ENZOOTIC TRANSMISSION OF DEER TICK VIRUS IN NEW ENGLAND AND WISCONSIN SITES

GREGORY D. EBEL, ERIC N. CAMPBELL, HEIDI K. GOETHERT, ANDREW SPIELMAN, AND SAM R. TELFORD III

Laboratory of Public Health Entomology, Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts

Abstract. To determine whether rodents that are intensely exposed to the deer tick–transmitted agents of Lyme disease, human granulocytic ehrlichiosis, and human babesiosis are also exposed to deer tick virus (DTV), we assayed serum samples from white-footed mice (Peromyscus leucopus) and meadow voles (Microtus pennsylvanicus) in sites densely infested by deer ticks. To conduct serosurveys, we developed an enzyme-linked immunosorbent assay (ELISA) and Western blot assay by cloning, expressing, and purifying a portion of the DTV envelope glycoprotein (DTV rE) for use as test antigen. Sera from mice and voles trapped in Massachusetts, Rhode Island, and Wisconsin were screened by ELISA for IgG reactive to DTV rE. Samples that were positive or borderline by ELISA were subsequently analyzed by immunoblotting. Samples reactive in both assays were considered to be positive. Three percent of 264 mouse samples collected from sites in Rhode Island, 3.8% of 52 samples from mice trapped in Wisconsin, and 3.9% of 282 samples collected from mice trapped on Nantucket Island, MA were positive. No samples from either Great Island, MA, or voles from any study site were reactive. A reverse transcriptase–polymerase chain reaction yielded molecular evidence of DTV infecting questing adult deer ticks in sites where seroreactive mice were trapped, but not from ticks collected where serologic evidence of virus perpetuation was absent. White-footed mice appear to be exposed to DTV in certain sites where other deer tick-borne agents perpetuate. This virus may be maintained in the same enzootic cycle.

INTRODUCTION

Deer tick virus (DTV), an agent that appears to be a subtype of Powassan (POW) virus, has been repeatedly isolated from adult deer ticks (Ixodes dammini) collected in the northeastern and northcentral United States. These viruses are the sole recognized North American members of the tick-borne encephalitis (TBE) group of flaviviruses. Despite wide geographic distances between their points of isolation, DTV strains share a high degree of nucleotide similarity, but are distinct at the nucleotide level from the sole POW virus strain (LB) that has been characterized to date. Although deer ticks are competent vectors for POW virus, isolates of this virulent virus have not been made from field-derived deer ticks. Indeed, ticks infesting sciurids (woodchucks and squirrels) and medium sized mammals (weasels, skunks, and other mustelids; camids) are thought to maintain POW virus in nature. Because deer ticks most commonly feed on white-footed mice as immature forms, it may be that a POW virus subtype is maintained between deer ticks and these mice.

Diagnosis of flavivirus infections is often complicated by serologic cross-reactivity with other flaviviruses. Cross-reactions due to family-wide envelope protein epitopes may occur when classic techniques such as hemagglutination-inhibition (HI), complement fixation (CF) and immunofluorescence (ELISA and Western blot assays). We then sought to determine whether white-footed mice, mammals that may be intensely infested with immature deer ticks, are exposed to DTV in sites where seroreactive mice were infected. To conduct serosurveys, we developed an enzyme-linked immunosorbent assay (ELISA) for antibody to tickborne encephalitis (Immunon AG, Vienna, Austria; unavailable in the United States). While neutralization is mediated through antibodies directed against the envelope protein, re- active antibodies neutralize heterologous virus types less effectively than homologous virus. Therefore, the “gold standard” is the neutralization test. However, this technique requires high biocontainment levels for the necessary manipulations of live TBE-group viruses. Such facilities are unavailable to many researchers. Standard diagnostic methods are therefore not well suited to processing the number of samples required for conducting large-scale serosurveys of naturally exposed animals and humans. A sensitive, safe, rapid, inexpensive immunoassay facilitating high sample throughput would be essential in conducting epidemiologic and ecologic investigations of DTV and the closely related POW virus.

In recent years, recombinant-expressed viral proteins have been increasingly used as antigenic targets for diagnostic purposes. Such proteins have proven highly useful in ELISA and Western and strip immunoblot assays. The flavivirus envelope protein (E) is consistently recognized by sera from infected individuals: IgG and IgM reach high titers and remain long after acute infection. Accordingly, we cloned a portion of the DTV envelope gene, expressed it in Escherichia coli, and evaluated its usefulness in ELISA and Western blot assays. We then sought to determine whether white-footed mice, mammals that may be intensely infested with immature deer ticks, are exposed to DTV infection in sites where other deer tick–borne agents perpetuate. In addition, we determined whether host-seeking deer ticks in such sites were infected with DTV.

MATERIALS AND METHODS

Viral RNA. The RNA from the Ipswich strain of DTV (DTV-IPS) was isolated from infected suckling mouse brain and reverse transcribed as previously described. To obtain an anchor-free portion of the DTV envelope gene with in-frame Xho I and HinD III restriction endonuclease sites for subsequent cloning and expression, we added 5 μl of cDNA to a reaction mixture containing the primers Env-1 (5'-GA-GAAAGTTCAGAGGTGCACCGCATTTGTA-3') and Env-2 (5'-TGCTCGAGTCGGCCTACTGAGCCTTTGTGGTCCCA-3'). The mixture was heated to 94°C for 45 sec for initial
denaturing, followed by 40 cycles of denaturing at 94°C for 45 sec, annealing at 54°C for 45 sec, and extension at 72°C for 90 sec. Final extension occurred at 72°C for 10 min. Reaction products were separated by electrophoresis on a 2% agarose gel. An amplicon of the expected size was excised from the gel and purified using spin columns (Qiagen, Valencia, CA) as directed by the manufacturer.

**Cloning and expression.** The polymerase chain reaction (PCR) product was ligated by TA cloning (Invitrogen, Carlsbad, CA) into pCR 2.1 according to the manufacturer’s instructions. Initial plasmid amplification and isolation was also performed as directed (Invitrogen and Qiagen). Minipreps of candidate cultures were digested with Hind III and Xho I at 37°C for 1 hr. The digestion products were separated and desired fragments were purified as described earlier. Ligation of the purified fragments into pET23h (Novagen, Madison, WI) and subsequent transformation of competent BL21(DE3) (Novagen) *E. coli* was also performed as recommended. To test for expression, 10 ml of Luria broth supplemented with 100 µg/ml carbenicillin was seeded with candidate colonies. When the optical density of each culture reached 0.250 (600 nm) 5 ml of the culture was removed and plasmid DNA was isolated for sequencing. Isopropyl-β-D-thiogalactopyranoside was added to the remaining 5 ml to induce expression of the recombinant protein. Induced cultures were assayed for expression by electrophoretic separation of proteins on a polyacrylamide gel. One culture had a greatly enhanced band of the expected size (approximately 50 kD), and this culture was selected for further analysis. A miniprep of this culture was sequenced by the dideoxy chain termination method on an automated sequencer (Applied Biosystems, Foster City CA). A BLAST search returned recently characterized DTV strains as the best matches (100% homology). Production and purification of recombinant DTV envelope protein (DTV rE) was conducted as described and purity of DTV rE was ascertained by visual inspection of a polyacrylamide gel stained with Coomassie blue.

**ELISA conditions.** The ELISA was used as a screening assay in these studies. Fifty microliters of a 1.3 µg of DTV rE/ml of 0.1% bovine serum albumin/phosphate buffered saline (BSA-PBS) solution were added to each well of 96-well ELISA plates (Immulon 4; Dynex, Chantilly, VA) and incubated with crude *Escherichia coli* lysate, 2) recombinant Prospect Hill virus (PHV) nucleocapsid protein (lane 3, expression construct kindly provided by Dr. Brian Hjelle, University of New Mexico, Albuquerque, NM) for 30 min at 37°C. The sera were then tested for anti-DTV reactivity as described in the Materials and Methods. a, antisera and ascitic fluid raised against Powassan (POW) virus, Central European encephalitis virus, and Venezuelan equine encephalitis virus were tested for reactivity to recombinant DTV envelope protein in the Western blot assay, as described in the Materials and Methods.

**Western blot conditions.** Sixteen micrograms of DTV rE were combined with 2X Laemmli loading buffer and boiled for 5 minutes, and denatured proteins were electrophoretically separated on a polyacrylamide gel and transferred to nitrocellulose. Blots were briefly stained with Ponceau S to confirm transfer of proteins and then blocked in 1% milk/Tris-buffered saline with 0.5% Tween 20 (TBST) buffer at 4°C overnight. After a brief wash with TBST, the blots were placed in a C-shell miniblot apparatus (Immunetics, Cambridge, MA) and 50 µl of serum diluted 1:100 were applied and incubated with rocking at room temperature for 1 hr. Following four 1-min washes with TBST, blots were incubated with alkaline phosphatase-conjugated anti- *P. leucopus* IgG (Kierkegaard and Perry Laboratories) diluted 1:1,000 in 1% milk/TBST buffer for 30 min, and subsequently washed as above and additionally four times for 1 min per wash with distilled water. Blots were developed with BluePhos phosphatase substrate (Kierkegaard and Perry Laboratories) until the positive control wells were clearly visualized (approximately 7 min). The reaction was stopped with 3% trichloroacetic acid and washed with water. Blots were dried overnight in the dark and read the following morning. Any samples with a positive band at the correct location were deemed positive.

To determine whether observed reactivity was specific to DTV rE, and not native *E. coli* antigens or a generalized state of immune activation, we conducted competition experiments. Serum from a naturally infected white-footed mouse was incubated with either 1) crude *E. coli* lysate, 2) DTV rE, or 3) recombinant Prospect Hill Virus (PHV) N protein prepared identically to DTV rE (expression construct kindly provided by Dr. Brian Hjelle, University of New Mexico, Albuquerque, NM) at 37°C for 30 min. Each sample
was then tested for reactivity as described (Figure 1A). Pre-incubation with DTV rE ablated the Western blot signal, while the signal remained strong following pre-incubation with PHV N protein and crude E. coli lysate.

To determine whether observed Western blot reactivity may be due to infection with another arbovirus, and not to true infection with POW virus, we tested murine antisera or ascitic fluid raised against POW virus (Biological Reference Reagent #VS2200; Centers for Disease Control and Prevention, Atlanta, GA), central European tick borne encephalitis (TBE vaccine), and Venezuelan equine encephalitis (VEE) for reactivity to DTV rE protein in the Western blot assay as described (Figure 1B). Polyclonal sera against TBE and VEE were raised using commercial (TBE–ImmunoAG) or experimental vaccines (VEE–Tc83; United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick MD). Anti-POW virus ascitic fluid strongly recognized DTV rE at all dilutions tested, while anti-TBE and anti-VEE did not produce detectable reactions at any tested dilution. From these experiments, we concluded that the Western blot assay is specific to antibodies raised against DTV and POW virus infection, but do not know whether reactivity to POW virus may be distinguished from that to DTV.

**Tick assay.** Ticks were collected from vegetation as previously described. Male ticks were counted and gently homogenized using flame-sterilized glass rods in 50 μl of PBS in 96-well ELISA plates and frozen overnight. Only males were used because females were used in assays for other tick-borne infections, and to supplement our deer tick colony. Plates were thawed in the morning and 5 μl of the supernatant from each of six wells were pooled. For archived samples (those from Nantucket Island, 1996), dissected salivary glands from engorged females had been stored at −70°C in Hank’s balanced salt solution supplemented with 10% fetal bovine serum. These samples were thawed and pooled in groups of five. The RNA was extracted and reverse transcribed as previously described. The PCR was conducted using primers ns5-f (5'-GGCCATGACAGACACAA-CAGCGTTTG-3') and ns5-2 (5'-GAGCGCTCTTCA-TCCACCAGGTCC-3') designed to amplify an approximately 310-basepair region of the DTV nonstructural protein 5 (ns5) gene. This RT-PCR assay will detect the POW virus ns5 gene as well. Reaction products were separated by electrophoresis on a 2% agarose gel and samples with bands of the correct size were deemed positive (Figure 2). To determine the number of infected ticks in each pool, wells contributing to each pooled positive sample were extracted individually and assayed by RT-PCR as described. Steps were taken at all stages of RNA processing to decrease the risk of contamination: ticks were handled in a separate room from where RNA extraction took place, and reactions were set up in a dedicated hood using dedicated pipettors. The PCR products were kept separate from all other areas and reagents used in sample processing.

To ensure that the amplified product was truly DTV-derived, reaction products from positive wells were sequenced as described and aligned with known DTV sequences. In addition, to obtain sequence data from newly isolated DTV strains, we isolated putative virus (from RT-PCR-positive pools of ticks) by suckling mouse inoculation; RNA was extracted from brains of moribund suckling mice, and a fragment of the ns5 gene was amplified as described vide supra. Reaction products were separated by electrophoresis on a 2% agarose gel, and bands of the correct size were excised, purified using spin columns (Qiagen), and sequenced using the dideoxy-chain termination method using an automated DNA sequencer (Applied Biosystems). To determine the relationship between the newly isolated strains and those previously characterized, we aligned the new sequences with those accessioned in GenBank using the PILEUP program of the GCG software package (Wisconsin Genetics Computer Group, Madison, WI) and performed sequence-based phylogenetic analyses using phylogenetic analysis using parsimony (PAUP; version 4.0b3a for Windows; Sinauer Associates, Inc., Sunderland MA).

**Mammal sampling.** Mammals on Prudence Island, RI; Great Island, MA; and Nantucket Island, MA were sampled monthly from April to October in a capture-mark-release fashion. Unbaited Longworth (Penlon Inc., Abingdon, UK) traps furnished with oats and cotton were left on a 0.49-hectare grid overnight. Trapped animals were given a
Great Island, Spooner, WI
great Island, Spooner, WI

...during the study period. Of the seven mice reacting, one
...M. pennsylvanicus
...P. leucopus

...leucopus trapped on Prudence Island, RI between April 1998
...P. leucopus
...M. pennsylvanicus
...P. leucopus
...P. leucopus

...priced earlier. All the described work conformed to Nation-
...left out overnight. Animals were anesthetized with halothane and bled by cardiac puncture. Additionally, we made thin blood smears for microscopical examination and sampled various organs. To supplement trapping by this method, we left lines of baited snap traps out overnight and processed trapped animals as described earlier. All the described work conformed to National Institutes of Health guidelines for the humane use of laboratory animals.

RESULTS

We screened 264 serum samples collected from 132 P. leucopus trapped on Prudence Island, RI between April 1998 and October 1999 (Table 1). Forty-two samples (15.9%; 95% confidence interval [CI] = 11.7, 20.9) were positive by the ELISA test. Of these, eight samples representing seven mice were positive by Western blot. Three percent of the samples screened (95% CI = 1.3, 5.9) and 5.3% of the individuals trapped (95% CI = 2.2, 10.6) had been exposed to DTV during the study period. Of the seven mice reacting, one seroconverted during the course of the study, three lost their reactivity, and three were trapped only once or were seropositive when first caught. None of the 99 samples obtained from meadow voles (Microtus pennsylvanicus) on Prudence Island reacted. No samples collected from Great Island, MA during the same study period reacted. From October 1997 to October 1999, we collected 52 serum samples from 52 P. leucopus near Spooner, WI, a focus of DTV transmission that has been previously characterized.1 Two of these (3.8%; 95% CI = 0.5, 13.2) were reactive in the Western blot assay. From April 1994 to October 1997, we collected 282 serum samples from 215 white-footed mice on Nantucket Island. Forty-four of these (15.6%; 95% CI = 11.6, 20.4) were positive in the ELISA. Eleven of these, representing nine mice, were reactive in the Western blot assay. A total of 3.9% of the Nantucket Island samples screened (95% CI = 2.0, 6.9) and 4.2% of the individuals trapped (95% CI = 1.9, 7.8) had antibodies that reacted to recombinant DTV envelope protein. Eight of the nine mice with reactive samples seroconverted during the period of observation, no mice lost reactivity, and one seropositive mouse was captured only once. White-footed mice collected at three of four study sites reported here appear to have been infected with DTV or POW virus. Prevalence at all study sites where seroreactive animals were resident is similar, with about 4% of the mice exposed.

Deer ticks collected at each site were screened for infection by RT-PCR (Table 2). One of 180 assayed ticks (0.6%; 95% CI = 0.01, 3.1) collected during October 1999 on Prudence Island was positive. Ten of 792 assayed ticks (1.3%; 95% CI = 0.6, 2.3) collected from October 1997 to October 1999 from Spooner, WI were positive. Two of 191 ticks (1.0%; 95% CI = 0.1, 3.7) collected on Nantucket in 1996 and 1999 were positive. No ticks collected at Great Island during October 1999 were positive. Isolation attempts were successful for the two pools of Nantucket ticks that were RT-PCR positive, but we failed to isolate virus from the Prudence Island tick pool, perhaps reflecting low virus titer or reduced viral viability in the ticks. Isolation was not attempted for the pools of Spooner, WI ticks inasmuch as we have previously isolated virus from that site.1 Virus-infected deer ticks were collected at all three sites where seropositive mice were trapped, but not at the sole site lacking serologic evidence of infection. Prevalence in adult ticks at these sites was similar, with about 1% infected.

### Table 1

<table>
<thead>
<tr>
<th>Sites</th>
<th>Dates</th>
<th>Species</th>
<th>Prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prudence Island, RI</td>
<td>Apr 1998–Oct 1999</td>
<td>P. leucopus</td>
<td>3.0 (1.3, 5.9), n = 264</td>
</tr>
<tr>
<td>Great Island, MA</td>
<td>Apr 1998–Oct 1999</td>
<td>M. pennsylvanicus</td>
<td>0 (0, 3.7), n = 99</td>
</tr>
<tr>
<td>Nantucket Island, MA</td>
<td>Oct 1997</td>
<td>P. leucopus</td>
<td>0 (0, 3.4), n = 108</td>
</tr>
<tr>
<td>Nantucket Island</td>
<td>Oct 1997</td>
<td>P. leucopus</td>
<td>3.9 (2.0, 6.9), n = 282</td>
</tr>
<tr>
<td>Spooner, WI</td>
<td>1997–1999</td>
<td>P. leucopus</td>
<td>3.8 (0.5, 13.2), n = 52</td>
</tr>
<tr>
<td>Total in sites with reactive mice</td>
<td>Apr 1994–Oct 1999</td>
<td>P. leucopus</td>
<td>3.5 (2.2, 5.3), n = 598</td>
</tr>
</tbody>
</table>

* CI = confidence interval; P = Peromyscus; M = Microtus.
† One-sided 97.5% confidence interval.

### Table 2

<table>
<thead>
<tr>
<th>Sites</th>
<th>Dates</th>
<th>Prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prudence Island, RI</td>
<td>Apr 1998–Oct 1999</td>
<td>0.6 (0.01, 3.1), n = 180</td>
</tr>
<tr>
<td>Great Island, MA</td>
<td>Apr 1998–Oct 1999</td>
<td>0 (0, 3.8), n = 96</td>
</tr>
<tr>
<td>Nantucket Island, MA</td>
<td>Oct 1996–Oct 1999</td>
<td>1.0 (0.1, 3.7), n = 191</td>
</tr>
<tr>
<td>Spooner, WI</td>
<td>1997–1999</td>
<td>1.3 (0.6, 2.3), n = 792</td>
</tr>
<tr>
<td>Total in sites with PCR-positive ticks</td>
<td>Oct 1996–Oct 1999</td>
<td>1.1 (0.6, 1.9), n = 1,163</td>
</tr>
</tbody>
</table>

* CI = confidence interval; PCR = polymerase chain reaction.
† One-sided 97.5% confidence interval.
FIGURE 3. Phylogenetic relationships among tickborne flaviviruses based on a 292-basepair fragment of the nonstructural protein 5 (ns5) gene. Maximum parsimony analysis with branch numbers indicating bootstrapped confidence based on 500 replicates. GenBank accession numbers are in parentheses. DTV = deer tick virus; POW = Powassan virus; TBE (CEE) = tickborne encephalitis virus, Central European subtype; LI = tickborne encephalitis, louping ill subtype; NFS9601 = virus isolated from ticks collected at the Nantucket Field Station, Nantucket, MA.

Phylogenetic analysis of an ns5 gene fragment indicated that the Nantucket isolate comprised DTV as opposed to POW virus (Figure 3; Genbank accession number AF276311). Although serologic reactivity within the mouse population might have been interpreted as exposure to POW virus inasmuch as rDTV would cross-react, our isolation and subsequent molecular identification of DTV from ticks in the same Nantucket site argues otherwise. The partial ns5 sequence from the Prudence Island tick was identical to that of either the Ipswich or Connecticut strains of DTV, but larger portions of the ns5 gene were not obtained nor was that for env, perhaps because the env amplification assay is relatively insensitive. Further information on the putative Prudence Island DTV is unavailable because we failed to isolate it.

DISCUSSION

We developed, tested, and implemented a new serologic assay for detection of antibodies specific to DTV and POW virus. This assay, which uses ELISA and Western blotting techniques, allowed us to safely and efficiently screen hundreds of rodent serum samples. This constitutes an improvement over classical techniques that require specific technical expertise and higher levels of biocontainment than are available in most laboratories. While neutralization is likely to remain the definitive serologic tool in studies of arbovirus epidemiology, the methods presented here will facilitate studies that were not previously undertaken due to excessive technical burden. Additionally, it is an assay that may be adapted for human use as a rapid screening tool to rule out DTV or POW virus as the etiologic agent in cases of encephalitis with onset during the summer months. The ELISA results may be obtained within 2 hr, which is significantly faster than many classical techniques.

The recombinant envelope ELISA antigen was cloned from DTV. Given the similarity of DTV and POW virus, we do not expect to be able to distinguish reactivity of sera from animals or humans exposed to prototypic POW virus from those exposed to DTV. Although DTV strains and not POW virus have been isolated from the Spooner and Nantucket sites (isolation attempts from Prudence Island-derived material were unsuccessful), we cannot exclude the possibility that the reactivity we observed is due to prototypic POW virus. Indeed, a previous report suggested up to 30% exposure of Peromyscus spp. to POW virus. The seroreactivity that we have observed is most likely to have been due to DTV because ticks from the sites where mice were demonstrated to be seropositive were infected by DTV (as determined by PCR sequencing) and not POW virus. Seroreactivity of mice in areas that are heavily infested with deer ticks predicts tick-derived molecular evidence of virus perpetuation, and vice-versa.

In the four mice from Prudence Island whose serologic status changed during the course of the study, three lost their reactivity. These mice were in smaller weight classes (<20 grams) at the first capture, indicating the likelihood that the antibody we detected was maternally acquired, and faded as the mice aged. Conversion of some mice collected on both Prudence and Nantucket Islands, however, indicates that active transmission occurs in both of these sites. However, the prevalence of seroreactivity was insufficient to perform meaningful statistical analyses using the data collected so far. Interestingly, no voles trapped during the course of this study had antibodies reactive with DTV rE. It may be that because fewer immature deer ticks feed on voles than on white-footed mice, voles become infected with DTV too rarely to detect in our limited sample. Alternatively, DTV may not infect these hosts, or infections may terminate fatally thus removing them from the sample population.

We are unable at the present time to adequately describe the enzootic cycle of DTV, but our detection of this agent in a faunally depauperate site suggests that it differs from prototypic POW virus. In two of the sites where viral activity was detected, presumptive reservoirs of POW virus such as woodchucks, skunks, weasels, fox, or squirrels are present. Because our serologic assay cannot discriminate between exposure to POW virus or DTV, we may have detected prototypic POW virus activity. Indeed, in the Wisconsin sites, all known POW virus reservoirs frequent the areas where we trapped mammals and collected ticks. On Prudence Island, the fauna is more restricted, but eastern gray squirrel, chipmunks, woodchucks, mink, and fox are present. The fauna on Nantucket Island, however, is severely restricted: no
mustelids or canids are present, and sciurids (solely gray squirrels; no woodchucks or chipmunks) exceedingly rare. In contrast, nonhuman primates, the human-biting habits of deer ticks, and the increases in deer tick populations have driven the prolonged epidemics of Lyme disease, human babesiosis, and human granulocytic ehrlichiosis currently underway in the United States. The development of this rapid, safe, and simple serodiagnostic tool will facilitate investigations into the public health burden of DTV and POW virus, as well as the dynamics of enzootic transmission.

Acknowledgments: We thank Allan Beck (Prudence Island Biological Research Station) and Rulon Wilcox (Superintendent of Great Island) for their assistance in facilitating the fieldwork. This is a contribution of the University of Massachusetts Nantucket Field Station. We are grateful to Brian Hjelle for cloning and expression protocols. Patrick Skelly and Swati Patankar provided valuable advice on protein expression.

Financial support: This work was supported by grants from the National Institutes of Health (NIH) (ROI AI-39002, AI-37993, and AI-42402). Gregory D. Ebel and Heidi K. Goethert were supported, in part, by NIH Training Grants (NIH T32 AI-07530 and NIH T32 AI-07350) to the Harvard School of Public Health.

PREVALENCE OF DEER TICK VIRUS IN TICKS AND MICE


REFERENCES


