SEROPREVALENCE OF LYMPHOCYTIC CHORIOMENINGITIS VIRUS IN NOVA SCOTIA

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Abstract. An ELISA system was used to determine the rate of seropositivity to lymphocytic choriomeningitis virus (LCMV) among a random sample of 505 Nova Scotians. Twenty (4%) were positive. Complete questionnaire data were available on all 20 seropositive subjects and on 449 seronegative subjects. Seventeen (85%) of the seropositive subjects were females ($P = 0.03$). It is concluded that infection with LCMV is present in Nova Scotia and females are more likely to be infected than males.

Lymphocytic choriomeningitis virus (LCMV) is an arenavirus that causes meningitis and a flu-like illness characterized by malaise, myalgia, retroorbital headache, and photophobia. A variety of other manifestations including testicular pain, parotid pain, and rash may occur. Outbreaks of LCMV infection have followed exposure to infected mice and hamsters raised in research laboratories. An investigation by the New York State Department of Health revealed that 55 of 60 patients with LCMV infection had pet hamsters and four others were employees of hamster wholesalers. It has also been shown that the house mouse, Mus musculus, is commonly infected with this virus. Children and others found that 9% of 484 mice trapped in urban sites in Baltimore were seropositive for LCMV. It is thus likely that infection with LCMV is common among a variety of rodents. We have not recognized cases of LCMV infection in Nova Scotia. A study of a random sample of the Nova Scotian population was used to determine the rate of LCMV infection in this province.

POPULATION, MATERIALS, AND METHODS

Study population. The names of 6,000 persons 16–70 years of age were randomly selected from a comprehensive listing of all residents of Nova Scotia maintained on the Provincial Medical Services Insurance computer. Contact with 5,915 was made by mail (85 had incomplete mailing addresses), of whom 808 (14%) responded, and 590 (10%) agreed to participate. These individuals returned their signed informed consent and completed questionnaire, and a baseline blood sample was collected by their family physician. The ages and geographic distribution of the respondents did not differ from the population from which they were selected. Follow-up of these individuals was made by mail between 1988 and 1991, one and two years after enrollment. Each year, subjects were again asked to complete a questionnaire and provide a blood sample. Five hundred five subjects were tested in this study. Four hundred sixty-nine had complete questionnaire data. This study was approved by the Nova Scotia Department of Health and by the Research Review Committee of Dalhousie University.

Determination of the presence of antibodies to LCMV. Preparation of LCMV antigen. The LCMV strain Arm/53b was grown in BHK-21 cells. All supernatants were purified by sucrose density gradient centrifugation. Uninoculated cells treated in the same fashion as inoculated cells were used as negative control antigen. Nunc polysorb plates were used; half of the plates were coated with purified LCMV strain Arm/53b diluted to a concentration of 1.25 µg/ml in phosphate-buffered saline (PBS) and half with noninfected cell supernatants (to detect nonspecific antibodies). Plates were sealed and left overnight at 0°C. After washing with 1% PBS-Tween (Merck, Munich, Germany), free plastic sites were saturated with PBS plus 5% skim milk (Difco, Detroit, MI). The plates were then sealed and incubated at 37°C for 30 min. Serum specimens diluted 1:100 were added to the wells and further diluted to 1:800 on the plate. Following sealing and incubation at 37°C for 1 hr, the plates were washed with 1% PBS-Tween, and purified peroxidase-conjugated antihuman IgG (Dako, Glostrup, Denmark) was added. Following a 1-hr incubation at 37°C the plates were washed with 1% PBS-Tween and the enzyme substrate tetramethyl benzidine plus H2O2 (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added. The reaction was stopped after 2-5 min by adding 1 M H3PO4. The plates were then read at 450 nm using an ELISA plate reader (Labsystems Multiscan Bichromatic; Life Sciences International, Gergy Ponoise, France). Anti-LCMV IgG-negative and IgG-positive control sera were included. A positive test result was three standard deviations above the optical density (OD) of the negative control serum at 1:100 dilution. In this test system all titers were positive (≥ 0 SD above the OD of the negative control serum), those 1:100 and 1:150 were positive, and those ≤ 1:100 were borderline positive results (≥ 0 SD above the OD of the negative control serum). Six serum samples were included as positive and those that were close to but still 3 SD above the OD for the negative control sera were positive results. Positive control sera were from human animal workers infected with LCMV, and negative control sera were those samples that were negative by ELISA, indirect immunofluorescence antibody testing, and Western immunoblotting.

RESULTS

Twenty (4%) of the 505 serum samples tested were positive. Six were positive at a dilution ≥ 1:300, five at 1:150–1:200, three at 1:100, and six were borderline positive. The 20 seropositive patients were compared with the 449 seronegative patients from whom all questionnaire data were
available. The seropositive subjects were more likely to be females (17 of 20 versus 3 of 20; \( P = 0.02 \)). The seroprevalence for males was three of 192 (1.6%) versus 17 of 276 (6.2%) for females \( P = 0.02 \). There was no difference in the seroprevalence rate between Halifax County, which was the most urban county, and the other counties in Nova Scotia. The mean ± SD age of the seropositive patients of 41.25 ± 15.7 years was not significantly different from that of the seronegative patients (43.38 ± 14.4 years). Ten of the 17 seropositive females were less than 40 years old and six of them were in the prime reproductive age group and six of them were in the prime reproductive age group of 18–31 years.

Seropositive subjects did not have a higher rate of febrile illness or hospitalization in the past year compared with seronegative subjects. We did note that visiting a farm was associated with seropositivity but because of our study design we do not have details regarding these farm visits.

We conclude that LCMV infection is present in Nova Scotia and that the rate of infection is significantly higher among females than males. We were not able to determine the reason for the higher infection rate among females in this study.

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