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Letter to the Editor

Detection of viable SARS-CoV-2 on the hands of hospitalized children with COVID-19

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To the Editor,

Potential routes of SARS-CoV-2 transmission have been a matter of debate since the early phase of the current COVID-19 pandemic. Similar to other respiratory viruses, airborne droplets exhaled by an infected individual and inhaled by another susceptible person were identified as the dominant route of transmission [1]. However, indirect routes of transmitting SARS-CoV-2 are still under investigation [1–3]. Transmission by indirect contact has been reported for other respiratory viruses [1], and therefore, it was intuitive to place great emphasis on the importance of appropriate hand disinfection during the COVID-19 pandemic [4]. Yet, data providing firm evidence for the presence and quantity of potentially infectious virus carried on the hands of patients infected with SARS-CoV-2 remained scarce.

To assess the potential role of hand contamination for indirect transmission, we screened standardized palm swabs from 70 hospitalized children with SARS-CoV-2 infection for the presence and infectious capacity of the virus detectable on hands (see Supplemental Material and Methods for details). Of these, 67 palm swabs were evaluable by RT-qPCR analysis and 48 (71.6%) tested positive for SARS-CoV-2. The median viral RNA copy number detected in the transport medium of individual swabs was 2.8×10^3 (range 3.0×10^1 to 8.6×10^7), with thresholds of the first and third quartiles at 1.8×10^2 and 2.8×10^4, respectively (Fig. 1). Although viral concentrations exceeding 1.0×10^5 copies were documented in seven samples, most swabs revealed considerably lower virus copy numbers, possibly due to sample collection beyond the peak of respiratory infection. Identification of the virus variant was possible in 34 patients and the results reflected the predominant circulation of individual SARS-CoV-2 variants in Austria at that time, as registered in the GISAID database [5]: between the beginning of October and mid-December, 2021, the Delta variant

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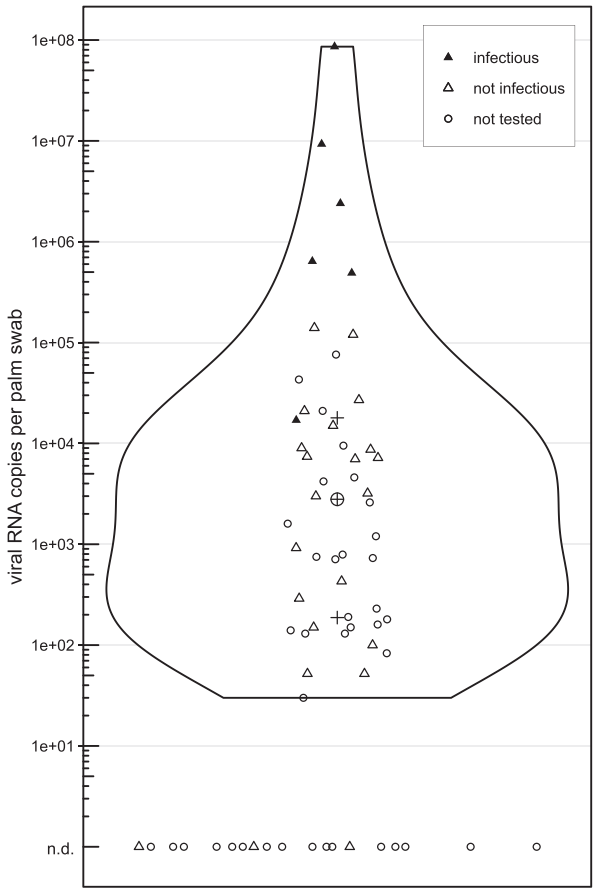

Fig. 1. Infectious potential of hand-borne SARS-CoV-2. Violin-plot showing the distribution of SARS-CoV-2 viral loads detected by RT-qPCR screening of positive hand swabs ($n = 48$), as specified by the scale on the ordinate. Hand swabs revealing no viral RNA ($n = 19$) are shown at the bottom and are not included in the violin-plot (n.d. – not detected). Triangles indicate samples selected for viability testing of the virus in cell culture ($n = 28$), and specimens with documented infectious particles are highlighted as filled triangles [▲] ($n = 6$). Circles [○] represent samples not tested in cell culture ($n = 39$). The plus symbols [+] mark the second and third quartiles of all viral RNA-positive hand swabs and the median is indicated by the encircled plus sign [⊕].

was detected in all patients tested, and Omicron variants were identified in all instances thereafter (Table S1).

The viability and replication capacity of SARS-CoV-2 detected in palm swab samples were determined in cell culture using the permissive cell line Vero E6. All patient samples displaying virus concentrations exceeding 1.0×10^5 viral RNA copies per swab transport medium ($n = 7$) were tested in cell culture. Moreover, 18 additional samples showing a wide range of virus concentrations and three negative samples as controls were randomly selected for further analysis by cell culture experiments (Fig. 1). The cell cultures were monitored by microscopy and tested by RT-qPCR over a period of 5 days. The positive control, a SARS-CoV-2-negative patient sample spiked with 1.2×10^6 viral RNA copies per mL cell culture medium, revealed a visible cytopathic effect even at the lowest multiplicity of infection tested (0.0003125). Successful replication of the virus was confirmed by RT-qPCR detection of SARS-CoV-2 RNA after only 1 day after inoculation at the indicated multiplicity of infection.

All patient samples displaying more than 5.5×10^5 viral genome copies per swab ($n = 5$) revealed a clearly identifiable cytopathic effect in cell culture—four of them as early as 2 days after inoculation. In addition, SARS-CoV-2 RNA was detected and successfully monitored throughout the time course of the experiment in all these specimens and in one additional sample (patient no. 9) with an initial viral load of 1.7×10^4 RNA copies per swab (Figs. 1 and Fig. S1). In addition to RT-qPCR-based monitoring of virus replication, intracellular detection of viral nucleocapsids by immunofluorescence staining of Vero E6 cells was successfully performed in three patients with high initial SARS-CoV-2 concentrations on day 5 after inoculation (patient nos 3, 22, and 46 in Table S1).

The present study was undertaken to assess the occurrence of hand contamination with SARS-CoV-2 in children with COVID-19, with a particular focus on the infectious potential of hand-borne virus. To our knowledge, we provide the first experimental evidence supporting the notion that hand-borne SARS-CoV-2 from infected patients can display infectious potential, thus confirming the possibility of virus transmission via an indirect route. The time span between the last hand hygiene measure and collection of the palm swab samples was documented, and it appears noteworthy that two of the samples displaying positive tests in cell culture were obtained from patients whose hands had been washed only 3 hours prior to sample collection. This observation emphasizes the role of frequent hand hygiene as a preventive measure and supports established recommendations—particularly during active infection. The observations presented should serve as a basis for further assessment of the potential role of SARS-CoV-2 transmission via virus-contaminated hands, particularly in epidemiologically critical settings, to facilitate the implementation of optimized strategies for preventing uncontrolled spread of the infection in the current pandemic and any future outbreaks.

Author contributions

Conceptualization: AZ, TL, JW, MH, JH; project administration: MH, PF; data curation: AZ, KK, PS, IA, VF, SD, CM, FG, MH, PF, JH, LU; formal analysis and investigation: MH, PF, JH, LU; visualization: MH, PF; funding acquisition, resources, and supervision: AZ, TL, JW; methodology: AZ, TL, MH, JH, LU, KO, SP, MF, CW, DS; validation: MH, PF, SP, MF, CW, DS; writing—original draft: MH, TL, JH; writing—review and editing: TL, MH, AZ, FG, KO, JW, VF, PF, PS, IA, KK, CM, SD, LU, SP, MF, DS, CW.

Transparency declaration

Conflict of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2023.06.012.

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