower proportion in the other variant cases (range 14.7% - 20.01% and 11.38% - 15.21%, respectively).

Figure 3. Proportion of cases in which more commonly reported symptom of interest was reported by SARS-CoV-2 variant. Common symptoms included cough, fever, anosmia, runny nose and sneezing. Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

INSERT FIGURE 3 AROUND HERE

The odds ratios derived from the fitted cough status model, fever status model, anosmia status model, runny nose status model and sneezing status model are reported in Table 4.

Whilst the odds of having a cough when infected with Alpha were increased, the odds were significantly lower for all Omicron variants, compared to Wild-Type, when holding all other model variables fixed. Most notably, for Omicron (BA.4) and Omicron (BA.5), the odds were 62% (OR = 0.38, 95% CI = [0.32, 0.44], $p < 0.001$) and 65% (OR = 0.35, 95% CI = [0.31, 0.40], $p < 0.001$) lower compared to Wild-Type infections.

There was a reduction in the odds of having a fever across all variants, compared to those infected with Wild-Type.

The odds of having anosmia were significantly lower for all variants, compared to Wild-Type. Omicron variants in particular demonstrated a marked reduction in odds, but most notable even among these, the odds of having anosmia when infected with Omicron (BA.4) and Omicron (BA.5) were 91% (OR = 0.09, 95% CI = [0.06, 0.12], $p < 0.001$; OR = 0.09, 95% CI = [0.07, 0.11], $p < 0.001$, respectively) lower compared to Wild-Type infections.

The odds of experiencing a runny nose were significantly lower for those infected with Omicron (BA.4) and Omicron (BA.5), 44% (OR = 0.56, 95% CI = [0.46, 0.69], $p < 0.001$) and 39% (OR = 0.61, 95% CI = [0.53, 0.70], $p < 0.001$) lower, respectively, compared to Wild-Type infections when holding all other model variables fixed. Conversely, there was an increase in the odds of experiencing a runny nose for all remaining variants, compared to Wild-Type infections.

Similarly, the odds of experiencing sneezing were significantly lower for those infected with Omicron (BA.4) or Omicron (BA.5), 53% (OR = 0.47, 95% CI = [0.37, 0.59], $p < 0.001$) and 49% (OR = 0.51, 95% CI = [0.44, 0.60], $p < 0.001$) lower, respectively, compared to Wild-Type when holding all other model variables fixed. Conversely, there was an increase in the odds of experiencing sneezing for all remaining variants, compared to Wild-Type infections.

Table 4. Odds ratios, 95% confidence intervals and p-values for the fitted models corresponding to the most commonly reported symptoms (cough, fever, anosmia, runny nose and sneezing**)**.*

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Less commonly reported symptoms status analysis

For the less commonly reported symptoms of diarrhoea, vomiting, nausea and loss of appetite, a brief summary of findings is provided below with further details given in the Supporting Information.

For these symptoms the proportion of cases in which the given symptom was reported was similar for all variants (range 1.97% - 15.27%). As the proportions reporting these less commonly reported symptoms were, however, markedly less than that of the more commonly reported symptoms, the reader can find further corresponding results in the supplementary information (Supporting Information S1: Figure 3).

Odds ratio tables derived from the models fitted to the less commonly reported symptoms are reported in Supporting Information S1: Tables 1-4.

Briefly, there was a significant reduction in the odds of having diarrhoea across all variants, compared to those infected with Wild-Type, whilst holding the other model variables fixed (smallest OR = 0.35, smallest 95% CI = [0.27, 0.45], $p < 0.001$).

There was also a significant reduction in the odds of experiencing nausea across all variants, compared to those infected with Wild-Type, whilst holding the other model variables fixed (smallest OR = 0.49, smallest 95% CI = [0.39, 0.59], $p < 0.001$).

Similarly, there was a significant reduction in the odds of experiencing a loss of appetite across all variants, compared to those infected with Wild-Type, whilst holding the other model variables fixed (smallest OR = 0.42, smallest 95% CI = [0.35, 0.50], $p < 0.001$).

Delta (AY.4.2) was the only variant to yield a statistically significant effect on the odds of having vomiting, which was a reduction in odds, compared to those infected with Wild-Type, whilst holding the other model variables fixed.

Discussion

We conducted a comprehensive analysis of the symptomatology of SARS-CoV-2 variants. This revealed that Omicron variants had a greater proportion of cases which were reported as asymptomatic compared to the other variants considered. There was also variation within the Omicron variant sub-lineages, with some variants associated with markedly lower symptomatic infection rates. In particular, approximately half the proportion of Omicron (BA.4 and BA.5) cases were reported as symptomatic compared to Alpha and Delta cases. Omicron (BA.4 and BA.5) cases were also markedly less likely to be symptomatic compared to Wild-Type. Across all variants cough, fever, anosmia, runny nose and sneezing were the most commonly reported symptoms, although anosmia was reported in non-Omicron variants approximately three times more frequently than in earlier Omicron (BA.1 and BA.2) variants and five times more frequently than in later Omicron (BA.4 and BA.5) variants.

Our dataset is one of the largest to date for assessing differences in variants' symptom patterns and uniquely includes the newer Omicron sub-lineages BA.4 and BA.5, for which little research on clinical presentation currently exists. By capturing data from a broad timeframe and multiple variant waves, our work expands upon earlier studies of variant-linked symptom differences (e.g., Rodríguez et al., 2023; Sumner et al., 2023; Wang et al., 2023) by using a substantially larger population and including these recent variants.

The increased proportion of asymptomatic cases in Omicron variants vs. non-Omicron cases is in line with recent work by (Peña Rodríguez et al., 2023; Yu et al., 2022). The finding of cough, fever and anosmia were among the most commonly reported symptoms, in support of the work by (Mattiuzzi et al., 2022; Sumner et al., 2023). However, the current study also

finds that less common symptoms were still present in up to 15.27% of cases, resulting in a significant portion of symptomatic cases which may have failed to seek testing. The present study also finds that sneezing is reported in up to 24.94% of cases despite it not being included in the official symptom list. The lower proportion of cases reporting anosmia in Omicron variant cases is in line with previous research (Pacchiarini et al., 2022; Peña Rodríguez et al., 2023; Wang et al., 2023; Whitaker et al., 2022). Anosmia was previously considered pathognomonic for COVID-19, so this symptom pattern shift presents a challenge in distinguishing SARS-CoV-2 cases from other respiratory virus cases.

Across all variants, diarrhoea, vomiting, nausea and loss of appetite were found to be reported less commonly, in line with (Whitaker et al., 2022). The proportion of cases in which the given symptom was reported was slightly lower in the present study compared to those observed by (Whitaker et al., 2022), possibly due to the difference in sample size. In addition, the maximum reported proportion for both runny nose and for sneezing was 32.69% in the current study, which is lower than that of (Whitaker et al., 2022) where sneezing and runny nose were reported in ~ 44-50% of Omicron (BA.2) cases and ~36-40% of Omicron (BA.1) cases. Again, these differing results may be related to our enhanced sample size - 86,243 sequenced BA.1/BA.2 cases were included in our study compared to 6,395 cases included by (Whitaker et al., 2022) - though such differences could also potentially be related to differences in sample characteristics, e.g., age structure.

The current literature on the symptom profiles of SARS-CoV-2 generally do not analyse the data on the Omicron variant into its sub-lineages, specifically, BA.4 and BA.5. Therefore, in addition to the present study, further supporting research regarding BA.4, BA.5 and subsequent variants' symptomatology should be conducted.

Limitations

There are minor limitations around missing data, including incomplete availability of symptom data. Symptomatic status, for example, was unknown for 4.49% of the study

population overall, and when missingness differs by variant, estimated odds ratios could potentially be biased by a complete case analysis that implicitly assumes data are missing completely at random (MCAR). However, a missing data sensitivity analysis that leveraged multiple imputation under less stringent missing at random assumption (MAR) produced results for our overall symptomatic status that were near identical to those reported above, providing some reassurance regarding the potential impact of the relatively small proportion of missing data in the present study (see Supporting Information S1: Table 7).

Data may also have been biased by individuals reluctant to disclose symptoms in order to end TTP phone calls sooner. Responder bias may also have occurred if cases were reluctant to report particular symptoms, for example failure to report symptoms for which stigma is associated e.g. diarrhoea. Conversely, responder bias may also have occurred if individuals reported symptoms to secure a test, during a period where this was a requirement for testing. Another limitation is the availability of data for only nine symptoms, which did not include symptoms that other publications reported as differing between variants such as sore throat and myalgia, nor all the symptoms in the latest version of the official list of COVID-19 symptoms, which may have resulted in the loss of patterns of symptomatology between variants (Mahase, 2021).

The results are not fully generalisable to the population due in part to a tendency to target testing for symptomatic cases during the study period (Welsh Parliament, 2023) There was also incomplete control of confounding in the present study. We did not have data pertaining to cases' comorbidities or treatment with antivirals and so could not estimate their influence upon the probability of experiencing symptoms. This is important as antiviral usage could reduce the odds of symptom expression. However, only a small number of individuals were eligible to receive antivirals and hence it is likely only a very small subset of cases included in our study were being treated with antivirals (NHS England, n.d.; Welsh Parliament, 2023). Comorbidities could instead be associated with increased symptom expression. Also important to note in this context, is the fact that vaccination was also prioritised for

vulnerable individuals, e.g., those with comorbidities/immunosuppression. There is therefore scope for an omitted variable bias in our estimated effect of vaccination on symptom expression (i.e., the increased odds of symptom expression among the vaccinated relative to unvaccinated that we report likely reflects lack of accounting for cases' comorbidities).

A number of potential temporal confounders, such as changes in public behaviour, e.g., with respect to adherence to social distancing guidance, and sampling/testing policy changes over time, could also have influenced the present findings (Welsh Parliament, 2023). For instance, free community PCR testing ended on 31 March 2022 (for a policy/response timeline see (Welsh Parliament, 2023), which overlaps with the emergence of Omicron BA.4/BA.5, and reduced testing could arguably inflate the observed proportion of asymptomatic cases. Whilst such temporal confounders are challenging to account for, to explore this possibility we repeated our overall symptomatic status regression model after restricting the data to sequenced samples obtained between the end of 2020 and the end of December 2021, a shorter period associated with relatively stable community testing, and found that the odds of overall symptom expression still differed between variants (see Supporting Information S1: Table 6 for details). This suggests that the general finding that symptom expression differs between variants is valid even though these temporal confounders may have influenced our results (e.g., fitted odds ratios).

Our study compared symptom reporting rates for successive variants compared to the initial Wild-Type strain. Importantly, however, overall, population immunity – associated with both natural infections and vaccination – increased throughout the study period, with serosurveillance data showing approximately 60% of the Welsh population had been infected by July 2022 (Public Health Wales, 2023). Population immunity therefore increased across successive variant waves, and a rapid increase in natural immunity was observed between April and July of 2022 with April being the month that Omicron (BA.4) and Omicron (BA.5) were declared variants (Public Health Wales, 2023; UKHSA, 2022). Notwithstanding the fact that the differences in symptom reporting rates were more striking for some

symptoms compared to others (e.g., anosmia), any moderation in symptom reporting across successive variants could therefore be attributable to the gradual increase in population immunity rather than virological/genomic changes per se. Although we attempted to account for the effect of reinfection (as well as vaccination status) in our estimated models, it is important to note that our binary reinfection status variable was derived based on identification of multiple records for each individual in our sequencing linelist. This variable would therefore only capture reinfections for cases who had multiple PCR-confirmed infections with subsequent sequencing of the associated samples. It is also important to note that the fixed-effects regression analyses reported above did not formally account for the nonindependence of a small proportion of observations comprising our dataset due to reinfection. However, complementary mixed-effects logistic regression analyses incorporating an additional random intercept for individual-specific ID codes produced near identical results. Model selection criteria (AIC) indicated, however, that the fixed effects-only model specifications reported here were preferred (see Supporting Information S1: Table 8).

Although differences in reinfection and vaccination status - which can be considered a proxy for acquired immunity - were accounted for in the present analyses, further research is therefore warranted to disentangle the relative contributions of genomic and immunological factors to the symptom profile effects detected here. One approach would be to compare symptom and/or other outcome rates between variants that were cocirculating in a given time period (ideally with cases matched for known confounders – see above). For example, (Jassat et al., 2023; Nyberg et al., 2022) report Omicron was associated with a lower hospitalisation rate than Delta and was also likely less severe than Wild-Type (for a systematic review and meta-analysis see also (Relan et al., 2023)). This complementary evidence suggests genomic differences as well as changes in population immunity could contribute to our findings of differential symptom reporting rates between variants in the Welsh population.

Conclusion and recommendations

Based on the findings of our large comprehensive study spanning a broad timeframe and multiple variant waves, Omicron variants had a greater proportion of cases which were reported as asymptomatic compared to the other variants considered. Moreover, there was also variation within the Omicron variant sub-lineages, with some variants (BA.4 and BA.5), associated with markedly lower symptomatic infection rates. Further, anosmia was reported at an approximately three-to-five times greater proportion of cases in non-Omicron variant cases compared to Omicron variant cases. The lower prevalence of anosmia - a distinctive feature of SARS-CoV-2- in Omicron variants, may have resulted in cases being unaware of their infection status, resulting in greater transmission. It may also have resulted in an underestimation of the burden of these variant cases. The reduction in proportion of symptomatic cases and proportion of recent Omicron variants cases reported with anosmia , coupled with the vast reduction in testing, presents significant difficulties for detection of SARS-CoV-2 positive cases. This allows for the potential for new variants to become well established or for increased transmission of existing variants to result in substantial waves that could take hold before public health action is able to be initiated.

Indeed, there are challenging implications of reduced symptom severity for clinical management and guidance on self-isolation based on variant symptom profiles. On the one hand, reduced symptom severity may ease burden on healthcare systems and arguably justify less restrictive self-isolation policies in general. However, individuals in high-risk groups may still require stricter isolation advice. Self-isolation guidance may therefore need updating based on circulating variants that may be associated with milder but nevertheless transmissible infections. Public health communication strategies must also strike a balance between providing reassurance about reduced symptom severity and emphasising caution regarding ongoing transmission risk, particularly for vulnerable groups.

The finding of cough, fever and anosmia as among the most commonly reported symptoms indicates that the Government’s first two drafts of the official list of COVID-19 symptoms may

have been sufficient to alert a large proportion of symptomatic cases to be tested (GOV.UK, 2022b). However, in order to increase awareness about the diverse clinical presentation of SARS-CoV-2, and due to our finding that runny nose and sneezing are also among the most commonly reported symptoms, we conclude expansion of the COVID-19 symptoms list should have occurred sooner than April 2022 and that this should have been more regularly reviewed to account for the emergence of new variants. We suggest, given the vast reduction in PCR testing and subsequent WGS (genomic surveillance), symptom surveillance should continue, to provide an approximation of burden of SARS-CoV-2 infection and to inform rapid updating of clinical case definitions in the event of further evolution in the symptom profile. Future symptom surveillance should, however, ideally cover a broader list of symptoms as the present analyses did not include several common symptoms of more recent variants such as sore throat and myalgia (Menni et al., 2022). This recommendation could be implemented in practice through a range of mechanisms, including app-based self-reporting, sentinel GP networks, and routine reporting from pharmacies and hospitals. Additional research could also potentially help disentangle the contributions of genomic, immunological and sampling changes to shifts in the SARS-CoV-2 symptom profile, clarifying the potential for symptom surveillance to be leveraged alongside existing epidemiological methods, as an aid in emerging variant detection.

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Contributions

Nicole Pacchiarini: Conceptualization; supervision. Felicity Simkin: Data curation; software; formal analysis; data visualisation. Mark Postans: Software; formal analysis; data visualisation. Simon Cottrell: Supervision. Jiao Song: Supervision. Christopher Williams: Supervision. Thomas R. Connor: Supervision. Catherine Moore: Supervision.

Ethics Statement

The study presented encompasses two elements. The first of these does not require specific ethical approval, as it focuses on public health/surveillance questions that make use of sequence data and other metadata that is already shared with the wider world as part of the activities of the COG-UK consortium (https://www.cogconsortium.uk/). COG-UK data are released and are publicly available via the ENA, GISAID and the COG-UK website. The element of the work that would/could require ethical approval is the specific examination of outcome data. The use of named patient data in the investigation of communicable disease outbreaks and surveillance of notifiable disease is permitted under Public Health Wales' Establishment Order. Data were held and processed under Public Health Wales' information governance arrangements, in compliance with the Data Protection Act, Caldicott Principles and Public Health Wales guidance on the release of small numbers. No data identifying

protected characteristics of an individual were released outside Public Health Wales. The use of the genomic dataset for research purposes is also covered as part of the COG-UK project protocol, which was approved by the Public Health England Research Support and Governance Office (RSGO) following review by the PHE Research Ethics and Governance Group (REGG).

Conflict of interest

None.

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Data Availability

Aggregate data available on request due to privacy/ethical restrictions (the surveillance datasets contain Personal Identifiable Information used for data linkage and derivation of individual-specific reference codes, e.g., name and date of birth). Requests for data should be directed to PHW.GenomicEpi@wales.nhs.uk.

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