Risk factors, immune response and whole-genome sequencing of SARS-CoV-2 in a cruise ship outbreak in Norway

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ABSTRACT

Objective: To improve understanding of SARS-CoV-2-transmission and prevention measures on cruise ships, we investigated a Norwegian cruise ship outbreak from July to August 2020 using a multidisciplinary approach after a rapid outbreak response launched by local and national health authorities.

Methods: We conducted a cross-sectional study among crew members using epidemiologic data and results from SARS-CoV-2 polymerase chain reaction (PCR) of nasopharynx-oropharynx samples, antibody analyses of blood samples, and whole-genome sequencing.

Results: We included 114 multinational crew members (71% participation), median age 36 years, and 69% male. The attack rate was 33%; 32 of 37 outbreak cases were seropositive 5-10 days after PCR. One PCR-negative participant was seropositive, suggesting a previous infection. Network-analysis showed clusters based on common exposures, including embarkation date, nationality, sharing a cabin with an infected cabin-mate (adjusted odds ratio [AOR] 3.27; 95% confidence interval [CI] 0.97-11.07, p = 0.057), and specific workplaces (mechanical operations: 9.17 [1.82-45.78], catering: 6.11 [1.83-20.38]). Breaches in testing, quarantine, and isolation practices before/during expeditions were reported. Whole-genome sequencing revealed lineage B.1.36, previously identified in Asia. Despite extensive sequencing, the continued transmission of B.1.36 in Norway was not detected.

Conclusions: Our findings confirm the high risk of SARS-CoV-2-transmission on cruise ships related to workplace and cabin type and show that continued community transmission after the outbreak could be stopped by implementing immediate infection control measures at the final destination.

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SARS-CoV-2 was first reported from Wuhan, China, in 2019, leading to outbreaks of COVID-19 and rapid spread worldwide. Cruise ships are ideal incubators for respiratory viruses because of crowded places, close-contact settings, and confined and enclosed spaces (the 3 C's) (WHO, 2021). Consequently, a number of SARS-CoV-2 outbreaks have occurred on cruise ships causing considerable morbidity and mortality (Expert Taskforce for the COVID-19 Cruise Ship Outbreak, 2020; Kordsmeyer et al., 2021). Cruise operators need to ensure that cruises do not pose unacceptable health risks to passengers, crew, and the general public (ECDC, 2021). Early recognition and an effective outbreak management strategy are crucial to controlling SARS-CoV-2 transmission on board (Kordsmeyer et al., 2021, Walker et al., 2021). To our knowledge, this is the first study of a SARS-CoV-2 outbreak in northern European waters on a cruise ship registered in a Nordic country.

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Outbreak Setting

In June 2020, the MS Roald Amundsen became the first cruise ship to return to service in Norway after a 3-month suspension because of the pandemic after being in storage in a small coastal municipality with a multinational crew on board. The ship had recently updated, but not fully implemented, outbreak prevention, control, and response plans (Wiersholm AS and DNV GL SA Law Firm, 2020). At the time, the national incidence of COVID-19 was low (3.7 cases/100,000 population/14 days), and no cases were reported from this municipality to the Norwegian Institute of Public Health (NIPH). The ship had a total capacity of 528 passengers. From July 6-17, 2020, approximately 90 crew members, recruited through a foreign staffing company, arrived in Norway, primarily from the Philippines. Before starting service, they had to provide COVID-19 certificates confirming negative results for tests taken 3-4 days before departure but were not tested in Norway before boarding the ship. They underwent 10 days of initial restrictions on board, including social distancing, respiratory and hand hygiene measures, and isolation in a single cabin if any symptoms developed. However, they were allowed to work during quarantine, except those employed in the kitchen.

Passengers and crew members had cabins on separate decks. Single or twin cabins were assigned to the staff based on job and rank; however, some were quarantined in twin cabins together with staff with an earlier embarkation date.

The MS Roald Amundsen completed two one-week voyages from Tromsø and around the Svalbard archipelago at 75° north and back; Expedition 1, July 17-24, 2020, included 210 passengers and 160 crew, and Expedition 2, July 24-31, 2020, included 181 passengers and 160 crew. Most passengers were Norwegian citizens, whereas 5% were from other European countries. Most crew members attended both expeditions.

Outbreak detection and public health response

On July 26, 2020, 2 days after returning home from Expedition 1, a passenger developed symptoms and had a SARS-CoV-2 positive test result 2 days later. The timeline was consistent with probable exposure on board (Lauer et al., 2020). On July 29, 2020, the local municipal medical officer notified the NIPH about the case, who then immediately contacted the ship and recommended alerting and testing of passengers, but no action was taken. During Expedition 2, several symptomatic crew members were reported unable to work but were not tested nor isolated.

After finishing Expedition 2, the ship arrived back in Tromsø on the morning of July 31, 2020. At midday, the NIPH was alerted by the Tromsø municipal chief medical officer that 4 sick crew members had been hospitalized and subsequently diagnosed with COVID-19. By then, all passengers had left the ship. An outbreak response was immediately launched by Tromsø municipality with support from the NIPH and Norwegian Directorate of Health. The objectives were to determine the origin and extent of the outbreak and implement infection control measures to prevent further spread in Norway.

On July 31, 2020, and during the following days, all passengers and crew on both expeditions had a nasopharynx-oropharynx swab taken, either in Tromsø or at their current location, which was tested by real-time polymerase chain reaction (PCR) for SARS-CoV-2. All close contacts of cases on both expeditions were traced and tested. After July 31, 2020, crew member cases were isolated in single cabins or at the hospital, whereas those who tested negative were quarantined onboard or in hotels in Tromsø.

The attack rate among crewmembers was 25.2% (42 of 167) compared with 7.2% (28 of 391) among passengers, with an increase in affected passengers from 1.4% on Expedition 1 to 13.8%

on Expedition 2. All crew members who tested positive had attended both expeditions. Reported dates for symptom onset for crew members ranged from July 6 to August 11, and for passengers, from July 26 to August 8. Ten crew members were hospitalized because of severe illness (no fatalities).

After the outbreak response, we conducted a study to determine when and how the virus was introduced and to examine risk factors for transmission onboard. To do so, we examined samples and exposure data collected from the crew members while they were in quarantine or isolation in Tromsø, as they had a significantly higher attack rate than the passengers and were still in one geographic area. As only a quarter of the crew was infected, we also aimed to assess if the noninfected crewmembers were protected by immunity from earlier infections.

Methods

Study Design and Population

We conducted a cross-sectional study among the crew members present in Tromsø from August 9-11, 2020. The study coordinator gave a video-streamed informational talk to all crewmembers in quarantine or isolation on the ship on August 9, 2020, which they shared in real-time with their colleagues staying in hotels or the hospital. Afterward, the attendees received written study information and consent forms.

Data Collection

The coordinator and a laboratory team collected signed consent forms and drew venous blood samples from crew members agreeing to participate on August 10-11, 5-10 days after the nasopharynx-oropharynx sampling (NOPS) on July 31, 2020, to August 6, 2020. Participants staying in hotels or the hospital were sampled on-site. A case was defined as a participant attending Expedition 2 with a SARS-CoV-2-positive PCR result.

The NIPH received crew and passenger lists from the ship, including data on sex, age, country of origin, embarkation date, workplace, cabin number and type, symptom onset date, clinical symptoms, test date, and results that were included in the NIPH outbreak registry and used in our study. Type of work was categorized as administrative, passenger services, outdoor work, mechanical operations, and catering occupations (Table 1).

Laboratory Methods

NOPS were analyzed with a SARS-CoV-2 real-time reverse transcription PCR test with primers and probe in the E-gene (Corman et al., 2020), which was considered positive when the cycle threshold (Ct) value was ≤37. Plasma was tested for SARS-CoV-2 antibodies with the Abbott Architect SARS-CoV-2 immunoglobin G (IgG) and immunoglobin M (IgM) assays, detecting antinucleocapsid antibodies, and with the Liaison SARS-CoV-2 S1/S2 IgG and IgM assays, detecting antispike antibodies (Supplementary Table 1). Of note, IgG antibodies against spike is a correlate of neutralizing activity (Dispinseri et al., 2021). To avoid unspecific results, only samples positive in at least 2 different assays were considered seropositive. For selected samples, SARS-CoV-2 IgG was in addition analyzed by immunofluorescence staining of SARS-CoV-2 infected Vero cells (Henriksen et al., 2020).

We performed whole-genome sequencing (WGS) of SARS-CoV-2 using the Artic Network nCoV-2019 V3 protocol (Corona Methods Development Community, 2020) and an Illumina MiSeq sequencer, with some supplementary genome sequences obtained using the Swift Amplicon SARS-CoV-2 Panel (Swift Bioscience,

Table 1

Demographics of study participants in a cruise ship outbreak of SARS-CoV-2, Tromsø, Norway from July 2020 to August 2020

Characteristics	n	%
Total number of participants	114	100.0
Sex		
Women	35	30.7
Men	79	69.3
Age group, years		
20-29	23	20.2
30-39	47	41.2
40-49	33	29.0
≥50	11	9.7
Country of origin		
Norway / Europe*	33	29.0
The Philippines	81	71.0
Type of work†		
Administrative	11	9.6
Passenger services	22	19.3
Outdoor work	21	18.8
Mechanical operations	10	8.8
Catering	50	43.9
Cabin type		
Single	60	52.6
Twin	54	47.4
Embarkation date		
5 July 2020 and earlier	33	29.0
6-8 July 2020	45	39.4
9-24 July 2020	36	31.6
SARS-CoV-2 PCR test		
Negative	77	67.5
Positive	37	32.5

* A few European countries.

† Administrative: officers and managers. Passenger services: reception, store and medical/spa. Outdoor work: expedition team and work on deck. Mechanical operations: engine work, electricity, and carpentry. Catering: kitchen, bar and restaurant

Table 2

Reported clinical symptoms among 37 cases in a cruise ship outbreak of SARS-CoV-2, Tromsø, Norway from July 2020 to August 2020

Characteristics	n	%
Total no. SARS-CoV-2 cases	37	100.0
Clinical symptoms		
Upper respiratory tract infection (URTI)*	18	48.6
Lower respiratory tract infection (LRTI)†	7	18.9
Unknown symptoms‡	7	18.9
Other symptoms§	2	5.4
Asymptomatic	3	8.1

* 18 SARS-CoV-2 cases reported URTI, among which 4 had lost the sense of smell and taste and 2 others in addition reported diarrhea and abdominal pain.

† 7 cases reported LRTI, among which 2 had lost the sense of smell and taste.

‡ 7 cases reported date for symptom onset but no symptoms ("unknown symptoms").

§ One case reported fatigue only and another reported tachypnea only.

Ann Arbor, United States) on Illumina NovoSeq at the Norwegian Sequencing Center. The resulting sequences were assembled into consensus genome sequences using pipelines available on GitHub (pipelines for the Artic data: https://github.com/folkehelseinstituttet/fhi-ncov-seq-pipelines). The most similar sequences in the global initiative on sharing all influenza data (GISAID) database EpiCoV were identified using Basic Local Alignment Search Tool (BLAST) and included in the phylogenetic analysis by neighbor-joining of maximum composite likelihood distances using the MEGA X software (Kumar et al., 2018).

Statistical Analyses

We linked outbreak registry data to laboratory data from the University Hospital of North Norway and WGS results from the NIPH. We used STATA Statistical Software Version 16.1 (StataCorp LLC, Texas, United States) for multivariable logistic regression analysis with outcome-variable SARS-CoV-2 test result and calculated adjusted odds ratios (AORs) with 95% confidence intervals (CIs). Variables with p-value >0.1 were included in the model. We considered 2-sided p-values <0.05 statistically significant. Collinearity was not a problem with variance inflation factor <2.0 for all variables. Interaction was assessed by including cross-product terms between all independent variables and gender. There was no statistically significant interaction in the full model. We used nonparametric tests to compare participants' IgG titers in different embarkation periods and to compare Ct-values between IgG-positive and IgG-negative participants. The descriptive network-analysis was conducted using STATA, whereas the network was visualized and modularity determined using Gephi 0.9.2, an open-source, free software for network visualization and exploration (https://gephi.org). The network was weighted by the number of

shared characteristics using the following four: grouped date of embarkation, sharing a cabin, workplace, and country of origin (weight 0 to 4).

Results

Crew member study population

In total, 71% (114 of 160) of eligible crew members with a median age of 36.4 years participated in the study, of whom 69% were male, 29% were Norwegian/European, and 71% Filipino (Table 1). The median number of days on board before PCR-testing was 24 (interquartile [IQR] 18-37). Most Norwegian/European participants (88%, n = 29) had single cabins compared with 38% (n = 31, p <0.001) of Filipino participants. Fifty (62%) Filipino participants shared a twin cabin with a fellow citizen, whereas only 4 (12%, p <0.001) Norwegian/European participants did so.

Outbreak study analyses

The attack rate among crew member participants was 32.5% (37 of 114), similar to that of the entire crew (25.8%, p = 0.182). The distribution of cases by date and symptom onset shows a slowly increasing outbreak with a sharp peak on July 31, and then decreasing incidence (Figure 1). Participants' confirmed PCR-positive tests had mean a Ct value of 24.9 (range 15.4-36.8).

Respiratory infection symptoms were reported by 25 (68%) cases, including 6, in addition, having lost their sense of smell and taste, and 2 reported gastroenteritis. Nine had "other" or unknown symptoms, and 3 were asymptomatic (Table 2).

SARS-CoV-2 spread among the participants in most workplaces on board. The first cases retrospectively reporting symptom onset between July 20-22, 2020, worked in catering occupations and mechanical operations (Figure 1). Nine days later, symptoms among administrative and passenger service staff were reported. No cases were identified among participants working on deck, with electricity and carpentry, or with medical and spa services.

The attack rate was higher among Filipino participants (41%) than among those from Norway/Europe (12%, p = 0.006) (Table 3). In multivariable analyses, being defined as a case was associated with working with mechanical operations (AOR 8.26, 95% CI 1.54-44.16) and catering occupations (6.06, 1.78-20.67). Sharing a twin cabin with an infected cabin-mate was strongly associated with being a case in crude analysis (OR 7.20, 2.48-20.41) but was attenuated to 3.27 (0.97-11.07) in the full model. The mean serial
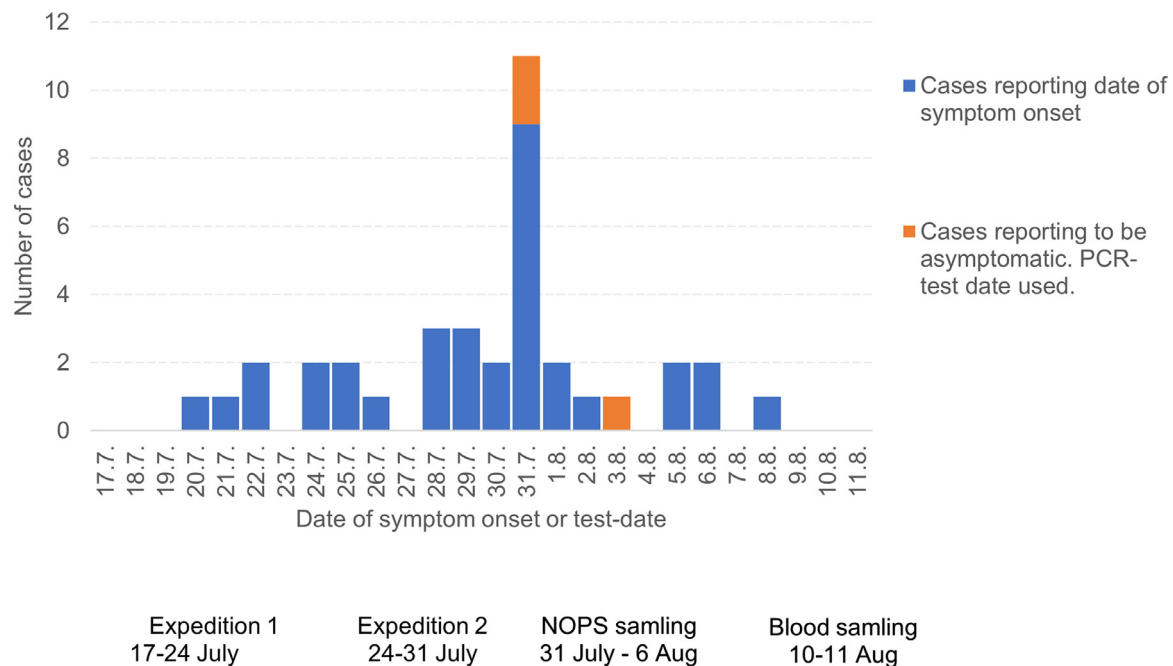
Figure 1. Epidemiologic curve of 37 SARS-CoV-2 cases by date of symptom onset, July 2020 to August 2020.

Table 3

Characteristics associated with being a case of SARS-CoV-2 in a cruise ship outbreak, Tromsø, Norway from July 2020 to August 2020

Characteristics	Cases n (%)	OR	95% CI	p-value	AOR	95% CI	p-value	N
Sex				0.149				114
Women	8 (22.9%)	1.00						35
Men	29 (36.7%)	1.96	0.79-4.87					79
Age group, years				0.579				114
20-29	5 (21.7%)	1.00						23
30-39	15 (31.9%)	1.69	0.53-5.41					47
40-49	13 (39.4%)	2.34	0.70-7.86					33
≥ 50	4 (36.6%)	2.06	0.42-9.97					11
Country of origin				0.006			0.812	114
Norway / Europe*	4 (12.1%)	1.00			1.00			33
The Philippines	33 (40.7%)	4.98	1.60-15.51		1.20	0.26-5.47		81
Type of work†				<0.001			0.005	114
Admin./passenger services/outdoor	5 (9.3%)	1.00			1.00			54
Mechanical operations	5 (50.0%)	9.80	2.09-45.89		9.17	1.82-45.78		10
Catering	27 (54.0%)	11.50	3.93-33.71		6.11	1.83-20.38		50
Cabin type				<0.001			0.057	114
Single, or twin with neg. cabin-mate	23 (24.5%)	1.00			1.00			94
Twin with pos. cabin-mate	14 (70.0%)	7.20	2.48-20.91		3.27	0.97-11.07		20
Embarkation date				0.006			0.525	114
5 July 2020 and earlier	10 (30.3%)	1.00			1.00			33
6-8 July 2020	22 (48.9%)	2.20	0.86-5.66		1.52	0.51-4.55		45
9-24 July 2020	5 (13.4%)	0.37	0.11-1.23		0.67	1.46-3.12		36

The multivariable model included country of origin, type of work, cabin type, and embarkation date.

OR = odds ratio; AOR = adjusted OR; N = denmoninator.

* A few European countries

† Administrative (officers, managers), passenger services (reception, store, medical/spa) and outdoor work (expedition team, work on deck) were combined as they had similar COVID-19 rates. Mechanical operations: engine work, electricity, and carpentry. Catering occupations: kitchen, restaurant and bar.

interval between symptom onset in twin cabins with both persons infected was 4.3 days (range 1-8 days).

The network-analysis displayed four distinct clusters (Figure 2). The purple cluster (n = 42) had an attack rate of 50%, of which 71% had catering occupations, all had the embarkation date July 6-8, 2020, most were Filipino (98%) and stayed in twin cabins (76%) (Figure 2, Table 4). Two clusters (green, n = 31 and blue, n = 10) had an attack rate similar to that of the study population as a whole, and most participants were Filipino. The orange (n = 31) had an attack rate of 10%, well below the study population, and most participants were Norwegian or European (97%), had outdoor work (55%), single cabin (90%), and late embarkation (84%). Cases were neither more central in the network nor had more links than non-cases.

Immunologic Responses

Antibody analysis of the 37 outbreak cases demonstrated SARS-CoV-2 seropositivity in 32 (87%), confirming their COVID-19 diagnosis (Figure 3). Except for one seropositive case with only IgG and 5 cases with only IgM, all seropositive cases had IgM and IgG antibodies. The quantitative antispike IgG assay revealed antibody
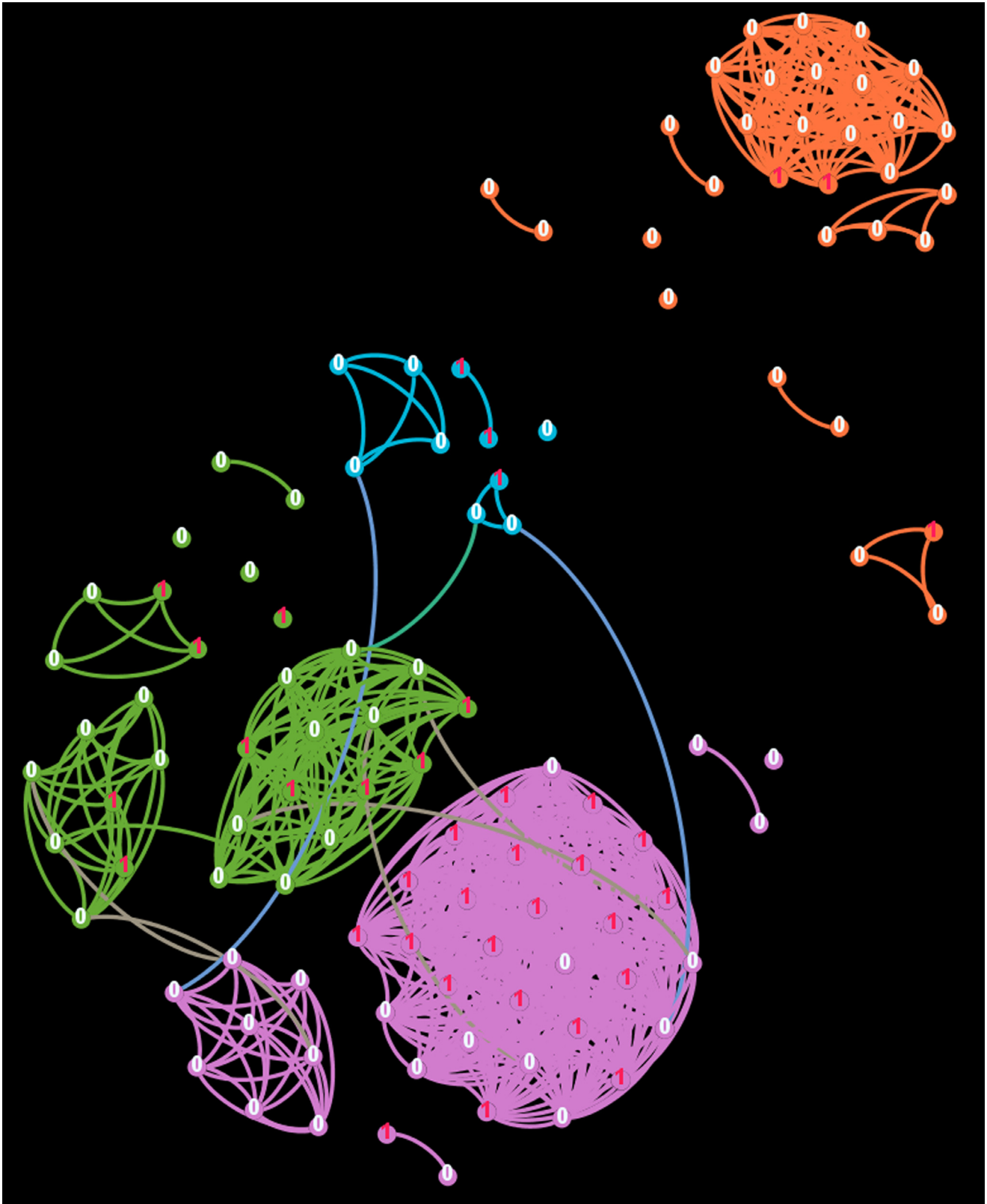
Figure 2. Social network-analysis of 114 study participants, July 2020 to August 2020. The network was split into four different communities displayed in different colors: purple cluster (n = 42), green cluster (n = 31), orange cluster 2 (n = 31) and blue cluster (n = 10). Nodes with a red "1" indicates a case; white "0" indicates a noncase. Only links between participants with at least three different connections are shown. Four possible connections were included: sharing twin cabin, same country of origin, same work place, and same embarkation date.
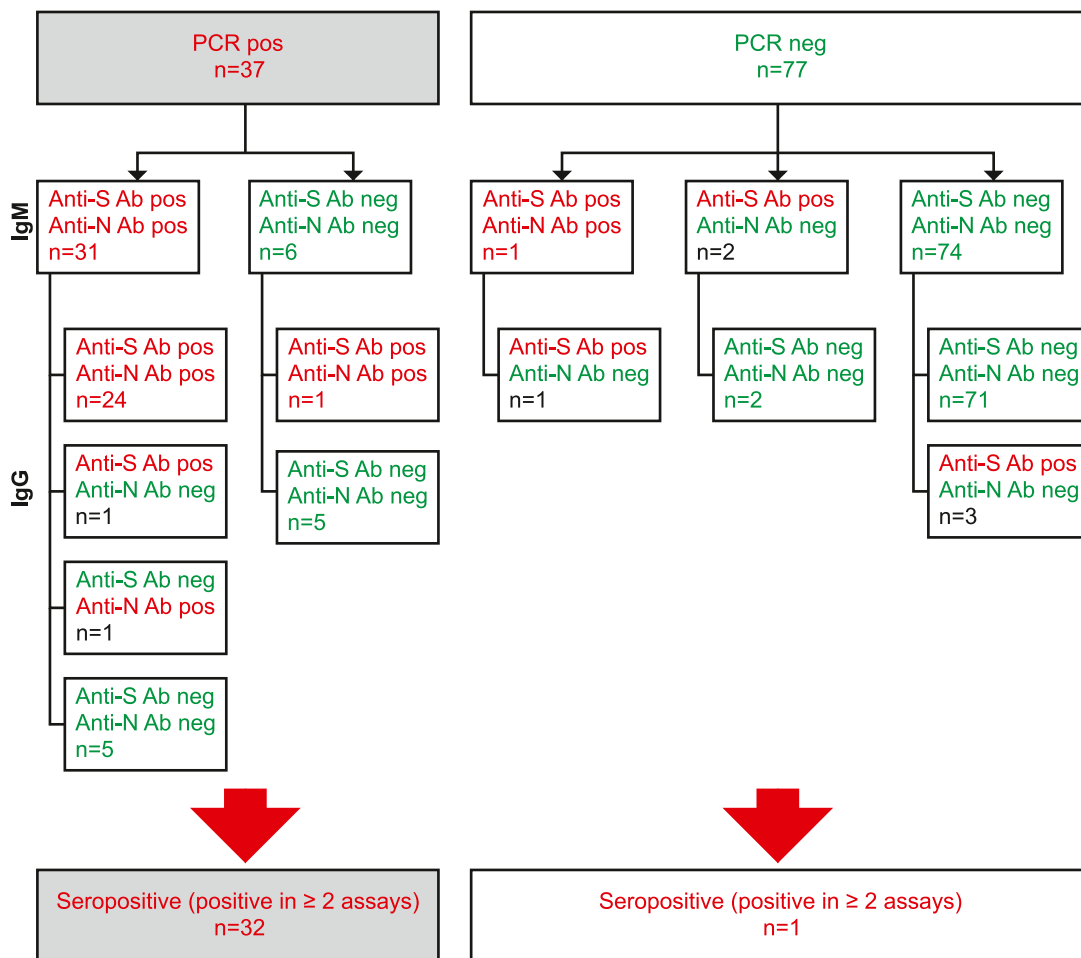
Figure 3. Serology results from 37 PCR-positive SARS-CoV-2 cases and 77 PCR-negative participants using 2 IgM and 2 IgG assays, July-August 2020.

titers from 18.5-166 AU/ml (mean value 70 AU/ml). There was a nonsignificant increase in antibody titers with increasing time after reported symptom onset (p = 0.092), and only samples taken more than 7 days after symptom start had titers above 100 AU/ml (Figure 4). The median Ct value of NOPS was 7.9 Ct-values lower in the 10 IgG-negative than the 27 IgG-positive cases (p = 0.130), indicating a 100-fold higher viral load among IgG-negative participants (Figure 5). Cases with a high viral load and a low or lacking IgG titer were presumably in an early phase of infection. In agreement with this, 10 of the 11 antispike IgG-negative cases reported symptom onset less than 14 days (2-12 days) before blood sampling (Figure 4).

To detect participants that may have recovered from COVID-19, antibody analysis was performed for all 77 PCR-negative participants. Only one participant (from Asia) had both SARS-CoV-2 IgM and IgG, the latter detected by 2 assays (Figure 4). In 5 others, only IgM or IgG antibodies against the spike-protein were detected. These 6 plasma samples were further analyzed by immunofluorescence staining of SARS-CoV-2 infected Vero cells. Similar to positive control plasma (Figure 6, panel A and B), the IgM and IgG-positive sample gave cytoplasmic staining of infected but not uninfected cells (Figure 6, panel C and D). For the other samples, only weak background staining was observed (Figure 6, panel E and F).

We concluded that only one PCR-negative participant was seropositive for SARS-CoV-2, indicating a previous infection with COVID-19. Noteworthy, the two different IgM and IgG assays used
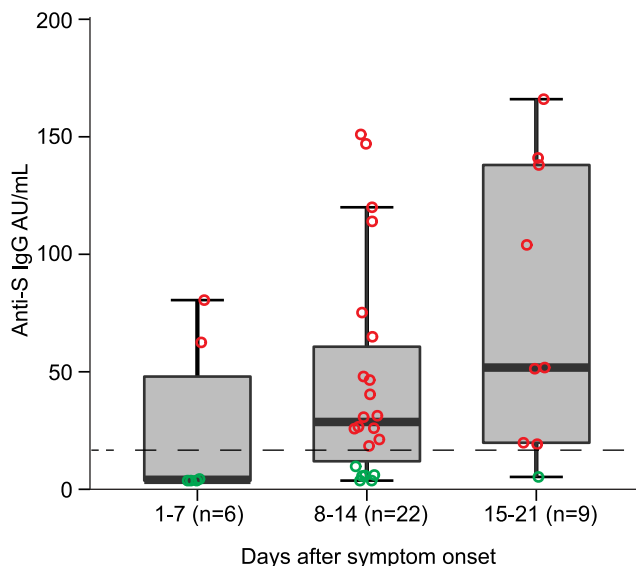

Figure 4. Antispike (Anti-S) IgG titer (AU/ml) from all 37 PCR-positive cases plotted based on the number of days between reported symptom onset and blood sampling. Samples with titers ≥ 15.0 AU/ml (dashed line) are defined as positive (red circles), and samples with titers < 12.0 AU/ml are negative (green circles). No equivocal samples with titers < 15.0 but ≥ 12.0 AU/ml were detected.

Table 4

Characteristics of 4 clusters in a social network-analysis of participants in a cruise ship outbreak of SARS-CoV-2, Tromsø, Norway from July 2020 to August 2020

Modularity class (cluster)*	0	1	2	3	Total
Cluster color code	Purple	Green	Orange	Blue	
Number of participants	42	31	31	10	114
Characteristics	%	%	%	%	%
Sex					
Women	31	23	39	30	31
Men	69	77	61	70	69
Age groups, years					
20-29	26	10	23	20	20
30-39	40	42	39	50	41
40-49	24	39	26	30	29
≥50	10	10	13	0	10
Country of origin					
Norway / Europe†	2	6	97	0	29
The Philippines	98	94	3	100	71
Type of work‡					
Administrative	0	3	29	10	10
Passenger services	19	29	3	40	33
Outdoor work	5	6	55	0	13
Mechanical operations	5	13	6	20	9
Catering	71	48	6	30	44
Type of cabin					
Single	24	58	90	40	53
Twin	76	42	10	60	47
Embarkation date					
July 5, 2020 and earlier	0	100	6	0	29
July 6-8, 2020	100	0	10	0	39
July 9-24, 2020	0	0	84	100	32
SARS-CoV-2 PCR test result					
Negative	50	68	90	70	68
Positive	15	32	10	30	32

* The color codes correspond to the 4 clusters in the network-analysis in Figure 2.
† A few European countries.
‡ Administrative work: officers and managers. Passenger services: reception, store and medical/spa. Outdoor work: expedition team and work on deck. Mechanical operations: engine work, electricity, and carpentry. Catering: restaurant, bar and kitchen.
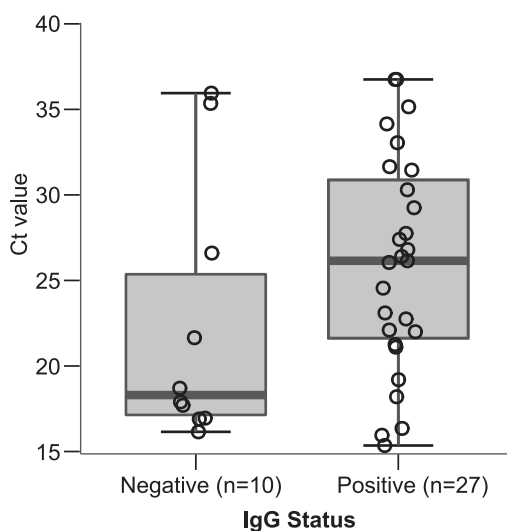

Figure 5. SARS-CoV-2 RNA in a nasopharynx-oropharynx swab (NOPS) from all 37 PCR-positive cases grouped by the presence of SARS-CoV-2 IgG, July 2020 to August 2020. Individual PCR results are shown as Ct-values. Median Ct-values are indicated with a horizontal line within the box.

gave identical qualitative results for 98% (112 of 114) and 94% (108 of 114) of samples (Figure 3).

Whole-genome Sequencing

WGS yielded near-complete (>97% of the genome) sequences for 29 of the 37 PCR-positive samples, 90% coverage for 1 sample, low-coverage (17%-59%) for three, and no sequences for four samples because of low viral RNA (Ct >35). One common virus, lineage B.1.36, was identified (lineage attribution as of September 13, 2021) (GISAID EpiCoV, 2021). The sequences constituted a highly similar group within this lineage as only two mutations in the whole-genome, C14760T and G26828T, separated it from other B.1.36 viruses (Figure 7). The only other viruses belonging to this outbreak genetic group in Norway were viruses received in the National Reference Laboratory from non-participant crew members (n = 5), cases among passengers (n= 10), and their close contacts (n = 2). Apart from these, the closest sequence matches were viruses mostly detected in India and Saudi Arabia from April 2020 to February 2021. Within the outbreak cluster, further subgrouping based on single nucleotide substitutions could be discerned.

Discussion

The findings from the epidemiologic, serologic, and WGS investigations are consistent with an evolving COVID-19 outbreak in this multinational, nonimmune study population, caused by a single virus introduction that was identified in all cases with a successful WGS result. Efficient infection control measures were quickly implemented just after the ship's arrival in Tromsø, and the outbreak was successfully contained.

We found that type of work, mechanical operations (i.e. engine work) and catering, were the main outbreak driver as also reported in other ship outbreaks (Expert Taskforce for the COVID-19 Cruise Ship Outbreak, 2020; Kakimoto et al., 2020; Kasper et al., 2020). The finding that sharing a cabin with an infected cabinmate increased the odds of being a case agrees with a study among passengers aboard the Diamond Princess (Plucinski et al., 2021). We detected only a few cases among outdoor work, reinforcing the evidence that SARS-CoV-2 transmission is lower outdoors (Bulfone et al., 2021, Kasper et al., 2020).

The increased odds for COVID-19 among Filipino participants was strongly attenuated in the multivariable analysis suggesting that workplace and sharing a cabin were stronger risk factors than the country of origin. This may reflect the segregated community on cruise ships with a crew from low-income countries living in shared facilities and working in confined spaces, making them more vulnerable to infections.

The outbreak was probably initiated by an Asian crew member prior to the first expedition and subsequently spread among crew and passengers on two expeditions in Arctic waters, illustrating how increased international mobility results in the fast spread of infectious diseases. Although no crew member participants reported symptom onset between July 6-19, 2020, there was likely ongoing transmission during this period, either by unreported illness or asymptomatic SARS-CoV-2 transmission (Payne et al., 2020). A nonparticipant crew member reported symptom onset on July 6, 2020, and could have been the primary case. The increasing number of cases from Expedition 1 to 2 shows that there was more extensive transmission on the second voyage with likely spill-over from crew to passengers. Given a typical incubation period of 4-5 days (Lauer et al., 2020), this would suggest that exposure occurred simultaneously for many people onboard. It is likely that breaches in several of the intended control measures led to unde-
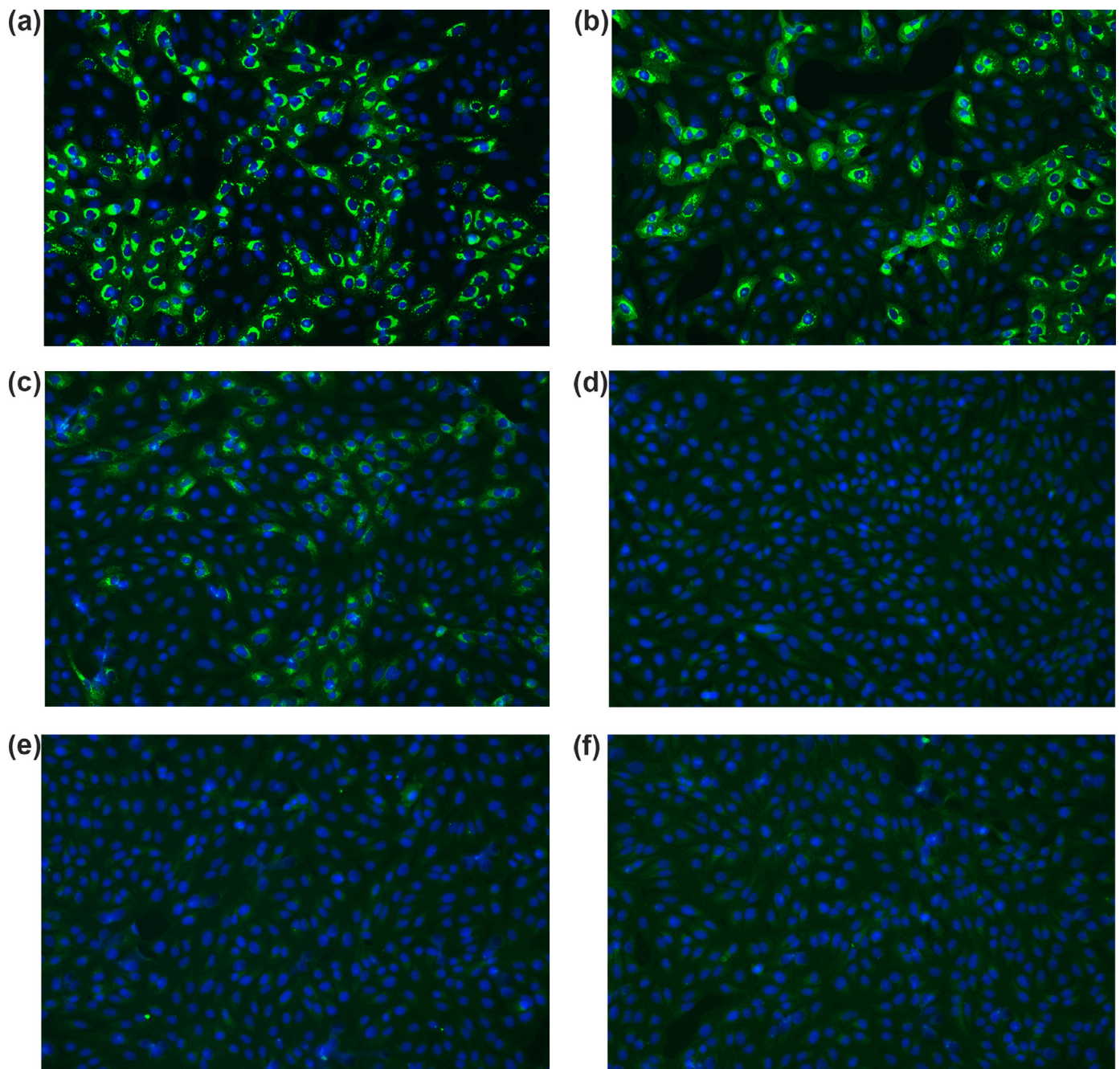
Figure 6. Panel A to F. Microscope images showing immunofluorescence staining of SARS-CoV-2 infected Vero cells using serum as primary antibody, August 2020. A) Positive control 1: serum from the case with the highest antinucleocapsid IgG titer. B) Positive control 2: Serum from the case with highest antispike IgG titer. C) Serum from PCR-negative participant with antispike IgG and antinucleocapsid and antispike IgM. D) The same serum as in C) used on uninfected Vero cells. E) Serum from PCR-negative participant with only positive antispike IgG. F) The same serum as in E) used on uninfected Vero cells. Similar images were obtained from the 4 other PCR-negative participants. As secondary antibody goat antihuman IgG-Alexa Fluor 488 was used. DNA was stained with Draq 5. Cytoplasmic staining is observed in A-C.

tected transmission onboard in early to mid-July when preparing for departure.

With the exception of 5 cases, all cases had detectable levels of SARS-CoV-2 specific IgM and/or IgG antibodies, confirming their COVID-19 diagnosis. The measured antibody response seems to agree with other studies. SARS-CoV-2 IgM is usually detected by day 5-7 from symptom onset and IgG by day 7-10, with peak levels approximately by day 49 (Stephens and McElrath, 2020).

SARS-CoV-2 viral load in the upper respiratory tract peaks in the first week of illness and declines about 1000-fold within 10 days after diagnosis, contemporary with an increasing level of SARS-CoV-2 antibodies (Cevik et al., 2021, Leuzinger et al., 2021a,

Leuzinger et al., 2021b). As our 10 IgG-negative cases (5 were also IgM negative), had median RNA load >100-fold higher than the 27 IgG-positive cases, they were most likely in an early phase of infection. In agreement with this, 10 of 11 cases that were lacking detectable IgG antibodies against the spike-protein reported symptom onset only 2-12 days before sampling, giving us confidence in the retrospectively reported symptom data.

SARS-CoV-2 IgM and IgG were only convincingly detected in one of 77 PCR-negative participants, increasing the number of seropositive participants to 33. This may have been a crew member with an infection before the outbreak or one of the first outbreak cases.
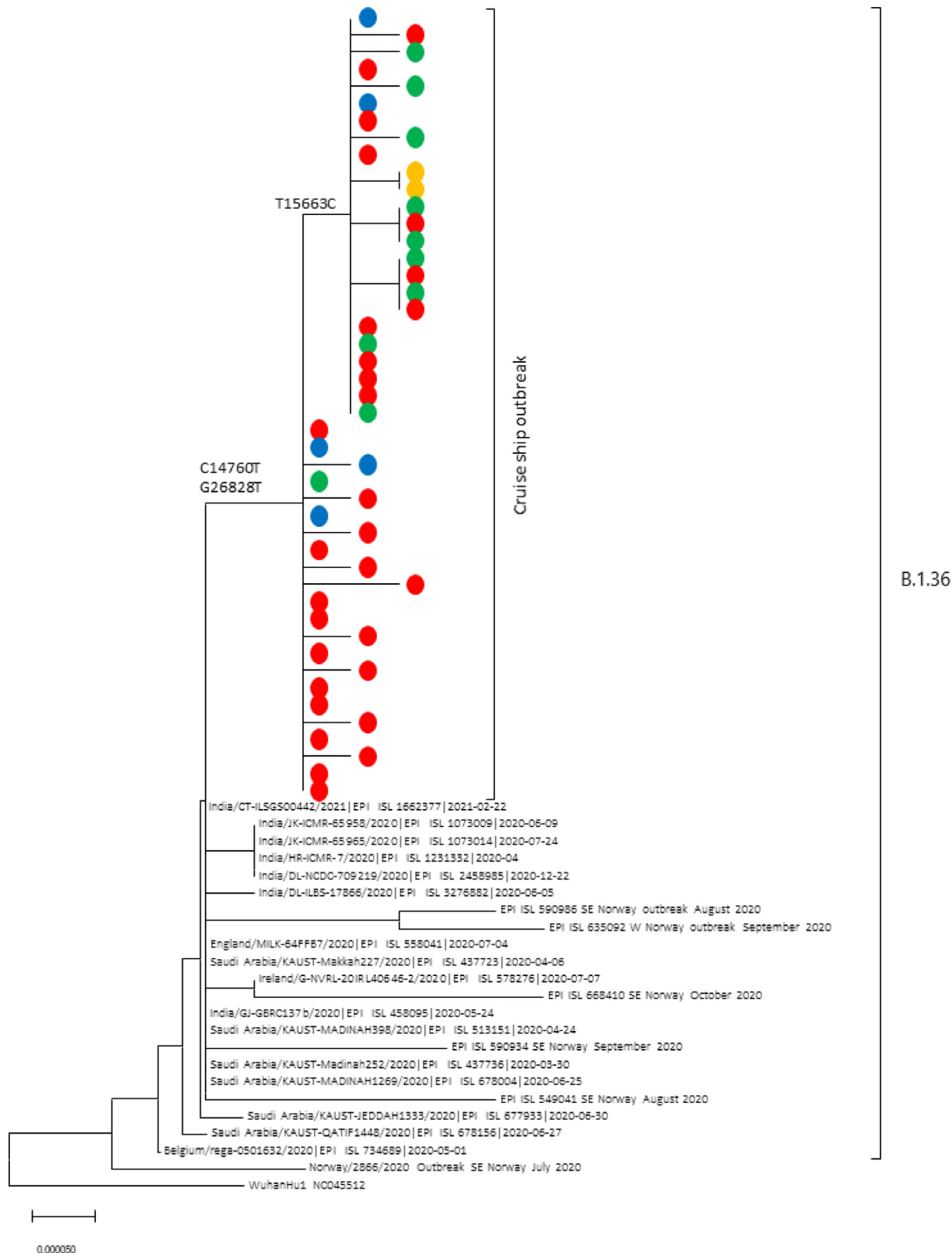
Figure 7. Phylogenetic analysis of SARS-CoV-2 whole genomes (coverage >98%) from cases in the outbreak on *MS Roald Amundsen* in Tromsø, Norway in July 2020 to August 2020. Outbreak associated viruses from cases among crew member participants (red dots, n = 29), crew member nonparticipants (blue, n = 5), passengers (green, n = 10) and their close contacts (gold, n = 2) were compared with the most similar sequences in the GISAID EpiCoV database, as well as to representatives of other lineage B.1.36 viruses in Norway during 2020. The scale bar corresponds to a distance of 0.00005 nucleotide substitutions per site (approx. 1.5 substitutions per viral genome).

In 5 other PCR-negative participants, single-assay positive antispike IgM or IgG results were likely because of cross-reactivity of preexisting antibodies against seasonal coronavirus or other microbes, as previously described (Manthei et al., 2021). Since only one PCR-negative participant was seropositive, preexisting SARS-CoV-2 immunity did probably not contribute to limit the spread of the virus. Instead, cross-reactive T cells from previous infections with common human coronaviruses (Perez-Potti et al., 2021) or a strong innate immune response (Park and Iwasaki, 2020) may have offered some protection.

The first documented COVID-19 case outside China was reported in Thailand in mid-January 2020, only 6 months before Expedition 2. Using a combination of serology and PCR, we assume to have detected all previous and ongoing SARS-CoV-2 infections among the participating crew.

The rapid action from the municipality, NIPH, and the Norwegian Directorate of Health efficiently limited the extent of the outbreak. No onward transmission of viruses belonging to this outbreak genetic group has been detected in Norway after the outbreak, despite extensive nationwide sequencing of SARS-CoV-2. Although other B.1.36 lineage viruses subsequently appeared in Norway, only cases with a direct epidemiologic link to this outbreak had sequences that matched the outbreak strain. The most similar sequences are viruses from Asia, and the sequence data are consistent with the introduction from this region (GISAID EpiCoV, 2021).

Strengths and weaknesses of the study

The strengths include the multidisciplinary approach to generating detailed epidemiologic data and the inclusion of WGS data from most PCR-positive participants. Our access to the National Reference Laboratory database enabled comparison of the outbreak strain to the Norwegian WGS database to map any onward transmission in Norway, which, to our knowledge, has not been done in previous studies. The study was limited by the fact that only three-quarters of crew members consented to participation, no passengers were included, and data on social gatherings were not available, implying potentially significant missing links for studying virus transmission dynamics. Furthermore, symptom onset dates were obtained retrospectively for the early cases, which may have introduced selection and recall bias, and immune response was measured only once.

Implications of the study

We found that the combined use of epidemiology, serology, and WGS can describe a SARS-CoV-2 outbreak in detail. The study is important in understanding the dynamics of an outbreak to prevent and control further outbreaks on cruise ships. The outbreak occurred early in the pandemic as one of the first reported on a Norwegian vessel. Norwegian legislation related to the COVID-19 pandemic has since been amended, and national recommendations for coastal cruise vessels, including crew change, accordingly revised (Norwegian Maritime Authority, 2021). Current legislation and guidance explicitly state that cruise ship companies need to implement infection control measures as described in their safety management system that includes risk assessment, responsibility, resources needed, communication, education, and test strategies, and this is in accordance with the European recommendations (ECDC, 2021).

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Contributors

KG, GSS, OK, and PE designed the study. KG wrote the study protocol and applied for ethical approval. KG, SH, and GSS contributed to the collection of blood samples. KG, KS, and HS conducted the epidemiologic analyses. SH and CHR conducted and interpreted the serologic analyses. OH conducted the WGS sequence analysis. OH, KSJ, and KB interpreted the WGS. KG and CHR drafted the manuscript. All authors contributed to interpreting the analysis, developing the manuscript, and approving the final draft.

Ethical Approval

The study was approved by the Regional Committee for Medical and Health Research Ethics, North Norway (reference: 2020/170968) and the Data Protection Officer at UNN (2020/9635). The study complied with the Declaration of Helsinki. All participants signed an informed consent form before participation.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.02.025.

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