BITING FLY — ARBOVIRUS PROBE IN INTERIOR ALASKA (CULICIDAE) (SIMULIIDAE) — (SSH:*CALIFORNIA* COMPLEX) (NORTHWAY:BUNYAMWERA GROUP)

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ABSTRACT. A probe was undertaken to determine what viruses in the *California* complex, of the California group, occurred in interior Alaska, and if biting flies were hosts. An insect net was used instead of traps, and an LD-40 wide mouth liquid nitrogen container was used for asphyxiating, freezing, and storing the specimens in the field. Biting flies were collected at intervals during the summer from June, 1970 through August, 1972 at five localities: Northway, Dot Lake, Fairbanks, New Minto and Tanana. Snowshoe hare (SSH) virus of the *California* complex was recovered from *Aedes cinereus* Meigen, *A. communis* (DeGeer), *A. excrucians* (Walker), *A. fitchii* (Felt & Young), *A. hexodontus* complex, *A. intrudens* Dyar, *A. punctor* complex, and from a black fly, *Simulium* n. sp?. There were 48 recoveries, some from all localities. This is the only virus of the *California* complex known to occur in Alaska. Therefore California encephalitis disease caused by CE virus, the type virus of the complex, is now believed not to occur in Alaska. Northway virus, of the Bunyamwera group, was discovered from *A. excrucians, hexodontus* complex, *punctor* complex and *Culiseta alaskaensis* (Ludlow). There were 5 recoveries from three localities: Northway, Dot Lake, and New Minto. One recovery was from pool 0234 containing the Northway type virus. The viruses were recovered by the Virology Unit at the Arctic Health Research Center and identified by the Arbovirus Section at the Center for Disease Control in Atlanta, Georgia. The information concerning the viruses and mammals has been published.

INTRODUCTION AND ACKNOWLEDGMENTS. In 1969 the staff of the Arctic Health Research Center found evidence that virus of the *California* complex: California group, occurred in people in east central Alaska (Feltz et al. 1972). Later that year snowshoe hares (*Lepus americanus*) were found with antibody titers of 1:8 against virus (prototype BFS 283) of the *California* complex. California encephalitis virus (CE), the causal agent of California encephalitis disease was thought responsible. The Entomology and Virology Units endeavored to determine if biting flies were involved. Snowshoe hare virus (SSH), a subtype of the *California* complex, was the only virus of that complex recovered from biting flies or mammals, so it is now believed CE virus was not involved. The classification nomenclature used here is that suggested by the Subcommittee on Immunological Relationships among Catalogued Arboviruses (Sudia et al. 1971) and differs from that used by Feltz et al. (1972) and Ritter and Feltz (1974).

Since this work could not have been done without the collaboration of others, my sincere appreciation and thanks are extended to the following: Elmer T. Feltz and Donald G. Ritter for suggestions and related technical assistance regarding field and laboratory preservation of insects for virus recovery; Esther Bushway, Fernanda Jacobs and Greg Dudas for the initial virus recovery from pools of biting flies, using the mouse-litter technique (one litter per pool); Betty List-Young for host identification by serologic means, of the

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blood meals of engorged biting flies; Donald Hartbauer, in charge of the mouse colonies; and the following, then at the Center For Disease Control, Atlanta, Georgia: Dr. Charles H. Calisher and his staff in the Arbovirus Section, for identification of the virus isolates; and Dr. W. D. Sudia for an introduction to field and laboratory techniques.

Since hares were known to be involved, biting fly study sites with virus potential could be selected. The criteria for site selection usually included: habitats of biting fly larvae and adults, the presence of hares and voles, and previous evidence of virus in humans, hares or indicator rabbits (laboratory reared *Oryctolagus caniculus*) (Ritter and Feltz 1974). The location of the rabbits at the sites depended upon a family willing to care for them. The virologists tested the rabbits before bringing them to the sites, and then repeated tests at intervals to determine if and when infection occurred.

LOCALITIES AND SITES. All the sites are in interior Alaska (Fig. 1) in or near the lowlands where marshes and bogs are interspersed throughout moderate to sparse forests consisting of willow (*Salix*), alder (*Alnus*), spruce (*Picea*), larch (*Larix*), birch (*Betula*), cottonwood and aspen, (both *Populus*). The dominant species reflected the stage of succession.

The frost-free period approaches 90 days, but frost can occur any time. Average annual precipitation is 12 in., and humidity is low too. The summer of 1970 was the 3rd consecutive dry summer throughout much of the interior and many mosquito breeding pools, especially temporary ones, again dried before the larvae and pupae completed development, reducing populations of the species favoring such pools. The population had not returned to "normal" even by 1972.

The daily change in sunset time is approximately 3 min so daylight, including twilight, is continuous from late May to mid-July at the more northern sites. As elsewhere at lower latitudes, weather permitting, mosquitoes are most active the 1st 30 min after sunset. Also toward sunset and in moonlight hare activity increases in the open and along roadsides, but voles are active day and night.

Five localities were involved: Northway, Dot Lake, Fairbanks, New Minto and Tanana.

NORTHWAY had 2 sites. Site 1 was a cleared, unnatural, people-dominated area on an extensive fill by Airport Lodge. The indicator rabbits were in the open by a large pond. Site 2, about 0.5 mi away, at the southeast end of the airfield, had reverted to a natural area with all the desired features. The 1970 visits, usually at both sites, were July 8–9, 13–14, 22–23, 27–28, Aug. 5–6, 10–11, 19–20, 24–25, Sept. 2–3, and 8 when heavy snow stopped activity. In 1971 Site 2 was visited Aug. 4–5, 25–26; and in 1972, Aug. 16–17.

DOT LAKE had 2 sites. Site 1 was by the Indian village at the lake. Besides the usual habitats there was a Diamond willow grove, floodlit lawn, landing strip, and small wooded campground. Site 2, where the indicator rabbits were in 1970, was much drier and about a mile down the highway in a cleared rural yard and field in aspen woods. The 1970 visits were July 7–8, 15–16, 20–22, 29–30, Aug. 3–4, 12–13, 17–18, 26–27, 31–Sept. 1, and 9. Thereafter only Site 1 was visited Aug. 2–3, 23–24, 1971 and Aug. 14–15, 1972.

FAIRBANKS had 6 sites, some in the lowlands, others on higher ground. At Dome the indicator rabbits were in a cleared rural yard and the site extended about 1 mi along the railroad at the base of a wooded slope. Visits in 1970 were June 24 and July 17; and 1971 June 22,

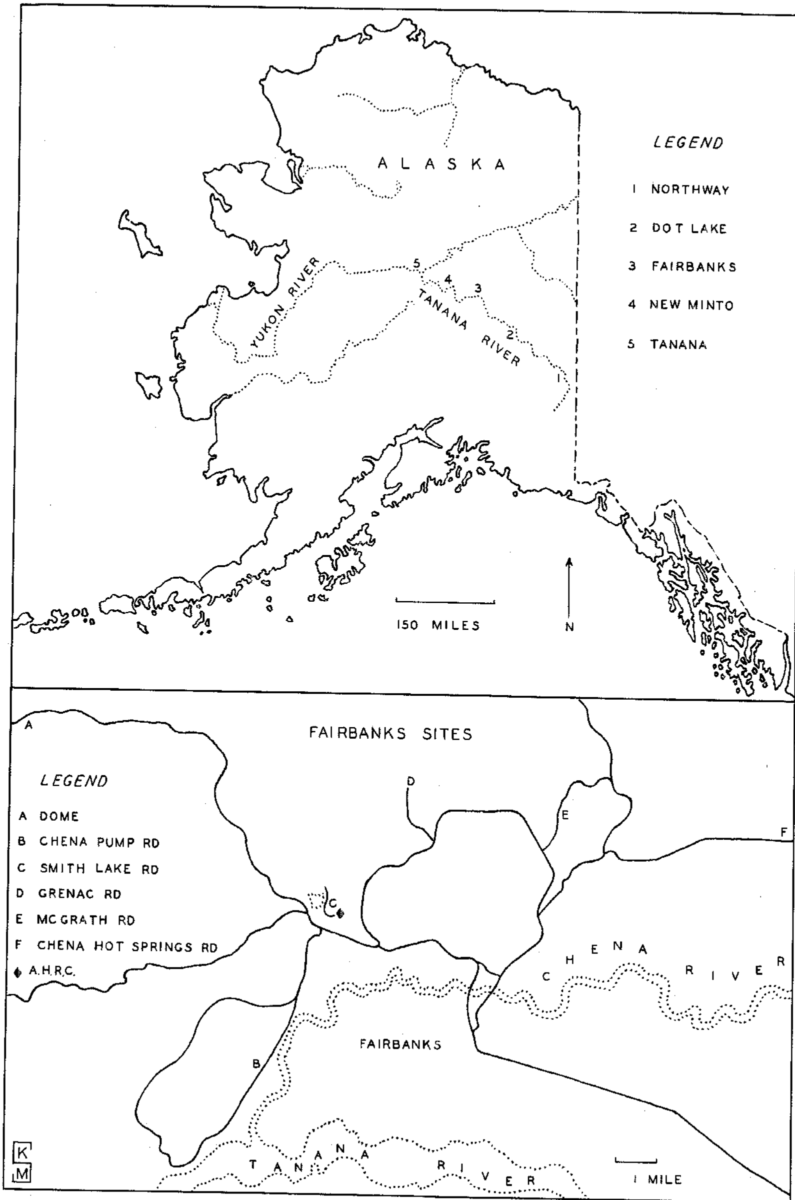

Fig. 1. Map of Alaska showing localities concerned, followed by an enlargement locating the Fairbanks sites.

July 8, 14, 19, Aug. 14, 15, 19, 31, and Sept. 7, 13 after frost when no mosquitoes were seen. The site on Chena Pump Road was a small, cleared front yard, in a wet, lowland wooded area. The indicator rabbits were beside other domestic animals: rabbits, ducks, chickens and large mammals. The only visit was July 19, 1971. The site on Smith Lake Road extended almost a mile along a canopied dirt road through mixed woods, and it lacked indicator rabbits. Visits were June 13, 19, 23 in 1970; and July 8, 16, 19 in 1971. The rural site on Grenac Road was a dry, wooded area at the upper end of a small valley. Evidence of temporary pools in a clearing by the indicator rabbits were the only habitats of biting fly larvae at the site. Visits were June 23 and July 12, 1970; and July 22, 1971. The site on McGrath Road was in a wooded (birch-spruce) residential area. The indicator rabbits were within a few yards of several semipermanent pools, and not far from a marshy clearing. The visits were June 23, 1970, before the indicator rabbits became infected; and in 1971, July 8, 16, 21, 29, Aug. 12, 18, and 31 after frost when no mosquitoes were seen. The rural site on Chena Hot Springs Road had all the desired features. The indicator rabbits were in mixed woods. The June 19, 1970 visit occurred before the indicator rabbits became infected. The 1971 visits were July 15, 21, 30, Aug. 13, 21, 30, and Sept. 10 after frost when no mosquitoes were seen.

NEW MINTO is an Indian village in the spruce forest bordering a vast expanse of aquatic habitats. The indicator rabbits were within the edge of the village (largely uncleared), and the site extended to mixed forest, open spruce-bog and grassy firebreak. Visits in 1971 were June 30, July 26–27, Aug. 16–17, Sept. 8; and July 31–Aug. 2, 1972.

TANANA, an Indian village on the bluffs of the Yukon River, is not accessible by road. Forest extends from the riverbank up the ridge, except where cleared at the village. Strong winds often sweep across the bluff preventing insect flight. The indicator rabbits were in an exposed area along the edge of the bluff. At the outskirts of the village a few semipermanent pools occurred, and cattails (*Typha*) were in small wet depressions. Visits in 1971 were June 30, Aug. 11–12, and Sept. 8.

PROCEDURES. Collecting was partly focused on the habitats most productive at the time; and at sites still negative, on those habitats producing species known to be infected in Alaska or elsewhere (Hoff et al. 1969, Iversen et al. 1969, Whitney et al. 1969, McLean et al. 1970, Newhouse et al. 1971 and Sudia et al. 1971).

CDC light traps, and hanging, CO_2-baited non-mechanized traps (Sommerman and Simmet 1967) were unsuitable because of the scarcity of low strong branches, arid conditions, high cost of dry ice (then 50¢/lb = $100/wk), and space required during transport. Therefore an "LD–40" wide mouth liquid nitrogen container, recharged once a week, was used for asphyxiating, freezing and storing the specimens, and an extra long aerial net of nylon organza was substituted for traps. After a few selected strokes the lower half of the net bag was put in the liquid nitrogen container for a few seconds, with the top and frame hanging out while the cover of the container was in place. Then the insects were emptied into a white porcelain pan and transferred by battery-operated aspirator to a screw-cap vial that was numbered and put in a small carton in the liquid nitrogen container. Related data were recorded.

When biting flies were scarce or the habitats far from the vehicle, the live insects were transferred by mechanical aspirator to 5 x 5 x 5 in. cages resting on a layer of freshly-pulled grass in a closed corrugated carton kept in the shade. Then the cages were put in the liquid nitrogen container momentarily and their contents emptied, etc. Caged specimens were brought back alive from Tanana and the Fairbanks sites, with almost no mortality, and freeze-killed in an electric freezer (Revco®).

The frozen specimens were stored at minus 65°F until removed for identification in winter. Then relative humidity in the laboratory was less than 5%, so very little frost accumulated on glassware. Engorged specimens were identified and processed for host-identification of their blood meal.

Mosquitoes were identified on the basis of characters used in the key developed for this purpose (Sommerman 1966). But in several localities there were many intergrades between *Aedes punctor* (Kirby) and *Ae. hexodontus* Dyar. These specimens, with characters of both species, were put with the species they most closely resembled superficially. For that reason it is inaccurate to refer to these "species" simply as *Ae. punctor* or *Ae. hexodontus*, since each is a complex. *Ae. excrucians* (Walker), as used here, might be a complex too if differentiation were carried beyond the claw character, because there was a wide range of variation in color-pattern and size. If *Ae. barri* Rueger does occur in Alaska it would have keyed out to *fitchii* (Felt & Young).

Most black flies were identified to species. In 1973 adults of the infected species, tentatively designated *Simulium malyschevi* ?, were compared with specimens of *S. malyschevi* Dorogostajakij, Rubzov and Vlasenko, reared from pupae from the Chatanika River in 1972, where larvae were also taken. The infected species, which is very distinctive, is definitely not *S. malyschevi* as inadvertently reported by Ritter and Feltz (1974).

During identification and pooling all glassware was in contact with cans of freeze-gel from the freezer so the specimens usually did not thaw. The collecting vials were emptied into clean petri dishes, and the specimens sorted and counted, one species at a time, under a magnifying lamp (5X) or the microscope. The usual maximum number of specimens per vial and per pool was: mosquitoes, 100 (except *Culiseta*, 50); black flies, 100; *Culicoides* about 1,000; and tabanids, 3. The data were recorded, and the vials were code-numbered and returned to the freezer until pooling. Content of the pools was predetermined on the basis of data recorded on the identification sheets: code-number, locality and site, date collected, identification, and number of specimens. During pooling the frozen specimens for each pool were dumped into a vial, the pool number attached and the vial returned to the freezer until processing.

One deviation from the described routine concerned the 1972 collections. Those from New Minto were handled routinely through pooling, but only the pools of previously infected species (all sites) were processed for virus recovery. Of those from Dot Lake and Northway, except for *Ae. canadensis* (Theobald) and *Ae. riparius* Dyar and Knab, only the species from which virus had previously been recovered (all sites) were sorted, identified, counted, pooled and processed. Therefore some information on species present, total numbers collected, and perhaps additional infected species, was forfeited. That procedure was necessitated by the sudden announcement in March, 1973 that the Arctic Health Research Center was to be closed June 30.

The virus recovery procedures are published (Ritter and Feltz 1974).

RESULTS. The Virology Unit recovered two viruses from the biting flies, snowshoe hare (SSH) virus of the

California complex : California group; and Northway virus of the Bunyamwera group, a new virus recently described (Calisher et al. 1974), and here indicated by BN.

As shown in Table 1, a total of 63,689 biting flies (15,603 mosquitoes, 34,467 black flies, 13,527 *Culicoides*, and 92 tabanids) were collected and pooled; and 991 pools were processed for virus recovery. Of these, 52 pools of mosquitoes and 1 pool of black flies contained virus—presumably from at least 1 infected specimen in each pool. The highest seasonal recovery rate (number of infected specimens/number of specimens taken) occurred in 1971 at Fairbanks when one of every 117 mosquitoes captured was infected; and New Minto was a close second with one of 133.

As shown in Table 2, both viruses were recovered in successive years from several species and localities. The species producing the most recoveries varied by locality, but it was the one taken most frequently at a given locality. Likewise at a given site, when several species were positive they were usually some of the 4 most abundant.

Of the 53 virus recoveries, 48 were SSH and 5 BN. Forty-one of the SSH recoveries came from 7 species of *Aedes* mosquitoes, 3 *cinereus* Meigen, 4 *communis* (DeGeer), 16 *excrucians* (Walker), 1 *fitchii*, 1 *hexodontus* complex, 1 *intrudens* Dyar, 14 *punctor* complex, and 1 from *Simulium* n. sp?.

Of the 8 virus recoveries from *Aedes* specimens too rubbed to identify, 7 (6 SSH, 1 BN) from dark legged specimens were probably from the *punctor* or *hexodontus* complexes; and the SSH recovery from banded legged specimens was most likely from *fitchii*.

Besides the one BN recovery mentioned in the preceding paragraph, there was one each from *excrucians*, and *hexodontus, punctor* complexes, and *Culiseta alaskaensis* (Ludlow).

As shown in Table 3, as the season progressed the mosquito population generally decreased and virus recoveries increased. Both viruses were

Table 1. Summary of field and laboratory results for each locality each year

		Number of Specimens				Number of Pools	
Locality	Year	Mosquitoes	Black Flies	Culicoides	Tabanids	Processed	Infected
NORTHWAY	1970	3694	13223	5277	54	313	8M
	'71	933	2169	1867	8	57	2M
	'72	807#	11#	-#	-#	17#	2M#
DOT LAKE	1970	3178	13784	1282	16	291	4M 1B
	'71	1059	1603	952	0	44	4M
	'72	929#	645#	-#	-#	21#	2M#
FAIRBANKS	1970	348	135	0	5	21	0
	'71	1288	2159	1515	7	145	11M
NEW MINTO	1971	1068	458	260	0	42	8M
	'72	1714	78	2151	2	21#	9M#
TANANA	1971	585	202	223	0	19	2M
Total		15603	34467	13527	92	991	53

see explanation at end of "Procedures", M mosquitoes, B black flies

Table 2. Yearly virus recovery rates for each species at each site

Locality & Site	Year	Number of: Infected specimens / Specimens processed											
		A. punctor complex	Rubbed Aedes dark legs	A. hexodontus complex	A. excrucians	A. communis	A. cinereus	A. fitchii	Rubbed Aedes banded legs	A. intrudens	Culiseta alaskaensis	Other mosquitoes	Simulium n. sp.?
NORTHWAY - 1	1970	187	154	402	2	38	2	3	0	6	1•/59	30	184
NORTHWAY - 2	'70	2/916	3/1098	1•/582	21	1/61	37	17	0	9	4	66	378
	'71	1/299	1•/186	304	11	5	62	2	0	11	5	48	30
	'72	1/438	213	25	57	3	1/48	8	2	3	0	10#	11
DOT LAKE - 1	1970	1/1742	1/346	1/209	20	1/174	7	9	0	15	94	69	1/1115
	'71	4/809	160	35	5	21	3	1	0	9	9	7	81
	'72	1•/685	1/156	38	7	8	16	16	0	1	0	2#	645
DOT LAKE - 2	1970	222	101	30	13	86	7	10	0	3	4	17	378
FAIRBANKS													
Dome	1970	1	13	1	104	9	0	0	0	0	0	0	0
	'71	26	1/12	6	71	53	95	2	0	0	0	14	0
Chena Pump Rd	'71	4	0	0	1/18	2	2	9	0	1	0	1	0
Smith Lake Rd	'70	1	5	3	11	149	0	2	0	0	0	1	0
	'71	30	0	4	1/52	28	12	7	0	0	1	13	0
Grenac Rd	'70	0	1	0	7	2	0	2	0	0	2	0	0
	'71	4	0	0	1/15	14	0	1	0	0	0	0	0
McGrath Rd	'70	0	0	1	0	4	14	0	0	0	0	0	0
	'71	1/120	6	27	2/95	1/42	14	16	0	1	0	1	0
Chena Hot Spgs Rd	'70	0	1	0	12	2	0	0	0	0	0	0	0
	'71	1/100	62	39	85	1/100	1/20	44	0	16	1	2	0
NEW MINTO	1971	1/83	57	34	6/682	21	18	72	0	1/97	1	3	0
	'72	2/408	89	48	5+1•/950	20	1/114	35	4	18	0	28	0
TANANA	1971	70	128	0	28	22	23	1/212	1/72	0	0	30	0
Total		6145	2788	1788	2266	864	494	468	78	190	180	342	2822
SSH Recoveries		14	6	1	16	4	3	1	1	1	0	0	1
•BN Recoveries		1	1	1	1	0	0	0	0	0	1	0	0

- see explanation at end of "Procedures"

Table 3a. Virus recovery rates, population trend and conditions each pooling period

Locality Site Year	Pooling Period	Infected specimens / Specimens processed: A. punctor complex	Rubbed Aedes dark legs	A. hexodontus complex	A. excrucians	A. communis	A. cinereus	A. fitchii	Rubbed Aedes banded legs	A. intrudens	Culiseta alaskaensis	Other mosquitoes	Simulium n. sp.?	Mosquito average per ten minutes	Conditions (legend at end)
NORTHWAY-1															
1970	July 8-14	23	35	34	0	16	0	1	0	2	25	1	4	18	RT
	July 22-28	146	112	349	2	19	0	1	0	4	1*/34	7	15	22	W
	Aug. 5-11	3	7	11	0	3	2	0	0	0	0	4	6	2	RW
	Aug. 19-25	0	0	0	0	0	0	0	0	0	0	0	0	-	S
	Sept. 2-3	15	0	8	0	0	0	1	0	0	0	1	159	3	RW
NORTHWAY-2															
1970	July 8-14	4	20	9	3	1/27	0	1	0	1	1	3	0	4	RT
	July 22-28	58	36	46	1	25	22	5	0	5	2	4	1	3	RSW
	Aug. 5-11	1/33	1/55	1*/38	1	5	10	2	0	2	0	5	4	4	RW
	Aug. 19-25	782	2/987	469	16	3	5	8	0	0	1	52	343	20	G
	Sept. 2-3	1/39	0	20	0	1	0	1	0	1	0	2	30	4	RW
1971	Aug. 4-5	1/250	137	271	8	4	59	2	0	9	3	9	9	14	CW
	Aug. 25-26	49	1*/49	33	3	1	3	0	0	2	2	39	21	5	R
1972	Aug. 16-17	1/438	213	25	57	3	1/48	8	2	3	0	10	11	#	ERW
DOT LAKE-1															
1970	July 7-8	2	2	0	0	18	0	0	0	0	6	14	2	-	RTW
	July 15-21	289	29	36	2	47	0	1	0	0	84	23	295	8	RT
	J. 29-A. 4	757	121	74	6	1/59	6	4	0	14	4	26	6	10	RW
	Aug. 12-18	1/394	1/193	73	6	38	1	3	0	1	0	3	301	11	GV
	A. 26-S. 2	300	1	1/26	6	12	0	1	0	0	0	3	1/500	5	G
	Sept. 9	0	0	0	0	0	0	0	0	0	0	0	11	0	K
1971	Aug. 3	4/546	25	19	0	15	1	0	0	8	9	7	8	18	EW
	Aug. 23-24	263	135	16	5	6	2	1	0	1	0	0	73	8	RVW
1972	Aug. 14-15	1*/685	1/156	38	7	8	16	16	0	1	0	2	645	#	EG
DOT LAKE-2															
1970	July 7-8	1	0	0	0	13	0	1	0	0	1	6	8	-	RTW
	July 15-22	19	17	0	1	25	0	0	0	1	3	4	51	2	RT
	J. 29-A. 4	65	33	13	1	16	1	2	0	1	0	1	63	8	SW
	Aug. 12-18	100	51	14	9	31	5	6	0	0	0	9	205	8	G
	A. 26-S. 1	37	0	3	2	1	1	1	0	1	0	0	24	4	RS
	Sept. 9	0	0	0	0	0	0	0	0	0	0	0	27	0	K

Table 3b. Virus recovery rates, population trend and conditions each pooling period

Locality Site Year	Pooling Period	Infected specimens / Specimens processed: A. punctor complex	Rubbed Aedes dark legs	A. hexodontus complex	A. excrucians	A. communis	A. cinereus	A. fitchii	Rubbed Aedes banded legs	A. intrudens	Culiseta alaskaensis	Other mosquitoes	Simulium n. sp.?	Mosquito average per ten minutes	Conditions (legend at end)
FAIRBANKS															
Dome															
1970	June 24	0	13	0	103	8	0	0	0	0	0	0	0	14	G
	July 17	1	0	1	1	1	0	0	0	0	0	0	0	1	G
1971	June 22	1	0	0	34	47	5	0	0	0	0	5	0	15	G
	July 8-19	8	2	4	34	3	82	2	0	0	0	1	0	6	CW
	Aug. 14-19	14	3	2	3	3	8	0	0	0	0	4	0	1	R
	Aug. 31	3	1/7	0	0	0	0	0	0	0	0	4	0	1	G
Chena Pump Rd															
1971	July 19	4	0	0	1/18	2	2	9	0	1	0	1	0	6	G
Smith Lake Rd															
1970	June 13	0	0	0	0	69	0	0	0	0	0	0	0	11	G
	June 19-23	1	5	3	11	80	0	2	0	0	0	1	0	17	G
1971	July 8-19	30	0	4	1/52	28	12	7	0	0	1	14	0	8	CG
Grenac Rd															
1970	June 23	0	1	0	5	2	0	2	0	0	2	0	0	4	G
	July 12	0	0	0	2	0	0	0	0	0	0	0	0	1	G
1971	July 22	4	0	0	1/15	14	0	1	0	0	0	0	0	6	G
McGrath Rd															
1970	June 23	0	0	1	0	4	14	0	0	0	0	0	0	8	G
1971	July 8-16	3	1	3	1/70	26	14	5	0	0	0	0	0	8	W
	July 21-29	21	0	14	1/19	12	0	7	0	1	0	0	0	4	G
	Aug. 12-18	1/96	5	10	6	1/4	0	4	0	0	0	1	0	4	G
Chena Hot Spgs Rd															
1970	June 19	0	1	0	12	2	0	0	0	0	0	0	0	5	RW
1971	July 15	12	26	23	41	56	1/18	30	0	6	1	1	0	14	G
	July 21-30	4	3	5	38	37	2	8	0	2	0	0	0	6	G
	Aug. 13-21	1/78	22	11	5	5	0	6	0	6	0	1	0	4	G
	Aug. 30	6	11	0	1	1/2	0	0	0	2	0	0	0	1	R

Table 3c. Virus recovery rates, population trend and conditions each pooling period

Locality Site Year	Pooling Period	A. punctor complex	Rubbed Aedes dark legs	A. hexodontus complex	A. excrucians	A. communis	A. cinereus	A. fitchii	Rubbed Aedes banded legs	A. intrudens	Culiseta alaskaensis	Other mosquitoes	Simulium n. sp.?	Mosquito average per ten minutes	Conditions (see legend)
		Infected specimens / Specimens processed													
NEW MINTO															
1971	June 30	10	2	1	21	4	0	0	0	38	0	0	0	13	G
	July 26-27	1/47	14	29	4/490	14	10	66	0	54	1	3	0	13	R
	Aug. 16-17	26	41	4	2/171	3	8	6	0	1/5	0	0	0	5	R
	Sept. 8	0	0	0	0	0	0	0	0	0	0	0	0	0	KW
1972	J. 31-A. 2	2/408	89	48	5+1*/950	20	1/114	35	4	18	0	28	0	18	GM
TANANA															
1971	June 30	0	0	0	1	0	0	1	0	0	0	0	0	<1	W
	Aug. 11-12	70	128	0	27	22	23	1/211	1/72	0	0	30	0	14	G
	Sept. 8	0	0	0	0	0	0	0	0	0	0	0	0	0	KW

Legend * BN virus, C favored cinereus habitat, E new emergence, G good weather, K killing frost, M moonlight, R rain, S favored other site, T traps, V vehicular trouble, W wind, # see explanation at end of "Procedures"

active in mosquitoes from July until killing frost, but the Virology Unit found evidence of June activity, as indicated by the SSH conversion of an indicator rabbit at New Minto June 30 and three at Fairbanks July 7, and by the recovery of BN virus from blood of an indicator rabbit at Northway June 10, 1970. The Virology Unit also recovered SSH virus from varying hares, and both viruses from red-backed voles (*Clethrionomys rutilus*) and indicator rabbits, suggesting BN may also infect hares.

Ae. punctor is the major pest species, widely distributed and most often encountered throughout the interior. The *punctor* complex accounted for at least 39% of the captured mosquitoes (more than twice as many as the next most abundant). It occurred at all sites and was the dominant species at Northway and Dot Lake. It frequents many habitats from open areas to dense forest and, early in the season, some females migrate up the sparsely forested hillsides. It was especially active just before sunset and afterwards when it became the dominant species and finally the last one active. There were 15 virus recoveries (14 SSH, 1 BN) from all localities except Tanana, and this complex may have been responsible for several of the 6 recoveries from unidentified dark legged *Aedes*. Single-habitat infected pools were from aspen grove, firebreak, lawn and willow grove. The overall (all sites) virus recovery rate was 1/409 specimens.

Ae. hexodontus has a somewhat restricted distribution in the interior because it is typically a tundra species. The adults were taken in treeless habitats such as open marshland, borrow pit, landing strip and lawn. They were especially active after sunset and were strong fliers, the last to take shelter from the wind. This complex furnished 11% of the mosquito catch. Only 2 infected pools (1 SSH, 1BN) were definitely *hexodontus* complex. But this complex may have been responsible for some of the infections in the 7 pools of unidentified dark legged *Aedes*, especially the 1971 BN-infected pool. This was pool 0234 containing the type virus on which the name Northway is based. The contents of all 3 pools were collected in August. The two BN-infected pools were both from open marsh and borrow pit at Northway-2, in 1970 and 1971. The other (SSH) was from landing strip and lawn at Dot Lake-1 in 1972. The overall virus recovery rate was 1/894, the lowest of all.

Aedes excrucians is widely distributed throughout the lowlands and occurred at all sites. It comprised 15% of the mosquito catch. Like *punctor* it migrates, and on warm sunny days it was one of the more active species in the shade. At forest edge and in the woods there was activity about mid-morning, then again in the late afternoon, peaking at sunset, and continuing to a lesser extent in moonlight. There were 17 virus recoveries from 2 localities: 5 SSH from Fairbanks, and 11 SSH and 1 BN from New Minto. Infected pools from single habitats came from woods, forest edge, and grassy firebreak. The overall virus recovery rate was 1/133, the highest of any species.

Ae. communis, a woods mosquito, was present at all the sites and accounted for 5% of the mosquito catch. Most specimens were taken in the woods where collecting was focused during periods of wind and light rain. Very few were captured in the open, especially after sunset. There were 4 SSH virus recoveries from 3 localities, Northway, Dot Lake and Fairbanks. Only one infected pool came from a single habitat, a field at forest edge. It contained only 2 specimens taken August 30. The overall virus recovery rate was 1/216.

Ae. cinereus was taken at all the sites except Grenac Road, which is upland, and too far from a breeding place. The females tend to stay in the vegetation (especially *Equisetum*) and woods by pools where they develop, though at these habitats some were taken in flight shortly before sunset, but after sunset only in moonlight. This species accounted for 3% of the mosquito catch but a definite effort was made to collect them. There were 3 SSH virus recoveries: from Northway, Fairbanks and New Minto. The contents of the infected pools were collected along the interface of woods and marsh. The adults have a short flight range which suggests that an infected host was in the immediate vicinity. The overall virus recovery rate was 1/164.

Ae. fitchii occurred at all the sites and accounted for 3% of all mosquitoes captured. More than half of the *fitchii* were from Tanana. This was the only locality where it was predominant in the collections and where it was known to be infected. It frequents forest edge and open areas. The two SSH-infected pools came from fields and open spruce bog, and from a thin row of willows and alders at the margin of the airfield. The overall virus recovery rate was rather low, 1/468.

Ae. intrudens, a forest mosquito, often of pest significance in the woods of the Tanana watershed, comprised only 1% of the mosquito catch, and none was taken at Tanana or 3 of the Fairbanks sites where *communis* occurred. More than half of the *intrudens* were from New Minto where *communis* was rare. The SSH-infected pool was taken there August 17 from a grassy firebreak and spruce bog. The *intrudens* population was approaching its demise, and this partly explains why 1 of 5 specimens was infected. The overall virus recovery rate was 1/190.

Cs. alaskaensis probably occurred at all the sites in spring, but the overwintered populations were well on the decline at the time of these collections. It comprised only 1% of the mosquito catch, and appreciable numbers were taken only in 1970 at Northway-1 and Dot Lake-1, where its habitats were more plentiful. The females were most often taken in flight in the open during the hour-period, 30 min before and after sunset. But in spring when it is cooler they are active during the day. The contents of the BN-infected pool were taken at Northway-1 July 22–23 between 8:45 and 11:00 pm. The overall virus recovery rate was 1/180, rather high, probably because the *alaskaensis* population was approaching its demise.

Simulium n. sp? was taken only at Northway and Dot Lake. There is probably but one generation a year. This species accounted for 8% of the black flies captured. They were taken in flight during daylight from early July until September 8 at temperatures from 68° to 42°F, from lowland aspen woods, forest edge, grassy marshes, roads, borrow pits, airstrip and lawns; they were attracted to humans, dogs and automobiles. The SSH-infected pool was taken August 26–27 at Dot Lake-1 in the open between 6:00 and 8:20 pm. The overall virus recovery rate was 1/2,822, very low probably because this species is attracted to larger animals from which it cannot acquire the virus.

DISCUSSION. The quantity of biting flies captured depended to some extent upon weather and other conditions during a visit, as shown on Table 3. But insect behavior was also involved. By 80°F mosquito activity had ceased in the open and only *punctor, excrucians* and *cinereus* were still active in the shade. During wind and light rain mosquito activity and collecting were limited to woods and sheltered places. On two consecutive moonlight nights insect activity continued at New Minto long after sunset. The temperature span was 62° to 48°F the first night and in a comparable period 66° to 60°F the second. Yet three times more mosquitoes (including at least 4 infected specimens) and 40 times more no-see-ums were captured the first night than on the second, when insects swarming in almost contiguous swarms, out of reach along the upper periphery of the trees, blanketed the forest and produced a loud hum until a light breeze disrupted their activity.

As shown in Table 3, the incidence of virus infection and period of virus dispersal are related to species habits and abundance. Those species infected are the ones most frequently encountered in interior Alaska and the most troublesome pests, according to Gjullin et al. (1961). Frohne (1954) recognized two kinds of life cycles, in part differentiated by the over-wintering stage,—inseminated females or eggs, e.g. *Culiseta* and *Aedes* respectively. The overwintered *Culiseta* population peaks shortly after breakup, and the *Aedes* around the summer solstice. Therefore the period of virus dissemination may be extended a month or so earlier in spring, as shown by the June 10 BN conversion of an indicator rabbit at Northway, implicating *C. alaskaensis.* All mosquitoes have but one generation a year in Alaska so there is no threat of repeated large-scale increases in the mosquito population or in subsequent virus dissemination during the short summer. But late emergence of some *Aedes* occurs sometimes in conjunction with late submergence of the eggs, as shown at Dot Lake-1. Except for overwintered females, species appearance in spring follows a sequence determined by water temperature and kind of pool in which the larvae develop—temporary pools warming first, then semipermanent and permanent (Gjullin et al. 1961). As indicated sporadically on Table 3, successive collections at a site showed that population peaks of various species also tended to follow the sequence of adult emergence. And in 1970 at Northway and Dot Lake the first virus recoveries occurred in that order too—*alaskaensis*, *communis*, and *punctor* and *hexodontus* complexes. So by the time *excrucians* et al. emerge from semipermanent and permanent pools, infected hosts (mammalian and mosquito) are already on the increase.

Blood meal identifications and published information (Hopla 1964–5, 1970) indicate all the infected species are known to feed on humans, and all except *Simulium* n. sp? on hares or indicator rabbits. The presence of dogs or people may reduce mosquito feeding on smaller mammals from which the virus can be obtained. At Dome and Dot Lake-2 where the rabbits did not convert, the dogs were almost always being attacked during my visits while the rabbits were not. Early SSH conversion of the indicator rabbits in 1971 at New Minto and Chena Pump Road (by June 30 and July 7 respectively) may have increased the virus recovery rates there because the biting flies had easy access to the viremic rabbits. This also may have happened at McGrath Road and Grenac Road where conversion dates were not given by Ritter and Feltz (1974).

With reference to mosquitoes, the virus dispersal pattern may be partly dependent upon the feeding and egg-laying habits of the species involved. Species such as *excrucians*, *fitchii* and *alaskaensis* that return to semipermanent and permanent pools to deposit eggs are then ready to feed again, so if infected would tend to increase the incidence of virus infection in hosts in the vicinity of those pools. Whereas species such as *intrudens*, *communis*, *punctor* and *hexodontus* that deposit eggs entirely or largely in depressions where temporary pools will form, would be able to deposit eggs and possibly feed again, farther from semipermanent and permanent pools. This seems to be substantiated to some extent by the lower virus recovery rates from *punctor* complex at Northway (1/460) and Dot Lake (1/576) where it dominated the collections, than at Fairbanks (1/143) or New Minto (1/164) where it was subdominant to *excrucians*. And where *excrucians* dominated the collections its virus recovery rates were still higher, e.g., Fairbanks (1/94) and New Minto (1/136). Also the adults of these two species frequented the greatest variety of habitats, and together were responsible for 60% of the virus recoveries. Other species such as *hexodontus*, *intrudens*, *communis* and *cinereus* would tend toward intrahabitat virus dissemination because of each's preference for one kind of habitat.

The wide variety of species involved, their staggered occurrence and different habitat preferences insure survival and dispersal of the viruses during the summer, but their overwintering hosts are still unknown. If both viruses are maintained in a simple small mammal-mosquito cycle of transmission perhaps SSH virus overwinters in a small hibernating mammal such as the meadow jumping mouse (*Zapus hudsonius*) that occurs throughout the range of SSH from the interior southward. Otherwise the virus maintenance cycles may be more complex and perhaps involve fleas, ticks or mites. A few leeches (Hirudinea) were processed but neither virus was recovered definitely, nor were muskrat (*Ondatra zibethica*) tissues processed.

Snowshoe hare virus is now known to be the causal agent of a mild, usually subclinical, non-contagious disease of humans that occurs in northern United States, north to Alaska. The biting flies obtain the virus when feeding on viremic small mammals such as hares or red-backed voles. They cannot acquire the virus by biting an "ill" person. In

Alaska the most sensible preventive action is use of insect repellents and repellent treated clothing.

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