

Phylogenetic and epidemiologic evidence of multi-year incubation in human rabies

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention

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Abstract:

Eight years after emigrating from Brazil, an otherwise healthy man developed rabies. An exposure prior to immigration was reported. Genetic analysis revealed a canine rabies virus variant found only in the patient's home country, and the patient had not traveled internationally since immigrating to the United States. We describe how epidemiological, phylogenetic, and viral sequencing data provided confirmation that rabies encephalomyelitis may present after a long, multi-year incubation period, a consideration that previously has been speculated without the ability to exclude a more recent exposure. Accordingly, rabies should be considered in the diagnosis of any acute encephalitis, myelitis, or encephalomyelitis.

Case report.

A 40-year-old Brazilian man presented to the emergency department with painful arm and leg paresthesias accompanied by myalgias; he was given analgesics and discharged. Over the following week he developed fever, chills, severe headache and intermittent confusion. His weakness evolved from predominantly upper extremities to significant gait dysfunction. Three weeks after symptom onset, he was admitted to a hospital with fever, areflexia, and weakness. Lumbar puncture revealed elevated protein (159 mg/dl), normal glucose, and mild leukocytosis with mononuclear predominance (33 cells/cu mm, 87% mononuclear). Broad-spectrum antibiotics were administered. Electromyography revealed absent right F responses. Brain MRI was unremarkable, but MRI of the spine showed abnormal T2 signal from T8 to the conus without T1 enhancement (Figure 1). He was transferred to a tertiary facility. On arrival he appeared toxic with a temperature of 38.9 C. Cardiopulmonary examination was unremarkable. No rash or edema was present.

The patient was awake, but confused. No cranial neuropathy was evident. Strength testing revealed symmetric distal weakness. Sensation was intact. Reflexes were absent except for 1+ triceps reflexes.

The subsequent 72 hours yielded progressive obtundation requiring intubation. The patient was transferred to the neurocritical care unit where he was unresponsive to noxious stimuli with intermittent down-beating nystagmus. Pupils were 5-mm, nonreactive; corneal reflexes were present. He had diffuse stimulus-induced facial contractions but no gag or cough reflex. Extremities remained flacid to nailbed pressure, and reflexes were absent. He had spontaneous respiratory effort and was hemodynamically stable. Continuous EEG monitoring showed generalized slowing (Figure 1).

MRI of the brain and spine revealed a new T2 signal abnormality in the cervical cord. Lumbar puncture revealed a lymphocytic CSF leukocytosis (71 cells/mm³, 55% lymphocytes) and elevated protein (81 mg/dL). Toxicology and an extensive infectious disease workup were negative. Intracranial pressure was normal by fiberoptic monitoring. Antibiotics were discontinued after microbiological testing was negative. Immunosuppressive therapy including steroids and IVIg was administered to treat possible autoimmune post-infectious encephalomyelitis. His neurological status continued to decline with loss of remaining brainstem reflexes by hospital day 5.

On day 5, EEG showed diffuse slowing with frequent waxing and waning of bifrontal sharp wave discharges with triphasic morphology, evolving by day 11 to diffuse background attenuation with no reactivity to further attenuation by day 15, albeit not meeting brain death criteria for electrocerebral silence.

Visual evoked potentials were present and reproducible on hospital day 8. Day 9 EMG demonstrated fibrillation potentials, absent sensory and motor responses, and positive sharp waves, consistent with severe sensorimotor peripheral neuropathy. On day 12, a third MRI of the brain showed new diffuse restricted diffusion of cerebral white matter with leptomeningeal enhancement, progressing to prominent temporal and perisylvian cortical edema by day 15. On day 13, a frontal brain biopsy showed diffuse subacute ischemic damage with minimal perivascular and parenchymal chronic inflammation, consisting predominantly of CD3+ T-cells, occasional CD138+ plasma cells and rare CD20+ B-cells. No vasculitis, demyelination, micro-organisms, or features of viral encephalitis were found (Figure 2). A sural nerve biopsy performed on the same day showed extensive peripheral nerve Wallerian degeneration. The patient continued to receive maximal support without signs of improvement. Rabies virus-neutralizing antibodies were retrospectively detected in CSF 24 days before death (0.56 IU/ml), and in serum 8 days before death (22.0 IU/ml), by rapid fluorescent focus inhibition testing.³ Unfortunately saliva and nuchal biopsy samples collected at that time were non-informative because of delay in sample arrival resulting in a break in the cold chain for these samples. The patient died on hospital day 28 after the family elected comfort care and terminal extubation.

An unrestricted autopsy was performed. The brain was dusky gray and liquefied with obliteration of all anatomic landmarks, consistent with severe hypoxic/ischemic injury. Histology revealed disseminated encephalomyelitis with widespread chronic inflammation, gliosis and diffuse hypoxic-ischemic damage.

The spinal cord was better preserved with striking grey matter perivascular lymphocytic cuffing, microglial nodules and loss of alpha-motor neurons. Negri bodies were identified in a few anterior horn cells. Dorsal root and multiple sympathetic nervous system ganglia showed moderate ganglionitis with mild ganglion cell loss but no viral inclusions. Peripheral nerves showed widespread severe axonal loss and demyelination

with patchy mild endoneurial chronic inflammation. Autopsy also revealed mild lymphocytic myocarditis with focal intra-cardiac lymphocytic neuritis (Figure 3).

Fresh frozen brain tissue obtained at autopsy was submitted to the CDC where presence of rabies virus antigen was detected by direct fluorescent antibody (DFA) testing.¹ Antigenic typing with anti-nucleocapsid monoclonal antibodies revealed a Latin American dog rabies virus variant.² Viral RNA was amplified by the reverse transcription-polymerase chain reaction, and complete viral genome sequencing, as described elsewhere,⁴ revealed a sequence with retained identity typical of canine rabies viruses, without alteration of known antigenic sites (Genbank: KC737850). With 99.3-100% identity based upon partial nucleoprotein gene sequences available for comparison, phylogenetic analysis showed a canine rabies virus variant from the patient's home state of Rondônia, Brazil (Figure 3), coinciding with the timing of his residency there. Further history from the patient's family indicated he was a carpenter with no recent exposures. Of note, 30 days prior to admission he had started using anabolic steroids. There had been no travel outside of Massachusetts for the prior 8 years or contact with animals transported from Latin America. However, a family member reported that the patient had been in contact with an abnormally behaving dog prior to his immigration, striking the animal in the head with a piece of wood and dragging the carcass without gloves, though without a known bite or scratch.

The Massachusetts Department of Public Health was contacted and post-exposure prophylaxis and vaccination was provided to a number of social and occupational contacts as well as to several healthcare workers.

Discussion:

We present a case of paralytic rabies, with phylogenetic and epidemiologic evidence of a prolonged incubation period before initial presentation. Once an exposure has occurred, rabies virus may remain locally at the inoculation site before movement to the CNS along peripheral nerves. The usual incubation period is 1-6 months. Incubation periods up to 6 years post-exposure have been reported with supportive

genetic or antigenic data^{5,6,7}. Other reports hypothesize that longer incubation periods may occur (up to 25 years); however, these cases occurred in regions where rabies is endemic and the possibility of re-exposure cannot be excluded^{8,9}. In the United States, enzootic canine rabies virus variants are eliminated,¹⁰ although unvaccinated dogs may be infected with other rabies virus variants. The patient reported here had lived in Massachusetts for 8 years without any known foreign travel and had genetic viral sequencing demonstrating a virus present only in Latin America. In addition, this patient experienced a prolonged prodrome, longer than the typical 7-10 days. Others have hypothesized that patients with prolonged incubations may have slowed viral replication¹¹, which if present could also result in a protracted prodrome.

Additionally, epidemiologic evidence supports that there had been no re-exposure from unreported travel. According to the Pan American Health Organization, cases of rabies in dogs in Brazil decreased from 289 to 5 between 2003 and 2011. Human cases of rabies attributable to a canine virus variant declined from 14 to 2 over the same time period. By surveillance activities, the last identification of a rabid dog in the patient's city of origin was 2003, and the last rabid dog in Rondônia State was documented in 2008. This decrease indicates that the alternative explanation of a more recent exposure to this virus, either through undocumented travel to the patient's state of origin, or through importation of a dog from that area, is similarly unlikely. This epidemiologic data together with the aforementioned phylogenetic identification of the virus provide support for a prolonged incubation period of greater than 8 years in our patient.

This case is also unusual because of the extent and striking severity of parenchymal inflammation in the spinal cord, the degree of which is unusual for most rabies virus infections. Although intense inflammation has been described in previously reported cases of paralytic rabies, this tends to be more restricted in distribution than seen in the current case. While differences in inflammatory patterns exist between paralytic and classic syndromes¹², it is unknown if inflammatory patterns vary with the incubation period or if they resulted from the prolonged hospital course and supportive care. The patient's anabolic steroid supplementation beginning one month before hospitalization is of uncertain significance. Though anabolic steroids have been reported to reduce maturation and stimulation of lymphocytes and decrease antibody

formation,^{13, 14} it is unclear whether the patient's steroid intake played a role in the activation of rabies virus.

In conclusion, this case represents the longest confirmed incubation period of rabies known to date. The extensive phylogenetic and epidemiological workup conducted strengthens the data and provides multiple methods of support that rabies may present after multiple years of incubation, previously a speculative hypothesis. Consideration of rabies in the evaluation of rapidly progressive encephalitis, myelitis or encephalomyelitis is critical for appropriate risk assessment of patient contacts and to guide prognosis, although the treatment outcomes of delayed progression remain unknown.

Legends to Figures

Figure 1: Clinical Studies: MRI Brain and Thoracic Spine, EEG

MRI Thoracic and Lumbar spine, T2 sequence, showing a non-enhancing T2 hyperintensity extending from the conus to the T8-9 level (A). Admission MRI Brain, FLAIR sequence, showing normal anatomy without signal changes (B). EEG on admission with diffuse slowing (C) EEG on day 5 with triphasic morphology (D) EEG on day 11 with diffuse attenuation (E).

Figure 2: Pre-mortem brain, sural nerve biopsy and post-mortem spinal cord, myocardium

Brain biopsy showing widespread subacute ischemic damage with rarefied neuropil and perivascular lymphocytic infiltrates (A, H&E). Sural nerve biopsy showing breakdown of myelin (B, Luxol H&E) with Wallerian degeneration on a teased nerve fiber preparation (C, osmium fixed nerve). Spinal cord ventral horn with neuronal loss and prominent perivascular and parenchymal lymphocytic infiltrates (D, Luxol Fast blue/H&E). Negri body in a residual spinal anterior-motor neuron, arrow (E, Luxol Fast blue/H&E). Inter-atrial cardiac septum with lymphocytic inflammation (F, H&E).

Figure 3: Phylogenetic tree of rabies virus lineages documented in the Americas

Phylogenetic tree based on the nucleoprotein gene sequences (complete and partial, with pairwise gap deletion), demonstrating position of the human isolate (MA human 2011) in the context of rabies virus lineages documented in the Americas. Significant bootstrap values (>70) are shown for key nodes.

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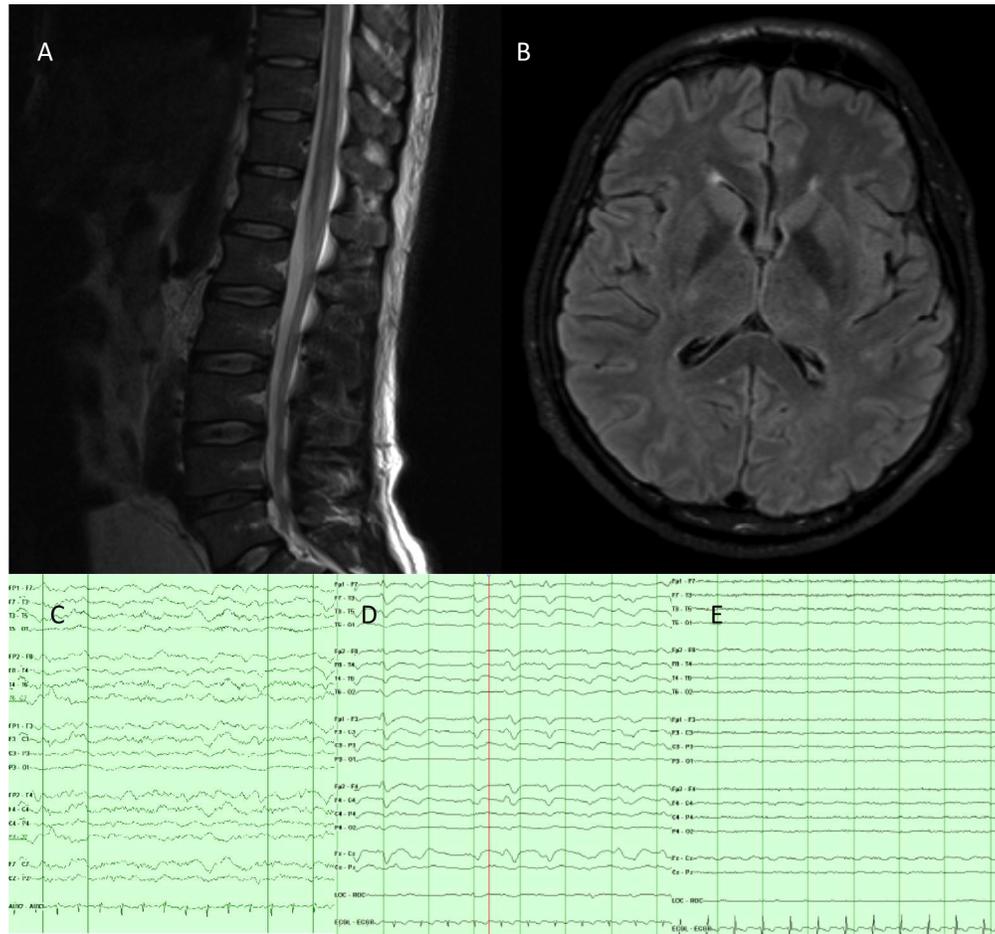


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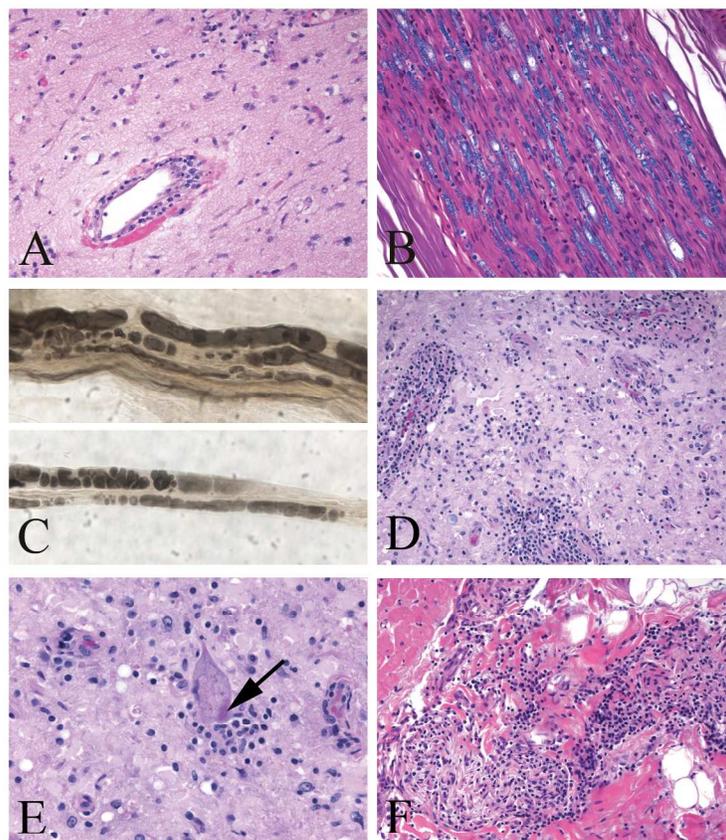


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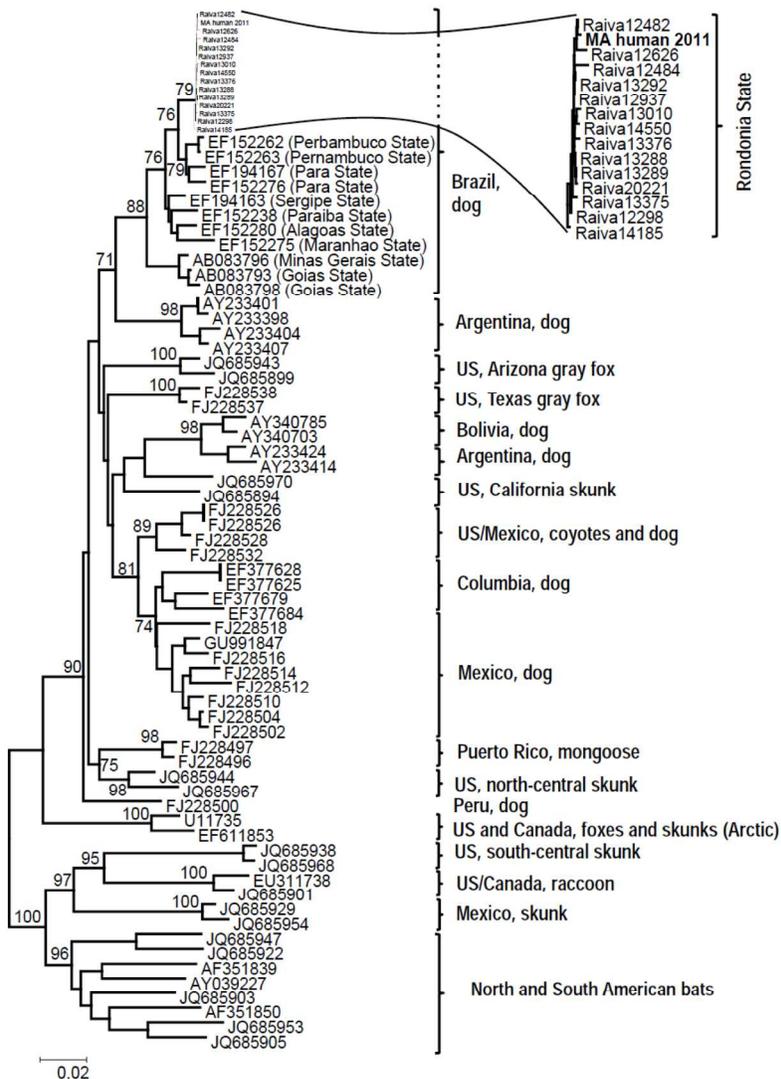


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