

Detection of Coronavirus in the Central Nervous System of a Child With Acute Disseminated Encephalomyelitis

E. Ann Yeh, MD, MA*; Arlene Collins, PhD‡; Michael E. Cohen, MD*; Patricia K. Duffner, MD*; and Howard Faden, MD§

ABSTRACT. We present a case in which human coronavirus was detected in the cerebrospinal fluid of a child presumed to have acute disseminated encephalomyelitis. In murine models, coronavirus has been found to cause a chronic demyelinating condition that resembles multiple sclerosis. Additionally, there is *in vitro* evidence of human coronavirus's ability to infect neural cells. This case report provides additional support for the hypothesis that coronavirus may be an important etiologic factor in the pathogenesis of demyelinating disease in humans. *Pediatrics* 2004;113:e73–e76. URL: <http://www.pediatrics.org/cgi/content/full/113/1/e73>; coronavirus, HCoV, acute disseminated encephalomyelitis, ADEM, postinfectious encephalitis, demyelination, child.

ABBREVIATIONS. ADEM, acute disseminated encephalomyelitis; CNS, central nervous system; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; PCR, polymerase chain reaction; IgG, immunoglobulin G; RT, reverse transcription; HCoV, human coronavirus; MS, multiple sclerosis.

Acute disseminated encephalomyelitis (ADEM) is a monophasic, demyelinating disease of the central nervous system (CNS) that primarily affects children and young adults. It is characterized by high-signal-intensity lesions in the white matter of the brain and spinal cord on T2-weighted magnetic resonance imaging (MRI). These lesions may be independent of the clinical findings. Children may present with diffuse encephalopathy, seizures, optic neuritis, hemiparesis, and/or symptoms suggestive of spinal cord transection.

The epidemiology of the condition is unknown. A review of cases presenting to a children's hospital suggested a prevalence of ~4.5 cases per 10 000 pediatric hospital admissions, exclusive of newborns.¹

The etiology of the illness is cryptogenic, although the disorder is generally thought to be due to a parainfectious process. Certainly, many case reports in the literature suggest an infectious process before the onset of CNS symptoms, and some have identified specific infectious agents in cases of ADEM.^{2–4} However, in most cases, a specific etio-

logic infectious agent is not identified. For example, in a recent retrospective review of cases of ADEM, clinicians were able to identify an infectious agent in only 1 of 18 cases.¹ ADEM has also been described after immunizations.^{5,6}

Despite reports of the possible association between infection and ADEM, there is no clear understanding of the relationship between the infectious agent and the onset of demyelination. There is experimental evidence in mice for a relationship between coronavirus and CNS demyelination.^{7–9} Little is known, however, about this virus's relationship to demyelinating disease in humans. Indeed, there have been no case reports of this virus in relation to ADEM. We report a case of demyelinating disease in a child in which cerebrospinal fluid (CSF) and nasopharyngeal specimens were positive for human coronavirus (HCoV) by polymerase chain reaction (PCR) and in whom a fourfold rise in antibody titer was documented. All other testing for infectious agents was negative.

CASE PRESENTATION

A 15-year-old previously healthy boy presented in January 2003 to the Children's Hospital of Buffalo after a 5-day history of numbness in the lower extremities. The numbness started in the distal lower extremities and progressed to the umbilicus. He reported difficulty walking ~1 day before admission. He also reported clumsiness in his right hand. His mother noted increased irritability. The patient and his mother denied visual changes, seizure, headache, mental status changes, bowel or bladder dysfunction, or speech or language difficulties. There was no history of toxin ingestion. There was a history of an upper respiratory tract illness ~1 week before his first symptom. His brother had experienced a sore throat 1 to 2 weeks before the hospitalization.

The patient's developmental milestones were normal, and he was an honor student at school. He was, however, enrolled in a special behavioral class. He was employed part-time as a bus boy at a local restaurant and lived with his mother and brother. There were three pet dogs, one of which was a puppy, in the home. The patient's father was not involved in the family. All family members were reported to be healthy, although little was known about the father's medical history.

The patient's general physical examination was unremarkable. His vital signs were stable, and he was afebrile. He was alert, oriented, and cooperative. Speech was fluent. His neurologic examination revealed normal optic discs and cranial nerve function. Sphincter tone was normal. Reflexes were present and symmetric in all extremities. Plantar reflexes were normal. There was mild distal weakness in the right hand and foot (4/5 in the finger extensors and extensor hallucis longus and 4/5 in the remainder of the upper and lower right limb). Sensory function above T10 was normal. There was patchy loss of vibration and temperature sensation below T10. Proprioception and pinprick sensation were normal below T10. Cerebellar testing revealed mild dysmetria of the left hand. Heel-to-toe walking was poor. The patient's gait was

From the Departments of *Neurology, ‡Microbiology, and §Pediatrics, State University of New York at Buffalo, School of Medicine and Biomedical Sciences, Buffalo, New York.

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Address correspondence to E. Ann Yeh, MD, MA, Women's and Children's Hospital of Buffalo, Department of Neurology, 219 Bryant St, Buffalo, NY 14222. E-mail: ann.yeh@sympatico.ca

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antalgic, likely due to discomfort associated with his sensory dysfunction. The Romberg test was negative. The patient's symptoms resolved over several weeks without any therapeutic intervention.

An MRI of the brain and spinal cord was performed the morning after admission. It demonstrated lesions on T2-weighted imaging at C4–C5 and at T7–T8. The spinal cord lesions were non-enhancing. The MRI of the brain revealed patchy areas of hyperintensity in the white matter tracts, particularly in the centrum semiovale (Fig 1). There was an area of hyperintensity in the left cerebellum adjacent to the superior aspect of the left brachium pontis (Fig 2). There was enhancement of some lesions, including that in the left centrum semiovale.

Coronavirus OC43 was detected in the CSF and nasopharyngeal secretions by PCR technology. Antibody titers to coronavirus OC43 rose from 1:160 in acute serum to 1:640 in convalescent serum (3 weeks later). Throat, stool, and CSF cultures for viruses were negative. A nasopharyngeal aspirate was negative for respiratory syncytial virus, parainfluenza virus, influenza virus, and adenovirus. Serologic studies for respiratory syncytial virus, mycoplasma, influenza viruses, parainfluenza viruses, adenoviruses, Epstein-Barr virus, and *Borrelia burgdorferi* were negative. Cerebrospinal fluid examination demonstrated 10 red blood cells and 38 white blood cells with 92% lymphocytes. Protein was 40 mg/dL (0.4 g/L), and glucose was 58 mg/dL (3.2 mmol/L). CSF culture for bacteria was negative. The immunoglobulin G (IgG) index was 0.77 (normal: <0.70). Somatosensory, brainstem, and visual evoked potentials were normal.

A follow-up MRI of the brain ~6 weeks after the onset of symptoms showed improvement of the lesions in the brain and cerebellum. However, a follow-up MRI performed 3 months after the onset of symptoms showed a possible new lesion in the left hemisphere of the cerebellum. The periventricular lesion in the right cerebral hemisphere appeared brighter and larger. There was no gadolinium enhancement of the lesions. The spinal cord lesions had resolved. Despite these changes, the patient had not complained of additional symptoms.

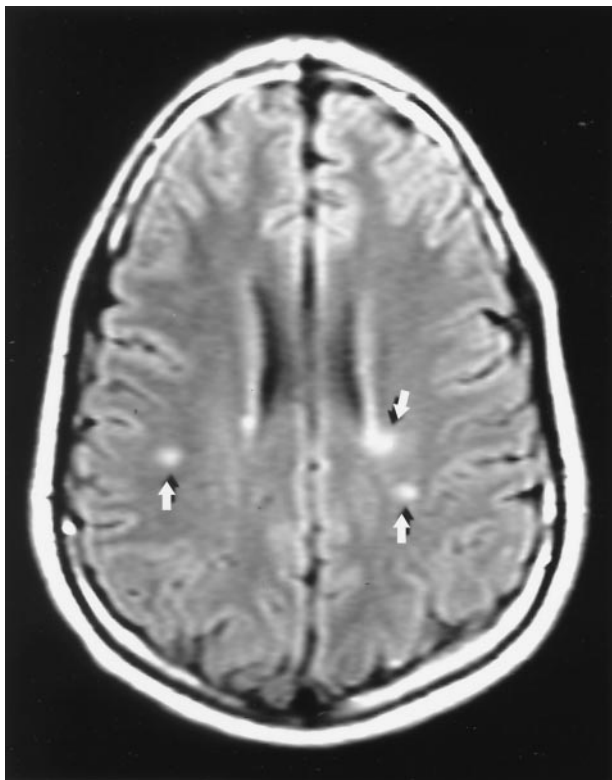


Fig 1. FLAIR (TR 8000) axial image of the brain showing multiple high-signal lesions in the area of the centrum semiovale (white arrows).

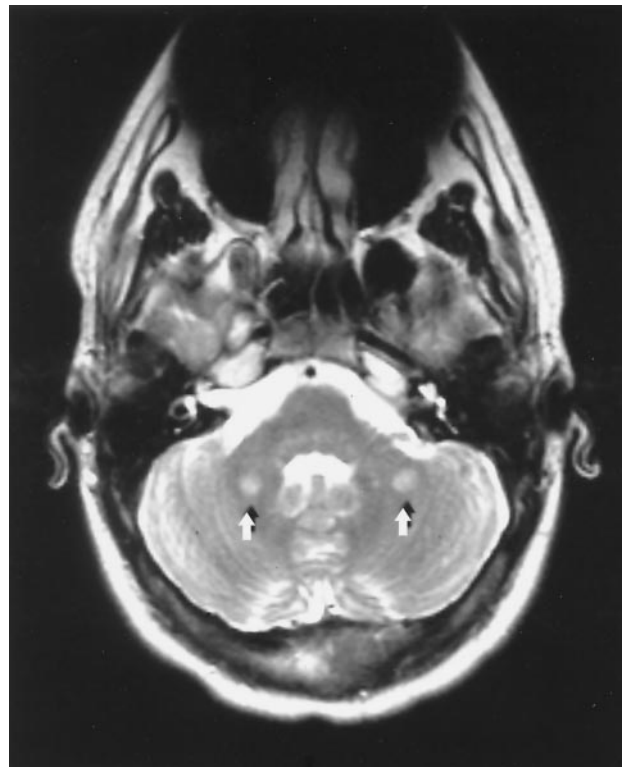


Fig 2. T2-weighted (TR 5000) axial image of the brain showing high signal in the area of the cerebellar white matter (white arrows).

LABORATORY METHODS

Coronavirus Reverse Transcription-PCR

Reverse Transcription (RT)-PCR for HCoV was performed by methods described elsewhere with minor modifications.^{10,11} Total RNA was extracted from 250 μ L of sample with TriReagent LS (MRC, Inc, Cincinnati, OH) according to the manufacturer's protocol. The resulting RNA was dissolved in 30 μ L of diethylpyrocarbonate-treated water (Ambion Inc., Austin, TX) and stored at -80°C . A 2- μ L aliquot of the RNA was tested in duplicate in a single-tube nested RT-PCR assay using the EZrTth RNA-PCR enzyme in a 50- μ L total volume (Applied Biosystems, Foster City, CA) with second-round inner primers (5 μ L) suspended in a hanging droplet in the lid.¹¹ The assay was performed by using HCoV OC43 outer primers O1 (5'-CCCAAGCAAACCTGCTACTCTCAG-3') and O3 (5'-GTAGACTCCGTC AATATCGGTGCC-3') and inner primers O1.1 (5'-CATCAGGAGGGAATGTTG-TACC-3') and O3.1 (5'-TACTGGTCTTTAGCATGCGGTC-3') in 25 mM Mn^{2+} .¹⁰ For HCoV 229E, the assay was performed by using outer primers E3 (5'-GGTACTCCTAAGCCTTCTCG-3') and E5 (5'-GACTATCAAACAGCATAGCAGC-3')¹² and inner primers E7 (5'-TCTGCCAAGAGTCTTGCTCG-3') and E9 (5'-AGCAT-AGCAGCTGTTGACGG-3')¹ with reduced (20 mM) Mn^{2+} concentration. All assays included Amperase (Applied Biosystems) and 2'-deoxyuridine 5'-triphosphate instead of thymidine 5'-triphosphate for prevention of carryover of amplicon contamination. Cycling conditions were as described.¹⁰ Positive controls were tissue culture-derived (rhabdomyosarcoma cell-grown) 229E and OC43 viruses.¹³ The sensitivity of the RT-PCR assay for HCoV OC43 was 1 infected cell. For HCoV 229E, the endpoint was 100 infected cells. There was unique specificity of each coronavirus type with its specific set of primers, which was verified by absence of bands in RT-PCRs with the other virus. The RT-PCR negative control was water in place of RNA in the reaction mix. The other negative control was to assay each RNA sample in the nested PCR (without the RT step). The integrity of the RNA was verified by RT-PCR with primers for the cellular gene triose phosphate isomerase. Ethidium-bromide staining was performed to visualize PCR amplicons after electrophoresis on 1.5% agarose gels. Samples that showed a band of the appropriate size on agarose gels

were reamplified with thymidine 5'-triphosphate to replace 2'-deoxyuridine 5'-triphosphate, cloned into TOPO-TA cloning vector (Invitrogen, Carlsbad, CA), and sent for sequencing (Roswell Park Cancer Institute, Biopolymer Facility, Buffalo, NY). Nasopharyngeal and cerebrospinal samples were considered as positive when sequencing revealed a >95% sequence correspondence to standard virus.

Immunofluorescence

Adherent rhabdomyosarcoma (ATCC CCL136) cells were infected with 0.3 mL of viral suspension providing a multiplicity of infection of 0.1 and incubated for 2 hours at 37°C with periodic agitation. Cell monolayers were washed with phosphate-buffered saline and incubated for 24 hours at 33°C and fixed in acetone at -20°C. The uninfected control cells were fixed in acetone immediately after the 2-hour adsorption period. For viral antigen control, the primary antibody, monoclonal antibody 4B-6.2, reacting with HCoV-OC43 nucleocapsid antigen at 1:500 dilution was used on infected cells. Fluorescein-conjugated goat anti-mouse F(ab')₂ (Cappel, Durham, NC) at 1:20 dilution was used as a secondary antibody.¹⁴

Fc receptors were blocked with normal goat serum before incubation with primary antibodies. Acute (January 22, 2003) and convalescent (February 11, 2003) sera were diluted twofold 1:40–1:1280, and each dilution was incubated on infected cells for 1 hour at 37°C. After washing, fluorescein-conjugated rabbit anti-human IgG F(ab')₂ (Dako, Carpinteria, CA) at 1:20 dilution was used as a secondary antibody. The test was validated with human serum having a neutralizing antibody titer to HCoV OC43 of 320 by plaque assay in MRC-5 cells and a titer of 1:200 in the immunofluorescence assay. For the negative control, IgG-deficient (134 mg/dL) human serum at a dilution of 1:40 was used. Fluorescence was observed on a Leitz (Leitz, Germany) epifluorescence microscope at ×40 magnification.

DISCUSSION

This is the first reported case of coronavirus associated with demyelinating disease in a pediatric patient. The patient's presumed diagnosis is ADEM, although multiple sclerosis (MS) cannot be ruled out.

ADEM is presumed to be the result of a post- or parainfectious process. Approximately 75% of patients are reported as having had a recent upper respiratory tract infection or vaccination before the onset of ADEM.^{1,15–17} Some studies have shown seasonal variation, with the majority of cases occurring in the winter and spring, suggesting a seasonal respiratory virus.¹ Although associations with numerous infectious agents have been reported, a specific etiologic agent has not been identified, nor has the pathogenesis been clarified.

Coronaviruses are common causes of upper respiratory tract infections in adults and children and generally produce mild disease. Most recently, a newly described variant of coronavirus has been associated with severe acute respiratory syndrome (SARS).¹⁸ Coronaviruses are distributed worldwide. They are RNA viruses, enveloped with lipid-soluble coats, and are pleomorphic. Outbreaks tend to occur in winter and spring, with young children having the highest infection rates. These viruses are difficult to diagnose because they cannot be cultured easily.

In a murine model, the coronavirus mouse hepatitis virus has been associated with demyelination in the CNS. It produces a chronic demyelinating condition that resembles MS. This disorder is thought to be the outcome of an immune-mediated process.^{7–9}

HCoV has been examined as a possible contributing factor in the pathogenesis of MS. HCoV RNA has

been detected in the CSF of MS patients and in the brains of MS patients on autopsy.^{19–21}

In vitro, this virus has been shown to cause acute and persistent infections in human neural cell lines. The astrocytoma cell lines U-87 MG, U-373 MG, and GL-15 as well as neuroblastoma SK-N-SH, neuroglioma H4, and oligodendrocytic MO3.13 have been found to be susceptible to acute infection by HCoV OC43 and 229E. CHME-5 immortalized fetal microglial cell lines have also been found to be susceptible to acute infection by HCoV 043. Persistent infection by HCoV 229E and 043 has been observed in the MO 3.13 and H4 cell lines. HCoV 043 also has been linked to persistent infections in the U-87 MG and U-373 MG cell lines.^{10,22–24}

Thus far, clinical studies have not shown a direct relationship between human demyelinating disease and HCoV. A recent study of patients with acute, monosymptomatic optic neuritis, for example, showed only 4 of 37 patients with this disorder with a positive CSF PCR for HCoV 229E, with 1 of the 15 controls testing positive.²⁵ Similarly, in an autopsy study, patients with MS were no more likely to have HCoV RNA than controls without neurologic disease.²⁶

CONCLUSIONS

This is the first reported association between HCoV and ADEM, providing support for the hypothesis that coronavirus may represent an important etiologic factor in the pathogenesis of demyelinating disease in humans. The murine counterpart of HCoV has been implicated in chronic demyelinating disease, and HCoV itself has been shown to infect neural cells.

Does coronavirus play a role in the pathogenesis of ADEM or MS? What is the mechanism by which demyelination might occur after infection with coronavirus? The etiology of ADEM and other demyelinating disorders, of course, is probably multifactorial, with both environmental and genetic factors playing important roles. Certainly, in the case of demyelination associated with MS, multiple factors including human leukocyte antigen types, T-cell-mediated mechanisms, and myelin basic protein have been implicated as etiologic factors. Controlled studies are needed to clarify the role of coronavirus in demyelinating disease and, additionally, to explore which qualities distinguish ADEM, a self-limited disorder, from MS.

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