JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Coronavirus Pandemic

Post-mortem diagnosis of human rabies in SARS-CoV-2 coinfected patient with minimally invasive autopsy in northeastern Brazil

Luis Arthur BG Farias[1,2], Marcos M Sousa[2], Ruth Maria O de Araujo[2], Kelma M Maia[2], Madalena Q de Azevedo[2], Nancy C de Oliveira Caetano[2], Karene F Cavalcante[3], Liana P Mello[3], Larissa Leao F de Sousa[3], Sami de Andrade C Gadelha[4], Deborah N de Melo[4,5,6], Ana Karine B Carneiro[7], Tânia Mara S Coelho[5,7], Antônio Silva L Neto[7], Francisco Edson B Abreu[2], Kellyn Kessiene de S Cavalcante[5,7], Silvia F Costa[1], Luciano P de Goes Cavalcanti[5,6,7], Lauro V Perdigão Neto[1,2]

[1] *Department of Infectious Diseases of Hospital das Clínicas and Laboratório de Investigação Médica – LIM 49, University of São Paulo, São Paulo, Brazil*
[2] *São José Hospital for Infectious Diseases, Fortaleza, Brazil*
[3] *Central Public Health Laboratory (LACEN), Fortaleza, Brazil*
[4] *Death Verification Service, Fortaleza, Brazil*
[5] *Graduate Program in Collective Health, Federal University of Ceará, Fortaleza, Brazil*
[6] *Graduate Program in Pathology, Federal University of Ceará (UFC), Fortaleza, Brazil*
[7] *Secretary of Health of the State of Ceará (SESA), Fortaleza, Brazil*

Abstract

Introduction: Human rabies (HR) is a lethal zoonotic disease caused by lyssaviruses with increase in the number of cases post-coronavirus disease 2019 (COVID-19) pandemic.

Methodology: We report a case of human rabies in a patient from a rural area of Ceará, northeastern Brazil in 2023, who was bitten by a white-tufted-ear marmoset (*Callithrix jacchus jacchus*). The patient was co-infected with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and was diagnosed by minimally invasive autopsy (MIA).

Results: MIA offers many advantages related to biosafety, and speed of sample acquisition; and markedly reduces disfigurement of the body compared with complete autopsy. It is a great alternative in COVID-19 patients.

Conclusions: New methods such as MIA are a promising tool for diagnosis, and have the potential to improve family cooperation and support rabies surveillance.

Key words: human rabies; marmoset; autopsy; COVID-19.

J Infect Dev Ctries 2024; 18(7):982-986. doi:10.3855/jidc.19427

(Received 21 October 2023 – Accepted 04 December 2023)

Introduction

Human rabies (HR) is a lethal zoonotic disease caused by the rabies virus (RABV), a type of lyssavirus [1]. Every year approximately 59,000 people from 150 countries die due to RABV infection [1]. Most cases occur in low to middle-income countries in Asia and Africa [1,2]. The RABV has been frequently detected in wild animals, primarily belonging to the orders Carnivora and Chiroptera [3,4]. In Latin America, the incidence of HR transmitted by dogs decreased between 2013 and 2016, while the transmission by wild animals became more common. In Brazil, there has been a significant reduction in mortality rates due to HR in the last 40 years after the implementation of canine vaccination campaigns and the intensification of post-exposure prophylaxis (PEP) [3].

Clinical laboratory diagnoses are based on clinical findings, and non-invasive samples (such as saliva or biopsies of skin); and more recently on polymerase chain reaction (PCR) analysis and autopsy of a brain biopsy using immunofluorescence techniques [1-4]. Due to the high level of lethality presented by rabies, the diagnosis is frequently achieved only through conventional autopsy (CA). CA is often not well accepted by the families due to disfigurement of the body [5]. The inconveniences associated with CA include the need for transporting the body to an appropriate location, large incisions to remove vital organs, and the contamination risks related to infectious diseases [5]. Minimally invasive autopsy (MIA) has been used as an alternative to CA with promising results [6]. MIA is a needle-based approach aimed at collecting samples of the main organs and fluids without opening the body, and reduces disfigurement of the body [6]. MIA has been widely used in the context of the coronavirus disease 2019 (COVID-19) pandemic as a fast and non-disfiguring method with minimal biological risk for the personnel performing the procedure, as well as an effective way for diagnosing other tropical diseases such as arboviruses [6,7].

Herein, we report a case of HR in a patient from a rural area of Brazil, who was co-infected with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), after being bitten by a white-tufted-ear marmoset (*Callithrix jacchus jacchus*) and was diagnosed by MIA.

Case report

A 36-year-old man from the city of Cariús, Ceará state, northeastern Brazil, and with a history of stab trauma, sought medical assistance at a primary care facility in May 2023. He had no previous comorbidities and had received two COVID-19 vaccine (Coronavac, Sinovac Biotech, Hong Kong, China) doses.

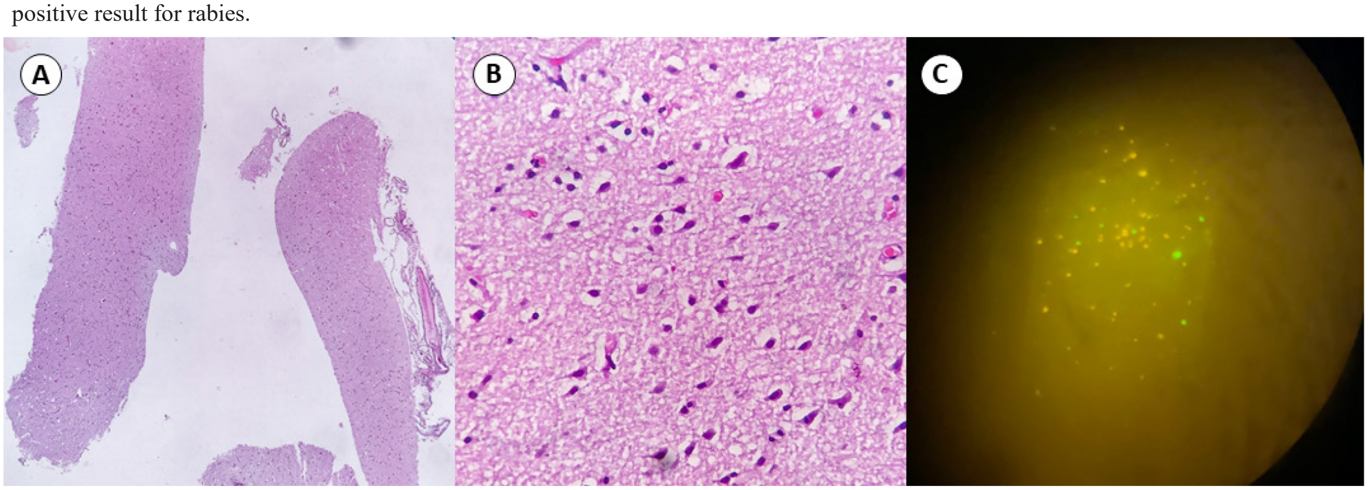
He had paresthesia and pain in his right upper limb. Initially, a diagnosis of carpal tunnel syndrome was considered. The patient received analgesics and was discharged. Few weeks later, he developed severe pain in his right shoulder and elbow.

During the medical history interview, he disclosed a previous incident of being bitten by a marmoset two months prior and stated that he had not received any rabies prophylaxis since then. The circumstances of the incident involved a chance encounter with an injured marmoset near the patient's home. Upon deciding to assist the marmoset with its wounds, the individual was bitten, and right after, the marmoset who presented previous signs of rabies did not survive.

The individual arrived at the medical facility conscious, oriented, and able to speak and walk. However, after 24 hours, he started experiencing diaphoresis, psychomotor agitation, muscle spasms, as well as dysarthria, dysphonia, and sialorrhea. The family members also reported that the patient had difficulty swallowing liquids. Two days later, he presented mild cyanosis, respiratory distress, and sensory loss, and required mechanical ventilation and intensive support.

The physician considered the possibility of rabies as the diagnosis. Samples of saliva, serum, and nuchal biopsy were collected. The patient was initiated on intravenous vitamin C (1 g/day) and amantadine (100 mg every 12 hours), along with sedation using midazolam (2 mg/kg/h) and ketamine (2 mg/kg/h). A lumbar puncture was performed, revealing clear cerebrospinal fluid (CSF) with a glucose level of 46 mg/dL, protein level of 181 mg/dL, and a cell count of 68 cells/mm³ with predominantly lymphomononuclear cells. Multiplex PCR (FilmArray Meningitis Encephalitis Panel, BioMérieux, Marcy l'Étoile, France) of CSF was negative for *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, herpesvirus, varicella-zoster virus, cytomegalovirus, and *Cryptococcus* spp. Following the admission protocol of our intensive care unit (ICU), a nasopharyngeal swab test was performed despite the absence of respiratory symptoms. The swab tested positive for SARS-CoV-2 RNA. Chest computed tomography (CT) was unremarkable. Nasopharyngeal swab samples were used for COVID-19 diagnoses

Figure 1. A. Brain fragments showing the presence of gliosis, indicated by the representation of gray and white matter. Hematoxylin and eosin (HE) staining, original magnification 40x. **B.** Neurons displaying reduced volume and intensely eosinophilic cytoplasm, accompanied by pyknotic nuclei. Gliosis, primarily involving oligodendrocytes, is observed in the surrounding area. HE staining was used; original magnification 400x. **C.** Immunofluorescence analysis of histopathological samples, demonstrating the presence of green dots indicating a positive result for rabies.



based on the amplification of the *betacoronavirus E* gene and the specific SARS-CoV-2 *RdRp* gene using PCR. Then, the patient was provided with a private ICU room which had negative pressure and HEPA filters, with powered air-purifying respirator (PAPR). All the healthcare providers involved with the patient's care reinforced protective measures with airborne precaution personal protective equipment (PPE), as well as contact precautions. On the sixth day, the patient developed dysautonomia and refractory bradycardia, which eventually led to death.

Due to the distance between the Cariús and the capital city Fortaleza, where the complete autopsy (CA) could be performed, the family members did not agree to a full autopsy, but consented to the collection of samples within the hospital. The MIA team from the death verification service (DVS) was contacted, and brain tissue samples were collected at the location of death. These samples were then sent to the Central Laboratory of Public Health of Ceará (LACEN-CE) for further analysis. Histopathological findings of brain samples were pathognomonic for rabies (Figure 1A-B). The samples were submitted to direct immunofluorescence antibody staining (DIF) and tested positive (Figure 1C). This result was confirmed by DIF of the neck biopsy collected before death. Direct immunofluorescence of CSF and reverse transcriptase PCR (RT-PCR) of saliva and serum samples collected before the fatal outcome were negative.

Methodology

Study site

This study was conducted at São José Hospital of Infectious Diseases (HSJ), a public reference hospital for infectious diseases located in Fortaleza, Ceará state, northeastern Brazil. The HSJ has 100 ward beds and 28 ICU beds. The HSJ has been responsible for the care of most HR cases in the state since 1970 [3,8]. The marmoset-bite occurred in the municipality of Caríus, in central-southern region of Ceará, which is located 405 km from Fortaleza.

MIA

Two professionals (a pathologist and a biopsy technician) performed the MIA and sent the samples to the hospital. Appropriate PPE was available for each person participating in the procedure. The equipment included a scrub suit, waterproof suit with hood, waterproof apron, hat to protect hair, mask, eye protection (goggles), and waterproof shoe covers; as recommended for MIA during the COVID-19 pandemic [6]. The mask used was a filter face piece N95 mask.

The MIA was conducted in the ICU immediately after the patient's death. The room was clean and had proper lighting. All the necessary equipment was transferred to the ICU prior to the procedure. Previously prepared COVID-MIA kit boxes were used, which included three needles, and pre-labelled formalin jars and cryovials. Cryovials for PCR were pre-filled with lysis buffer solution (ATL buffer, Qiagen, Hilden, Germany).

Rabies sample collection

The samples were collected according to the Laboratory Diagnosis Manual of Rabies from the Ministry of Health [9]. CSF, saliva, and serum samples were collected before death. CSF was collected through lumbar puncture, serum through blood collection, and saliva collected directly from the patient's mouth.

After death, samples were collected for DIF and RT-PCR from CSF and brain during MIA procedure. CSF was collected through needle aspiration by an occipital approach to the cisterna magna. A core needle-biopsy was necessary to collect brain samples by a trans-nasal approach through lamina cribrosa.

Epidemiological investigation

During epidemiological investigation, four animals were identified to be bitten by the marmosets (two dogs, and two horses). Two wild marmosets were captured and tested for rabies. All family members were studied, and none was positive for SARS-CoV-2 or had similar exposure to the RABV.

Ethical considerations

This study was approved by the Ethical Review Board of HSJ, Fortaleza, Brazil (CAAE Nº 69569223.7.0000.5044).

Discussion

In the last decade, most HR cases in Brazil occurred due to accidental encounters of animals of economic interest and wild animals. Historically, HR incidence in Brazil was high, but after vaccination campaigns and PEP, HR has become a rare disease with few cases each year [8]. Ceará state is located in northeastern Brazil, and in one of the poorest areas of the country. It is characterized by the semi-arid climate, and is rich in wildlife such as marmosets and bats [9]. Marmosets are commonly captured in the country, and are frequently kept as pets, which increases the risk of HR [10]. The most recent HR cases in this region took place in the municipalities of Camocim in 2008, Ipu in 2010, and Jati in 2012. Additional HR cases in the region were associated with encounters with wild dogs and bats, and reported in 2010 and 2016, respectively [11]. Many studies reported the circulation of RABV in these animals, including new variants [10]. Two variants were obtained from humans, each bitten by a different marmoset (Brhm4097 and Brhm4108), and one from a rabid marmoset (Brsg4138) in 1998 [9]. Recent studies have shown an increase in spatial circulation of RABV in these mammals in many states such as Ceará, Bahia, and Pernambuco in northeastern Brazil [12].

In Brazil, current legislation prohibits the killing, persecuting, hunting, and catching of wild animals; and collecting and using specimens of wild fauna, either native or on the migratory route; without due permission, license, or authorization from the competent authority. Wildlife trade has been regulated by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) which only allows for the commerce of specimens bred by legalized breeders [13]. However, most captive marmosets in Ceará state, are not subject to regulation and are typically not maintained by licensed breeders. They are usually captured when they encounter humans while seeking food or shelter, and kept in captivity.

This case also highlights the importance of the measures necessary to control HR. Human PEP and mass dog vaccination campaigns are not the only methods that can control HR worldwide. The control of rabies in the wild environment, as well as its circulation in nature, seems to be necessary, given that most HR cases in Brazil occur due to wild animal bites [3,8]. An alternative described since 1984 is the use of oral vaccines with baits. Currently, the widespread acknowledgment of immunizing wildlife using oral vaccines is a crucial element in a comprehensive rabies management program. This strategy adds an extra layer of defense for domestic animals and humans, in preventing the transmission from infected reservoirs to humans [14].

Another important aspect of this case was the diagnostic methods performed. MIA offers many advantages related to biosafety, speed, and sample acquisition; and markedly reduces disfigurement of the body compared with complete autopsy [6]. This patient had confirmed SARS-CoV-2 infection and all measures to reduce transmission and protect the professionals were taken according to the literature [6]. Interestingly, the patient's family members were negative for COVID-19, which may indicate the possibility of nosocomial transmission of SARS-CoV-2. Nosocomial transmission of SARS-CoV-2, as well as outbreaks in ICUs, have been previously described. COVID-19 may exhibit high infection rates, which can facilitate nosocomial transmission [15]. On the other hand, no other COVID-19 cases were reported in the ICU, which may indicate the possibility of contamination during the emergency admission or transportation. We do not know if SARS-CoV-2 may interfere in the pathogenesis of HR as in other diseases. In fact, this case may emphasize the importance of investigating COVID-19 when considering zoonotic diseases. HR already represents a severe and fatal illness, but COVID-19 may also exacerbate the underlying conditions during coinfection. More studies are necessary to better understand the role of SARS-CoV-2 in zoonotic diseases [16].

In this case, the method provided sufficient sample collection to perform DIF. Data regarding the application of MIA in HR is absent. Most HR diagnoses are confirmed through CA, when the diagnosis is not achieved in vivo, after days of waiting for the laboratory results [5]. This report reinforces the efficacy and safety of this method in different settings. More studies are needed to understand the applicability of MIA in HR. MIA can prove to be an alternative, mainly in scenarios where there is not great acceptability of CA by families, or in places where there is no DVS.

Conclusions

To the best of our knowledge, this is the first report on the association between HR and COVID-19. New strategies like MIA have the potential to enhance family cooperation and support rabies surveillance as a promising tool for diagnosis. Further studies are needed to explore the complete application, efficacy, and safety of this method in other tropical and infectious diseases.

Acknowledgements

The authors would like to acknowledge the staff at São José Hospital for Infectious Diseases (HSJ) for their valuable support. We are indebted to representatives of the Secretary of Health of the State of Ceará (SESA) who provided data and support for this study.

References

1. Fisher CR, Streicker DG, Schnell MJ (2018) The spread and evolution of rabies virus: conquering new frontiers. Nat Rev Microbiol 16: 241–255. doi: 10.1038/nrmicro.2018.11.
2. Liu C, Cahill JD (2020) Epidemiology of rabies and current US vaccine guidelines. R I Med J 103: 51–53.
3. Duarte NFH, Pires Neto RDJ, Viana VF, Feijão LX, Alencar CH, Heukelbach J (2021) Clinical aspects of human rabies in

the state of Ceará, Brazil: an overview of 63 cases. Rev Soc Bras Med Trop 54: e05092021. doi: 10.1590/0037-8682-0509-2021.
4. Kotait I, Oliveira RN, Carrieri ML, Castilho JG, Macedo CI, Pereira PMC, Boere V, Montebello L, Rupprecht CE (2019) Non-human primates as a reservoir for rabies virus in Brazil. Zoonoses Public Health 66: 47–59. doi: 10.1111/zph.12527.
5. Pathak S, Horton DL, Lucas S, Brown D, Quaderi S, Polhill S, Walker D, Nastouli E, Núñez A, Wise EL, Fooks AR, Brown M (2014) Diagnosis, management and post-mortem findings of a human case of rabies imported into the United Kingdom from India: a case report. Virol J 11: 63. doi: 10.1186/1743-422X-11-63.
6. Rakislova N, Marimon L, Ismail MR, Carrilho C, Fernandes F, Ferrando M, Castillo P, Rodrigo-Calvo MT, Guerrero J, Ortiz E, Muñoz-Beatove A, Martinez MJ, Hurtado JC, Navarro M, Bassat Q, Maixenchs M, Delgado V, Wallong E, Aceituno A, Kim J, Paganelli C, Goco NJ, Aldecoa I, Martinez-Pozo A, Martinez D, Ramírez-Ruz J, Cathomas G, Haab M, Menéndez C, Ordi J (2021) Minimally invasive autopsy practice in COVID-19 cases: biosafety and findings. Pathogens 10: 412. doi: 10.3390/pathogens10040412.
7. Melo DN, Mara Coelho T, Rolim Pinheiro Lima G, Gomes Fernandes C, Cavalcante Fales de Brito Alves B, Montenegro de Carvalho Araújo F, Aparecida de Almeida Monteiro R, Ordi J, Hilário do Nascimento Saldiva P, Pamplona de Góes Cavalcanti L (2022) Post-mortem diagnosis of pediatric dengue using minimally invasive autopsy during the COVID-19 pandemic in Brazil. Trop Med Infect Dis 7: 123. doi: 10.3390/tropicalmed7070123.
8. Duarte NFH, Pires Neto RDJ, Viana VF, Feijão LX, Abreu KG, Melo IMLA, Sousa AQ, Alencar CH, Heukelbach J (2021) Epidemiology of human rabies in the state of Ceará, Brazil, 1970 to 2019. Epidemiol Serv Saude 30: e2020354. doi: 10.1590/s1679-49742021000100010.
9. Brazil. Ministry of Health (2008) Laboratory diagnosis manual of rabies. Brasília. Available: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/svsa/raiva/manual-de-diagnostico-laboratorial-da-raiva.pdf/@@download/file. Accessed: 29 December 2023.
10. Favoretto SR, de Mattos CC, Morais NB, Alves Araújo FA, de Mattos CA (2001) Rabies in marmosets (*Callithrix jacchus*), Ceará, Brazil. Emerg Infect Dis 7: 1062–1065. doi: 10.3201/eid0706.010630.
11. Ceará Health Secretary (2009) Epidemiological bulletin. Rabies. Available: https://www.saude.ce.gov.br/wp-content/uploads/sites/9/2018/06/boletim_raiva_15052019.pdf. Accessed: 29 December 2023.
12. Benavides JA, Raghavan RK, Boere V, Rocha S, Wada MY, Vargas A, Voietta F, de Oliveira E Silva I, Leal S, de Castro A, Arruda MF, Peterson AT, Megid J, Carrieri ML, Kotait I (2022) Spatio-temporal dynamics of rabies and habitat suitability of the common marmoset *Callithrix jacchus* in Brazil. PLoS Negl Trop Dis 16: e0010254. doi: 10.1371/journal.pntd.0010254.
13. Kuhnen VV, Kanaan VT (2014) Wildlife trade in Brazil: a closer look at wild pets' welfare issues. Braz J Biol 74: 124–127. doi: 10.1590/1519-6984.18912.
14. Maki J, Guiot AL, Aubert M, Brochier B, Cliquet F, Hanlon CA, King R, Oertli EH, Rupprecht CE, Schumacher C, Slate D, Yakobson B, Wohlers A, Lankau EW (2017) Oral vaccination of wildlife using a vaccinia-rabies-glycoprotein recombinant virus vaccine (RABORAL V-RG®): a global review. Vet Res 48: 57. doi: 10.1186/s13567-017-0459-9.
15. Andrés M, García MC, Fajardo A, Grau L, Pagespetit L, Plasencia V, Martínez I, Abadía C, Sanahuja A, Bella F (2022) Nosocomial outbreak of COVID-19 in an internal medicine ward: probable airborne transmission. Rev Clin Esp (Barc) 222: 578–583. doi: 10.1016/j.rce.2022.04.001.
16. Prapty CNBS, Rahmat R, Araf Y, Shounak SK, Noor-A-Afrin, Rahaman TI, Hosen MJ, Zheng C, Hossain MG (2023) SARS-CoV-2 and dengue virus co-infection: epidemiology, pathogenesis, diagnosis, treatment, and management. Rev Med Virol 33: e2340. doi: 10.1002/rmv.2340.

Corresponding author
Luís Arthur Brasil Gadelha Farias, MD.
Hospital São José de Doenças Infecciosas (HSJ) Nestor Barbosa St. 315,
Postal Code 60455-610, Fortaleza, Brazil
Tel: + 55 85 99680-3802
Email: luisarthurbrasilk@hotmail.com

Conflict of interests: No conflict of interests is declared.