Infective SARS-CoV-2 in Skull Sawdust at Autopsy, Finland

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We assessed the distribution of SARS-CoV-2 at autopsy in 22 deceased persons with confirmed COVID-19. SARS-CoV-2 was found by PCR (2/22, 9.1%) and by culture (1/22, 4.5%) in skull sawdust, suggesting that live virus is present in tissues postmortem, including bone. Occupational exposure risk is low with appropriate personal protective equipment.

Autopsies afford simultaneous access to all tissues and body compartments. The unique opportunity for extensive sampling during autopsy enables several research questions to be addressed. Early in the COVID-19 pandemic, autopsies were rare, mainly because of presumed transmission risk and shortage of personal protective equipment (PPE), and suspicions that autopsies might be of limited value (*1*,*2*).

Autopsies pose an occupational infectious hazard to the personnel involved in a pathogen-dependent manner. For example, *Mycobacterium tuberculosis* deserves particular attention as a major cause of airborne infections in autopsies that puts pathologists at a 100–200-fold risk for infection compared with the general public (*3*). Viable SARS-CoV-2 has been detected in tissues for prolonged periods after death from COVID-19 (*4*). However, to our knowledge, no confirmed occupational cases of COVID-19 transmitted at autopsies have been reported.

Protection against aerosols remains a challenge in autopsy settings. Bone sawing is a major source of aerosols that can carry pathogens. Sawing of the skull is a standard procedure in every routine autopsy to enable access to the brain. SARS-CoV-2 has previously been documented in bone tissues in 2 reported cases, neither of which were in the skull (*5*). Here, we present results of SARS-CoV-2 analyses from 22 deceased persons with PCR-confirmed COVID-19 and detail our experience of managing the occupational hazards associated with COVID-19 autopsies.

Our study belongs to the Clin_COVID-19 master study approved by the Helsinki University Hospital Ethics committee (approval no. HUS/1238/2020). All autopsies were clinical (nonforensic) and conducted in compliance with research laws and regulations in Finland, after consent from the next of kin.

The postmortem examinations were conducted in the pathology department of the HUS Diagnostic Center in Meilahti, Helsinki, Finland. The series comprised 22 PCR-confirmed cases (any positive airway sample from nasopharynx, bronchi, lungs, tonsils, sclera, or airway-associated cervical or parabronchial lymph nodes) of SARS-CoV-2 identified during 2021–2022 that had skull sawdust sampled during autopsy. Testing was carried out in the pathology and virology laboratories by using accredited and previously published methods (*6*) (Appendix, https://wwwnc.cdc.gov/EID/article/30/8/24-0145-App1.pdf). All autopsies encompassed a neuropathological examination and a collection of swabs/fresh tissues from airway, nonairway, and central nervous system (CNS) categories. In addition, swab samples were collected from skull sawdust and the contaminated autopsy table with the organ block. Each tissue was sampled with separate sterile equipment. PCR-positive samples were cultured using VeroE6 cells to assess for infective SARS-CoV-2.

We detected SARS-CoV-2 by reverse transcription PCR in 22/22 (100%) airway, 10/22 (45.5%) nonairway, 0/22 CNS, 2/22 (9.1%) skull sawdust, and 13/22 (59.1%) autopsy table samples (Table). The virus was culturable in 13/22 (59.1%) airway, 2/22 (9.1%) nonairway, 1/22 (4.5%) skull sawdust, and 3/22 (13.6%) autopsy table samples.

Among the personnel present at COVID-19 autopsy procedures, no cases of COVID-19 resulting from occupational exposure were identified. Serologic screening results of all persons involved in COVID-19 autopsies (n = 5) in June 2020 were negative, and none showed PCR positivity when tested during symptoms.

Our findings revealed that SARS-CoV-2 was detectable by PCR in 9.1% and by viral culture in 4.5% of skull sawdust samples, suggesting the presence of live virus and a risk, although low, of infective viruses becoming aerosolized. We could not identify previous

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Table. SARS-CoV-2 distribution among cohort of 22 autopsied deceased persons with COVID-19 who had skull sawdust sampling, Finland*

	Airway		Nonairway		CNS PCR	Skull sawdust		Autopsy table	
Case no.	PCR	Culture	PCR	Culture	and culture	PCR	Culture	PCR	Culture
1	+	–	–	–	–	–	–	+	–
2	+	–	–	–	–	–	–	–	–
3	+	–	–	–	–	–	–	–	–
4	+	–	–	–	–	–	–	–	–
5	+	–	–	–	–	–	–	–	–
6	+	+	–	–	–	–	–	+	–
7	+	+	+	–	–	–	–	+	–
8	+	+	+	–	–	–	–	–	–
9†	+	+	+	–	–	+	+	+	–
10	+	+	–	–	–	–	–	+	–
11	+	+	+	+	–	–	–	+	–
12	+	+	–	–	–	+	–	–	–
13	+	–	–	–	–	–	–	–	–
14	+	+	+	+	–	–	–	+	–
15	+	+	+	–	–	–	–	+	+
16	+	+	+	–	–	–	–	+	+
17	+	–	–	–	–	–	–	+	–
18	+	+	+	–	–	–	–	+	–
19	+	–	+	–	–	–	–	+	–
20	+	+	+	–	–	–	–	–	–
21	+	–	–	–	–	–	–	–	–
22	+	+	–	–	–	–	–	+	+
Positive samples/total no. samples (%)	22/22 (100)	13/22 (59.1)	10/22 (45.5)	2/22 (9.1)	0/22	2/22 (9.1)	1/22 (4.5)	13/22 (59.1)	3/22 (13.6)

*The pooled sample category per patient was considered positive if a single positive tissue sample of that category was found (copy number cutoff value 10; Appendix, https://wwwnc.cdc.gov/EID/article/30/8/24-0145-App1.pdf). Airway refers to tissues relating to the airway system (i.e., nasopharynx, bronchi, lungs, tonsils, sclera, and airway-associated cervical and parabronchial lymph nodes). Autopsy table refers to the contaminated autopsy table and the outer surfaces of the organ block, representing the main working area and target of showering with water. Only a limited number of cases showed culture positivity (Ct value reduction after culture compared with initial Ct value; Appendix) in general; skull positivity was scarce, whereas the autopsy table was more often positive by both PCR and viral culture.
†Test results showed culture positivity in the cranial sawdust sample and low-level nonairway PCR-positivity limited to skeletal muscle and salivary gland tissues, indicating limited systemic viral involvement (Appendix Table).

work examining cranial sawdust for the presence of pathogens, but our results align with a previous study showing SARS-CoV-2 PCR positivity for 4.5% of goggles and no masks tested after autopsy (*7*).

The sample size for our study was limited but represents a consecutive and nonselected series of cases at a single institution. We did not directly assess aerosols, but given that bone sawing is the only high-energy technique used, and considering the findings from a previous study (*7*), the presence of concomitant other sources of infective aerosols in the autopsy room is unlikely. The personnel present during COVID-19 autopsies were not systematically tested, but symptomatic persons were extensively PCR tested for SARS-CoV-2 during the study period (2020–2022). In addition, skull sawdust samples might not consist solely of bone and could contain adjacent tissues because of anatomy, particularly the frontal sinus, which is lined with respiratory epithelium. Skullcap sawing has the potential to generate infective aerosols, but in our experience, general autopsy safety measures are effective. The absence of positive findings in our CNS samples give confidence in the sterility of our sampling technique, thereby making other sources of contamination in the skull sawdust samples less likely.

Pandemic preparedness should encompass plans for early, rapid autopsies to acquire vital data at the onset. General safety measures appear adequate for most pathogens encountered during autopsy, including SARS-CoV-2 (*3*). However, early testing for pathogens in skull sawdust, along with other tissues, could prove beneficial in further assessing the risk for occupational infections resulting from autopsies during future pandemics.

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About the Author

Dr. Kantonen is a certified pathologist and medical doctor performing research at the University of Helsinki, Finland. His research interests focus on the use of autopsies for medical research.

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Novel Genotypes of Highly Pathogenic Avian Influenza H5N1 Clade 2.3.4.4b Viruses, Germany, November 2023

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Several subtypes and many different genotypes of highly pathogenic avian influenza viruses of subtype H5 clade 2.3.4.4b have repeatedly caused outbreaks in Germany. Four new highly pathogenic avian influenza genotypes emerged in November 2023 after reassortment with low pathogenicity precursors, replacing genotype BB, which had dominated in Europe since 2022.

Germany has experienced repeated outbreaks of highly pathogenic avian influenza (HPAI) viruses (HPAIVs) of clade 2.3.4.4b of the H5 goose/Guangdong lineage since 2016, causing devastating losses to wild bird biodiversity and the poultry production sector (*1*). Since 2016, seasonal outbreaks or cases increased during the winter season and decreased to zero in summer. Seasonality terminated in 2021, when HPAIV H5 became endemic in wild birds in Germany and the rest of Europe (*2*). Along with an increasing incidence, genetic diversity expanded, resulting in a high number of new genotypes (*3*).

During summer 2023, genotype Ger-02-23-N1.1 (BB based on the European Union nomenclature [*4*,*5*]), a reassortment with a gull-derived H13 virus, dominated HPAI cases caused by outbreaks in colony breeders (*6*). Sporadically, older genotypes (Ger-10-21-N1.5 and Ger-12-22-N1.1) were identified, accompanied by some viruses that could not be assigned to a proper genotype because of incomplete genome covering. After the breeding season ended, incidence decreased (84 cases in July, 16 in August, 10 in September, and 3 in October). In addition, increasing numbers of low pathogenicity avian influenza (LPAI) viruses (LPAIVs) were detected during active and passive wild bird monitoring, representing the autumnal, bird migration–related upsurge of avian influenza virus infections in Germany. Since November, the number of HPAIV H5 cases has increased to a still moderate but substantially higher level (29 in November).

We analyzed the genotypes of HPAI and LPAI viruses by using full-genome sequencing. Sequencing

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Appendix

Autopsy facilities and practices

The autopsy facilities at our department include three autopsy suites. All three have negative air pressure, a minimum of 15 air changes per hour, with one suite dedicated to infectious diseases. The dedicated room uses a HEPA-filter for the exhaust air, and all autopsy suites offer a local HEPA-filtered air purifying unit for aerosol control. Two of the COVID-19 cases were done in routine autopsy suites due to unknown infection status, but these cases did not show skull positivity. All the identified COVID-19 cases were autopsied in the dedicated autopsy suite for infectious cases.

The autopsy protocol used en bloc removal of the viscera, and all autopsies included assessment of the central nervous system via craniotomy. The skull was opened using an oscillating saw, under the hood of the local air purifying unit for aerosol control. Cold water was used sparingly, with minimal pressure to avoid aerosol formation.

The personnel involved in the COVID-19 autopsies included five autopsy technicians and four pathologists. One to two pathologists and autopsy technicians were present in the autopsy suite for a single case. The duration of autopsies varied from 1–4 hours per case.

Personal protective equipment (PPE) used routinely consisted of an FFP3 mask, cap, goggles, water resistant gown, double pairs of gloves and boots. A powered air purifying respirator (VersaFlo, 3M) was used in some cases instead of the FFP3 mask and goggles.

SARS-CoV2 tissue processing, virus isolation and quantitative RT-PCR

For SARS-CoV2+ cases fresh tissue samples, swabs (including swabs of skull sawdust and swabs of the contaminated autopsy table with the organ block), cerebrospinal fluid (CSF) and blood were collected at autopsy and transferred to the biosafety level 3 laboratory (BSL-3) for further processing. For virus isolation, tissue samples were homogenized using mortar and pestle and sterile sand with ice-cold PBS. Cleared tissue samples were collected, inoculated on Vero E6 cells, and incubated for 1 h at +37°C 5% CO2, after which the cells were washed with PBS and virus growth media containing 2% FCS was added. Virus growth was followed by cytopathic effect (CPE) formation and confirmed by quantitative RT-PCR on day 0 and between days 4–11, with >2-log value increase considered positive. See Appendix Table for culture positive samples per sample category.

RNA was extracted from the tissue samples using TRIzol reagent (Invitrogen) according to the manufacturer's instructions, and from cell culture supernatants using the QIAamp Viral RNA Mini Kit (Qiagen). Quantitative SARS-CoV-2 RT-PCR was performed according to previously published protocol (*1*). See Appendix Table for the tissue with highest SARS-CoV-2 copy number per category.

High SARS-CoV-2 copy numbers correlated with positive viral culture (rank biserial correlation for airway samples $r_{rb} = 0.783$ (n=22) and all cultured samples $r_{rb} = 0.646$ (n=47), both $p < 0.01$), as reported in other studies (*2*,*3*). The data in this cohort does not allow for reliable evaluation of the effect of postmortem delay on culture positivity. However, our experience is similar to the findings seen by others, with surprisingly long postmortem delays showing positivity (*4*).

Serological testing

The autopsy personnel were tested for presence of antibodies after the first wave of infections in June 2020. The tested cohort included all 6 individuals that had known exposure to COVID-19 autopsies at the time of testing. None of the individuals either had symptomatic disease warranting PCR-testing, or their PCR-tests were negative in the time prior to antibody testing. A total of five COVID-19 autopsies were performed during the spring of 2020, with these cases not being part of the cohort reported on here.

Experience on occupational hazards

In our experience FFP3 masks as part of aerosol controlling autopsy precautions seem to protect from airborne infections during autopsy. No cases of autopsy-related COVID-19 were identified among the personnel in Helsinki during the years 2020 – 2023. In addition, no cases of TB have been identified while using the same safety procedures during the years 2011 – 2023. The addition of local exhausts as a source-control with HEPA-filters helps further reduce the amount of potentially infective aerosols in the autopsy room, easing the workload of masks as the single method for aerosol filtering.

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Appendix Table. Highest SARS-CoV-2 copy number values per sample category, with culture positive tissue type where applicable, ranked according to PCR copy number*

Case no.	Airway PCR	Airway culture	Non-airway PCR	Non-airway culture	CNS PCR & culture	Skull PCR	Skull culture	Table PCR	Table culture
1	493000 (lung)	-	-	-	-	-	-	1090 (table)	-
2	27570 (tonsil)	-	-	-	-	-	-	-	-
3	1114000 (lung)	-	-	-	-	-	-	-	-
4	41170 (tonsil)	-	-	-	-	-	-	-	-
5	176100 (tonsil)	-	-	-	-	-	-	-	-
6	111400 (bronchi)	bronchi, lung, tonsil	-	-	-	-	-	6840 (table)	-
7	18180000 (lung)	lung, tonsil, nasopharynx	15260 (heart)	-	-	-	-	206 (table)	-
8	15000000 (cervical lymph node)	cervical lymph node	1028 (pancreas)	-	-	-	-	-	-
9	5476000 (nasopharynx)	nasopharynx, bronchi, tonsil	8465 (salivary gland)	-	-	74830 (skull)	skull	36.87 (table)	-
10	3552000 (nasopharynx)	bronchi	-	-	-	-	-	8625 (table)	-
11	7929000 (bronchi)	bronchi	182700 (gut)	gut	-	-	-	1201 (table)	-
12	1504000 (nasopharynx)	nasopharynx	-	-	-	53360 (skull)	-	-	-
13	3006 (parabronchial lymph node)	-	-	-	-	-	-	-	-
14	365400 (bronchi)	nasopharynx, sclera, parabronchial lymph node	13980 (heart)	muscle	-	-	-	10360 (table)	-
15	134500000 (lung)	lung	1249 (salivary gland)	-	-	-	-	9470 (table)	table
16	44870000 (lung)	lung, bronchi	14810 (spleen)	-	-	-	-	3492 (table)	table
17	455200 (lung)	-	-	-	-	-	-	32387 (table)	-
18	2486000 (nasopharynx)	lung	135.3 (salivary gland)	-	-	-	-	13550 (table)	-
19	591700 (nasopharynx)	-	828.5 (thyroid)	-	-	-	-	33320 (table)	-
20	6553000 (lung)	lung, tonsil	21480 (salivary gland)	-	-	-	-	-	-
21	24200 (parabronchial lymph node)	-	-	-	-	-	-	-	-
22	917600 (nasopharynx)	nasopharynx, bronchi	-	-	-	-	-	23120 (table)	table

*The airway samples showed systematically higher copy numbers of SARS-CoV-2 when compared to other sample categories. Positive skull samples were found in cases showing high copy numbers in the nasopharynx. For statistical testing the copy number data were ranked, and culture was only deemed positive if the sample showing the highest copy number showed culture positivity. Using rank biserial correlation we obtained correlation coefficients $r_{rb} = 0.783$ ($n=22$, $p < 0.01$) for airway samples and $r_{rb} = 0.646$ ($n=47$, $p < 0.01$) for all cultured sample categories, showing correlation between high copy number and viral culture positivity. Airway refers to tissues relating to the airway system (i.e. nasopharynx, bronchi, lungs, tonsils, sclera, and airway-associated cervical and parabronchial lymph nodes). Skull refers to skull sawdust. Table refers to the contaminated autopsy table and the outer surfaces of the organ block, representing the main working area and target of showering with water.