This is an Accepted Manuscript for *Infection Control & Hospital Epidemiology* as part of the Cambridge Coronavirus Collection.
DOI: 10.1017/ice.2021.224

Article Type: Research Brief

SARS-CoV-2 infection in asymptomatic vaccinated-healthcare workers

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Word count: 900

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection caused more than 2.7 million,[1] devasting health systems end economies worldwide. Since the first months of the pandemic, a rapid and massive effort has been performed by the scientific community to develop a safe and effective vaccine against SARS-CoV-2.[2] In Italy the first category to receive the vaccine was represented by healthcare workers (HCWs) who are principally exposed to SARS-CoV-2 infection during the management of COVID-19 patients.[3] Several doubts remain as concerning the neutralizing properties of antibodies produced after vaccination.[4] Moreover little is known about transient infections in vaccinated subjects that therefore could be potential carriers of the disease.[5] Finally, it is important to understand the actual efficacy of the approved vaccines against SARS-CoV-2 variants, which have led to enhanced virus transmissibility, morbidity and mortality.[6,7]

Here we report several asymptomatic vaccinated-HCWs, who were tested positive for SARS-CoV-2 during surveillance testing.

METHODS

Samples

About 500 nasopharyngeal swab specimens of HCWs and hospitalized patients were daily collected at the Hospital Ss. Annunziata of Chieti, Italy, and analysed by the Laboratory of Molecular Genetics - Test Diagnosis Covid-19 of the Center for Advanced Studies and Technology (CAST), Gabriele d'Annunzio University of Chieti-Pescara (Italy).

RNA extraction and qRT-PCR

RNA was extracted from nasopharyngeal specimens, using MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit on the automated KingFisher processor (Thermo Fisher Scientific, USA). The extracted RNA underwent real-time reverse transcription polymerase chain reaction (qRT-PCR) with two commercial kits: the TaqPath™ COVID-19 CE-IVD RT-PCR Kit assay (Thermo Fisher Scientific, USA) and Allplex™ SARS-CoV-2 Variants I Assay (Seegene, Korea).

Next-generation sequencing (NGS)

For whole viral genome sequencing, total RNA was reverse transcribed using Invitrogen™ SuperScript™ VILO™ cDNA Synthesis Kit (Thermo Fisher Scientific, USA). cDNA libraries were prepared using the Ion AmpliSeq SARS-CoV-2 Research Panel (Thermo Fisher Scientific, USA). Sequencing was performed on the Ion GeneStudio™ S5 System (Thermo Fisher Scientific, USA). The Consensus sequences were aligned with the Wuhan-Hu reference SARS-CoV-2 genome using the Torrent Suite platform. For phylogenetic analysis the whole genome sequences of the isolates were uploaded on Pangolin COVID-19 Lineage Assigner.[8]

RESULTS

In the period from January to March 2021, we were informed that among the positive tested subjects seven were HCWs, who had received BNT162b2 vaccination (Table 1). All were contacted and gave informed consent to use the material for the study.

Six of these subjects had both doses of the vaccine while one had only received the first dose. HCW 2, 4, and 5 resulted positive between three and eight days after administration of the second dose. Instead, the remaining three cases (HCV 3, 6 and 7) resulted positive between 23 and 36 days after the administration of the second dose of vaccine.

All subjects resulted completely asymptomatic and had undergone testing because included in a routine surveillance schedule for HCWs. In three cases we were able to re-test the HCWs the day after the positive qRT-PCR result and show they were already negative. The remaining cases were tested after 10 days, and all were negative for SARS-CoV-2 infection.

In all seven cases, molecular test evidenced the presence of the Δ69/70 deletion of the S gene, found in the B.1.1.7 lineage known as the UK variant, since the TaqPath™ Kit fails to amplify the S gene in the presence of the Δ69/70 deletion [9].

NGS of the viral genome of HCWs 1, 2 and 3 confirm that the infections belonged to the B.1.1.7 lineage. The remaining cases (HCWs 4, 5, 6 and 7) were tested using Allplex^{TM} Assay that confirmed the presence of Δ69/70 deletion and of the N501Y mutation assigning reasonably also these cases to the B.1.1.7 lineage.

DISCUSSION

Data collected in this study confirm that infection is possible following vaccination. In one case this is clearly expected since the infection occurred only a few days after the administration of the first dose, likely before the production of antibodies. Indeed, it has been reported that neutralizing antibody levels start to raise eight days after the first dose of vaccine reaching the peak two weeks after the boost of the second dose.[4] Reasonably, the infection that occurred in three HCWs a few days after the administration of the second dose is due to the weak titre of neutralizing antibodies produced.

The last three cases, instead, show that some subjects can still be infected after having completed the vaccination schedule and after sufficient time has passed allowing for the peak of antibody production to occur. Unfortunately, we were not able to evaluate antibody levels at the time of infection and therefore attempt to correlate infection with low antibody titre.

Finally, it is interesting to note that all the cases we observed in this report were due B.1.1.7 lineage infection. Although we cannot draw conclusions due to the limited numbers of cases so far

observed, it can be confirmed that the vaccine has effect also on this variant, since none of the infected showed symptoms and all became rapidly negative to the infection.

In conclusion, although further data and observation of a larger cohort of cases are needed, we strongly believe that continue attention should be devoted to the problem of infection in vaccinated people: these data demonstrate that some people can have transient asymptomatic infections following vaccination and can therefore be potentially infectious, thus suggesting the necessity of precaution to be maintained particularly for HCWs to avoid spreading of the virus particularly among hospitalized people.

Conflicts of Interest: all the authors have no conflict of interest to declare.

Funding: this study received no external funding.

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Table 1. Vaccination status, qRT-PCR results and NGS summary.

ID	Date of Vaccination		Date of Testing		Results of TaqPath Positive Test			Lineage	Detection Method	Mutations		
	First Dose	Second Dose	Positive	Negative	ORF 1ab	N	S			E484K	N501Y	Δ69/70
HCW 1	13/01/21	-	19/01/21	20/01/21	28.949	29.201	ND	B.1.1.7	NGS	No	Yes	Yes
HCW 2	05/01/21	28/01/21	01/02/21	02/02/21	31.656	31.720	ND	B.1.1.7	NGS	No	Yes	Yes
HCW 3	04/01/21	26/01/21	01/03/21	12/03/21	18.318	17.463	ND	B.1.1.7	NGS	No	Yes	Yes
HCW 4	10/01/21	31/01/21	06/02/21	17/02/21	35.823	34.976	ND	B.1.1.7	Allplex	No	Yes	Yes
HCW 5	13/01/21	03/02/21	06/02/21	17/02/21	36.236	36.911	ND	B.1.1.7	Allplex	No	Yes	Yes
HCW 6	03/01/21	24/01/21	16/02/21	17/02/21	31.645	31.213	ND	B.1.1.7	Allplex	No	Yes	Yes
HCW 7	05/01/21	26/01/21	02/03/21	13/03/21	15.894	15.866	ND	B.1.1.7	Allplex	No	Yes	Yes

SARS-CoV-2 qRT-PCR results are shown as ct values. ND denotes not determined ct value.

NGS: indicates that next generation sequencing of the entire viral genome was performed

Allplex: indicates that the presence of Δ69/70, N501Y and E484K were evaluated using Allplex^{TM} SARS-CoV-2 Variants I Assay