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Integrated surveillance of human respiratory viruses in addition to SARS-CoV-2 in a public testing facility in the Netherlands

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ABSTRACT

Background: SARS-CoV-2 prevention measures impact the circulation of other respiratory viruses. Surveillance in the network of general practitioners is hampered by widespread testing for SARS-CoV-2 in public testing facilities.

Objectives: To evaluate integrated community surveillance of SARS-CoV-2 and other respiratory viruses and describe epidemiological trends.

Study design: Respiratory surveillance was set up within an existing SARS-CoV-2 public testing facility. Community-dwelling (a)symptomatic persons provided consent for completion of a questionnaire and additional testing on residual material from swabs taken for SARS-CoV-2 RT-PCR (Allplex Seegene). Daily, a random subset was tested for sixteen respiratory viruses by multiplex realtime PCRs (Seegene).

Results: Between October 6th (week 40) 2021 and April 22nd (week 16) 2022, 3,969 subjects were tested. The weekly median age ranged from 23 to 39 years. The prevalence of respiratory symptoms ranged from 98.5% (week 40) to 27.4% (week 1). The prevalence of detection of any respiratory virus (including SARS-CoV-2), ranged from 19.6% in week 49 to 75.3% in week 14. SARS-CoV-2 prevalence ranged from 2.2% (week 40) to 63.3% (week 14). Overall, SARS-CoV-2 was detected most frequently (27.3%), followed by rhinoviruses (14.6%, range 3.5–47.8%) and seasonal coronaviruses (3.7%, range 0–10.4%, mostly 229E and OC43). Influenzavirus was detected in 3.0% of participants from week 6 onwards.

Conclusions: Integrated respiratory viral surveillance within public testing facilities is feasible and informative. Prevalences may be affected by changes in SARS-CoV-2 prevention and testing policies. Population characteristics help to interpret trends over time. Integrated surveillance may inform policymakers and hospitals for adequate response measures during respiratory seasons.

1. Background

In 2020, the coronavirus disease 2019 (COVID-19) pandemic urged countries globally to implement interventions to mitigate the transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Public health interventions aimed at restricting the spread of SARS-CoV-2 probably contributed to an earlier end of the 2019/20 influenza season and reduced circulation of other respiratory viruses [2], also during the winter of 2020/21 [3]. Due to waning of immunity and genetic drift of viruses, it is likely that the low level of circulation in the past two years has led to diminished population immunity to respiratory viruses [3]. Easing of restrictions on social distancing could then result in an increase of circulation of respiratory viruses other than SARS-CoV-2 [4]. Indeed, several countries have observed a peak in
respiratory syncytial virus (RSV) infections in spring/summer 2021 instead of the typical fall and winter months, following relaxing of restrictions [5–7].

Due to high levels of vaccination for SARS-CoV-2 and the reduced virulence of the omicron variant, many European countries eased restriction measures, increasing the chance for co-circulation of other respiratory viruses in winter 2021/2022. An increase in the number of cases of especially SARS-CoV-2, influenza and RSV could confer extra pressure on the healthcare system. When making projections about the expected occupancy of hospital and intensive care beds, policymakers should take the prevalence of these respiratory viruses into account. This prevents delay of regular care and limits the growing backlog [6,9].

Since the outbreak of COVID-19, the outcome of the regular annual surveillance of respiratory viruses performed by the existing Sentinel General Practice Network (SGPN) is no longer comparable to pre-pandemic populations. The introduction of large scale SARS-CoV-2 testing, changes in medical advice, and changed behaviour of patients with respiratory symptoms, who no longer consulted a general practitioner with mild respiratory symptoms, resulted in an underrepresentation of the community in this surveillance [10].

In order to continue community surveillance of human respiratory viruses during the COVID-19 pandemic, we piloted a respiratory virus surveillance system in a public testing facility in Utrecht, the Netherlands, where individuals testing for SARS-CoV-2 were simultaneously tested for a panel of respiratory viruses.

2. Objectives

The aims of the study are 1) to evaluate the feasibility of an integrated surveillance of SARS-CoV-2 and other respiratory viruses and 2) to describe the epidemiological trends during the respiratory season starting from early October 2021.

3. Study design

3.1. Study population and sampling

The respiratory surveillance was set up within a public health facility for COVID-19 testing (Fig. 1). Community-dwelling symptomatic and asymptomatic individuals visiting this facility were informed about the study upon arrival and asked for verbal informed consent for participation. Participants first completed a questionnaire about the reason for COVID-19 testing, symptoms (if any), date of symptom onset, vaccination status for SARS-CoV-2 and influenza and recent travel. Subsequently, a combined throat and nasopharyngeal flocked swab (Copan, Italy) was taken and transferred into universal transport medium (UTM, Copan Italy) for SARS-CoV-2 PCR. A random subset of approximately 30 residual nucleic acid eluates was selected daily for PCR-based testing on sixteen different respiratory tract viruses. Since there is no infringement of the physical integrity of the participants as only residual material was used for additional testing, the Medical Research Involving Human Subjects Act (WMO) does not apply for this study.

3.2. Laboratory testing

SARS-CoV-2 diagnostics was performed using real-time PCR (RT-PCR) including three genes of SARS-CoV-2 (Allplex 2019-nCoV assay, Seegene, Seoul, South Korea). Residual nucleic acid eluate was used to test a panel of nineteen targets defining sixteen respiratory viruses in three multiplex RT-PCR (Allplex Respiratory Panel 1, 2 & 3, Seegene, Seoul, South Korea). The sixteen respiratory viruses are Human adeno-virus, Human enterovirus, Human bocavirus, Influenza A, Influenza B, Human metapneumovirus, Parainfluenza type 1 through 4, RSV A, RSV B, Rhinovirus and seasonal Coronavirus OC43, NL63 and 229E. Automated extraction and PCR setup were performed on a Seegene Startlet liquid handling system. Amplification was performed using a CFX96 thermocycler (Biorad, Hercules CA, USA). Sample tracking, extraction, PCR setup, amplification and data analysis were managed by Seegene Viewer software. For each virus, a PCR Ct value <36 was used to define positivity, to exclude low viral load detections that are of uncertain clinical relevance. For SARS-CoV-2, samples were reported positive in line with clinical reporting. The turnaround time for a run of thirty samples was approximately 5 h, including extraction and testing for the complete set of respiratory pathogens. Time from sample collection to reporting was approximately 8–10 h.

3.3. Data analysis and reporting

Descriptive statistics were performed to describe the population that consented to participation, and the final study population (selected for additional testing). Per week, the proportion of individuals experiencing symptoms associated with COVID-19 and the proportion of positive samples were analysed. Weekly prevalences of respiratory viruses including SARS-CoV-2 were calculated and presented together with the

Fig. 1. (A) Regular procedure at a public health facility for COVID-19 testing versus (B) Additional procedure in the facility for COVID-19 testing combined with respiratory surveillance. 1) Individuals with symptoms and without symptoms associated with COVID-19 present at the test lane, 2) All individuals are informed about the respiratory surveillance study, 3) Participants provide verbal informed consent, 4) Participants fill in a short questionnaire about amongst others: symptoms and vaccination status, 5) From all participants a combined throat and nasopharyngeal swab is collected in the same way as for non-participants, 6) All specimens are used for additional testing, the Medical Research Involving Human Subjects Act (WMO) does not apply for this study.
changes in public health measures taken by the government to contain SARS-CoV-2. Numbers of viral respiratory infections and persons tested negative for any virus were reported anonymously to the regional and national public health authorities on a weekly basis.

4. Results

4.1. Study population

From October 6th 2021 until April 22nd 2022, a total of 7,045 individuals provided informed consent and completed the questionnaire (consent population). Samples of 3,696 subjects were randomly selected for respiratory surveillance (study population). The mean number of tested participants (i.e. study population) was 127 per week and ranged from 22 (week 52) to 237 (week 41). The study population is representative for the entire population that consented to participation (Table 1). The median age of the study population was 28 years, 2,537 (68.6%) experienced any symptoms suggestive for COVID-19, and 2,964 (80.2%) persons were at least fully vaccinated against SARS-CoV-2. Missing data was <0.5% for person characteristics including SARS-CoV-2 vaccination status, and 0.7%–4.2% for the nineteen specified symptoms.

4.2. Characteristics of study population over time

Some characteristics of the study population showed week-by-week variation. During the 29 weeks of surveillance, the proportion of the study population reporting symptoms decreased from 98.5% in week 40 to 27.6% in week 1 (Fig. 2A). From week 46, we were informed about the primary reason for testing (Fig. 2B). Average weekly prevalence of participants reason for testing were in order of priority 1) a positive SARS-CoV-2 selftest (range: 0.9% (week 46)) - 36% (week 14), 2) contact with a COVID-19 case (range: 18.7% (week 16)) - 75.6% (week 1)) and 3) symptoms suggestive of COVID-19 (range: 8.1% (week 1) - 55.4% (week 47)). A minority of participants tested for recovery after a prior positive test or for unclear reasons.

4.3. Public health measures, testing policy, Omikron and booster uptake

During the study period, national containment measures and testing policies were subject to several changes, of which the most important ones are described in Fig. 2C. At the start of the study period, containment measures were limited to the use of masks in public transport, an isolation policy for positive individuals, quarantine for unvaccinated contacts, advise to work from home when possible, and the use of a coronavirus entry pass - with a negative test result, proof of vaccination or proof of recovery (3G) - for access to social events. From week 46 until week 2, containment measures were intensified with partial and major-lockdowns of schools and shops. In week 2, 3 and 9 measures were gradually lifted. At study start, everyone with suggestive symptoms or a COVID-19 contact was advised to test in a public testing facility. Importantly, the testing criteria changed from week 49 onwards, when a negative antigen self-test became an accepted alternative to PCR-testing, whilst individuals with positive self-tests were requested to undergo confirmation PCR to keep track of the number of COVID-19 cases. From week 14, both a positive and negative self-test were considered accepted alternatives for most persons. The omikron variant became dominant (>50%) from week 51 onwards. Between week 49 and 5 booster uptake reached >50% for all birth cohorts between <1940 and 1980.

4.4. Respiratory surveillance results

Weekly prevalence of the different viruses is presented in Fig. 3. Monthly prevalences per viral subtype, as well as prevalences amongst symptomatic and asymptomatic individuals, are presented in Table 2.

The prevalence of detecting at least one respiratory virus in any subject was 50.4%, and it was 63.5% vs. 21.2% in symptomatic vs. asymptomatic individuals respectively. Double infections with two or more respiratory viruses occurred in 140 (3.8%) individuals. SARS-CoV-2 was detected most frequently (27.3%), followed by rhinovirus (14.6%) and seasonal coronaviruses (3.7%, mostly 229E and OC43).

The weekly prevalence of per subject detection of at least one respiratory virus including SARS-CoV-2, ranged from 19.6% in week 49 to 75.3% in week 14. SARS-CoV-2 prevalence changed in time and ranged from 2.2% (week 40) to 63.3% (week 14). Influenza was detected in weeks 40 through 42 with a prevalence of 5.9%, but did not re-occur until one case in week 46. From week 6 forward, influenza was found systematically until the end of the surveillance period. Overall, most influenza detections were Influenza A subtype H3 (N = 101), Influenza A subtype H1 was found sporadically (N = 4 in week 14 and 15 of 2022). Both RSV A and RSV B were detected, with a relatively low but stable prevalence of maximum 1% (week 42). Parainfluenza type 2 and type 4 were detected each week until week 47, with type 4 being most prevalent in weeks 40 to 43 and type 2 in weeks 44 to 46. After week 48, Parainfluenza type 2 prevalence became sporadic, while Parainfluenza type 4 disappeared. The second most commonly detected virus was rhinovirus, which occurred in 47.8% of samples in week 40, followed by a decline to 4.7% in week 46, and stabilisation around 5–10% afterwards (with the exception of week 9).

Table 1

| Characteristic                  | Informed consent (N = 7,045) | Study population (N = 3,696) |
|--------------------------------|------------------------------|-----------------------------|
| Median age [IQR]               | 28.0 ([22.0 - 34.0])         | 28.0 ([22.0 - 34.0])        |
| Gender, nr. of males (%)       | 2,982 (42.3%)                | 1,595 (43.2%)               |
| Symptoms suggestive for COVID-19†| 4,827 (68.6%)                | 2,537 (68.6%)               |
| Median number of days from symptoms to test, if any [IQR] | 2.0 ([1.0 - 3.0]) | 3.0 ([3.0 - 6.0]) |
| Common cold                    | 2,917 (41.4%)                | 1,552 (42.0%)               |
| Sore throat                    | 2,441 (34.6%)                | 1,337 (36.2%)               |
| Cough                          | 1,703 (24.2%)                | 930 (25.2%)                 |
| Rhinorrhea                     | 1,139 (16.2%)                | 622 (16.8%)                 |
| Headache                       | 1,010 (14.3%)                | 526 (14.2%)                 |
| Fever                          | 714 (10.1%)                  | 359 (9.7%)                  |
| Travel abroad in past 14 days  | 725 (10.3%)                  | 405 (11.0%)                 |
| SARS-CoV-2 vaccination status  |                              |                             |
| Fully vaccinated               | 958 (13.6%)                  | 556 (15.0%)                 |
| Fully vaccinated (primary series, not informed about booster)** | 4,650 (66.0%) | 2,408 (65.2%) |
| Partially vaccinated           | 528 (7.5%)                   | 290 (7.8%)                  |
| Not vaccinated                 | 893 (12.7%)                  | 434 (11.7%)                 |
| Unknown if vaccinated          | 13 (0.2%)                    | 8 (0.2%)                    |

The study population consisted of a random selection of the consent population that was tested for respiratory viruses (in addition to SARS-CoV-2 testing). Missing data was <0.5% for person characteristics including SARS-CoV-2 vaccination status, and 0.7%–4.2% for the nineteen specified symptoms.

† The table includes the six most prevalent symptoms. Other symptoms evaluated included all frequent and less frequent symptoms associated with COVID-19 as listed by the Dutch National Institute for Public Health and the Environment (RIVM) [15]. Prevalence in study population: fatigue (6.5%), malaise (5.4%), myalgia (4.7%), dyspnoea (4.1%), nausea (1.7%), loss of smell and taste (1.5%), loss of appetite (1.5%), stomach ache (1.0%), arthralgia (0.6%), t: vomiting (0.4%), diarrhoea (0.4%), eye pain (0.1%), and skin rash (0.1%).

** Fully vaccinated was defined as at least 2 doses Pfizer/Moderna/AstraZeneca or 1 dose of Janssen at least 14 days and 28 days respectively before symptom onset (or test date, if symptoms were absent [Protocol bron- en contactonderzoek COVID-19 | LCI richtlijnen (rivm.nl), version December 20th 2021]).

Table 2

| Virus Type | Study Population (%) |
|------------|----------------------|
| Influenza A H1, 2 | 19 (0.6%) |
| Influenza A H3 | 101 (2.9%) |
| Influenza B | 4 (0.1%) |
| Parainfluenza type 1 | 1 (0.0%) |
| Parainfluenza type 2 | 4 (0.1%) |
| Parainfluenza type 3 | 1 (0.0%) |
| Parainfluenza type 4 | 2 (0.0%) |
| Rhinovirus | 2,714 (7.5%) |
| Coronavirus | 7,045 (100.0%) |

The weekly prevalence per subject detection of at least one respiratory virus including SARS-CoV-2, ranged from 19.6% in week 49 to 75.3% in week 14. SARS-CoV-2 prevalence changed in time and ranged from 2.2% (week 40) to 63.3% (week 14). Influenza was detected in weeks 40 through 42 with a prevalence of 5.9%, but did not re-occur until one case in week 46. From week 6 forward, influenza was found systematically until the end of the surveillance period. Overall, most influenza detections were Influenza A subtype H3 (N = 101), Influenza A subtype H1 was found sporadically (N = 4 in week 14 and 15 of 2022). Both RSV A and RSV B were detected, with a relatively low but stable prevalence of maximum 1% (week 42). Parainfluenza type 2 and type 4 were detected each week until week 47, with type 4 being most prevalent in weeks 40 to 43 and type 2 in weeks 44 to 46. After week 48, Parainfluenza type 2 prevalence became sporadic, while Parainfluenza type 4 disappeared. The second most commonly detected virus was rhinovirus, which occurred in 47.8% of samples in week 40, followed by a decline to 4.7% in week 46, and stabilisation around 5–10% afterwards (with the exception of week 9).
5. Discussion

Integrated respiratory viral surveillance within public health facilities is feasible and informative. Even though test facilities aim at handling high throughput, it is possible to use the facility for extended surveillance, including the administration of a questionnaire, which takes approximately two to three minutes of time for both participant and administrator. Residual eluates allowed testing for sixteen additional viruses without the need for additional sample collection. Data from the questionnaire provided significant value to interpret the dynamics of the respiratory viruses over time, since they gave insight in age, reason for testing, the proportion of symptomatic individuals, symptomatology and vaccination status. On average, more than 240 individuals provided weekly data which allowed for more data collection than is seemingly feasible in any primary healthcare facility.

Weekly prevalence of respiratory viruses was dynamic for some and less dynamic for other viruses. The prevalence of rhinovirus was highest in the first weeks of the surveillance period, which is in line with the seasonal pattern for this virus of being especially prevalent in early autumn [11]. We detected an atypical seasonal influenza pattern with a relatively late onset from week 6 and onwards. This trend is in line with influenza surveillance data from hospitals and primary care in the Netherlands [12]. RSV was detected sporadically throughout the entire study period (0.6%), with highest prevalence being detected in October (1.6%); both RSV A and B were found. In the Netherlands a RSV surveillance amongst 20 laboratories and several hospitals is in place, which reported that RSV had already been endemic from week 23 of 2021, and peaked in week 29 of 2021, after which low numbers of cases continued to be detected until at least the end of our study period [13].

Besides seasonal effects, virus prevalence may be affected in
response to measures to prevent SARS-CoV-2 transmission (which probably explains the late start of the influenza season in winter 2021/2022) and in response to SARS-CoV-2 circulation itself. In the first nine weeks of the surveillance, we saw the prevalence of symptoms suggestive of SARS-CoV-2 decline from 98.5% to 50.4% whilst the prevalence of SARS-CoV-2 increased until week 47, suggesting that more and more participants came to test because of contact with a case of COVID-19. Following this observation, we added a question on the reason for testing, allowing better interpretation of trends observed. From week 49 onwards.

There are a few limitations to our study. First, the population in the testing facility involved in our study contained a relatively large number of individuals aged 19 to 24 years and fewer children compared to the population in public testing facilities across the entire region of Utrecht (median age in study 28, median age in region 31). The most likely reason for this is that the testing facility was initially located near the University of Utrecht student faculty and student housing for most of the region of Amsterdam, who both implemented similar integrated surveillance systems [14]. Future studies could evaluate the optimal number and distribution of surveillance structures to generate sufficient and representative data for trend analysis in a certain country or region.

Second, during the first phase of the integrated surveillance on the testing facility, we experienced that the set-up is vulnerable to variation in inclusion rate due to changes in logistics, workload and personnel.
Low numbers of consent and/or samples tested were related to relocation of the public health facility (N = 43, week 46), relocation of the testing to a different hospital (N = 58, week 50) and shortage of personnel. This variation is expected to decrease with more experience with the surveillance system.

Thirdly, the proportions of symptomatic and asymptomatic individuals fluctuated (Fig. 2A) together with changes in viral circulation, public health measures, and changes in testing policy and reason for testing (Fig. 2B, i.e. COVID-19 contact). We detected almost all viruses both in symptomatic and in asymptomatic individuals, with rhinovirus and SARS-CoV-2 being most prevalent in both subgroups, and Influenza and RSV being mostly present in symptomatic individuals. These results suggest that testing of asymptomatic individuals for respiratory viruses other than SARS-CoV-2 has no additional value to testing of symptomatic individuals.

The ultimate aim of this surveillance is to investigate whether the integrated respiratory surveillance in the public test facility can be used as early warning for healthcare facilities and prepare them for increased burden. Weekly data sharing with the RIVM allowed for timely detection of the start of increased influenza activity. Moreover, we plan to combine our data with data on number of patients hospitalised with influenza, SARS-CoV-2 and RSV.

Whether integrated respiratory surveillance is rewarding on the long term, depends on the benefits and costs of such a strategy. Benefits are that it provides a more complete picture of the circulating viruses in the event of a future pandemic with large scale testing in a public facility. In addition, it allows for the collection of additional information to assess for example vaccine effectiveness (e.g. SARS-CoV-2, Influenza), with no additional burden in data collection for healthcare providers. However, when countries implement strategies that lead to selection of the population in the testing facility, e.g. by accepting a (negative) SAR-CoV-2 antigen self-test as alternative to PCR-testing, it seems hardly possible to obtain prevalences representative of circulating viruses in the entire community. In that scenario the economic burden of integrated surveillance might not outweigh the benefits.

In conclusion, setting up integrated surveillance of SARS-CoV-2 and other respiratory pathogens within a public testing facility proved feasible, with a minimal burden for participants, sufficient willingness to participate, and therefore a high number of participants. Timelines of the laboratory work and data processing allowed weekly national reporting of reliable, robust and real-time data on seventeen different respiratory viruses. By combining the number of viral infections with population characteristics and containment measures, the results can be interpreted in the right context. Preferably, such surveillance system should be set-up in an unselected population (i.e. without the use of self-tests). Trends in prevalence of viruses over time can prepare policymakers for a new viral surge and inform hospitals about the expected occupancy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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