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Seroprevalence of Crimean-Congo Hemorrhagic Fever Virus and Rift Valley Fever Virus in human population in Senegal from October to November 2020

Safietou Sankhe[1,2,*], Cheikh Talla[3,*], Mareme Seye Thiam[3], Martin Faye[1], Mamadou Aliou Barry[3], Maryam Diarra[3], Moussa Dia[1], Oumar Ndiaye[1], Pape Mbacke Sembene[2], Boly Diop[4], Gamou Fall[1], Oumar Faye[1], Ousmane Faye[1], Moussa Moise Diagne[1,*,#], Cheikh Loucoubar[3,*]

[1] *Arboviruses and Hemorrhagic Fever Viruses Unit, Virology Department, Institut Pasteur de Dakar, BP220 Dakar, Senegal*
[2] *Animal Biology Department, Faculty of Sciences and Techniques, Cheikh Anta Diop University of Dakar, Dakar, Senegal*
[3] *Epidemiology, Clinical Research and Data Science Department, Institut Pasteur de Dakar, BP220 Dakar, Senegal*
[4] *Epidemiological Surveillance Division, Prevention Department, Ministry of Health, Dakar, Senegal*

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ABSTRACT

Objectives: Rift Valley Fever and Crimean-Congo Hemorrhagic Fever are two infections classified among the emerging diseases to be monitored with highest priority. Studies undertaken in human and animals have shown endemicity of these two arboviruses in several African countries. However, most of the investigations were carried out on domestic cattle and the studies conducted on human populations are either outdated or limited to a small number of well-known endemic areas. It is then critical to better evaluate the burden of these viruses in Senegal at a national scale.

Methods: This work relies on a previous seroprevalence survey undertaken in all regions of Senegal at the end of 2020. The existing biobank was used to determine the immunoglobulin G [IgG] Rift Valley Fever and Crimean-Congo Hemorrhagic Fever seroprevalences by indirect enzyme-linked immunosorbent assay.

Results: The crude seroprevalences of Rift Valley Fever and Crimean-Congo Hemorrhagic Fever were 3.94% and 0.7% respectively, with the northern and central part of the countries as the main exposed areas. However, acute infections reported in both high and low exposed regions suggest sporadic introductions.

Conclusions: This study gives updated information and could be of interest to support the stakeholders in the management of these zoonoses.

Introduction

Rift Valley Fever Virus (RVFV) (*Phenuiviridae* family; *Phlebovirus* genus) and Crimean-Congo Hemorrhagic Fever Virus (CCHFV) (*Nairoviridae* family; *Orthonairovirus* genus), are two arboviruses belonging to the *Bunyavirales* order and are among the ten emerging infectious diseases to monitor with the highest priority [1].

RVFV causes Rift Valley Fever (RVF), an African viral zoonosis mainly affecting domestic ruminants and capable of being transmitted to humans. An epidemic causing abortions in cattle was notified for the first time in 1930 near Lake Naivasha in Kenya [2] and RVFV was isolated from mosquitoes as the causal agent. The virus can be transmitted to ruminants and humans through mosquito bites and to humans by contact with infected tissues and fluids [3]. In humans, the disease is a mild self-limiting febrile sickness, with a higher mortality rate in cases of hemorrhagic fever, meningoencephalitis, and hepatitis [4]. In livestock, RVF is mainly characterized by abortions in pregnant females and a high mortality rate in offspring [5].

Corresponding author
E-mail addresses: safietou.SANKHE@pasteur.sn (S. Sankhe), cheikh.TALLA@pasteur.sn (C. Talla), mareme.THIAM@pasteur.sn (M.S. Thiam), martin.FAYE@pasteur.sn (M. Faye), aliou.BARRY@pasteur.sn (M.A. Barry), maryam.DIARRA@pasteur.sn (M. Diarra), moussa.DIA@pasteur.sn (M. Dia), oumar.NDIAYE@pasteur.sn (O. Ndiaye), mbacke.sembene@ucad.edu.sn (P.M. Sembene), diopboly@yahoo.fr (B. Diop), gamou.FALL@pasteur.sn (G. Fall), oumar.FAYE@pasteur.sn (O. Faye), ousmane.FAYE@pasteur.sn (O. Faye), moussamoise.DIAGNE@pasteur.sn (M.M. Diagne), cheikh.LOUCOUBAR@pasteur.sn (C. Loucoubar).
* These authors contributed equally to this work.

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CCHFV is a tick-borne virus causing Crimean-Congo Hemorrhagic Fever (CCHF), first described in Soviet soldiers in Crimea in 1944-1945 [6] and in ticks belonging to the *Hyalomma* genus [7]. Symptoms of CCHF in humans include a sudden onset of high fever, headache, myalgia, joint pain, and vomiting, as well as visible symptoms such as red eyes, red face and throat, and petechiae (Sahak, 2019). Bruising and severe bleeding may be observed when the disease worsens [8]. Transmission routes in humans include indirect transmission by infected tick bites, direct contact with contaminated blood or tissues from animals, and human-to-human transmission through virus-containing body fluids, mainly in a nosocomial context. CCHFV infection in animals is asymptomatic, but viremia and antibodies have been observed in domestic and wild vertebrates [9]. The disease shows a wide spectrum of symptoms ranging from mild non-specific febrile syndrome to hemorrhagic signs [10].

RVFV and CCHFV are endemic in several West African countries such as Senegal and Mauritania [6]. In Senegal, RVFV was isolated in 1974 and 1983 from *Aedes dalzieli* mosquitoes [11]. Near the village of Rosso (southern Mauritania) on the Senegal River, almost 200 human deaths related to the only documented RVF outbreak have been reported [12]. Previous studies have shown high RVFV seroprevalence in both healthy human and livestock populations of northern Senegal [13,14]. RVFV strains have also been found in mosquitos [15]. In 1987, the first epidemic of RVF was notified at the Senegalese-Mauritanian border with nearly 1500 cases of human infection and more than 200 deaths [12]. Senegal reported an RVF outbreak between the end of 2013 and the beginning of 2014. Eleven people and 52 animals were infected in several places, including Saint-Louis, Dakar, Linguere, Mbour, and Kedougou [16]. More recently in 2019, the Syndromic Sentinel Surveillance Network in Senegal allowed the detection of several RVF cases in different parts of the country [17].

Less is known about the spread of CCHF in Senegal. However, CCHFV was found in ticks and goat samples from the Mbour area in 1989 and 1996 (Wilson et al., 1990) followed by the diagnosis of three human cases in 2003 [18]. More recently in 2019, a human case has been recorded in the Matam region in the north of the country [10]. It was previously shown that seroprevalence in both ruminants and humans significantly decreased from the arid north to the south [19]. A more contemporary study on different animals shows the same pattern, indicating the infection is always prevalent in the country's northern part [9].

Since recurrent RVF/CCHF outbreaks and sporadic human cases are increasingly reported in Senegal [10,6,17], it is crucial to better evaluate the burden of these two viruses at a national scale. Overall, only a few investigations have been conducted in human populations and most of them are either outdated or limited to a small number of well-known endemic areas. In addition, most of the studies on RVFV and CCHFV seroprevalence were focused on animals.

Updated data will be of interest to support the stakeholders in RVF/CCHF management. This study is a nationwide human RVF/CCHF seroprevalence study conducted in all regions of Senegal at the end of 2020.

Methods

Study design

Our study relies on a work carried out by Talla et al. [20] who aimed to perform a cross-sectional SARS-CoV-2 seroepidemiological survey at a national level conducted in all fourteen regions of Senegal in the period from October to November 2020. The extensive sampling effort allowed for the creation of an interesting biobank for future research. A multistage cluster sampling was applied as previously described [20]. Briefly, areas were first selected across Senegal by identifying the number of clusters by region according to the population size and using sampling proportional to the probability by size (PPS), then a systematic random sampling to select 10 households in each selected area was performed. In each of the sampled households, two individuals over 5 years old and another one under five years old were randomly enrolled.

Ethical considerations

This study was approved by the Senegalese National Ethics Committee for Research and Health. As described by Talla et al. [20], all persons who fulfilled the inclusion criteria were included in the study with their informed consent. For minors (under 18 years), a legal representative guaranteed consent to participate in the study.

Sampling and Data collection

Blood collection was carried out under optimal conditions into dry vacutainer tubes by standard venipuncture technique, and samples were immediately stored at 4°C and then quickly directed to the Institut Pasteur de Dakar within 24–72 hours. After a centrifugation step, sera were aliquoted into cryotubes and stored at -20°C. Metadata such as age, sex, region, and symptoms during the last 6 months before the survey were also recorded.

In parallel, aggregated data on the number of RVFV/CCHFV severe acute infections detected in the different parts of the country through the Syndromic Sentinel Surveillance [21] in Senegal from 2019 to early 2022 were collected from both the WHO Collaborating Center for arbovirus & hemorrhagic fever viruses and the Epidemiology, Clinical Research & Data Science department in IPD.

Indirect Elisa IgG RVF and CCHF

For indirect IgG ELISA, standard ELISA plates (Immulon II 96-well microtiter plates; Dynatech laboratories, Inc., El Paso, TX, USA) were coated with 100 µL of in house-prepared mouse hyper immune ascitic fluids specific to either RVFV or CCHFV at 1/1000 dilution in phosphate buffered saline (PBS) solution at 0.135 M and coated overnight at 4°C. After the initial three washing steps with 300 µL of wash buffer (PBS 1X-Tween20 0.05%), 100 µL of in house-prepared specific corresponding mouse brain antigens to either RVFV or CCHFV were diluted at 1:100 in dilution buffer (PBS 1X-Tween20-1% skimmed milk) and incubated at 37°C for 1 h. For each sample, 100 µL of 1:100 diluted negative antigen control (obtained from non-infected mice brain) was also added. After 1 h incubation time, plates were washed three times with 300 µL of wash buffer and 100 µL of samples, negative and positive controls were added at 1/100 dilution in dilution buffer.

After 1 h incubation at 37°C and three washing steps with 300 µL of wash buffer, 100 µL of goat anti-Human IgG horseradish peroxidase conjugated (Seracare, Milford, MA, USA) diluted at 1 µg/mL in dilution buffer was added to the plates. After 1 h incubation at 37°C and subsequent washing steps with 300 µL of wash buffer, specific binding was revealed by addition of 100 µL of ready to use 3,3',5,5'-Tetramethylbenzidine (TMB) (Catalog number T0440-100ML, Sigma-Aldrich, Saint Louis, MO, USA) and subsequently stopped using 100 µL of 2 N Sulfuric acid (H2SO4). Plates were later read on the spectrophotometer at 450 nm wavelength and 620 nm as passive reference. Sera were considered positive when the difference of optical density between the specific and non-specific (from mock mice brain) antigens was > 0.20 and the ratio (R) between the sample and the negative control was > 2.

Statistical analyses

Crude and standardized seroprevalence were assessed using 95% Confidence Interval (CI). The Clopper-Pearson technique was utilized to calculate 95% confidence intervals for seroprevalence. We evaluated weighted prevalence estimates based on 2020 population data from The

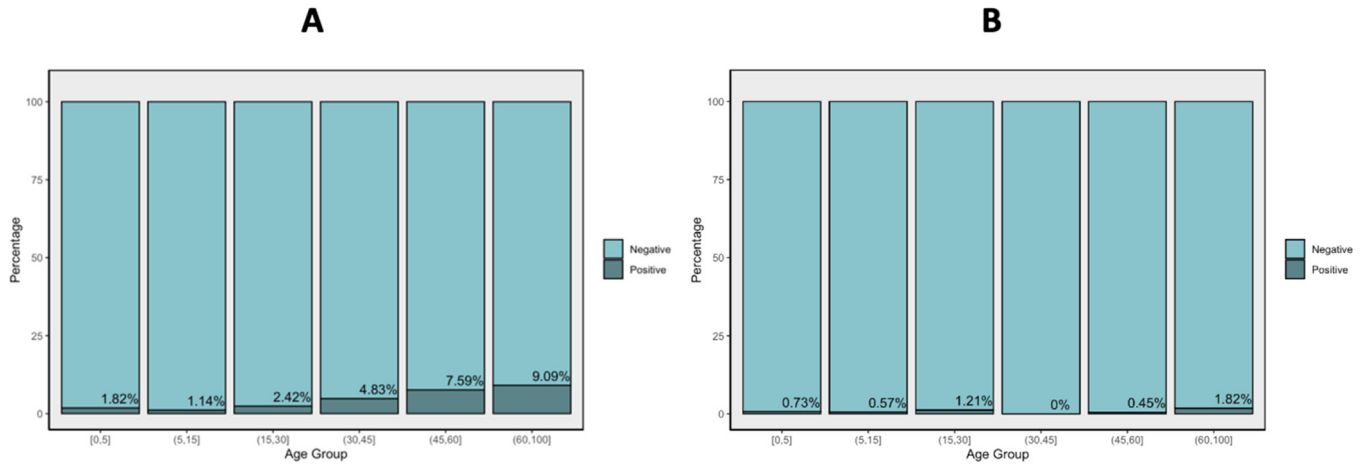

Figure 1. Rift Valley Fever (A) and Crimean-Congo Hemorrhagic Fever (B) Ig-G seroprevalence according to age group from October to November 2020 in Senegal.

Table 1
Characterization by age groups of the study population.

Age Group	Number (percentage)
[0,5]	274 (19.5%)
(5,15]	175 (12.5%)
(15,30]	331 (23.6%)
(30,45]	290 (20.7%)
(45,60]	224 (16.0%)
(60,100]	110 (7.8%)

National Agency for Statistics and Demography (ANSD) and direct standardization of observed seroprevalence and population weights by age-sex strata. A multiple regression analysis was performed. We assumed statistical significance at p-value <0.05. All statistical analyses were performed using R 4.0.4. Using the "mapplots" R package, the geographic distribution of seroprevalence has been plotted.

Results

Study population characteristics

Overall, 1421 individuals were included in this study with basic epidemiological data and an eligible blood sample for serological analysis, as previously described [20]. The participants had an average age of 29 years, ranging from 0.2 to 84.8 years. Women were more represented with 59% (n=829) versus 41% (n=575) for men (sex ratio = 0.69).

Seroprevalence of anti-RVFV and anti-CCHFV antibodies

Overall, the crude seroprevalences of RVFV and CCHFV were 3.94% and 0.7% respectively.

Seroprevalence according to age group

With 331 persons, the 15-30-year-old age group was the largest, followed by the 30-45-year-old (n=290) and the 0-5-year-old age group (n=274) (Table 1).

RVFV seroprevalence was highest in the 60-100-year-old age group (9.09%), followed by the 45-60-year-old age group (7.59%), and the 30-45-year-old age group (4.83%) (Figure 1A). The lowest RVFV seroprevalence was found in 5-15 years old with 1.14%. No significant differences were observed in seroprevalence data between age groups.

Regarding CCHFV, lower antibody titers compared to RVFV at the level of the age groups were obtained (Figure 1B). Moreover, there was no significant difference between the seroprevalences of people aged 60–100 and those aged 15–30, which were 1.82% and 1.21%, respectively.

Seroprevalence according to sex

The results showed that the seroprevalence of RVFV was higher in men with 5.3% than in women with 3% (Figure 2A). This trend was also observed for CCHFV, for which the antibody rate was 0.86% in men and 0.6% in women (Figure 2B).

Seroprevalence by region and acute infection

RVFV and CCHFV seroprevalences were assessed for all regions of Senegal. No RVFV IgG antibodies were found in the Ziguinchor, Matam, and Diourbel areas. In contrast, the region of Saint-Louis had the greatest RVFV seroprevalence at 14.44%, followed by Tambacounda, Fatick, and Kolda at 10.38%, 8%, and 7.42%, respectively. The Sedhiou region had an intermediate seroprevalence of 7.14% followed by the Kaffrine region with 5.06% (Figure 3A, Supplementary Material S1-S2). While no CCHFV specific IgG was found in the other regions, Kaolack had the highest CCHFV seroprevalence of 1.8%, followed by Diourbel, Fatick, Kaffrine, and Saint-Louis with 1.74%, 1.33%, 1.27%, and 1.11%, respectively (Figure 3B, Supplementary Material S3-S4).

From 2019 to early 2022, respectively 10 and 16 cases regarding CCHF and RVF were reported by the surveillance system, mainly in five regions (Saint-Louis, Matam, Fatick, Kaolack and Dakar) as shown in the supplementary material S5-S6.

4. Discussion

Our study is the first that assess the nationwide seroprevalence of RVFV and CCHFV in human populations in Senegal. Indeed, the previous studies were mainly done in specific areas of interest in the northern part of the country such as the border along the left bank of the Senegal River [22]. In our study, seroprevalence for both viruses was correlated with age, with the lowest IgG levels recorded in younger people. This is in agreement with previous studies where RVF or CCHF prevalence was associated with age [23,24]. It seems that men were slightly more exposed than women even if the difference was not significant. The same observation was made in several research studies conducted in locations other than Senegal [25,24].

Saint-Louis and Tambacounda had the highest RVFV seroprevalence, between 10% and 15%. In Kedougou, Kaolack, Dakar, Thies, and Louga, the seroprevalence was between 0% and 5%. Our findings corroborate those of Seck et al. [26] indicating greater risk estimates in western small ruminant populations compared to eastern populations, as well as a high transmission rate in North-Central Senegal. Seroprevalence rates in our study are lower than previous studies done in the Podor district (on the border with Mauritania) where around 30% anti-IgG RVF was reported in a Fulani population, in an endemic area, shortly after a significant

Figure 2. Rift Valley Fever (A) and Crimean-Congo Hemorrhagic Fever (B) Ig-G seroprevalence according to sex from October to November 2020 in Senegal.

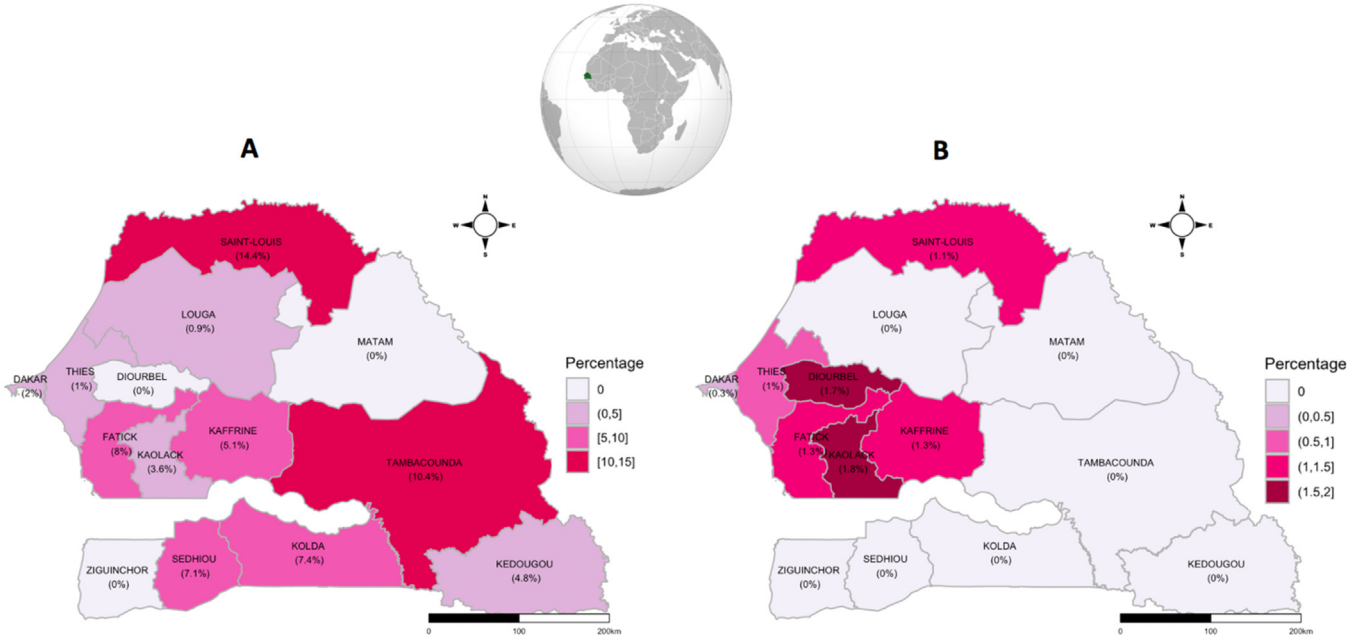

Figure 3. Rift Valley Fever (A) and Crimean-Congo Hemorrhagic Fever (B) Ig-G seroprevalence per region from October to November 2020 in Senegal.

epizootic event in 1987 [13]. Thonnon et al. [22] observed a continuous decrease of RVF seroprevalence in human populations from the same area between 1988 and 1996 with anti-IgG RVF antibodies dropping to 15%. Our results show the same trend with a seroprevalence in North Senegal of less than 15%, indicating limited or non-existent exposure to RVFV since the 1987 epizootic event. Overall, the current study involves a larger sample of the population from Senegal with both endemic regions and other areas with less RVF circulation in animals, highlighting a heterogeneous risk of exposure within the country. Unfortunately, the provided data did not allow additional analysis of the effect of socioeconomic and cultural activities on exposure risk.

The nationwide distribution of CCHFV seroprevalence was substantially less pronounced. In fact, the regions of Kaolack and Diourbel (1.5-2%) had the highest rates of CCHFV seroprevalence. With a seroprevalence rate between 1% and 1.5% in Fatick, Kaffrine, and Saint-Louis, the distribution of CCHFV in human populations appears to be restricted to the northern and central parts of the country. Studies conducted on animal [9] and human populations in Senegal [19] showed a similar trend. In addition, it was found that areas with long periods of low rainfall and low humidity were associated with a higher risk of CCHF [27]. These conditions are those observed in the northern and central parts of the country but not in the south demonstrating that climatic factors may have a significant role in the pattern shown in our investigation.

In some areas, such as Thies and Matam where no evidence of CCHFV exposition in the populations was found during our study, a number of human cases have been repeatedly reported in recent years [28,29,10] (Supplementary material S5). This is potentially due to a sampling bias in certain areas, meaning that the true prevalence may be underestimated. Indeed, movements of human and animal populations can establish a complex circulation dynamic of CCHFV strains within a given area. Moreover, CCHFV infection may be subclinical or asymptomatic in some people [30], according to earlier studies that showed little to no evidence of its circulation in the southern most parts of the country [19,31]. In addition to research on human populations, the combination

of data from tick screening and animal surveys is necessary to identify high-risk regions for CCHF [32,33].

In the same way, a growing number of RVFV severe acute infections have been reported in different parts of the country through the Syndromic Sentinel Surveillance in Senegal from 2019 to early 2022 [17] (Supplementary material S6). Our study highlights that transmissions occur in areas where exposure within the general population seems low or even non-existent, suggesting either punctual introductions of viral strains in these areas where the population is relatively naïve, resulting in isolated cases, or a spatial and/or temporal sampling bias. According to this study, the virus is circulating in places with low human concentrations. Further studies are required to identify the specificity of these areas as well as the transmission dynamics.

Overall, the two viruses of interest in this study could circulate silently within populations of human, animals and arthropod (mosquito, tick) vectors with potential undetected emergences, particularly in areas where the risk is underestimated and surveillance less sustained, resulting in large-scale epidemics after long amplification phases. This highlights the need for studies integrating the One Health concept with active surveillance of multiple vector and reservoir species.

To our knowledge, this investigation is the first nationwide RVFV and CCHFV seroprevalence study in the human population in Senegal. It will help work toward a better mapping of the distribution risk of these diseases. Using this information, the most exposed regions should be prioritized in the surveillance network even if additional holistic prospective studies are still required at a national level to determine the risk factors and dynamics of the emergence of these two zoonotic viruses. It is then important to continue surveillance at least in areas with low seroprevalence where sporadic cases have already been reported and which could constitute viral dissemination channels in the country and potentially in neighboring regions.

Conflict of interests

The authors declare no conflicts of interest.

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Author contributions

MMD, CT, MF, BD, OusF, CL conceived and designed the study. CT and MAB led the samples and metadata acquisition. CT handle data curation. OF, MD, ON managed the biological resources conservation. SS performed the serological analysis. MMD validated the experiment results. MST and SS analysed the data. CT, MMD, MF validated data analysis. SS and MMD drafted the manuscript. CT, MST, MF, MaD, PMS, GF, OF, OusF, CL reviewed the first draft. All authors approved the final version of the work.

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Ethical Approval statement

This study was approved by the Senegalese National Ethics Committee for Research and Health.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2023.03.016.

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