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Serologic Evidence of Jamestown Canyon Virus Infection in White-Tailed Deer Populations from Connecticut

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ABSTRACT: We determined the prevalence and distribution of Jamestown Canyon (JC) virus antibody in white-tailed deer (*Odocoileus virginianus*) populations in Connecticut, USA. Sera were collected from hunter-killed deer during 1993. Antibody to JC virus was detected by enzyme-linked immunosorbent assay (ELISA) in 92 (21%) of 446 deer sera, and was uniformly distributed among geographic sites. Twenty-one ELISA-positive sera were tested and confirmed positive by plaque reduction neutralization testing. This represents the first serologic evidence of JC virus in a reservoir host population from the northeastern United States. No cross-reactivity was seen with California encephalitis, Keystone, or snowshoe hare viruses, but a varying degree of cross-reactivity was obtained with Guaroa, Jerry Slough, La-Crosse, San Angelo, and trivittatus viruses. We conclude from this investigation and previous isolations of JC virus from mosquitoes in the state that JC virus occurs enzootically in Connecticut.

Key words: Jamestown Canyon virus, California serogroup, *Bunyavirus*, antibody, white-tailed deer, *Odocoileus virginianus*, enzyme-linked immunosorbent assay, plaque reduction neutralization.

Jamestown Canyon (JC) virus is a vector-borne California serogroup bunyavirus (Bunyaviridae: *Bunyavirus*). It is transmitted by the bite of infective mosquitoes (mostly snowpool *Aedes* species); white-tailed deer (*Odocoileus virginianus*) are generally recognized as the primary vertebrate reservoir host (Grimstad, 1988). Jamestown Canyon virus has been increasingly implicated as an etiological agent of emerging human disease that appears to be expanding with increasing deer populations in many regions of North America (Deibel et al., 1983; Grimstad, 1983; DeFoliart et al., 1986; Lederberg et al., 1992). In Michigan (USA), for example, human infection with JC virus has a distribution pattern that closely parallels the distribution of white-tailed deer (Grimstad et al., 1986) and in New York (USA) significant increases in the isolation of JC virus from mosquitoes coincided with warm winters and a surge in wild deer populations (Grayson et al., 1983). In addition, serological evidence of JC virus infection in humans was reported (Deibel et al., 1983). In Indiana (USA), where JC virus infection in white-tailed deer is common, high antibody prevalences (up to 67%) in deer populations from the northern portion of the state were correlated with higher antibody prevalences (2% to 15%) in human residents (Boromisa and Grimstad, 1987).

Knowledge of JC virus in Connecticut (USA) is limited. The virus was repeatedly isolated from field-collected mosquitoes whenever arbovirus surveillance was conducted (Whitman et al., 1968; Sprance et al., 1978; Main et al., 1979; Andreadis et al., 1994). However, the prevalence and distribution of JC virus in potential reservoir vertebrate host populations is completely unknown. Meanwhile, white-tailed deer populations have increased substantially throughout many urban and suburban areas of the state (Christie et al., 1987; Curtis and Richmond, 1992), thus leading to speculation that the virus may be enzootic (Andreadis et al., 1994). Our objective was to determine the prevalence and distribution of JC virus antibody in white-tailed deer populations in Connecticut.

Sera from 446 deer were tested for antibody to JC virus. These samples were obtained from the Connecticut Agricultural Experiment Station's Lyme disease surveillance program and were kindly provid-

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ed by Dr. Louis A. Magnarelli (The Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA). They were collected from five white-tailed deer check stations during the regulated hunting season (November and December) of 1993 and had been stored at −20 C. Three deer check stations were in the northwest region of the state (Litchfield County): Litchfield Station, Litchfield (41°45′N, 73°12′W); Housatonic Meadows State Park, Sharon (41°50′N, 73°23′W); and People's State Forest, Barkhamsted (41°57′N, 73°00′W). Two check stations were in the south-central region (Middlesex County): Devil's Hopyard State Park, East Haddam (41°28′N, 72°22′W) and Cockaponset State Forest, Haddam (41°27′N, 72°28′W).

The samples were initially screened using an enzyme-linked immunosorbent assay (ELISA) to identify the presence of immunoglobulin G (IgG) antibody to JC virus. This assay was adapted from that of Rift Valley fever antibody detection (Meegan et al., 1987) as follows. Flexible 96-well microtiter plates made of polyvinyl chloride (Dynatech Labs, Inc., Chantilly, Virginia, USA) were coated with 100 µl of a 1:16,000 dilution of JC rabbit antibody in phosphate buffered saline (PBS). These were incubated overnight at 4 C. The next day, the plates were washed three times with a washing buffer. Jamestown Canyon virus antigen and a negative control antigen (yellow fever) were added to the odd and even numbered columns, respectively, at a dilution of 1:320 and a volume of 100 µl. Antigens were incubated for 1 hr at 37 C. Plates were again washed three times and 100 µl of the serum sample diluted 1:100 was added in duplicate wells. Plates were incubated for 1 hr and washed three times. The anti-deer conjugate, a peroxidase-labeled affinity purified antibody to deer IgG that was produced in rabbit, (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, Maryland, USA) was then added (100 µl per well) at a dilution of 1:100 and the plates were again incubated for 1 hr and washed three times. We added 100 µl per well of 2.2′-azino-di[3-ethylbenzthiazoline sulfonate (6)] (ABTS) peroxidase substrate and H_2O_2 in equal volumes (Kirkegaard and Perry Laboratories, Inc.) and the plates were incubated at room temperature (20 C) for 10 min. Plates were read with a Titertek Multiskan plate reader (Flow Laboratories, McLean, Virginia) with a 414 nm filter.

Using a known positive deer sample, it was determined that 1:100 was the optimal dilution of conjugate for screening. Jamestown Canyon mouse brain antigen and a negative control yellow fever mouse brain antigen were produced by the sucrose-acetone method (Clarke and Casals, 1958). A dilution of 1:320 gave the most conclusive resultant readings as calculated from the absorbance values using a Titertek Multiskan with a 414 nm filter. Three negative and one positive control sera were included in each assay. A sample was considered positive if the difference in optical density between the positive and negative antigen wells was more than the mean background of the three negative control sera plus two standard deviations. In the first three tests the positive control was serum from an Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) (No. 83105, Yale Arbovirus Research Unit (YARU) that had previously been identified by plaque reduction neutralization (PRNT), ELISA and immunofluorescence assays. A consistently positive sample that was identified from Connecticut deer was included in all subsequent tests.

Five positive samples with a range of optical densities and a negative control were additionally screened against other California group antigens (YARU collection) to test for cross-reactivity. The following antigens were used: California encephalitis (CE) virus, LaCrosse (LAC) virus, Guaroa (GUA) virus, Jerry Slough (JS) virus, Keystone (KEY) virus, snowshoe hare (SSH) virus, San Angelo (SAN) virus and trivittatus (TVT) virus.

Samples were confirmed as either neg-

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ative or positive using an 80% PRNT (Earley et al., 1967). Sera were heated in a 56 C waterbath for 30 min. A 1:5 dilution of serum: PBS with 5% fetal calf serum (FCS)(Gibco, Life Technologies, Inc., Grand Island, New York) and 1% antibiotic/antimycotic consisting of 10,000 units/ml penicillin G sodium, 10,000 μg/ml streptomycin sulfate, and 25 μg/ml amphotericin B as Fungizone (Gibco) was made. Sera were further diluted serially two-fold, then mixed with a previously determined dose of virus resulting in 100 plaque forming units in a 96-well plate. The dose of virus necessary to form 100 plaque forming units had been previously tested by titration of the virus onto a confluent monolayer of Vero (green monkey kidney) cells. The virus-serum dilutions were incubated for 1 hr at 37 C, then 50 μl were transferred to duplicate wells of Vero cells confluent on a flat bottom 16 mm, 24-well tissue culture treated polystyrene plate (Corning Glasswares, Corning, New York). Final serum dilutions were titrated from 1:10 to 1:320. At this time, the virus was also re-titrated for a recalculation of the dose. The 24-well plates were incubated for 1 hr at 37 C and were rocked every 10 to 15 min for even adsorption on the cell monolayers. After the incubation, the cells were overlaid with 1.5 ml of equal volumes of a tragacanth/Minimum Essential Medium (MEM) Autopow (ICN Biomedical, Inc., Costa Mesa, California, USA) solution containing 4% heat inactivated FCS, 2% L-glutamine, 2% antibiotic/antimycotic and 6% sodium bicarbonate. The cultures were incubated in 5% CO_2 at 37 C. Four days post-inoculation, the overlay was removed and 1 ml of a second overlay was added, consisting of equal volumes of 1% agarose and the liquid medium described, containing 1:25,000 concentration of neutral red. Cultures were incubated at 37 C for 4 to 6 hr and were then scored; plaque reduction of 80% or more was considered positive.

Ninety-two (21%) of the 446 deer sera samples assayed by ELISA tested positive for JC virus antibody (Table 1). None of the three negative controls tested positive in 15 assays. Percent positive deer sera per site ranged from 18% to 24% and no significant differences were found at the five collection sites (chi-square analysis, $P < 0.05$) (SAS Institute, Vol. 6, Cary, North Carolina, USA). Based on these findings, infection of white-tailed deer with JC virus occurs enzootically in northwest and southcentral Connecticut. This represents the first serological evidence of JC virus in reservoir host populations from the northeastern United States, and adds to previous finding of JC virus antibody in *O. virginianus* populations in Indiana (Boromisa and Grimstad, 1987), Michigan (Grimstad et al., 1987), Minnesota (Neitzel and Grimstad, 1991), Wisconsin (Issel et al., 1972; Murphy, 1989) and the Delaware-Maryland-Virginia Peninsula of the USA (Watts et al., 1979). The result of our serologic survey is also consistent with the previous isolations of JC virus from local mosquito populations in Connecticut where the virus first was isolated nearly 30 years ago (Whitman et al., 1968).

TABLE 1. Prevalence of Jamestown Canyon virus antibody as determined by ELISA in white-tailed deer in Connecticut, 1993.

Collection station	Total serum samples tested	Number (%) positive
Devil's Hopyard, East Haddam	60	11 (18)
Cockaponset, Haddam	117	22 (19)
Litchfield Station, Litchfield	148	35 (24)
Housatonic Meadows, Sharon	107	21 (20)
People's, Barkhamsted	14	3 (21)
Total	446	32 (21)

No cross-reactivity by ELISA was seen with CE, KEY and SSH antigens with the five positive samples that were tested. A varying degree of cross-reactivity was obtained, however, with GUA, JS, LAC, SAN, and TVT. The absorbance readings were virtually identical for JS, which is considered to be indistinguishable from JC virus. Deer number 120, however, had

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cross-reactivity with JS, LAC and TVT. Thus, this deer may have been infected with either LAC or TVT. Antibody to multiple California serogroup viruses has typically been reported in other serosurveys of deer (Issel et al., 1972; Murphy, 1989; Neitzel and Grimstad, 1991). Neither LAC nor TVT has been isolated from Connecticut mosquitoes. Cross-reactivity seen with GUA and SAN was considered non-specific because neither virus is known to occur in or near this region. Therefore, the two remaining cross-reacting viruses, LAC and TVT remain possible candidates for past infection, and infection with more than one California serogroup virus may have occurred in at least one deer.

Twenty-one (23%) of the 92 JC virus antibody positive and three negative controls were assayed by PRNT to confirm the presence of specific antibody to this virus. All 21 ELISA-positive sera tested positive, thus supporting the reliability of ELISA for survey of prior JC virus infection. The present experiment was not designed to determine the magnitude of amplification of JC virus nor its potential human infection in Connecticut.

In this study we sought to determine the presence of JC virus in Connecticut through antibody detection in white-tailed deer. Using both the ELISA and PRNT, antibody to JC virus was detected in white-tailed deer of Connecticut for the first time. The virus was previously found in Connecticut through mosquito surveys; although the virus isolation attempts were limited, each time investigators looked for JC virus, it was found. Whether a 21% seroprevalence is high enough to amplify and cause substantial clinical illnesses remains unknown. That 21% antibody prevalence in Connecticut deer is lower than for other states and may be evidence that JC virus is not as significant a threat to human populations as in other regions of the United States. However, the increase in the deer herd and an associated increase in other vector-borne diseases such as Lyme disease, may be indicative of the emerging importance of JC virus. Surveys to examine human sera for evidence of exposure are needed.

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