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BRIEF REPORT

Detection of SARS-CoV-2 RNA by PCR in a series of corpses sent for autopsy[☆]

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Abstract Following the emergence of SARS-CoV-2 in the world, the COVID-19 pandemic broke out, affecting 185 countries. There have been numerous scientific publications presenting epidemiological, clinical, statistical, and microbiological data. The high mortality has implied the need for increasingly accurate data regarding the virus and its behaviour in the environment and, on the other hand, faced health teams with a serious problem in relation to the safe handling of corpses and the risks of transmission that this implies. The objective of this study was to detect the presence of SARS-CoV-2 RNA in corpses sent to the National Judicial Morgue that were not included in the definition of a suspected case submitted by the Argentine Ministry of Health. Nasopharyngeal and oropharyngeal swabs were taken from 101 cadavers chosen randomly and based on inclusion criteria for detection of viral RNA using the RT-PCR technique. Of the cadavers included in the study, 16.8%, not classified as suspected cases of COVID-19, were tested for the presence of viral RNA in the samples collected.

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PALABRAS CLAVE

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 Autopsia

Detección de ARN del SARS-CoV-2 por PCR en una serie de cadáveres remitidos para autopsia

Resumen A partir de la emergencia del SARS-CoV-2 en el mundo se desató la pandemia por COVID-19 que afectó a 185 países; aparecieron numerosas publicaciones científicas presentando datos epidemiológicos, clínicos, estadísticos y microbiológicos. La elevada mortalidad ha implicado la necesidad de contar con datos cada vez más precisos con relación al virus y a su comportamiento en el medio y, por otro lado, enfrentó a los equipos de salud con un grave problema con relación al manejo seguro de cadáveres y a los riesgos de transmisión que ello implica. El objetivo del presente estudio ha sido detectar la presencia de ARN del SARS-CoV-2 en cadáveres remitidos a la Morgue Judicial de la Nación que no estuvieran comprendidos en la definición de caso sospechoso propuesta por el Ministerio de Salud de la Nación de la República Argentina. Se realizaron hisopados nasofaríngeos a 101 cadáveres elegidos aleatoriamente y basándose en criterios de inclusión para la detección de ARN viral mediante técnica RT-PCR. Se detectó ARN viral en el 16,8% de los cadáveres incluidos en el estudio.

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Introduction

In December 2019 an outbreak of pneumonia due to an unknown cause was reported in the city of Wuhan, the provincial capital of Hubei, in China. On 7 January 2020 Chinese scientists isolated a new coronavirus, coronavirus 2, which causes severe acute respiratory syndrome or SARS-CoV-2 as the cause of the disease due to coronavirus 2019 (COVID-19) as the World Health Organisation (WHO) would determine¹. Clinically the disease includes asymptomatic cases, mild upper airway disease and pneumonia with respiratory failure that may be life-threatening². By 25 December 79,489,000 cases and 1,744,563 deaths had been recorded worldwide³; after 3 March 2020 in Argentina there had been 1,571,680 positive cases and 42,392⁴ deaths, with a lethality rate of 2.71%. The percentage of positivity in the country stands at 41.72%. The median age of confirmed cases is 38 years, with a lethality rate which increases over the age of 60 years. Its distribution according to sex is similar, at 49.7% in women and 49.9% in men. The National Health Ministry has reported that 4,597,960 diagnostic tests have been performed since the start of the outbreak, representing 101,328 samples per million inhabitants⁵. The city of Buenos Aires reported 167,978 positive cases on 23/12/2020, with 5,806 deaths and a lethality rate of 3.46%. On 11 September 2020 the Ministry of Health of the Republic of Argentina set a new definition of suspected cases of COVID-19 which includes everyone with two or more symptoms: fever of 37.5°C, cough, odynophagia, respiratory difficulty, cephalgia, diarrhoea and/or vomiting, anosmia or dysgeusia, without any other disease that could explain the symptoms. The definition also covers all health workers, those who live or work in closed institutions or subject to prolonged admission, essential workers and those who live in working class or native quarters, who have been in close contact with a confirmed case of COVID-19, and who have one or more of the symptoms described within 14 days of the contact. It also included post-COVID-

19 multisystemic inflammatory syndrome in paediatric cases⁶.

It is hard to estimate the prevalence of SARS-CoV-2 infection within this context, as it is only possible to count individuals who were infected and subjected to a test due to their symptoms or because they fulfilled the above criteria. Specific data do exist on the number of deaths in hospitals or medical institutions. This situation is worrying⁷, as the transmission of the virus in these cases determines the increase in new cases. It is vitally important to make it possible to determine the real prevalence of infection by SARS-CoV-2 more exactly, given that "the fact that asymptomatic individuals are potential sources of infection by 2019-nCoV may justify a re-assessment of the dynamic of transmission of the current outbreak"⁸.

The National Judicial Morgue, which is controlled by the National Justice Forensic Medicine Corps, receives the bodies of individuals who have died within the jurisdictional area of the Autonomous City of Buenos Aires, in cases analysed by the federal legal authority with the aim of establishing the causes and mechanisms of death by the necessary means of examination.

In the context of the COVID-19 pandemic measures were adopted to prevent the propagation of the disease by bodies received, according to international and national recommendations for the safe handling of the same and the performance of autopsies⁹⁻¹¹.

Basic factors should therefore not be omitted, such as the performance of medical-forensic autopsies and the data on mortality due to COVID-19, in connection with the minimisation of risks, reducing the handling of cadavers within this context to the minimum¹². The process of dispatching cadavers, admitting and storing them, performing autopsy, taking samples and then returning them was made suitable. Recommendations were established for the use of personal protective equipment by the personnel involved and for entry into the establishment by those individuals who needed to do so. In the case of cadavers with known

infection by COVID-19 measures were adopted to minimise the risk of transmission, establishing a protocol for action within the framework of the tasks undertaken by forensic doctors. A complete external examination is performed in such cases: anthropometric, thanatological and traumatological. Additionally, photographs are taken of the whole body and a radiography of the thorax is taken. In cases where there are reasons to justify such actions, minimum incisions are made to take tissue samples and obtain blood or vitreous humour using puncture-aspiration¹³.

The aim of this study was to detect the presence of SARS-CoV-2 RNA in cadavers received by the National Judicial Morgue which were excluded from the definition of suspicious cases proposed by the National Ministry of Health of the Argentinian Republic.

Materials and methods

Samples were taken at random for the detection of viral SARS-CoV-2 RNA by PCR in nasopharyngeal swabs¹⁴.

Case selection

Cadavers were selected on the basis of the following inclusion criteria:

- Older than 20 years. Given the higher prevalence of COVID-19 disease a minimum age of 20 years old was set, according to information from the National Board of Epidemiology and Strategic Information from the national health authority (the National Ministry of Health of Argentina). Its report of 08/10/20⁵ shows a significant increase in cases in the age group corresponding to individuals aged from 20 to 29 years old.
- Deaths with a NON-violent cause. The cadavers of individuals who had died due to non-violent causes were selected. The majority of these had been “found lifeless” or their death was no directly linked to any known cause, to prevent altering any possible tests in subsequent legal proceedings.
- Found in the home. Individuals found dead in their home were included, when their deaths could not be explained by other causes that could be linked to cases of asymptomatic COVID-19, or which either had not shown any symptoms or when these had gone unnoticed.
- A time lapse since death of less than 36 hrs. This criterion was established due to the lack of sufficient evidence regarding the permanence of the SARS-CoV-2 virus or particles of its RNA in the body after death.
- Without the suspicion of disease caused by SARS-CoV-2 or any reported comorbidities. The lack of suspicion of disease caused by SARS-CoV-2 involved ruling out this prior to the autopsy in individuals who had been suspected of COVID-19 before their death, whether this had been validated or not by the health authority.

The exclusion criteria were: hospitalised individuals, cadavers found in public spaces, deaths due to a violent cause, individuals under the age of 20 years or putrefaction in a chromatic-emphysema stage.

The information used to define whether a cadaver was included or excluded was based on the data supplied by the body which sent the cadaver, usually the Buenos Aires City police force, in the form of the corresponding investigation report.

Sample taking and analysis

Samples were taken by forensic medical technicians when a cadaver was admitted to the National Judicial Morgue, following international and national recommendations for sample taking in the context of the coronavirus pandemic in terms of the use of personal protective equipment^{14,15}. These consisted of wearing a surgical jacket and trousers, glasses and a N95/FFP3 face mask and high rubber boots, together with disposable equipment: an impermeable surgical gown, a surgical cap and gloves. To prevent the contamination of samples, they were taken from cadavers in suspicious cases in an isolated environment.

Nasopharyngeal and oropharyngeal swabs were taken. Both swabs (nasal- and oropharyngeal) were taken during the first period (from 16/04/2020 to 24/04/2020), while in the second period (from 01/07/2020 to 03/09/2020) only a nasopharyngeal swab was taken due to a change in health authority directives for the diagnosis of COVID-19. The first collection period was initiated 30 days after the start of the preventive obligatory social lockdown (quarantine) in Buenos Aires and at the start of the increase in cases due to community transmission, which at the time stood at 16%, with 122 deaths and 2,669 positive cases. The second period in which samples were taken was selected due to the increase in cases and deaths, which at the time amounted to 67,197 positive cases and 1,351 deaths. 46% of cases were caused by community transmission at first, and at the end of the sample collection period there were 451,198 positive cases, 9,361 deaths and 62.65% of cases were due to community transmission.

Flexible sterile polyethylene terephthalate (Dacron) swabs with a plastic tubes were used. They were kept in 2 ml of sterile physiological saline solution and were transported to the reference laboratory in a refrigerated Sisteg® M6¹³ secure transport system for diagnostic samples and infectious substances. The reference laboratory which processed the samples was the *Instituto de Investigaciones Biomédicas en Retrovirus y Sida (INBRIS)* of the Medicine Faculty of the UBA-CONICET of the Autonomous City of Buenos Aires. Samples were processed for extraction of the viral RNA using RT-PCR columns (Qiagen, ThermoFisher brand or similar quality) in real time (GeneFinder™ kit, COVID-19 Plus RealAmp Kit).

Samples were sent registered with a chain of custody and by registration of the case in the National Health Vigilance System of the National Ministry of Health of the Republic of Argentina for the purposes of epidemiological monitoring and the relevant notification of the health authority. In all cases the transport and delivery of the bodies was undertaken according to the biosecurity standards which minimise viral transmission, as there was no evidence in this respect apart from the recommendation not to perform autopsies on the cadavers of individuals who had died due to COVID-19¹⁵, given the risks of aerosolization implicit in the procedure¹⁶.

Results

One hundred and one cadavers were studied, 51 males and 50 females. The average age was 72.4 years (with a minimum age of 43 years and a maximum age of 102). The minimum age of the males was 43 years and the maximum was 94 years, while for the females the minimum was 57 years and the maximum was 102 years.

SARS-CoV-2 RNA was detected in the samples from 17 cadavers, 16.8% of the total. 7 of the cadavers in which viral RNA was detected were female (41.2%) and 10 were male (58.8%). The average age of the detected cases was 70.4 years in the males and 84.2 years in the females.

The average time from death until sample taking in both periods was 20 hrs. 10 min. In the first period it amounted to an average of 14 hrs. 15 min., with a minimum interval of 9 hrs. and a maximum of 62 hrs. 45 min., obtaining viral RNA in the sample. In the second period the average time was 25 hrs. 10 min., with a minimum interval of 8 hrs. 40 min. and a maximum of 90 hrs. 30 min.

Discussion

Certain correlations may be established with international and national epidemiological studies, even though they were performed in live individuals. Here it must be underlined that although international recommendations state that cadavers are a risk for viral transmission, this has not been definitively established. It is known that the mechanism of human to human transmission is by respiratory droplets larger than 5 μ , hands and fomites which enter into contact with the mucus membranes (oral, nasal or ocular) directly or by the hands¹⁷. It is necessary to take into account that SARS-CoV-2 remains with a potential risk of transmission, i.e., in a viable state, on certain surfaces. The corresponding time is for 4 hrs. on copper, 24 hrs. on cardboard, 48 hrs. on stainless steel and 72 hrs. on plastic, if the temperature remains at an average of 22 °C and a humidity of 40% in the experimental model. In the same way and under these conditions the viability of the virus in aerosols has been established to stand at 3 hrs., with an average lifespan of 1.1 hrs¹⁸. We therefore estimate that during a medical-legal autopsy, where aerosols are produced by opening cavities, especially the cranium, viral transmission may occur from cadavers without any signs of putrefaction. We understand that putrefaction phenomena would deactivate the virus due to the denaturalisation of organ material. As putrefaction commences at from 24 to 36 hours in our field, this coincides with the average time the possibility of viral transmission lasts on certain surfaces. If we consider how long it remains viable in aerosols (A/L 1.1 hrs.) it would be far less. It should be made clear that the detection of viral RNA in this case does not necessarily imply that viable virus exists, given that samples were taken from cadavers. It has also been established that it is effectively deactivated by concentrations of bleach of 1:49 and 1:99, 70% ethanol, povidone iodine at 7.5%, chlorhexidine at 0.05% and a liquid soap solution concentration of 1:49¹⁹.

This is why one of the objectives in discovering the possibility of detecting viral RNA in cadavers is to determine the risk of transmission to which the personnel involved in

forensic transport and autopsies are exposed. Particles of viral RNA have been found up to 90 hrs. after death and at an average of approx. 25 hrs., although this does not necessarily imply viral activity, so that study by culture should be performed.

Based on the number of cases detected in cadavers, it may be said that there is a lack of medical visits due to suspicion of the disease, or that individuals who are symptom-free or have only mild symptoms go on to suffer aggravation of their symptoms and/or a fatal outcome without being admitted to a medical centre.

In the series of cases reported by the WHO in China, the average age was 51 years, with the highest incidence (77.8%) in the group aged from 30 to 69 years, of whom 51% were men²⁰. This study therefore showed no significant differences in terms of the incidence described in epidemiological reports and the situation in the Republic of Argentina. The sex ratios of the bodies in which virus was detected also corresponds to national and international epidemiological reports. When the series of cadavers without suspicion of SARS-CoV-2 disease is analysed, there are no clear indications in the previous clinical history of the individuals concerned. They were simply not included as being suspected of having COVID-19 according to the criterion of the relevant authority. Nor is there any information on their comorbidities, personal symptoms or their immediate environment. It has to be said that transmission in China was chiefly within families and through close contact²⁰. No cases connected with the individuals who had died were reported²¹⁻²³.

Conclusions

The positivity rate in the detection of viral SARS-CoV-2 RNA in the cadavers of individuals with no suspicion of infection by COVID-19 sent to the Judicial Morgue of the City of Buenos Aires amounted to 16.8%. These findings correlate with national and international epidemiological data in terms of age, with a lower rate of detection in female cadavers. This may correspond hypothetically to a reduced rate of mortality due to complications or comorbidities in the latter sex, considering that the average age of this group was higher than it was in the group of males²⁴⁻²⁸.

The data obtained do not make it possible to make any recommendations on testing all of the cadavers which are admitted to the Judicial Morgue, to detect viral RNA by nasopharyngeal swabbing followed by a RT-PCR test; the resulting recommendation is therefore to use personal protective equipment in all cases, as if they had proven to be positive for viral RNA.

Conflict of interests

The authors have no conflict of interests to declare.

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