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MODOC-LIKE VIRUS ISOLATED FROM WILD DEER MICE (*PEROMYSCUS MANICULATUS*) IN ALBERTA

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ABSTRACT. Small mammals were trapped in northeastern Alberta, Canada during 1976. Blood samples from these animals were tested for virus by inoculation of suckling mice. Blood clots from two deer mice yielded isolates of the same virus. The virus was related antigenically to a number of flaviviruses which have been isolated from mammals in Central America and North America and was related most closely to Modoc virus. Physical, chemical, and biological properties of the virus were similar also to those of Modoc virus. It did not produce illness or death in deer mice inoculated in the laboratory. Neutralization tests indicated that 1/38 (3%) red squirrels (*Tamiasciurus hudsonicus*), 3/35 (9%) least chipmunks (*Eutamius minimus*), 13/109 (12%) deer mice, and 3/50 (6%) humans were infected naturally. This is the first reported evidence of infection of red squirrels and chipmunks with a Modoc-like virus. These data extend the range of Modoc-like viruses northward by 1,500 km and comprise the first isolate from mammals in the boreal forest of Canada.

INTRODUCTION

Modoc (MOD) virus, a flavivirus, has been isolated exclusively from the deer mouse (Berge, 1975). There is serologic evidence of MOD virus infection associated with aseptic meningitis in a boy in California (Emmons and Reeves, pers. comm.). Syrian hamsters, laboratory mice, and deer mice are susceptible to experimental infection with MOD virus (Johnson, 1960, 1967, 1970; Davis and Hardy, 1974; Davis et al., 1974). The role of other animals in MOD virus maintenance is unknown.

Modoc virus has been isolated from deer mice in California (Johnson, 1960, 1970), Oregon, Colorado, and Montana (Berge, 1975). Deer mice are distributed widely, however, and occur throughout the United States except in the Southeast and throughout Canada except for tundra areas. If deer mice are the primary mammalian host of MOD virus, it is logical to assume that this virus may also occur in other parts of its range.

We conducted a study in northeastern Alberta, Canada to determine the effect(s) of extensive forest clearing on small mammal populations and the occurrence of viruses that these mammals harbor. This communication reports isolation and identification of a MOD-like virus from deer mice captured in this area, as well as the natural mammalian host range and ecology of this virus.

MATERIALS AND METHODS

The study area for this project was in the boreal forest 45 km north of Fort McMurray, Alberta, Canada (111°W longitude; 57°N latitude), an area described previously (Alberta Oil Sands Environmental Research Program, 1975).

Small mammal trapping grids were established in the three major terrestrial plant community types of the region: sandy jackpine ridge, aspen upland, and black spruce bog. A grid was also established in a 2,600-ha area that had been clear-cut for development purposes. This area had been a jackpine ridge before clearing. Live traps were placed around fallen trees or in animal runways. Traps were checked daily from 10 June through 18 August 1976. Snowshoe hares (*Lepus americanus*) were bled by cardiac puncture using Vacutainers (Becton, Dickinson and Co., Rutherford, New Jersey

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07070, USA). All other wild animals were bled with sterile 15-cm Pasteur pipettes from the retro-orbital sinus of the eye. Animals were never bled more frequently than once per week, regardless of frequency of recapture. Animals were tagged with fingerling tags (Salt Lake Stamp Co., Salt Lake City, Utah 84101, USA) for later identification, and then released.

On 21 September 1976, blood samples were taken from 50 residents of Fort MacKay, Alberta, an American Indian community of approximately 250 people located 15 km north of the study area.

Blood clots for all species except hares and humans were macerated and extracted with sterile diluent. Diluent consisted of: medium M-199 with 10% heat-inactivated fetal calf serum (International Scientific Industries, Inc., Cary, Illinois 60013, USA), 0.1% gentamicin sulfate (Schering Corp., Kenilworth, New Jersey 07033, USA), 1.0% sterile tricine buffer, and 2.0% sterile sodium bicarbonate. A 0.03-ml aliquot of the aqueous phase was inoculated intracerebrally (IC) into each of 10 Swiss Albino mice of age 0–2 days (Charmany Farms Colony, Department of Veterinary Science, University of Wisconsin–Madison, Wisconsin 53706, USA). Mice were observed for 14 days for signs of illness or death. Deaths within 24 hr postinoculation (PI) were considered to be a result of inoculation trauma and were not investigated further. For all other deaths, either brain tissue of the dead mice was further passaged, or (if the suckling mouse had been eaten by the mother) additional mice were inoculated with the original sample. All re-isolation attempts were performed as above in a separate room which was reserved strictly for isolations from field-collected specimens.

Approximate size determination of the virus was accomplished by passing a virus suspension through syringe filters (Gelman Instrument Co., Ann Arbor, Michigan 48106, USA) and inoculating the filtrate into suckling mice as described above. The chloroform sensitivity test of Feldman and Wang (1961) was used, with minor modifications. Chloroform sensitivity was determined by titration of treated and control suspensions in suckling mice (as described above). Hyper-immune mouse ascitic fluid (HI-MAF) was prepared for PM00160 virus (Feild and Kalter, 1972).

Second passage suckling mouse brain suspension of PM00160 virus was inoculated onto monolayers of the following cell cultures: (1) baby hamster kidney (BHK-21); (2) African green monkey kidney (VERO); (3) albino Swiss mouse whole embryo primary (Youngner, 1954); (4) deer mouse neonatal kidney primary (Rodgers et al., 1967); (5) bovine turbinate; (6) embryonic bovine kidney; (7) human fetal tonsil; (8) mallard duck embryo primary (McManus et al., 1974); (9) chick embryo primary (Youngner, 1954); (10) mouse L-929; and (11) Rhesus monkey kidney (LLC-MK_2). Inoculated cultures and controls were observed for a minimum of 14 days for signs of cellular abnormalities or death. At 5 and 7 days PI, growth medium was removed and inoculated into suckling mice, as above. Fresh medium was added to each flask. Virus was blind passaged three times in BHK-21 and VERO cells in an effort to stimulate or enhance any cytopathogenesis (CPE) caused by PM00160 virus.

Monolayers of BHK-21 cells were inoculated with a suspension of mouse brain infected with PM00160. After 14 days, one-half of these tubes were challenged with approximately 100 LD_{50} vesicular stomatitis virus in order to determine if the cultures had become challenge-resistant due to a non-cytopathogenic infection by PM00160 virus.

Adult deer mice (two animals per dilution for dilutions 10^{-2} through 10^{-7}) were inoculated with 0.2 ml of 10-fold dilutions of PM00160 by the subcutaneous (SQ), intraperitoneal (IP), or oral route. Mice were bled on day 36 PI. Mice were observed for 90 days for signs of illness or death. Three other adult deer mice each received PM00160 virus by the IC route. These mice were observed for 25 days before being exsanguinated. Log_{10} neutralization indices (LNI's) were determined in suckling mice by the method of Lennette and Schmidt (1969).

Hemagglutination (HA) and hemagglutination-inhibition (HAI) tests were performed with sucrose-acetone extracted PM00160 mouse brain antigen (Clark and Casals, 1958). Group-specific ascitic fluids for the major arbovirus groups, and many ungrouped viruses, were obtained from the National Institutes of Health (Cunningham and Nutter, 1975). The myxo- and paramyxovirus diagnostic HA test was performed with infected mouse brain which had not been extracted (U.S. Public Health Service, 1965).

Complement fixation (CF) (Palmer et al., 1974) and tissue culture serum neutralization (SN) tests (Lindsey et al., 1976) were performed with the HI-MAF (or sera) and antigens of the following viruses: Jutiapa, Modoc, Cowbone Ridge, Phnom-Penh bat, Montana Myotis leucoencephalitis, Rio Bravo, and two unidentified flaviviruses isolated from cotton rats (*Sigmodon*

TABLE 1. Complement-fixation test reactions of PM00160 and Modoc viruses with homologous and heterologous antisera.

Antigen	Antibody	
	PM00160	Modoc
PM00160	256[a]	32
Modoc	256	64

[a] Inverse of serum dilution which fixed complement in standard test.

TABLE 2. Plaque-reduction neutralization test reactions of PM00160 and Modoc viruses with homologous and heterologous antisera.

Antigen	Antibody	
	PM00160	Modoc
PM00160	>640[a]	40
Modoc	>640	40

[a] Inverse of serum dilution which neutralized ≧90% of plaque-forming units in virus test dose.

hispidus) in Texas which are designated 71V-1251 and R-1972.

Dilutions of the small mammal and human sera were mixed with a suspension of PM00160 virus containing approximately 100 $SMLD_{50}$. These suspensions were inoculated into suckling mice, as above. Both positive and negative controls were used. A serum sample which protected at least five of the 10 inoculated mice at a minimum serum dilution of 1:10 was considered positive. All other samples were considered negative.

RESULTS

The following species (in order of abundance) were captured during the study: deer mouse (*Peromyscus maniculatus*), red squirrel (*Tamiasciurus hudsonicus*), least chipmunk (*Eutamius minimus*), masked shrew (*Sorex cinereus*), pygmy shrew (*Microsorex hoyi*), red-backed vole (*Clethrionomys gapperi*), snowshoe hare (*Lepus americanus*), short-tailed weasel (*Mustela erminea*), and northern flying squirrel (*Glaucomys sabrinus*). Deer mice were found in all habitats (jackpine, aspen, and clearing) except spruce bog and were the only species captured in the cleared area. Population density of mice increased several fold (12 vs. 62 captures) in the clear-cut area as compared to the jackpine habitat. Red squirrels and chipmunks were found everywhere but in the bog habitat, with squirrels being most abundant in aspen habitat and chipmunks most abundant in jackpine. All three of these species (mice, squirrels, and chipmunks) were captured commonly in the jackpine habitat.

Blood clots from two deer mice (PM00133 and PM00160) which were captured in the cleared area yielded viral isolates. Re-isolation from the respective clot was successful in both cases. PM00160 was chosen as the prototype and was used in all further investigations.

PM00160 virus was less than 220 nm in size. Mouse infectivity was decreased by at least 5.8 logs following chloroform treatment. Incubation time in suckling mice was 8–12 days.

PM00160 virus caused CPE only in LLC-MK_2 and VERO cell cultures. A minimum of $10^{7.5}$ $SMLD_{50}$ of PM00160/0.03 ml was detected in the tissue culture medium of the BHK-21 culture on day 7 PI. This is at least 4.2 logs more virus than had been inoculated onto the culture initially. PM00160 did not produce challenge resistance in BHK-21 cells.

LNI's (in suckling mice) for PM00133 and PM00160 viruses with PM00160 HI-MAF were 4.02 and 4.28, respectively. LNI's (in suckling mice) for PM00160 virus versus antiserum prepared by inoculating adult deer mice with PM00160 virus by the IC, SQ, IP, and oral routes were 2.0, 2.6, 3.1, and 2.6, respectively. PM00160 caused no death or signs of illness in these deer mice.

The PM00160 HA antigen was inhibited only by flavivirus group antiserum. The optimum pH range for HA was 6.0–6.2. Among the small mammal flaviviruses, PM00160 was closely related to MOD by CF and SN tests (Tables 1 and 2). There

TABLE 3. Results of in vivo neutralization tests with PM00160 virus and mammal sera.

Common name	Scientific name	No. positive*/ no. tested	Percent positive	Titer	
				Geometric mean	Range
Deer mouse	*Peromyscus maniculatus*	13/109	12	22	14–65
Least chipmunk	*Eutamius minimus*	3/35	9	13	10–14
Red squirrel	*Tamiasciurus hudsonicus*	1/38	3	16	16
Snowshoe hare	*Lepus americanus*	0/11	0	—	—
Red-backed vole	*Clethrionomys gapperi*	0/11	0	—	—
Northern flying squirrel	*Glaucomys sabrinus*	0/2	0	—	—
Human	*Homo sapiens*	3/50	6	23	16–32

* Positive = serum titer ≧1:10.

were at least four-fold differences between homologous and heterologous titers of antisera with strain PM00160 and all other small mammal flaviviruses, other than MOD (Calisher, pers. comm.). Results of in vivo neutralization tests with PM00160 virus and mammal sera are presented in Table 3. Three of the 13 seropositive deer mice (Table 3) were captured in jackpine habitat. The remaining 10 were from the clear-cut area.

DISCUSSION

PM00160 virus was isolated from two wild deer mice. Validity of the isolation is supported by successful re-isolation of the same virus from both of the blood clots from which the original isolations were made. In addition, no virus with similar properties was present in our laboratory during the course of this project.

Serologic evidence of PM00160 virus infection was detected in other deer mice, as well as red squirrels and least chipmunks captured in the same general area and at the same time as the two viremic deer mice (Table 3). None of the sera which contained PM00160 antibody contained detectable levels of Powassan or St. Louis encephalitis virus antibodies. These were the only previously described flaviviruses known to exist in Alberta. The possibility that this was actually cross-reacting antibody to another unknown flavivirus cannot be ignored, however. The relatively low antibody titers detected in mammals from the study area are not surprising. In the present study, deer mice inoculated with large doses of PM00160 virus also developed low antibody titers.

HI tests revealed that PM00160 is a flavivirus. Chloroform sensitivity, incubation period in mice and cell cultures, and HA pH range for PM00160 are also similar to these particular characteristics of the other small mammal flaviviruses (Berge, 1975).

There was no detectable antigenic difference between strain PM00160 and prototype MOD virus as measured by CF or SN tests (Tables 1 and 2). However, antigens of PM00160 and MOD viruses differed in the extent of their CF and SN reactions with antisera to other small mammal flaviviruses.

This is the first reported isolation of a MOD-like virus from mammals in the boreal forest of Canada, and extends the geographic distribution of this virus by 1,500 km. MOD-like viruses appear to be maintained in very different habitats over their range, but always in association with deer mice.

Virus isolation and NT results indicated that deer mice were also the primary mammalian hosts of PM00160 virus on our study area (Table 3). Serologic data indicated that chipmunks and red squirrels were also infected. This is the first reported evidence for naturally occurring infec-

tion of least chipmunks or red squirrels with a MOD-like virus. However, the role of chipmunks and red squirrels in maintenance of the virus is unknown. The PM00160 virus antibody prevalence in deer mice captured in the jackpine habitat was not different significantly from that in the cleared area. Apparently, PM00160 virus transmission also occurred in the simplified habitat of the cleared area and (1) was independent of deer mice population levels and (2) did not require red squirrels or chipmunks for transmission.

Although the mode of transmission of PM00160 virus is unknown, the small mammal flaviviruses may not require arthropods for transmission. MOD has been isolated from mammary tissue, urine, milk, and salivary glands of various rodents (Johnson, 1960, 1967, 1970; Davis and Hardy, 1974; Davis et al., 1974). Rodents have been infected experimentally intranasally, orally, and transplacentally (Johnson, 1967; Davis and Hardy, 1974; Davis et al., 1974). Thus, MOD virus, and presumably PM00160 virus, could be transmitted either vertically from mother to offspring via milk, or horizontally via food contaminated by urine or by inhalation of aerosolized virus.

If PM00160 virus is transmitted directly between deer mice, it is difficult to explain why increased deer mouse population density did not result in increased PM00160 virus antibody prevalence. There were no significant differences in antibody prevalence between the various sex and age classes of deer mice ($P < 0.05$ by the chi-square test).

Experimental infection of deer mice with PM00160 virus produced no apparent disease within 90 days PI. This agrees with the findings of previous studies (Johnson, 1967, 1970; Davis and Hardy, 1974; Davis et al., 1974). Natural infection with either of these viruses is probably not a significant cause of mortality in deer mice.

Infection of humans with MOD virus appears to be related to the presence of infected deer mice. Humans with PM00160 antibody lived approximately 15 km from our study area, where the viremic deer mice were captured (Table 3). Deer mice are found commonly in human habitations in the area and are the only wild mice commonly found in homes. In the only known case of human disease as a result of MOD infection, a boy in California had handled a deer mouse, and it is believed that the infection was acquired via deer mouse urine contaminated with MOD virus. (Emmons and Reeves, pers. comm.).

Extensive clearing of the forest in the Oil Sands study area near Fort MacKay appeared to increase deer mouse populations. Increased populations of deer mice did not appear to increase the rate of PM00160 virus infection in the mice. There has been a considerable increase in human population in the area in the past 10 yr and continued growth is predicted for the near future. This increased human population combined with higher deer mouse densities (as a result of clearing effects) could lead to more mouse-human contacts and more exposure of humans to PM00160 virus infections. The public health consequences of such increased human exposure await study.

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