ARBOVIRUS ISOLATIONS FROM MOSQUITOES IN MANITOBA

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ABSTRACT. During the years 1975–1979 a total of 86 arbovirus isolations were made from 3030 pools of mosquitoes collected from various locations in Manitoba. Most of the virus isolations were western equine encephalomyelitis (WEE). The main vector of WEE in Manitoba is *Culex tarsalis* Coquillett; however, WEE virus was also isolated from other mosquito species. This is the first report of WEE virus isolation from *Aedes vexans* (Meigen) and *Culiseta inornata* (Williston) in Manitoba. As far as we know, these are the first isolations anywhere of WEE from *Coquillettidia perturbans* (Walker), *Ae. pionips* Dyar and *Anopheles earlei* Vargas. *Cq. perturbans* and *An. earlei* may be important vectors of WEE where they make up a significant proportion of the mosquito population. We report here the first isolation of Cache Valley Virus (CVV) and Hart Park Flanders virus (HPF) from mosquitoes in Manitoba.

INTRODUCTION

Arboviruses in Canada were reviewed by McLintock and Iverson (1975), McLintock (1976) and Artsob and Spence (1979). The most frequent arbovirus infection in Manitoba is western equine encephalomyelitis (WEE) (Medovy 1976). Each summer since a 1975 outbreak of WEE (with 14 and 227 confirmed cases in humans and in horses, respectively), the Province of Manitoba has supported an arboviral surveillance program aimed at predicting epidemics and epizootics and limiting the initiation of large area vector control measures to imminent outbreak situations. One of the indicators used in the surveillance program consisted of the isolation of arboviruses from mosquitoes collected from various locations during the period of May to August.

This report summarizes the results of the arbovirus isolations made during the years 1975–1979.

MATERIALS AND METHODS

Live mosquitoes were collected from 9 locations in Manitoba, and arboviruses were isolated as reported by Sekla and Stackiw (1976). The 9 locations were selected on the basis of human population centers and past history of WEE activity. The Delta, Manitoba area was chosen on the basis of an isolation of a WEE virus from *Culex tarsalis* Coquillett by McKay, et al. (1968). Of the other 8 locations selected, 6 are larger centers of human population: Winnipeg, Morris, Stonewall, Selkirk, Portage la Prairie and Brandon. The other locations were Bird's Hill Provincial Park, which is on the outskirts of Winnipeg, and Oak Hammock which is a waterfowl sanctuary 30 km north of Winnipeg.

RESULTS AND DISCUSSION

During the years 1975–1979 a total of 3030 pools of live mosquitoes were tested and 86 isolations of an arbovirus were made from them. The actual number of pools processed and viruses isolated each year are summarized in Table 1. In 1975 and again in 1977, the percentage of virus isolations reached 7%, while in the other 3 years the percentage was less than 2%. In both 1975 and 1977, Manitoba experienced small outbreaks of arboviral encephalitides in humans (respectively 14 and 5 cases of WEE and 1 and 2 cases of St. Louis encephalitis (SLE). With the ex-

Table 1. Arbovirus isolations from mosquitoes 1975–1979.

	1975	1976	1977	1978	1979
No. pools tested	72	882	1045	581	450
No. arbovirus isolations	5	0	73	1	7
% pools positive	7	0	7	0.2	1.5

ception of mosquitoes collected in 1975, all live mosquitoes processed for this study were sorted to sex and species wherever possible. In 1976 mosquito populations were low, and as a result the mosquito pools brought to the laboratory contained only a small number of adults. This may have contributed to the lack of virus isolation in that year. In 1977, 1978 and 1979, some of the mosquito pools consisted entirely of adults of the same species collected from different locations in Manitoba. The variety and number of arboviruses isolated during each of the 5 years is given in Table 2. Out of the 86 isolations, 2 were Cache Valley virus (CVV), 2 were California encephalitis virus (CEV, not serotyped) 1 was a Hart Park Flanders virus (HPF), 81 were WEE virus. WEE virus was isolated from *Aedes vexans* (Meigen) and *Culiseta inornata* (Williston) for the first time in Manitoba, and to the best of our knowledge this is the first time WEE virus has ever been isolated from *Cq. perturbans* (Walker), *Ae. pionips* Dyar and *Anopheles earlei* Vargas. In addition, this is the first report of the isolation of CVV and HPF from mosquitoes in Manitoba and the first isolation of CE virus from mosquitoes collected from the southern part of Manitoba.

Table 2. Types and numbers of arbovirus isolations from field-collected female mosquitoes in Manitoba.

	1975	1977				1978	1979					
Species	WEE	WEE	MIR	CVV	MIR	WEE	WEE	MIR	HPF	MIR	CEV	MIR
Cx. tarsalis		33 (9–80)[2]	1:347						1 (21)	1:1347		
Cx. spp.[1]		9 (25–60)	1:371									
Cs. inornata		8 (4–54)	1:706	2 (1–2)	1:2724		3 (80)	1:764			1 (8)	1:3057
Ae. vexans		8 (60–80)	1:711			1 (80)						
Ae. spp.[3]		8 (28–87)	1:761								1 (64)	1:5784
Ae. pionips		1 (60)	1:2773									
An. earlei		3 (4–6)	1:87				1 (14)	1:42				
Cq. perturbans		1 (7)	1:202									
Mixed spp.	5 (15–250)											

1) 97% *Cx. tarsalis*, 3% *Cx. restuans*.
2) ( ) range of adults/pools.
3) Primarily *Ae. vexans*.
WEE—Western Equine Encephalomyelitis.
CVV—Cache Valley Virus.
CEV—California Encephalitis Virus.
HPF—Hart Park Flanders.
MIR—Minimum Infection Ratio. This ratio assumes at least one mosquito is infected in each pool containing virus. This is compared to the total no. of adults tested.

The virus causing SLE has not been isolated from mosquitoes in Manitoba, although three human cases were diagnosed (1 in 1975, 2 in 1977) on the basis of serological findings. Conversely, no human cases of CEV or CVV infections have been diagnosed in Manitoba.

Cx. tarsalis is considered to be the main vector of WEE virus, and Henderson et al. (1979) demonstrated that a Manitoba strain of *Cx. tarsalis* was highly susceptible to infection with a Manitoba isolate of WEE virus. A range of mosquito species appears to be carrying viruses, especially WEE (Table 2). McLintock (1976) has suggested that various *Aedes* spp. may be involved in the amplification of the WEE virus early in the spring. Burton and McLintock (1970) indicated that in northern areas, where *Cx. tarsalis* is not abundant, the primary vector may be *Cs. inornata* or an *Aedes* sp.

From Table 2, we can see that *An. earlei* and *Cq. perturbans* may also be important vectors of WEE virus. *An. earlei* had the highest minimum infection ratio (MIR—assuming at least 1 infected mosquito per pool) of any species. In fact, in 1979, 4 pools containing a total of 42 mosquitoes yielded 1 WEE isolate. In 1977, 54 pools of *An. earlei* and 36 pools of *Cq. perturbans* were submitted for virus isolation. The minimum infection ratios were 1:87 and 1:202 compared to 1:347 for *Cx. tarsalis*, the primary vector of WEE (Table 2).

Of the 81 isolations of WEE viruses, 76 were obtained from mosquitoes identified as follows: *Cx. tarsalis* 33 (43.4%, *Cx.* spp. 9(11.8%), *Ae. vexans* 9(11.8%), *Ae. pionips* 1 (1.3%), *Ae.* spp. 8 (10.5%), *An. earlei* 4 (5.2%), *Cs. inornata* 11 (14.5%) and *Cq. perturbans* 1 (1.3%). Both of the Cache Valley viruses and one of the California encephalitis viruses were isolated from *Cs. inornata* while the 2nd CEV was isolated from *Ae.* spp.; the HPF was isolated from *Cx. tarsalis*. The number of mosquitoes in each of the pools from which arboviruses were isolated and the minimum infection ratio is shown in Table 2. From our experience, the recommended size of a mosquito pool submitted for virus isolation is 60 adult mosquitoes. However, as shown in Table 2, arboviruses may be isolated from small pools of only 1 or 2 mosquitoes.

The earliest virus isolation was from mosquitoes collected on 8 July and the latest from mosquitoes collected on 16 August, confirming the clinical impression that the infective season in Manitoba is a brief one. Before and after these dates, the dominant species of mosquitoes submitted for virus infection during the years 1977 and 1979 were *Cs. inornata* and *Ae. vexans*. In 1977, collections were begun on 17 May and terminated on 31 August; in 1979, they were begun on 28 May and terminated on 18 August.

The isolation of arboviruses from live mosquitoes appears to be a sensitive indicator system, capable of demonstrating the presence of a variety of arboviruses. Although not discussed here, viruses were present in mosquitoes collected from nearly all of the locations sampled.

ACKNOWLEDGMENTS

We wish to thank the Province of Manitoba for financial support and the Manitoba Arbovirus Surveillance Committee for their cooperation and advice. We are grateful to the Manitoba Youth Secretariat for providing the technical assistants, and to Drs. L. Spence and H. Artsob of the National Arbovirus Reference Center for supplying antigen and antisera and confirming our isolates.

References Cited

Artsob, H. and L. Spence. 1979. Arboviruses in Canada. pp. 39–65. *In* Kurstak, E. (Ed). Arctic and Tropical Arboviruses. Academic Press, New York, N.Y.

Burton, A. and J. McLintock. 1970. Further evidence of Western encephalitis infection in Saskatchewan mammals and birds and in reindeer in Northern Canada. Can. Vet. J. 11:232–235.

Henderson, L. P., R. A. Brust and F. C. Wong.

1979. Biological transmission of Western encephalomyelitis virus by *Culex tarsalis* Coquillett. Mosquito News 39:385–390.
McKay, J., W. Stackiw and R. A. Brust. 1968. Western encephalitis in Manitoba in 1966. Manitoba Med. Rev. 48:56–57.
McLintock, J. 1976. The arbovirus problem in Canada. Can. J. Public Health 67, Suppl 1:8–12.
McLintock, J. and J. Iversen. 1975. Mosquitoes and human diseases in Canada. Can. Ent. 107:695–704.
Medovy, H. 1976. The history of Western encephalomyelitis in Manitoba. Can. J. Public Health 67, Suppl 1:13–14.
Sekla, L. H. and W. Stackiw. 1976. Laboratory diagnosis of Western encephalomyelitis. Can. J. Public Health 67, Suppl 1:33–40.

SUSCEPTIBILITY OF *AEDES AEGYPTI* TO SYNTHETIC PYRETHROIDS COMPARED WITH A NEW INSECT GROWTH REGULATOR[1]

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ABSTRACT. Three synthetic pyrethroids and a new insect growth regulator (IGR) were evaluated in the laboratory against early 3rd instar *Aedes aegypti* (Linnaeus). Of all compounds tested the Atroban® permethrin formulation was the most effective. *Ae. aegypti* were more susceptible at the LC_{90} level to the synthetic pyrethroids than to the IGR Bay Sir 8514(2-Chloro-N-[[[4-(trifluoromethoxy) phenyl]amino]carbonyl]benzamide). LC_{90} values for Atroban, Ectiban®, fenvalerate, and Bay Sir 8514 were 0.44, 3.84, 38.17, and 51.42 ppb, respectively.

INTRODUCTION

The increasing threat of mosquito resistance to chlorinated hydrocarbons and organophospate larvicides has initiated research in alternative chemicals for mosquito control. The potential control value of insect growth regulators (IGR) and synthetic pyrethroids has been extensively tested in both laboratory tests and field trials (Jakob and Schoof 1971 and 1972, Jakob 1972, Hsieh and Steelman 1974, Lowe et al. 1975, Mulla et al. 1975, Dame et al. 1976, Mulla and Darwazeh 1976, Rogers et al. 1976, Mulla et al. 1978, Yap and Jamaludin 1979). Recently, one of the newer, IGRs, Bay Sir 8514 (2-chloro-N-[[[4-(trifluoromethoxy)phenyl]amino]carbonyl]benzamide), was tested against the house fly, *Musca domestica*, L. (Chang 1979). Reported here is an evaluation of the comparative laboratory susceptibilities of Bay Sir 8514 and 3 synthetic pyrethroids to *Aedes aegypti* (Linnaeus) larvae.

MATERIALS AND METHODS

The compounds tested against *Ae. aegypti* were:

Bay Sir 8514: tested as the technical formulation (95% pure) provided by Chemagro Agricultural Division, Mobay Chemical Corporation.

fenvalerate (SD 43775): tested as the technical formulation (95% pure) provided by Shell Development Company.

Atroban® (BW 21Z permethrin): tested as the technical formulation (96.3% pure) provided by Burroughs Wellcome Company.

[1] The investigation reported in this paper (No. 80–7–5) is in connection with a project of the Kentucky Agricultural Experiment Station and is published with approval of the Director.

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