

Detection of corona-like virus in a case of flaccid paraparesis in Belém, Pará State, Brazil

Detecção de vírus corona-like em um caso de paraparesia flácida em Belém, Estado do Pará, Brasil

DetECCIÓN de vírus corona-like en un caso de paraparesia flácida en Belém, Estado de Pará, Brasil

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ABSTRACT

In this study, we analyzed a fecal sample of a female infant with paralysis and other clinical symptoms that resembled poliomyelitis. Negative staining electron microscopy showed viral particles with a diameter of approximately 120 nm and displaying a crown-like appearance with surface projections. Ultrathin sections showed particles budding from the membranes of the Golgi apparatus. Based on these results, we propose the association of this virus with the neurological disorder and tentatively assign it to the *Coronaviridae* family. Further studies are required on this proposed relationship.

Keywords: *Coronaviridae*; Paraparesis; Transmission Electron Microscopy; Motor Disorders.

INTRODUCTION

The acute flaccid paraparesis (AFP) may be associated with various etiologies, including viral infections. Coronavirus, an agent of AFP, belongs to the order Nidovirales, the family *Coronaviridae* and the sub-family *Coronavirinae* that comprises three genera: *Alphacoronavirus*, *Betacoronavirus* and *Gamacoronavirus*¹. The origin of the term "corona" is from Latin and means "crown" because of the appearance of surface projections. The *Coronaviridae* family genome is non-segmented and is composed of positive-sense single-stranded RNA. The virions are spherical or pleomorphic, exhibit helical symmetry and are approximately 150 nm in diameter².

The incubation period can vary in most cases between 1-2 weeks. Transmission occurs through aerosols or fecal-

oral infection. The human symptoms can be enteric (diarrhea)² or associated with the respiratory tract (RT). The replication of this virus may also occur in the nervous system. The laboratory diagnosis of enteroviruses can be performed with the isolation and identification of the viruses in cell cultures, serological tests or polymerase chain reaction reverse transcription (RT-PCR)^{3,4}.

In this paper, we performed an ultrastructural identification of 89351 viral sample isolated from the fecal sample of a patient with AFP between 1998 and 2004 within the national surveillance network for acute motor deficiency (AMD).

MATERIALS AND METHODS

VIRUS ISOLATION

The sample studied was obtained through the national surveillance network for AMD, with the primary purpose of searching for the possible circulation of wild poliovirus in Brazil. A corona-like virus was analyzed from a fecal sample from a female infant with paralysis and other clinical symptoms that resembled poliomyelitis.

VIRUS INFECTION

To infect cell cultures with the corona-like virus, 1 mL of a 1:20 fecal suspension was inoculated into the center of a confluent (70% to 80%) monolayer of L-20B cells (a transgenic mouse cell line expressing the human poliovirus surface receptor). The viral particles were adsorbed to the

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cells for 1 h. Cell culture maintenance was performed according to Wanzeller et al⁵. When the cells showed a cytopathic effect (CPE), the supernatant was aliquoted and stored at -70° C.

TRANSMISSION ELECTRON MICROSCOPY

Negative staining

The procedure was previously described in Wanzeller et al⁵, and the sample was examined with a transmission electron microscope (TEM) (Zeiss EM 900).

Ultrathin sections

The L20B cell line was used for conventional electron microscopy. The method used was similar to that described in Wanzeller et al⁵. The sections were stained and observed with TEM (Zeiss EM 900).

RESULTS

CULTURE

The corona-like virus caused CPE in the L20B cell line 24 h p.i., and the cells were completely destroyed 48 h p.i., (Figure 1). The sample was negative when tested for poliovirus.

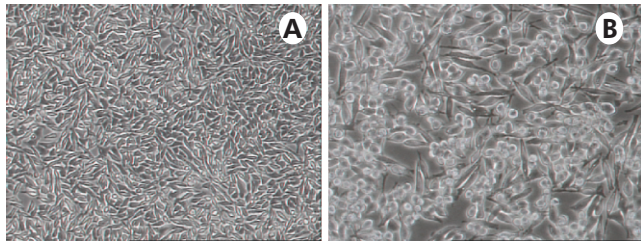


Figure 1 – Cell culture used for viral inoculation. (A) L20B cells without virus (control); (B) The corona-like virus caused CPE in the L20B cell line 24 h p.i. A: 20x; B: 40x

TRANSMISSION ELECTRON MICROSCOPY

The negatively stained viral particles were approximately 120 nm in diameter. These particles had an envelope that consisted of a bilaminar membrane covered with projecting structures on the surface (Figure 2). In figure 3, different stages of viral replication can be observed.

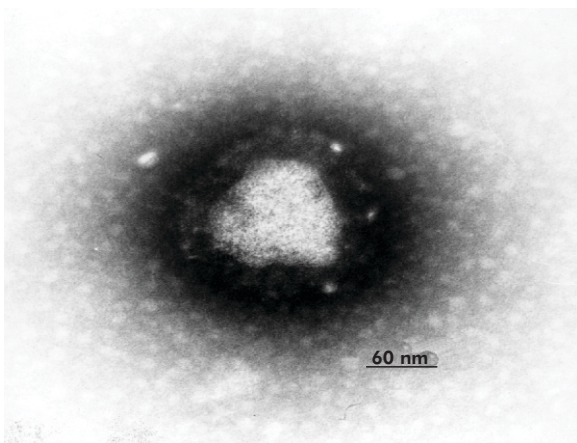
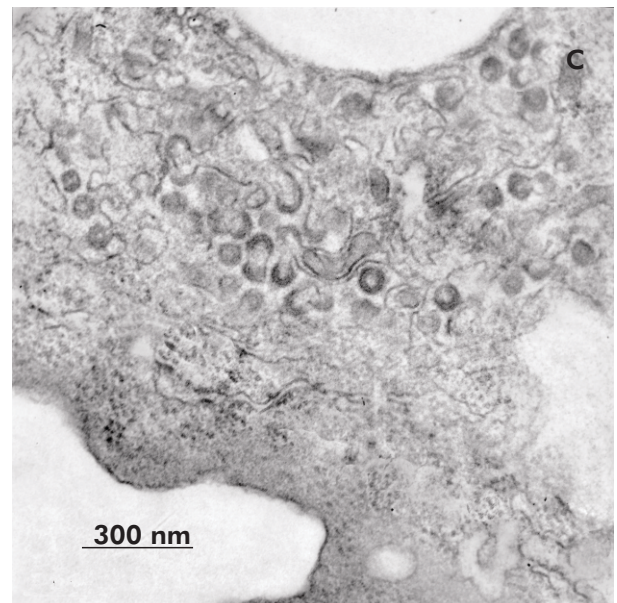
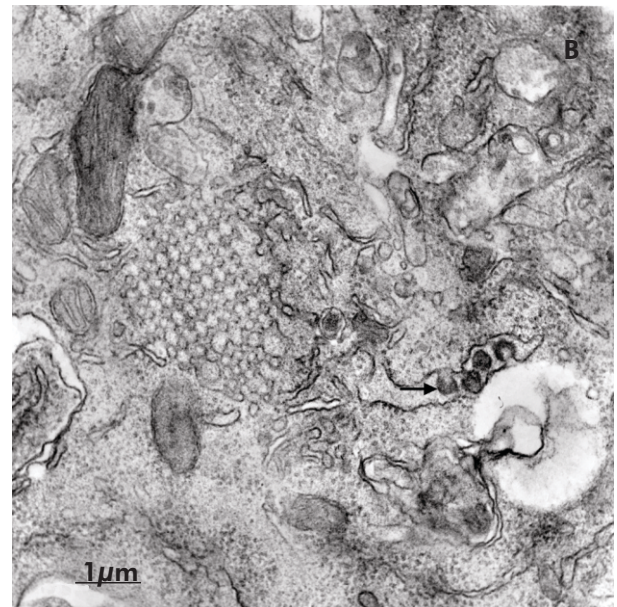
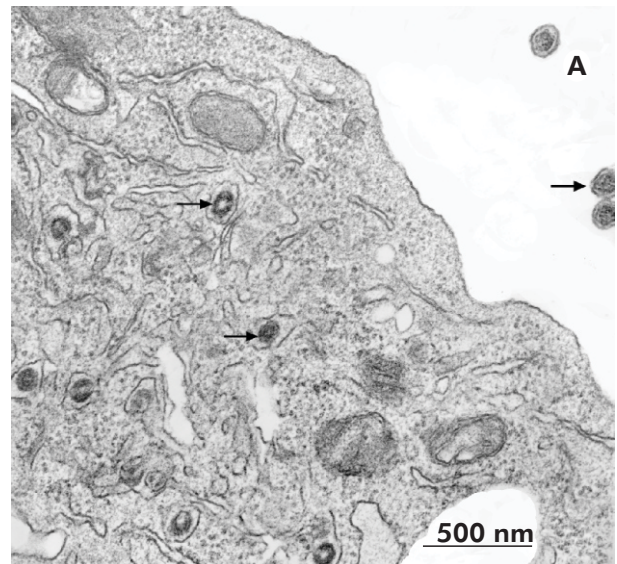


Figure 2 – negative staining of the supernatant of L20B cell cultures showed coated viral particles with a diameter of approximately 120 nm that were pleomorphic, with projections on the surface



A: Note virus particles within (stars) and outside the cell (arrows). **B and C:** Virus particles in different stages of maturation within the cell (arrows). The viral particles had a diameter of approximately 100 nm.

Figure 3 – Ultrathin section of viral replication in L20B cells 24 h p.i. showing different stages of viral particle maturation

The sample studied was tested with primers to *Coronaviridae* family, demonstrating a positive result by RT-PCR technique (data not shown).

DISCUSSION

A number of morphological features of this virus in L20B cells were similar to those previously described for other members of the *Coronaviridae* family^{2,6}.

The budding of the gastroenteric coronavirus and severe acute respiratory syndrome coronavirus (SARS-CoV)⁷ in all stages of maturation has been primarily observed to be inside membranes of the Golgi structure or endoplasmic reticulum and has been considered a hallmark of members of the *Coronaviridae* family^{7,2,8,9}.

The stages of viral replication observed in this study are in complete agreement with those of others coronavirus¹⁰. Lai et al² described changes that occur during the replication process: I) the viral proteins appear as an electron-dense layer in the cytoplasm; II) viral envelope proteins adhere to vesicular membranes within the cytoplasm; and III) an electron-dense area, possibly corresponding to viral spikes, can be observed in the lumen of vesicles.

In this study, we also found these characteristics^{7,8}, reinforcing the association of this virus with the neurological disorder presented by this patient. We have tentatively assigned this virus to the *Coronaviridae* family.

The association between coronaviruses and neuropathology in animals has been shown by Pasick et

al¹¹, who reported a variety of neurotropic strains that cause diseases, such as encephalitis, acute poliomyelitis and chronic inflammatory demyelination caused by mouse hepatitis virus (MHV4).

There are few reports in humans concerning the potential association of coronaviruses with neurological diseases. Among these studies, Yeh et al¹² detected human coronavirus in the cerebrospinal fluid of a child who was diagnosed with disseminated acute encephalomyelitis similar to that presented in diseases such as multiple sclerosis.

CONCLUSION

Based on the viral morphology presented in this study, we provisionally proposed the inclusion of this virus in the *Coronaviridae* family and attributed an association of this virus with neurological diseases, such as AMD.

This is the first detection of the association of a viral particle similar to coronavirus with patients with AFP in Brazil; however, it is still not possible to confirm this association, and future studies are necessary to determine this correlation.

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RESUMO

Neste estudo, analisamos uma amostra fecal de criança do sexo feminino com paralisia e outros sintomas clínicos que se assemelharam à poliomielite. A microscopia eletrônica (contrastação negativa) mostrou partículas com 120 nm de diâmetro, exibindo projeções na superfície semelhantes a uma coroa. Cortes ultrafinos mostraram partículas brotando do complexo de Golgi. Com base nesses resultados, propomos a associação deste vírus com o distúrbio neurológico e o associamos provisoriamente à família *Coronaviridae*. Estudos adicionais são necessários para esclarecer a relação proposta.

Palavras chaves: *Coronaviridae*; Paraparesia; Microscopia Eletrônica de Transmissão; transtornos motores.

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RESUMEN

En este estudio, analizamos una muestra fecal de niña con parálisis y otros síntomas clínicos semejantes a la poliomielitis. La microscopia electrónica (por contraste negativo) mostró partículas con 120 nm de diámetro, exhibiendo proyecciones en la superficie semejantes a una corona. Cortes ultrafinos mostraron partículas brotando del complejo de Golgi. Con base en esos resultados, proponemos la asociación de este virus al disturbio neurológico y lo asociamos provisoriamente a la familia *Coronaviridae*. Se necesitan estudios adicionales para aclarar la relación propuesta.

Palabras claves: *Coronaviridae*; Paraparesia; Microscopía Electrónica de Transmisión; transtornos motores.

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