Possible Tick-Borne Human Enterovirus Resulting in Aseptic Meningitis

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Enterovirus-specific genetic sequences were isolated from two Amblyomma americanum tick pools. Identical genetic sequences were later obtained from cerebrospinal fluid of a patient with aseptic meningitis and a recent history of tick attachment. These observations suggest the possibility of an emerging tick-borne human enterovirus associated with aseptic meningitis.

In 1999, several cases of possible enterovirus-associated aseptic meningitis occurred in middle Tennessee. The individuals, all local residents, presented with similar symptoms including fever, myalgias, nausea, malaise, headache, and nuchal tenderness lasting greater than 7 days. The patients were not related, and their histories revealed no shared potential for disease acquisition, with the exception of exposure to tick feeding. All four individuals had removed attached ticks approximately 1 to 2 weeks prior to symptom onset. All patients recovered without sequela. No ticks were retained for study.

Symptomatology and limited laboratory testing for one patient by a private firm resulted in a diagnosis of aseptic meningitis. Laboratory tests were run for ehrlichiosis, eastern equine encephalitis, western equine encephalitis, California encephalitis, St. Louis encephalitis, Lyme disease, Rocky Mountain spotted fever, murine typhus, and a panel of coxsackie A viruses that included CAV7, CAV9, CAV10, and CAV16. An elevated titer of complement-fixing antibody (1:16) was reported for coxsackievirus A9 alone; all other tests were negative. Ticks were collected from patients’ counties of residence and examined for the presence of enterovirus nucleic acid. During summer 2000, 192 ticks were collected from middle Tennessee counties Rutherford and Williamson using CO2 traps as previously described (11). Prior to dissection, adults and nymphs were identified as Dermacentor variabilis (40 ticks) and Amblyomma americanum (152 ticks) using appropriate keys (2, 10). Gut contents were pooled in groups of four (10 Dermacentor pools and 38 Amblyomma pools), digested using proteinase K, extracted with phenol-chloroform, and ethanol precipitated. Potential viral RNA was reverse transcribed and amplified by PCR. Primers were designed to bind to conserved enterovirus regions within the 5′ untranslated region. Primer sequences were as follows: forward, 5′ CAAGCAGTTCTGTCTCCC CGG; reverse, 5′ GAAACAGGAGCGGAAATG (Integrated DNA Technologies, Coralville, IA). PCR products were resolved by 1.25% agarose gel electrophoresis and visualized by ethidium bromide staining. All PCR products were applied to a nylon membrane (Hybond; Amersham Pharmacia Biotech, Piscataway, NJ), and an enterovirus-specific digoxigenin-labeled probe (Roche Applied Science, Indianapolis, IN) was allowed to hybridize according to the manufacturer’s instructions (DIG Nucleic Acid Detection Kit; Roche Applied Science). The probe sequence was 5′ CGATGCGTTGCGCT CAGCAC (Integrated DNA Technologies). Probe-binding amplicons were cleaned using a Quantum Prep cleaning column (Bio-Rad, Hercules, CA) and ligated into pGEM-T (Promega, Madison, WI). Plasmids were transformed into Escherichia coli JM109 (Promega), and transformants were selected by blue-white screening. Plasmids were prepared by standard alkaline lysis and bidirectionally sequenced on an Open Gene automated DNA sequencer using a dye-primer cycle sequencing kit according to the manufacturer’s instructions (Visible Genetics, Toronto, Ontario, Canada). None of the Dermacentor pools were amplified. Two of the 38 pools from A. americanum ticks resulted in successful amplification (Fig. 1A). Both positive pools were composed of A. americanum nymphs collected in Williamson County and demonstrated fragment sizes of approximately 400 bp (expected size, 402 bp; lanes 3 and 5, Fig. 1A). Hybridization with the enterovirus sequence-specific probe also occurred for both PCR-positive pools (spots 1 and 2, Fig. 1B). The two reactive tick pools were sequenced and were nearly identical (99%), differing at a single nucleotide (GenBank accession numbers AF409090 and AY123425). A BLAST search of enteroviruses in the GenBank database revealed that the greatest homology of the tick enterovirus sequences occurred with poliovirus 2 (91% sequence identity), polioviruses 1 and 3 (90%), coxsackievirus A11 (90%), and coxsackieviruses A17 and A15 (89%). This sequence identity is consistent with enterovirus nucleotide identity in the 5′ untranslated region (8).

In September 2001, a 29-year-old male was seen in the emergency room of a middle Tennessee hospital. The patient complained of a headache worsening over 1 to 2 days with photophobia, nausea, and vomiting, as well as neck pain. He exhibited a fever of 100.4°F, and initial physical examination revealed some meningismus and rigidity of the neck. His joints were not swollen, and a rash was not observed. The patient was admitted, and head computed tomography (CT) and cerebrospinal fluid (CSF) tests were run. The patient later reported...
experiencing tick attachment at least a week prior to the onset of symptoms. Additional tests were ordered from a private laboratory and included Lyme disease immunoglobulin M (IgM) and IgG screening for Borrelia burgdorferi and a coxsackievirus A antibody panel. Titors for all of the six coxsackie A virus antibodies were reactive (Table 1, limit of reactive detection for complement-fixing antibody, <1:8). Since complement-fixing antibody typically persists for only a few months, the elevated titers suggested recent infection. The patient recovered without event. Due to patient history of tick attachment prior to developing meningitis, the CSF sample was retained for more thorough evaluation for enterovirus genetic sequences. Vero cells (American Type Culture Collection certified cell line 81) in medium 199 supplemented with 10% fetal calf serum, 2 mM l-glutamine, and antibiotics (Sigma, St. Louis, MO) were inoculated with the remaining supernatant of the virus cultivation of the virus would have allowed more complete virus characterization; however, no cytopathogenic effect was observed after 10 days. The tissue culture medium-CSF sample was treated for extraction of nucleic acid and prepared for reverse transcription-PCR as already described. Tissue culture medium from non-CSF-exposed cells was also evaluated as a negative control. Tick samples were never prepared at the same time that the CSF sample was amplified; distilled-H₂O negative controls were always included. The forward primer was identical to the primer used for tick gut samples, but the reverse primer was 5'-CTTGTTCACTACTAGCGTCC (Integrated DNA Technologies). This primer was prepared in order to maintain complete sequence complementarity with the sequences detected in ticks. Amplification of the CSF-tissue culture extract resulted in a band of approximately 250 bp on an electrophoretic gel (lane 7, Fig. 1A) (expected size, 252 bp) that was bound by the enterovirus-specific probe (spot 3, Fig. 1B). The sequence obtained was identical to the tick pool sequences (GenBank accession number AF533014).

The titers of patient antibodies reactive to several coxsackie A viruses (Table 1) are likely the result of the cross-reactivity frequently noted within this group of viruses (7). Problems exist with heterotypic antibody responses, and antigenic variants exist among the enteroviruses, with several reported as being untypeable (5). The failure of the CSF sample to cause noticeable cytopathogenic effects in tissue culture has often been encountered with other enteroviruses, particularly the coxsackie A viruses (8). The enterovirus sequences reported here may represent a previously undescribed enterovirus. The cross-reactivity in patient sera, lack of cytopathogenic effect in tissue culture, and sequence similarity suggests that this virus may be a coxsackie A virus.

Blood-feeding arthropods have not been reported in the natural transmission of enteroviruses (7). Although no conclusive association has been established by this study, our results raise the possibility that this virus may be a new and important etiologic agent of meningitis in humans that could be transmitted by ticks. Others have reported viruses from the family Picornaviridae in blood-feeding arthropods. A Cardiovirus was...
isolated from a tick, *Ixodes persulcatus*, that had been feeding on a boar (3). In one laboratory study, mosquitoes were allowed to feed on mice infected with coxsackievirus A6. Mosquitoes maintained detectable virus levels for up to 8 days and were able to transmit the virus to other mice (4). It remains to be determined whether this virus is capable of replication within the tick.

This study relates to the marked seasonality of enterovirus infections that exists in temperate climates. A seasonal increase in enterovirus meningitis during the summer and early fall has been documented in temperate climates (6, 9). Cases of aseptic meningitis that occur during the late summer months or early fall have a particularly high probability of enterovirus etiology (7). It has been speculated that increased fecal-oral transmission during the summer and fall may occur when less clothing is worn (1). In view of the present study, however, it seems plausible that tick transmission may contribute to the seasonality of enterovirus disease. Such a hypothesis should be considered since the frequency of enterovirus infections during the summer and early autumn corresponds to increased tick activity in temperate climates.

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REFERENCES