Two Viruses Isolated from Rodents (Clethrionomys gapperi and Microtus pennsylvanicus) Trapped in St. Lawrence County, New York*

Authors: WHITNEY, ELINOR, ROZ, ALBERT P., and RAYNER, GEORGE A.

Source: Journal of Wildlife Diseases, 6(1) : 48-55

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-6.1.48

Two Viruses Isolated from Rodents (*Clethrionomys gapperi* and *Microtus pennsylvanicus*) Trapped in St. Lawrence County, New York*

ELINOR WHITNEY, ALBERT P. ROZ, and GEORGE A. RAYNER

From the Division of Laboratories and Research, New York State Department of Health, Albany

Received September 6, 1969

Abstract

Four strains of *C. gapperi* virus were isolated from 3 *Clethrionomys gapperi* and 47 strains of *Microtus* virus from 15 *Microtus pennsylvanicus* and 1 *Mus musculus*. One of the *Microtus* strains was isolated from a pool of 20 mites while the others were from rodent tissues. These agents were insensitive to ether and sodium desoxycholate, withstood freezing at —70 C for 3 years and lyophilization without loss of titer, and were not killed when heated at 60 C for 1 hour. Their size as determined by filtration was less than 50 mμ and greater than 20-35 mμ. The strains within each group appear to be similar. The illness induced in suckling mice by the *C. gapperi* agents had a 5-day incubation period followed by prostration and death with a histologic picture of extensive encephalomalacia. The incubation period in mice for the *Microtus* agents was 9 to 11 days followed by convulsions and death. Histopathology showed meningeal infiltration and necrosis of the molecular layer. No antigenic similarity was detected between the *C. gapperi* and *Microtus* viruses by cross complement-fixation test.

Introduction

An arbovirus survey of tissues and ectoparasites from live-trapped wildlife collected from both mainland and island sites in St. Lawrence County, New York, was conducted from 30 June, 1964, to 26 August, 1965.[12] Infectious agents were isolated from 3 of 20 *Clethrionomys gapperi* (red-backed mouse), from 15 of 96 *Microtus pennsylvanicus* (meadow voles) and 1 of 43 *Mus musculus* (house mouse). The properties of these virus agents are the subject of this report.

*Supported in part by grant 1-S01-FR-05247 from the National Institutes of Health, USPHS, Bethesda, Maryland.

Materials and Methods

Five hundred and sixty-one specimens from ectoparasites (Table 1), brain, kidney, spleen, liver, and blood, or pooled suspensions of the latter 3 tissues were investigated. The methods of collection, shipment, and subsequent storage of specimens, preparation of suspensions from tissue or arthropod specimens, maintenance of virus strains, preparation of hyperimmune sera, and technic of ether and sodium desoxycholate sensitivity tests have been recently described.[1,7-11]

Virus isolation attempts were performed in 1-day-old mice, Nylar strain. Two groups of 8 mice were inoculated intracerebrally (ic) with 0.03 ml of the tissue or ectoparasite suspensions.

In neutralization (N) tests, equal volumes of undiluted or 2-fold serum dilutions were mixed with a constant virus dilution calculated to contain 100 LD_{50}. The ic route of inoculation was used. Methods and interpretation of N tests were the same as recently published.[7]

The complement-fixation (CF) method of Kent and Fife[5] using 5 units of complement was adapted to a micromethod described by Sever.[6] The antigens were prepared according to the sucrose-acetone extraction technic of Clarke and Casals.[2]

In heat inactivation experiments, aliquots of 10% suckling mouse brain (smb) preparations were dispensed into glass vials and sealed before submersion in a waterbath held at stated temperatures for varying periods. They were then titrated in 2-day-old or weanling mice.

Isolation and Properties

Fifty-one infectious agents were recovered from 561 specimens. Four strains were isolated from 3 *Clethrionomys gapperi*, 2 from the spleen and liver of one animal, the other 2 from pooled blood, and liver and spleen suspensions. Fifty-two of 64 mice inoculated with preparations from which the 4 strains were isolated showed ruffled fur, arched back, lethargy, poor balance, paralysis,

TABLE 1. *Ectoparasites from* C. gapperi *and* M. pennsylvanicus *tested for virus isolation*

	No. animals	No. ectoparasites	No. isolates
SIPHONAPTERA			
Ctenophthalmus pseudagyrtes	2	3	0
Epitedia w. wenmanni	1	2	0
Nosopsyllus fasciatus	7	13	0
Orchopeas howardii	2	5	0
Orchopeas leucopus	9	12	0
Orchopeas sexdentatus pennsylvanicus	1	3	0
Peromyscopsylla catatina	2	3	0
Unidentified Siphonaptera	1	1	0
ACARINA			
Ixodes species	1	3	0
Unidentified Acarina	10	103+	1*

* Pool contained 20 mites.

and died 9 or 10 days postinoculation. On subsequent passage of 10% smb suspension the incubation period was reduced to 5 days and the LD_{50} titers were from $10^{9.1}$ to $10^{10.2}$ per gram of brain tissue. These agents are referred to in this paper as *C. gapperi* viruses. The lack of materials precluded any attempts at reisolation.

Of the 47 remaining strains, 46 were isolated from mites, kidney, brain, spleen, liver, and blood from 15 *Microtus pennsylvanicus* (Table 2). Another strain was isolated from the kidney of a *Mus musculus*. Five hundred and eighty-four of the 720 mice inoculated with these preparations developed convulsions 12 to 14 days postinoculation and died. The incubation period was reduced on further passage of smb tissue to 8 to 9 days; the LD_{50} titers ranged from $10^{7.2}$ to $10^{9.0}$ per gram. Successful reisolations were made from 5 original tissue suspensions frozen for from 4 to 6 weeks. In this report, these strains are called the *Microtus* agents.

TABLE 2. Microtus *virus isolated from* 96 Microtus pennsylvanicus

10% tissue suspension	No. strains isolated
Blood	2
Spleen	3
Liver	3
Kidney	15
Brain	10
Pool (spleen and liver)	1
Pool (spleen, liver and blood)	11
Ectoparasites in 1 ml volume	
One pool of 20 unidentified mites	1
TOTAL	46

Histologic sections of suckling mice infected with the *C. gapperi* virus showed encephalitis and severe extensive encephalomalacia. Tissue from animals infected with the *Microtus* agent demonstrated meningeal infiltration, encephalomalacia, and necrosis of the molecular

TABLE 3. *Physical properties of* C. gapperi *and* Microtus *agents*

	C. gapperi 64-7855		*Microtus* 64-7947	
	LD_{50}/gram	Passage level	LD_{50}/gram	Passage level
Size. By filtration				
LD_{50}/gram original	9.1	smb_3	8.3	smb_8
50 mμ*	8.4		8.1	
20-35 mμ†	no activity		no activity	
Sensitivity				
LD_{50}/gram control	9.9	smb_2	8.1	smb_5
Sodium desoyxcholate	9.9		8.8	
LD_{50}/gram control	10.2	smb_2	9.2	smb_5
Ether	10.5		8.8	
Stability				
Original	9.8	smb_4	8.9	smb_4
Lyophilization	not tested		9.0	smb_4
Stored frozen 3 years —70 C	9.8	smb_4	9.4	smb_4
Heat				
Unheated	10.1	smb_3	8.9	smb_0
1 hr 60 C	6.4		>8.4	

*Millipore Filter Corporation, Bedford, Massachusetts

†Carl Schleicher and Schuell, Keene, New Hampshire

layer of the central portion of the cerebellum. No nuclear or cytoplasmic inclusions were seen.

The incubation time, symptoms and histology suggest that the *C. gapperi* agents are different from the *Microtus* agents. There may be, however, similarity or identity among the strains within each of the two species.

The *C. gapperi* and *Microtus* agents appeared to have similar physical properties, such as size, their reaction to ether and sodium desoxycholate treatment, and the stability of storage at —70 C for 3 years without loss of titer. They differed in their resistance to heat. The *C. gapperi* strain showed a 4 $\log_{10}$ loss of infectious titer after 1 hour at 60 C (Table 3).

Host spectrum. Suckling mice were susceptible to the *C. gapperi* agents by both ic and intraperitoneal (ip) routes of inoculation but to *Microtus* agents only by the ic route. Both agents infected weanling mice by ic inoculation. With the *Microtus* strains deaths were irregu-

TABLE 4. *Neutralization tests in suckling mice with* C. gapperi *and* Microtus *strains*

Virus strains	Hyperimmune mouse sera															
	C. gapperi								*Microtus*							
	64-7855				Pool 452				64-7947				Pool 593			
	No. LD_{50} virus	Ser. dil.	Result		No. LD_{50} virus	Ser. dil.	Result		No. LD_{50} virus	Ser. dil.	Result		No. LD_{50} virus	Ser. dil.	Result	
C. gapperi																
64-7855	100	1:2	16/16*													
		1:8	10/16													
	502	θ	6/16		199	θ	6/8									
Pool 452	316	1:2	16/16		316	1:2	16/16									
		1:4	15/16			1:4	14/16									
		1:8	14/16			1:8	10/16									
		1:16	13/16			1:16	3/16									
Pool 497	398	θ	13/16													
Microtus																
64-7947									199	θ	12/14					
										1:4	10/15					
										1:16	1/15					
18 other strains									100—1000	1:2	10-15/16					
Pool 593									166	θ	5/8		166	1:2	9/16	
House mouse strain	200	θ	0/15		200	θ	0/10		200	θ	16/16		200	θ	14/14	

* Number of mice surviving/number of mice inoculated

θ = undiluted

lar at the higher dilutions. Convulsions began 8 to 9 days postinoculation and frequently persisted for 10 to 12 additional days.

Guinea pigs infected ic with 0.1 ml of a 10% smb suspension of *Microtus* strain 64-7947 showed no rise in temperature or other signs of infection during a 28-day observation period. Ten-per-cent chorioallantoic membrane (cam) suspensions from 3 serial passages of the *Microtus* strain 64-7947 on cam of 12-day-old embryonated hens' eggs failed to elicit any infection in suckling mice.

Cell cultures. Ten-per-cent smb suspensions of *C. gapperi* strain 64-7855 and *Microtus* strain 64-7947 failed to propagate in cell cultures of continuous lines of FL†, HEL‡, or BHK21¶. The intermediate and terminal passages were checked for virus by ic inoculation of suckling mice.

To rule out ectromelia, footpads of weanling mice were inoculated with 0.03 ml of a 10% smb suspension of *Microtus* strain 64-7947. There were 3 control animal groups: one received 0.03 ml of 10% normal smb suspension; the 2nd, 0.03 ml of diluent, 0.75% bovine plasma albumin; and the 3rd was uninoculated. In 2 experiments no swelling or redness was detected; in the third trial the test group showed slight transient swelling and redness on days 7 to 11.

TABLE 5. *Complement-fixation tests of three* Microtus *strains*

Sucrose-acetone antigens	Mouse sera	
	64-7947 4/13/65	Normal 5/18/62
64-7947	1024/16*	<16
64-8906	1024/16	<16
64-8912	1024/16	<16
Normal	<16	<16

*Reciprocal highest dilution of serum giving 50% hemolysis/reciprocal highest dilution of antigen giving 50% hemolysis.

Antigenic relationships between *C. gapperi* and *Microtus* virus strains were determined by N and CF tests. In N tests with hyperimmune sera prepared against 2 *C. gapperi* strains (64-7855 and pool 452), the 3 *C. gapperi* viruses appeared similar (Table 4).

The hyperimmune serum prepared with the *Microtus* strain 64-7947 in a 1:2 dilution neutralized not only 199 LD_{50} of the homologous strain but 100 to 1000 LD_{50} of 18 other *Microtus*

†FL = human amnion cell line originally established by Dr. Jørgen Fogh and Rosemary O. Lund (Proc. Soc. Exper. Biol. & Med., 1957, *94*, 532-537).

‡HEL = human embryonic lung received August 9, 1962 from Dr. E. V. Davis, Communicable Disease Center Field Station, Phoenix, Arizona.

¶BHK21 = clone 13 baby hamster kidney cells received from Dr. Sonja Buckley, Rockefeller Foundation Laboratory, N. Y. C. on April 17, 1964.

TABLE 6. *Cross complement-fixation test of* C. gapperi *and* Microtus *agents*

Sucrose-acetone antigens	Titers with mouse sera		
	C. gapperi 64-7855	*Microtus* 64-7947	Normal
C. gapperi — 64-7855	256/16*	<4	<4
Microtus — 64-7947	<4	512/16	<4
Normal	<4	<4	<4

*Reciprocal highest dilution of serum giving 50% hemolysis/reciprocal highest dilution of antigen giving 50% hemolysis

agents. Similarity of the strains is also indicated by cross-neutralization tests of *Microtus* Pool 593 with the two *Microtus* sera (Table 4).

The results of the CF test with 3 antigen preparations and serum 64-7947 indicate that the *Microtus* agents are at least similar if not identical (Table 5).

The infectious agent isolated from the kidney of the house mouse was shown to belong to the *Microtus* group of agents and not to the *C. gapperi* (Table 4).

Major antigenic differences between the *C. gapperi* and *Microtus* viruses were noted in cross CF tests and are shown in Table 6.

Sera prepared against the following infectious agents failed to neutralize *C. gapperi* and *Microtus* strains: eastern and western equine encephalomyelitis, Powassan, St. Louis encephalitis, Maguari, Cache Valley, Flanders, epizootic hemorrhagic disease (EHD) of deer, MM, herpes simplex, Theilers TO, lymphocytic choriomeningitis (LCM), and psittacosis. Immune sera to Colorado tick fever and Q fever also failed to neutralize the *Microtus* agents; sera against Modoc, California encephalitis virus, trivittatus, and reovirus type 3 failed to neutralize the *C. gapperi* strains.

Mouse hyperimmune sera prepared in September, 1965, against *C. gapperi* strain 64-7855 and in April, 1965, against *Microtus* strain 64-7947 were checked for antigenic similarity with several mouse agents by Microbiological Associates, Inc.** No reactivity was noted with antigens of reovirus type 3, Theilers GDVII, and K virus. Both sera reacted in hemagglutination-inhibition (HI) tests with antigen of minute virus of mice (MVM) with titers of 1:80 and 1:40, respectively. In the CF test with mouse hepatitis virus (MHV) antigen, both sera had a titer of 1:80. Two pools of

**Microbiological Associates, Inc., 4733 Bethesda Avenue, Bethesda, Maryland 20014.

TABLE 7. *Trapsites of animals yielding* C. gapperi *and* Microtus *agents*

Species	Number animals trapped	Site	Trapped Date
Clethrionomys gapperi	1	Mainland, Louisville, Wilson Hill Rd.	8/ 8/64
	1	Mainland, Norfolk, O'Brien Rd.	10/29/64
Microtus pennsylvanicus	1	Mainland, Norfolk, O'Brien Rd.	11/17/64
	1	Barnhart Island, Pole 37A	8/ 4/64
	1	Barnhart Island, Pole 37A	8/ 6/64
	1	Barnhart Island, Pole 42C	8/20/64
	2	Barnhart Island, Pole 44B	8/31/64
	1	Barnhart Island, Pole 44B	9/ 1/64
	1	Barnhart Island, Power Dam	8/19/64
	1	Barnhart Island, South Picnic	9/10/64
	1	Barnhart Island, South Picnic	11/17/64
	1	Barnhart Island, Pole 121C	3/ 8/65
	1	Mainland, Louisville, Swamp	10/17/64
	1	Mainland, Louisville, Swamp	10/23/64
	2	Mainland, Wonderland, Route 37	3/ 3/65
	1	Mainland, Wonderland, Route 37	3/ 5/65
Mus musculus	1	Mainland, Brasher, Keenan Farm	6/30/64

weanling Nylar strain mouse sera collected in September, 1965, and in July, 1967, from animals of approximately the same age as those used for immunization were submitted to Microbiological Associates for murine virus antibody determination. Again, no reactivity was noted with reovirus type 3, Theilers GDVII, and K virus, but both pools of sera reacted in HI tests with antigens of MVM with titers of 1:160 and 1:1280, respectively. In the CF test with MHV antigen, the titers were 1:10 and 1:20, respectively.

Neutralizing antibodies against agents isolated from either *Microtus* or *C. gapperi* were not detected among human residents of St. Lawrence County in 53 and 32 sera, respectively.

Discussion

Two different rodent viruses were discovered, one from *Clethrionomys gapperi*, the other from *Microtus pennsylvanicus* and *Mus musculus*. Tissues from 20 other mammalian species totalling 438 animals, also from 201 amphibians and 95 reptiles failed to yield any infectious agents. The *C. gapperi* viruses were isolated from animals trapped over a 4-month period at 2 different mainland sites. Two animals in the same area were caught 19 days apart (Table 7). The *Microtus* agents were from animals collected over an 8-month interval; three of the *Microtus* were trapped at Pole 44B on 8/31 and 9/1/64 (Table 7). The persons trapping reported no observations of sick animals.

Hamilton in 1937[1] described among the *Microtus* of New York State an illness with signs of twitching about neck and shoulders, thrusting of hind legs straight backward, followed shortly by death. Neither gross nor microscopic examination with particular attention to the brain revealed any evidence of toxoplasma or other contributing disease organisms. Mice inoculated with our *Microtus* viruses developed convulsions similar to those described by Hamilton.

It is not yet possible to classify the agents isolated from *Clethrionomys gapperi* and *Microtus pennsylvanicus*. Their small size and their apparent lack of envelope may place them in either the picorna or the parvovirus group.[3] Determination of the nucleic acid type, DNA or RNA, is essential to gain further information for their classification. Such studies are at present, however, not possible due to the failure of the viruses to propagate in tissue cultures.

The findings by Microbiological Associates of HI titers with MVM antigen and CF titers with MHV antigen not only in the hyperimmune mouse sera prepared with viruses of *C. gapperi* and *Microtus* but also in two pools of sera from uninoculated mice of the same age and strain collected in 1965 and 1967, indicate that these reactions are the result of latent infection of the Nylar mice with MVM and MHV viruses, rather than cross reactions with the *C. gapperi* and *Microtus* agents.

We failed to find serologic evidence of past infections with *C. gapperi* or *Microtus* viruses in a survey of a small group of residents in St. Lawrence County.

Acknowledgement

Grateful acknowledgement is made to Dr. Hugo Jamnback, Mr. Robert G. Means, and Mr. Thomas H. Watthews of the State Museum and Science Service of the New York State Education Department, Albany, for the identification and collection of the specimens, and to Dr. Doris Collins for histopathologic examinations.

Literature Cited

1. ANDREWES C., and HORSTMANN, D. M. 1949. The susceptibility of viruses to ethyl ether. J. Gen. Microbiol. 3: 290-297.
2. CLARKE, D. H., and CASALS, J. 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. & Hyg. 7: 561-573.
3. FENNER, F. 1968. The Biology of Animal Viruses. Academic Press, N.Y. and London. 845 p.
4. HAMILTON, W. J., Jr. 1937. The biology of the microtine cycles. J. Agricultural Res. 54: 779-790.
5. KENT, J. F., and FIFE, E. H., Jr. 1963. Precise standardization of reagents for complement-fixation. Amer. J. Trop. Med. & Hyg. 12: 103-116.
6. SEVER, J. L. 1962. Application of a microtechnique to viral serological investigations. J. Immunol. 88: 320-329.
7. THEILER, M. 1957. Action of sodium desoxycholate on arthropod-borne viruses. Proc. Soc. Exper. Biol. and Med. 96: 380-382.
8. WHITNEY, E. 1963. Serologic evidence of group A and B arthropod-borne virus activity in New York State. Amer. J. Trop. Med. & Hyg. 12: 417-424.
9. WHITNEY, E. 1964. Flanders strain, an arbovirus newly isolated from mosquitoes and birds of New York State. Amer. J. Trop. Med. & Hyg. 13: 123-131.
10. WHITNEY, E. 1965. Arthropod-borne viruses in New York State: Serologic evidence of Groups A, B and Bunyamwera viruses in dairy herds. Amer. J. Vet. Res. 26: 914-919.
11. WHITNEY, E., and JAMNBACK, H. 1965. The first isolations of Powassan virus in New York State. Proc. Soc. Exper. Biol. and Med. 119: 432-435.
12. WHITNEY, E., JAMNBACK, H., MEANS, R. G., and WATTHEWS, T. H. 1968. Arthropod-borne virus survey in St. Lawrence County, New York. Arbovirus reactivity in serum from amphibians, reptiles, birds, and mammals. Amer. J. Trop. Med. & Hyg. 17: 645-650.