forensic sciences

Perspective

Among Bodies: Portuguese Cemeterial Exhumations Three Years after a Pandemic

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Abstract: On 19 March 2020, a "state of emergency" was declared in Portugal due to the manifestation of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with an exponential rate of infection and high mortality and morbidity rates. Since then, the state of emergency was declared a further fifteen times until 30 April 2021, during which a total of 16,974 deaths associated with COVID-19 were acknowledged in the country. Over the aforementioned period, guidelines were followed regarding the handling of suspected cases in autopsy rooms, mortuaries, and cemeteries. However, no procedures have been established regarding the handling of human remains during and after cemeterial exhumations. Furthermore, little is known about the virus survival and its spatial distribution in postmortem human tissues. Given that the minimum Portuguese legal period of inhumation is ending and cemeteries have been facing limited burial space and soon will start exhuming buried individuals, the authors believe it is important to reflect on the matter.

Keywords: cemeteries; infectious diseases; inhumations; SARS-CoV-2

Citation: Silva-Bessa, A.; Ferreira, M.T.; Dinis-Oliveira, R.J. Among Bodies: Portuguese Cemeterial Exhumations Three Years after a Pandemic. *Forensic Sci.* **2023**, *3*, 293–301. https://doi.org/10.3390/forensicsci3020022

Academic Editor: Bruce Royston McCord

Received: 13 March 2023
Revised: 4 April 2023
Accepted: 10 May 2023
Published: 12 May 2023

1. Introduction

Coronaviruses are a large group of RNA viruses that mostly cause respiratory and gastrointestinal diseases [1,2]. However, before the 2002 and 2003 outbreak of severe acute respiratory syndrome (SARS) in China, coronaviruses were not deemed highly pathogenic to humans [1]. Infected individuals would present themselves with distinct symptoms such as dry cough, fever, dyspnoea (i.e., shortness of breath), and hypoxemia (i.e., low blood oxygen), with death resulting from continuous respiratory failure [3]. The clinical spectrum of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is similar, fluctuating from asymptomatic infection to fatal illness, with an exponential rate of infection and high mortality and morbidity rates [4]. According to the World Health Organization (WHO) COVID-19 global dashboard, by 8 February 2023, there had been more than 755 million confirmed cases, including 6,830,867 deaths (http://COVID19.who.int accessed on 8 February 2023). In general, the main portals of entry of SARS-CoV-2 into the body are the nasal and oral routes [5], entering the cells via the angiotensin-converting enzyme 2 (ACE2) receptor which can be found in several cell types besides the oral and nasal mucosae, including the blood vessels, brain, gastrointestinal tract, heart, and spleen [6,7]. Once internalised, the virus replicates and matures, causing an inflammatory response [8].

In Portugal, throughout the pandemic, precautionary actions were taken based on the WHO [9] and as recommended by the Portuguese Directorate-General for Health

(DGS: Direção-Geral da Saúde). As expected, regulations and guidelines on numerous subjects were released and constantly updated, such as for the technical specifications of personal protective equipment (PPE) when dealing with individuals with suspected, probable, and confirmed infection (Norm No. 007/2020 of 29 March 2020); the direct and indirect implications of regulations on mental health, especially in the most vulnerable citizens (Norm No. 011/2020 of 18 April 2020); the vaccination campaign, according to the vaccine administered (e.g., Norm No. 021/2020 of 23 December 2020); and the so-called post-COVID condition associated with a set of heterogeneous symptoms, which may persist or even occur after a patient's acute clinical picture (Norm No. 002/2022 of 17 March 2022). Additionally, the Norm No. 002/2020 of 16 March 2020 was released with procedures to be followed in autopsy rooms, mortuaries, and cemeteries. According to this norm, medical–legal autopsies should be dismissed; however, if carried out, the result of a laboratory test for detection of SARS-CoV-2 would have to be available before the postmortem examination. Another of the procedures to be followed would be the enclosure of the corpse of an infected individual in an impermeable shroud sprayed with a disinfectant solution, followed by the burial of the body in a sealed coffin, or proceeding with cremation. The body bag would have to be degradable, but resistant up to 150 kg, with a plasticised waterproof face inside, and would have to include an absorbent protective sheet. Although hard numbers are not available, it is common knowledge that most individuals were buried, as it is standard practice in the country, mostly due to religious beliefs. However, several studies have shown that the rate of human decomposition is heavily influenced by the type of burial (e.g., whether the body was buried in direct contact with soil or in a coffin, and the type of coffin) [10–12], and that the envelopment of the body in a cloth might create a micro-environment conducive to cadaveric preservation [13–15]. Hereupon, bodies might not be skeletonized and ready for exhumation. According to the Portuguese Decree-Law No. 411/98 of 30 December 1998, inhumations must take a minimum of three years, and if the bodies are not completely skeletonized when the burial graves are opened, inhumations must continue for successive periods of two years until full decomposition is achieved. Given that the minimum Portuguese legal period of inhumation is ending, and most Portuguese cemeteries have been facing limited burial space [16–18] and soon will start exhuming buried individuals, the authors believe it is important to reflect on this matter.

2. Postmortem Detection of SARS-CoV-2 in Human Samples

When death has been caused by an infection, or when such an event is suspected, postmortem microbiology (PMM) can be of great help in both clinical and forensic contexts, in spite of the continuous controversy surrounding this field of expertise [19,20]. Furthermore, unexpected findings may take place during the autopsy process, revealing unknown pathologies and changing the course of determining one's cause-of-death [21,22]. At the very beginning of the COVID-19 pandemic, several interests and aims emerged regarding the postmortem detection of SARS-CoV-2, even though little was known as to which human samples would be more advisable for virus detection in corpses [23]. Nevertheless, experts started to study the postmortem persistence of SARS-CoV-2 RNA in fresh cadavers. In Italy, oro- and rhinopharyngeal swabs were collected from a male individual who tested positive for COVID-19 by RT-PCR after remaining in a −4 °C freezer for thirty-five days whilst waiting for permission for cremation [24]. Though swabs tested positive, the authors considered that both refrigeration and non-antiviral therapy during hospitalization might have contributed to the persistence of the virus. In fact, PMM analyses are usually more efficiently implemented in properly refrigerated and well-preserved corpses [22]. Meanwhile, in Germany, 80 individuals (34 females and 46 males) who tested positive for SARS-CoV-2 ante- or postmortem were autopsied [25]. Even though the main goal of the study was not to assess the persistence of the virus, swabs from the lung tissue and from the naso- and oropharyngeral regions were collected from the first 30 individuals being studied. Here, the maximum postmortem interval (PMI) was twelve days, and the virus was detected in all individuals. Due to the lack of scientific knowledge regarding the spread of the virus at the beginning of the pandemic, a study was conducted on postmortem ocular tissues and swabs from a total of 43 fresh cadavers in order to understand the feasibility of transplants [26]. The individuals were either consenting research donors who died of COVID-19, or surgical-intended donors whose tissues were ruled out due to symptoms of COVID-19 and/or postmortem positive tests for the disease. Overall, SARS-CoV-2 RNA and proteins were detected in ocular tissues. However, researchers were unsure if the human eye was a portal of entry and transmission for the virus. A more recent study by a few elements of the same research team on two healthy eye donors and two eye donors infected with SARS-CoV-2 demonstrated that the virus can infect the conjunctiva and induce an antiviral response [27].

At the time of writing, several studies have also been published, aiming to evaluate extrapulmonary pathological changes caused by the virus infection [28–33]. Such papers are indeed of great importance to the scientific and lay communities to better understand (i) the means of infection and transmission of the virus within the human species; (ii) the persistence and stability of the virus in human tissues; (iii) the damage caused by the virus in the human body; and (iv) the consequences of infection for those living with the post-COVID condition. However, what most studies have in common is the short PMI of the deceased, with autopsies being conducted mostly in fresh cadavers. To the best of the authors' knowledge, little research has been conducted on corpses in the advanced stage of decomposition. This might be due to the lack of need for judicial exhumations and the consequent inability to study the persistence of the virus in cadaveric remains. Nonetheless, in 2021, six studies were published regarding the detection of SARS-CoV-2 in exhumed bodies [34–39]. In the work of Sablone et al. [34], five individuals (one female and four males) were autopsied 22 to 27 days after death, with the female individual being buried for 5 days before being stored in a −15 °C freezer with the remaining four corpses. Multifocal swabs and tissue blocks were collected for analysis and, regardless of the PMI, SARS-CoV-2 was detectable. In the research of Prasad et al. [35], a male individual who has died 2 days after testing positive for COVID-19 by RT-PCR was exhumed 15 days after being buried. The body was in putrefaction, and despite swab samples being collected from the nasopharynx and the oropharynx, tissues from the lungs, intestine, liver, and kidneys were also sampled. After 21 days stored in a −80 °C freezer, the samples were evaluated for SARS-CoV-2. The only biological samples that tested negative were the kidneys and lungs. Given that the individual showed no COVID-19 complications while alive, the authors believe the infection was still in its early stage when he died. For this reason, their study showed not only how the virus can be distributed in the body, but also that it can be detected up to 36 days after death. In another investigation, Gabbrielli and collaborators [36] exhumed an individual who was infected by SARS-CoV-2 and had been buried in a zinc coffin for 1 month. The lungs, heart, and kidneys were analysed, with the latter testing negative for the SARS-CoV-2 viral genome. Despite the fact that the trials to grow the virus in vitro were ineffective, the authors still hypothesised that transmission from a deceased individual was possible within a certain postmortem time window. Meanwhile, Musso and collaborators [37] collected lung samples from 16 long-term COVID-19 exhumed individuals 24 to 78 days after death, and from two COVID-19 patients within 48 h postmortem to work as positive controls. Samples were analysed according to three different chemistries and systems and, despite some differences in the results between them, the virus was detectable even after an extensive PMI. In the work of Manjula et al. [38], a male individual who tested positive for COVID-19 by RT-PCR antemortem died by self-inflicted hanging and was exhumed 16 days after burial for legal reasons. Nasopharyngeal swabs and tissues from the liver, kidney, small intestine, and stomach were sampled and analysed 22 days later. Though the virus was not detected in all samples, the authors were surprised by the wide extrapulmonary spread of the virus shortly after the symptoms appeared. Lastly, in the study of Plenzig et al. [39], two female individuals who supposedly died of COVID-19 were exhumed; one had been tested positive by RT-PCR while the other tested positive by an antigen test. Swabs were sampled from the lungs, pleural effusion, oropharynx, perioral, and perinasal regions, as well as inside the body bag, to assess the risk of infection for those managing the corpses. After four months of burial, it was still possible to detect SARS-CoV-2 in the oropharyngeal and lung areas. However, no swabs tested positive for infectivity, as verified by cell culture inoculation.

3. Suggested Guidelines

With the beginning of the pandemic and the high number of deaths across the globe, guidelines were published on how to plan, prepare, and manage a cemetery during COVID-19 [40]. Still, guidelines for post-pandemic exhumations appear to be undervalued by governments and other institutions. This might be due to the lack of government preparation given that the last great pandemic to overwhelm developed countries was in 1918 [41,42], and exhumations from previous pandemic times have been taking place in archaeological contexts long after the event [43,44]. In some situations, the location chosen to bury those who died during an epidemiological outbreak is even found accidentally during a preventive archaeological action. As demonstrated in Willmott et al. and Kacki et al. [45,46], archaeological excavations had previously ended up revealing mass and single graves associated with the Black Death plague of the 14th century.

Just as there are mortuary differences due to distinct religious beliefs, there are also worldwide legislative and cultural disparities concerning cemeterial exhumations [47–49]. As far as Portugal is concerned, the re-use of graves is a standard practice. That is to say that the dead are often inhumed in temporary soil graves, and exhumed after a three-year period if complete skeletonization is achieved. Skeletonized remnants can then be cremated or placed in an ossuary or family plot. At the time of writing, the minimum Portuguese legal period of inhumation for the first COVID-19 deceased individuals is ending, with Portuguese cemeteries struggling against the low rate of decomposition way before the pandemic [16,50]. Due to the burial conditions imposed by legal measures (i.e., an impermeable shroud sprayed with a disinfectant solution), it is possible that bodies might not be skeletonized and ready for exhumation. Regarding the hypothetical virological risk, the authors believe it is important to reflect on the procedures to be followed during the coming exhumations, and how the exhumed bodies should be managed. For this reason, the authors aim to purpose some guidelines for cemetery workers and the responsible authorities:

- *Safety around the grave*: Given that exhumations take place during the opening hours of cemeteries, a safety perimeter should be established around the grave to restrict the access of cemeterial visitors and the relatives of the deceased.
- *PPE*: Gravediggers must wear head-to-toe PPE as used during the pandemic burials (i.e., safety glasses or face shields, masks, gloves, full body suits, and footwear protection). To avoid cross-contamination, the PPE should be safely removed and disposed in appropriate containers for staff-only use and not on the containers accessed by cemeterial visitors. Reusable PPE should be avoided but, if necessary (e.g., safety glasses or face shields), they should be washed with clean running water and soap, and disinfected.
- *Impermeable shroud*: Gravediggers will have to open the body bag to ensure the body is completely decomposed. If the body is not skeletonized and the grave must be closed, the impermeable shroud should be safely removed and disposed of in an appropriate container for staff-only use to avoid cross-contamination. If the body continues to be wrapped in the bag, complete decomposition might hardly be achieved in the next two years, as expected by law.
- *Exhumed skeletonized remains*: As designated by law, bones must be placed in wood coffins for cremation or zinc coffins for ossuaries and vaults. Coffins should be sprayed with a disinfectant solution to avoid cross-contamination when handling, and cremation should take place on the same day of the exhumation.
- *Burial materials*: Once the body is exhumed, all the materials present in the grave (e.g., coffin boards and the deceased's garments) should be safely removed and disposed of

in an appropriate container for staff-only use to avoid cross-contamination. Nothing should be left inside the graves.

- *After the exhumation*: Gravediggers should thoroughly wash their hands with running water and soap, and use hand-sanitiser. All personal belongings (e.g., eyeglasses) should also be disinfected. Gravediggers should take a shower before leaving the cemetery as is already common practice.

4. Future Perspectives

The postmortem fate of human remains has been studied by several researchers for the past few decades to better understand how the complex human body decomposes in different scenarios [51–55]. Though there is still much to discover when it comes to human taphonomy, studies have shown that cadavers can display distinct states and patterns of decay even when sharing the same PMI and burial conditions [12,17,56,57]. According to the scientific literature, this can be explained by endogenous factors (i.e., cadaver-related variables) such as body mass [58,59], thanatomicrobiome (i.e., microbes colonising orifices and internal organs after death) [60], and the presence of traumatic injuries related to the cause-of-death or autopsy procedures [61,62]. As such, what gravediggers will find when exhuming those who died with or from COVID-19 disease is not clearly known. Still, the incomplete skeletonization of the bodies will for sure create a snowball effect of difficulties with respect to cemeterial management in the country. At this point, the authors would like to raise the question if it is reasonable to re-bury those who are not completely skeletonized when first exhumed, as established by law. The envelopment of the body in the impermeable shroud, and the possible retention of water and body fluids, might be favourable for adipocere formation [63–65]. If so, the superficial shell produced by it may not allow soft tissues to further decay. From the point of view of the authors, this scenario should be seen as an opportunity for the competent authorities to learn how to deal with future mortality crises, and to consider a re-evaluation of the existent outdated legislation.

Regarding SARS-CoV-2, little information is available about the persistence and stability of the virus so long after death, as demonstrated in Section 2. Even though the scientific literature demonstrates that microorganisms have a short lifespan after the host's death, and the risk of infection is reduced with time, studies on PMM are mainly focused on autopsies shortly after death, given that decay and a longer PMI can hinder the efficiency of the analyses [66,67]. Still, when it comes to pathogens, the former can be detectable and/or contamination can occur if human soft or hard tissues are available even after a long duration of time. Proof of this lies in studies previously published, showing the detection of the variola virus in mummies and skeletons from the 17th–18th centuries in Siberia, Lithuania, and France [68–70]; the discovery of *Mycobacterium tuberculosis* in a skeleton from the 10th–11th centuries in Argentina [71]; the identification of *Yersinia pestis* in skeletons from the 14th century in England and France [45,46]; and the detection of *Mycobacterium leprae* in skeletons from across Europe dated from the 6th to the 20th centuries [72]. It goes without saying that the postmortem analysis and detection of pathogens is of great value to the field of (paleo)pathology, providing new insight into the aetiology, geographical distribution, and pathogenesis of re-emerging infectious diseases of the former [73–75]. For this reason, the authors believe the upcoming cemeterial exhumations should also be seen as a research opportunity to better understand (i) the spread of SARS-CoV-2 in the human body; (ii) the viability and detection of the virus in human samples after all these years; (iii) the possible geographic coverage of the multiple strains identified; and (iv) the impact of the disease on bone metabolism, to name a few. With respect to this last topic, the authors would like to leave the question open as to whether future studies should not also be performed on bone tissue samples to check their infection through blood. If the bodies are skeletonized, their remains can either be cremated or placed in an ossuary, as stated by the Decree-Law No. 411/98 of 30 December 1998, providing another opportunity to conduct new lines of investigation. Besides the ethical concerns inherent to the development of such a study, the authors acknowledge the fragility of the situation due to the lack of a proper funeral and farewell from the relatives at the time of burial. However, in addition to the implied scientific purposes of future research in the field, special attention should be given to the public health matters associated with these exhumations, given that cemetery workers are going to continuously be exposed to undisclosed hazards. As mentioned by Plenzig and collaborators [39], the soil conditions (e.g., temperature) in their study might have influenced the virus stability and predisposition for it to be detected, given that the inhumations occurred during winter, and SARS-CoV-2 clearly shows a higher capacity for survival in lower temperatures [76,77]. It is common knowledge that soil properties (e.g., temperature and humidity) have in fact undergone numerous seasonal variations throughout this time, which might have led to the degradation of the virus. Nevertheless, the authors consider that it would be of great significance to likewise conduct analysis on soil from burial graves. The presence of human pathogens in soil can have a negative impact on human health [78–80] and, given that soil is re-used between burials, there may be an increased virological risk. On the other hand, Plenzig et al. [39] mention that the individuals analysed showed their first COVID-19 symptoms between 15 to 19 days before death, thereby bringing up the possibility that they were no longer contagious when they died. When it comes to the Portuguese context, cemetery workers have no access to an individual's medical history to know one's stage of disease when death occurs. Still, a future scientific investigation could also evaluate this hypothesis, given that researchers and ethics committees could ask for permission to access the medical records of the deceased. Yet, because there is no certainty if the individual was still in a contagious phase when he/she died, or if the virus can still be detected in exhumed bodies so long after their inhumation, gravediggers cannot be too careful.

In summary, this reflection intends to raise awareness of the importance of studying exhumed individuals to understand the postmortem viability of SARS-CoV-2. Besides the inherent scientific interest such research might have, governments and institutions cannot discard the social value of the knowledge to be gained in the future. Even though postmortem detection of the virus may pave the way for unknown justifications for the cause-of-death, it is of paramount importance to understand what cemetery workers might be dealing with, given that they are especially exposed to biological fluids, human remnants in distinct stages of decomposition, and possibly contaminated soil. Though the presence of the virus might not be indicative of the disease, it should not be overlooked. In either case, the authors would like to emphasise the high significance of this public health matter, in addition to its impact on cemeterial management. Although this manuscript presents a Portuguese perspective, the authors believe other countries might find value in these reflections, particularly those with similar legislation and funerary customs.

Author Contributions: Conceptualization, A.S.-B. and R.J.D.-O.; writing—original draft preparation, A.S.-B.; writing—review and editing, A.S.-B., M.T.F. and R.J.D.-O. All authors have read and agreed to the published version of the manuscript.

Funding: The first author was financed by FCT, grant number SFRH/BD/143242/2019. The second author was financed by the R&D Unit Centre for Functional Ecology—Science for People and the Planet (CFE), with the reference UIDB/04004/2020, financed by FCT/MCTES through national funds (PIDDAC). This work was supported by the Applied Molecular Biosciences Unit—UCIBIO, financed by national funds from FCT (UIDB/04378/2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank the anonymous reviewers for their valuable comments and suggestions.

Conflicts of Interest: The authors declare no conflict of interest.

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