ARBOVIRUS ISOLATIONS FROM SOUTHWESTERN UTAH AND NORTHWESTERN ARIZONA INSECTS, 1972–1975[1]

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ABSTRACT. Insects collected by light traps at Bloomington, Middleton, and Washington Field, near St. George, Utah, and at Beaver Dam Wash, Arizona, were pooled by species and assayed for arboviruses. Of 22,640 total insects, 61% were collected at Bloomington and Beaver Dam Wash and 11 of 13 virus isolations were from these 2 sites. There were 7 isolations of Western equine encephalitis virus, 1 from *Culex tarsalis* from Washington Field and 6 from Bloomington including 5 from *Cx. tarsalis* and 1 from a pool of rubbed *Culex* abdomens. One Main Drain virus was isolated from *Culicoides variipennis* from Middleton. Isolations from Beaver Dam Wash were a St. Louis encephalitis virus from *Cx. tarsalis* and a Jamestown Canyon virus from *Culiseta inornata* in 1973 and 1 Anopheles A Group and 2 Main Drain viruses from *Anopheles freeborni* in 1974. Precipitin results on 83 blood-engorged insects indicated 34 cow-feedings, 15 at Washington Field and 12 at Bloomington; and 31 rabbit-feedings, 16 at Middleton, 9 at Bloomington, and 6 at Beaver Dam Wash.

The 1971 epidemic of Venezuelan equine encephalitis in Mexico and Texas (Sudia et al. 1975a) prompted surveillance for the virus in 1972 in southwestern United States and Mexico (Bown and Work 1973, Elbel et al. 1973, Hayes et al. 1976, Sudia et al. 1975b, and Work 1973). Surveillance in southwestern Utah and northwestern Arizona continued through 1975. This paper presents data on arbovirus isolations from light-trapped insects obtained during the 4-year study.

With the help of Drs. Lewis T. Nielsen and Don M. Rees, Department of Biology, University of Utah, Salt Lake City, Utah, 5 sites were established in 1972 along the most likely path for virus entry into Utah (Elbel et al. 1973). At each site Dr. Richard O. Hayes, Center for Disease Control, Fort Collins, Colorado, helped select trap locations and plant dominants were determined by Dr. Andrew H. Barnum, Dixie College, St. George, Utah. He also suggested Washington Field as a site. On each monthly visit, light traps were operated for 1 or 2 nights at Bloomington and Beaver Dam Wash each year, at Middleton for 3 years, and at Washington Field for 2 years.

Bloomington, Middleton, and Washington Field were near St. George, elevation 2,760 feet. The Bloomington site was 3½ miles south of St. George and traps were along a road that paralleled irrigated pastures containing horses and cows. A small stream intersected the upper end of the road and vegetation near the traps consisted of tamarisk (*Tamarix pentandra*), Goodding willow (*Salix gooddingi*), bulrush (*Scirpus olneyi*), saltgrass (*Distichlis stricta*), common reed grass (*Phragmitis communis*), and foxtail (*Hordeum jubatum*). At Middleton, 2 miles northeast of St. George, traps were adjacent to a small stream flowing through a cow pasture of mesquite (*Prosopis juliflora*), screwbean (*P. pubescens*), rabbitbrush (*Chrysothamnus teretifolius*), saltbush (*Atriplex canescens*), snakeweed (*Gutierrezia lucida*), sagebrush (*Artemesia filifolia*), and lizardtail (*Anemopsis californicus*). At Washington field, 3 miles east of Bloomington and 4 miles south of Middleton, traps were along a road separating a cow pasture of spring-fed saltgrass from

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irrigated alfalfa fields. Dominant plants were tamarisk, common reed grass, cattail (*Typha latifolia*), sedges (*Carex* spp.), Johnson grass (*Sorghun halevense*), and 5-hooked bassia (*Bassia hyssopifolia*).

Beaver Dam Wash, Arizona, elevation 1,875 feet, was 24 miles southwest of St. George and near the Utah-Nevada border. Traps were along the stream bed just above the union with the Virgin River; tamarisk, Goodding willow, cattail, mesquite, cottonwood (*Populus fremontii*), Russian thistle (*Salsola kali*) and arrowweed (*Pluchea sericea*) were plentiful. The Virgin River and Beaver Dam Wash leave Utah to enter Arizona at 2,250 feet so that the Virgin River Basin, containing both the Beaver Dam Wash and the St. George subcenters, is the lowest elevation in the entire state and is alone in possessing elements of the Lower Sonoran Life Zone (Durrant 1952).

MATERIALS AND METHODS. Methods were similar to those described by Crane et al. (1970) and by Sudia and Chamberlain (1967). Each year CDC Miniature Light Traps supplemented with paper-wrapped dry ice were placed approximately 125 feet apart except that a few traps in 1975 were as close as 62 feet. At the St. George Veterinary Hospital of Dr. Richard E. Hazen, insects were sealed in vials, frozen on dry ice (−60°C), and then transported to the Dugway laboratory where mosquitoes and midges were pooled visually by species, date, and site on CDC chill tables. Pool sizes varied, the maximum being 50 for *Culiseta inornata* and 100 for other species. Using key characteristics described by Nielsen and Rees (1961) and Atchley (1967), all identifications were verified under a stereomicroscope on a chill table. Engorged specimens were removed for precipitin tests to determine the source of blood meals. Samples of unusual mosquitoes were identified by Dr. Lewis T. Nielsen and a few specimens of unusual midges were mounted for microscopic verification.

Each pool for assay was prepared immediately as a suspension in chilled diluent consisting of 10% normal rabbit serum in phosphate-buffered water containing 200 units of penicillin and 50 μg of streptomycin per ml. Suspensions were centrifuged at 2500 x G at 4°C for 20 minutes and the supernatant fluid was removed. Each of 6 suckling mice was injected intracerebrally with 0.02 ml of supernatant fluid and the mice were observed daily for 7 days. Brains of dead or moribund mice were prepared as suspensions and used for subsequent passage. Specimens were considered positive when death or sickness occurred in the second mouse passage. Isolates were identified by neutralization tests in suckling mice. Selected specimens were tested by complement fixation and/or by neutralization either in mice or by plaque reduction in primary Pekin duck embryo or Vero cell cultures.

RESULTS AND DISCUSSION. During 1972–1975, there were 22,640 insects collected and of these 61% were from Bloomington and Beaver Dam Wash. Of 6,984 specimens collected at Bloomington with 199 trap nights, 50% were *Aedes vexans* most of which were collected in June and August 1973 (Table 1). Of 6,885 specimens collected at Beaver Dam Wash with 197 trap nights, 30% were *Anopheles freeborni* and 27% were *An. franciscanus*; most of the *An. freeborni* were collected in 1974, and most of the *An. franciscanus* were collected in 1975 (Table 4). More specimens were obtained with 91 trap nights in 2 years at Washington Field than with 124 trap nights in 3 years at Middleton where 46% of 3,039 insects were *Culicoides hieroglyphicus*, collected in the spring of 1972 (Table 2). At Washington Field 72% of 5,732 mosquitoes were *Ae. dorsalis* most of which were collected in September 1975. Most of the "others pooled" shown in Tables 1, 2, and 4, were *Culex thriambus, An. freeborni,* and *An. franciscanus* from Bloomington, *Culiseta inornata* from Middleton, *Ae. nigromaculis* from Washington Field, *Cx. erythrothorax* and *Culicoides* spp. from Beaver Dam Wash. An additional 352 insects were obtained with 81 trap nights in 1972 and 1973 from Leeds, 15 miles northeast of St. George, and the St.

Table 1. Arbovirus isolations from insects collected by light traps at Bloomington, Utah, 1972–1975.

Year, Month	Number of pooled insects and (virus isolations)*								
	Aedes dorsalis	*Aedes vexans*	*Culex tarsalis*	*Culiseta inornata*	*Culicoides variipennis*	Others pooled	Total pooled	Trap nights	Averages/ trap night
1972									
April–May	376	20	130	121	628	18	1293	25	52
September	4	13	60 (1W)*	15	70	7	169	22	8
	380	33	190 (1W)*	136	698	25	1462	47	31
1973									
June	99	1831	55 (1W)*	27	8	25	2045	10	204
August	97	1466	76		15	30	1684	10	168
October	140	101	16 (1W)*	23	187	26	493	13	38
	336	3398	147 (2W)*	50	210	81	4222	33	128
1974									
July			56			95	151	21	7
August	40	11	235			27	313	24	13
September	18	11	327	7		171	534	26	20
	58	22	618	7		293	998	71	14
1975									
August	11	7	11 (1W)*			50 (1W)#	79†	24	0†
September	56	38	65 (1W)*	48		16	223	24	9
	67	45	76 (2W)*	48		66 (1W)#	302	48	..
Totals	841	3498	1031 (5W)*	241	908	465 (1W)#	6984	199	..

* Virus isolations: W—Western equine encephalitis.

\# Western equine encephalitis from a pool of 25 rubbed *Culex* abdomens.

† Container broken enroute, some specimens lost.

Table 2. Arbovirus isolations from insects collected by light traps in sourthwestern Utah, 1972–1975.

Area Year, Month	Number of pooled insects and (virus isolations)*								
	Anopheles franciscanus	*Anopheles freeborni*	*Culex tarsalis*	*Culicoides variipennis*	*Culicoides hieroglyphicus*	Others pooled	Total pooled	Trap nights	Averages/ trap night
Middleton									
1972									
April–May	1		1	94	1400	15	1511	8	189
September	55	41	69	110		40	315	20	16
	56	41	70	204	1400	55	1826	28	65
1973									
June	149	1	31	78 (1M)*		12	271	16	17
August	146	8	2			2	158	10	16
October	2	2		200		5	209	3	70
	297	11	33	278 (1M)*		19	638	29	22
1974									
July	29	66	35	17		7	154	22	7
August	41	33	18			9	101	21	5
September	144	127	20			29	320	24	13
	214	226	73	17		45	575	67	9
Totals	567	278	176	499 (1M)*	1400	119	3039	124	24

Table 2.—*Continued*

	Number of pooled insects and (virus isolations)*								
	Anopheles franciscanus	*Aedes dorsalis*	*Aedes vexans*	*Culex tarsalis*	*Culex erythrothorax*	Others pooled	Total pooled	Trap nights	Averages/ trap night
Washington Field									
1974									
August	63	1165	30	117	27	93	1495	21	71
September	54	224	17	279	58	47	679	24	28
	117	1389	47	396	85	140	2174	45	48
1975									
August	6	444	60	181 (1W)*	7	69	767	20	38
September	8	2324	194	100	8	157	2791	26	107
	14	2768	254	281 (1W)*	15	226	3558	46	77
Totals	131	4157	301	677 (1W)*	100	366	5732	91	63

* Virus isolations: M—Main Drain, W—Western equine encephalitis.

George Golf Course which were part of the original 5 sites (Elbel et al. 1973).

The highest and lowest averages per trap night were both in June 1973, 204 at Bloomington and 2 at Beaver Dam Wash., but the total precipitation was above normal at St. George and below normal at Beaver Dam in June 1973 (Tables 1, 4, and 5). At St. George 1974 was the only year with no precipitation in both May and June but with normal precipitation in July (Table 5) which may explain the lower average per trap night (ATN) in 1974 (Table 3). At Beaver Dam Wash 1973 and 1975 were the only years with total precipitation below normal May through October (Tables 4 and 5). Although the ATN for 1973 was the lowest of any year, that for 1975 was slightly higher than the ATN for the wettest year, 1972, when total precipitation was above normal in June, September, and October.

The following observations are based on the data in Tables 1, 2, 4, and 5. Eleven of 13 virus isolations were from Bloomington and Beaver Dam Wash. *Cx. tarsalis* comprised 15% of the total insects from Bloomington and 12% from Washington Field. However, *Cx. tarsalis* accounted for 6 Western equine encephalitis (WEE) virus isolations, 1 from Washington Field in 1975 and 5 from Bloomington, 1 in 1972 and 2 each in 1973 and 1975. Another WEE isolate was from a pool of 25 rubbed *Culex* abdomens collected at Bloomington in August 1975. All WEE isolations were from *Culex* spp. collected in the fall except for the isolation in June 1973. Graham et al. (1960) showed that *Cx. tarsalis* abundance and WEE in horses in Utah were associated with above normal precipitation in May or June and an unusually dry July and early August. So, it is not surprising that WEE was present in *Cx. tarsalis* only in 1972, 1973, and 1975 when total precipitation was above normal in May or June and below normal in July. Hess et al. (1963) stated that unusually cool, wet springs at Greeley, Colorado were favorable for high WEE transmission rates in avian sentinels. Thus, as expected, 4 of 7 WEE isolations from *Culex* spp. were in 1975, the only year with an unusually cool, wet spring. It is of interest that 5 of 7 WEE isolations were from *Culex* spp. collected in months with above normal precipitation, and 4 of these 5 isolations also came from months with below normal average temperatures. Only in September 1975 was a WEE virus isolated in a month with below normal precipitation and above normal temperature.

Table 3. Yearly totals for insects collected at Bloomington, Middleton, and Washington Field (data from Tables 1 and 2).

Year	Total pooled	Trap nights	Averages/ trap nights
1972	3,288	75	44
1973	4,860	62	78
1974	3,747	183	20
1975*	3,781	70	54

* Only September totals were used for Bloomington as August lacked an average/ trap night (Table 1).

The only St. George area isolate, other than WEE, was a Main Drain (MD) virus from 78 *Culicoides variipennis* collected at Middleton in June 1973, the only month May through October with above normal precipitation (Tables 2 and 5). *C. variipennis* comprised 16% of the total Middleton insects. Other Bunyamwera Group viruses have been isolated in southern Arizona, New Mexico, and Mexico (Hayes et al. 1976, Sudia et al. 1967 and 1975b).

One Anopheles A (ANA) Group and 2 MD viruses were obtained from *An. freeborni* collected at Beaver Dam Wash in September 1974, the first month May through October with above normal precipitation (Tables 4 and 5). Each MD virus was from a pool of 100 specimens, and the ANA Group virus was from a pool of 40 specimens. The similarity of this isolate to Tacaiuma virus, a member of the ANA group (Calisher et al. 1973), is of interest, and characterization studies will be published separately. No virus of the ANA Group has been found previously north of Guatemala where 2 subtypes were isolated

Table 4. Arbovirus isolations from insects collected by light traps at Beaver Dam Wash, Arizona, 1972–1975.

Year, Month	Number of pooled insects and (virus isolations)*								
	Anopheles franciscanus	*Anopheles freeborni*	*Culex tarsalis*	*Culex thriambus*	*Culiseta inornata*	Others pooled	Total pooled	Trap nights	Averages/ trap night
1972									
April–May	46	80	15		108	919	1168	22	53
September	54	58	300		37	73	522	11	47
	100	138	315		145	992	1690	33	51
1973									
June	1		15			2	18	10	2
August	155	5	33 (1S) *		1	4	198	17	12
October	57		12		53 (1J)*	11	133	16	8
	213	5	60 (1S)*		54 (1J)*	17	349	43	8
1974									
July	394	396	54			19	863	23	37
August	70	475	31	32		90	698	25	28
September	14	340 (2M1A)*	59	122	5	15	555	21	26
	478	1211 (2M1A)*	144	154	5	124	2116	69	31
1975									
August	257	490	209	234		120	1310	27	48
September	814	197	121	164	75	49	1420	25	57
	1071	687	330	398	75	169	2730	52	52
Totals	1862	2041 (2MIA)*	849 (1S)*	552	279 (1J)*	1302	6885	197	35

* Virus isolations: S—St. Louis encephalitis, J—Jamestown Canyon, M—Main Drain, A—Anopheles A Group.

Table 5. Climatological data for St. George, Utah and Beaver Dam, Arizona, 1972–1975 (Climatological data for Utah and Arizona, 1972–1975.

St. George Month	Total precipitation (inches)					Average temperature (degrees F)				
	1972	1973	1974	1975	Normals	1972	1973	1974	1975	Normals
May	0.00	0.31	0.00	1.25	0.38	69.1	70.7	72.1	65.8	68.9
June	0.55	0.32	0.00	0.05	0.19	78.2	78.1	81.2	76.7	77.1
July	0.00	0.32	0.61	0.15	0.61	85.2	84.5	84.6	85.2	84.3
August	1.26	0.34	0.59	0.80	0.64	79.7	82.6	81.8	81.4	82.6
September	2.06	0.00	0.08	0.06	0.48	70.5	72.9	78.5	76.7	74.9
October	2.33	0.00	2.43	0.34	0.57	57.9	62.6	65.3	61.7	62.9
Averages	1.03	0.21	0.62	0.44	0.48	73.4	75.2	77.2	74.6	75.1

Beaver Dam Month	1972	1973	1974	1975	Northwest Arizona Normals	1972	1973	1974	1975	Northwest Arizona Normals
May	0.04	0.10	0.03	0.03	0.24	73.1	75.3	76.2	67.9	67.7
June	0.33	0.12	0.00	0.01	0.18	83.6	83.7	86.2	80.7	76.2
July	0.00	0.49	0.02	0.08	0.88	90.3	90.0	87.7	88.7	83.2
August	0.80	0.77	0.25	0.39	1.49	85.8	85.2	85.9	84.4	81.2
September	1.78	0.00	0.65	0.11	0.63	76.9	77.8	83.0	82.3	75.2
October	1.57	0.30	1.67	0.58	0.66	65.1	67.5	69.4	65.5	64.5
Averages	0.75	0.30	0.44	0.20	0.68	79.1	79.9	81.4	78.2	74.7

recently by Gutierrez V. et al. (1975). There is no explanation of how the virus transferred from the tropics to Beaver Dam Wash in the Lower Sonoran Life Zone. Establishment of a tropical virus at Beaver Dam Wash is unlikely, and the ANA Group virus was not found again in 1975.

Cx. tarsalis comprised 12% of the total Beaver Dam Wash insects. However, the presence of St. Louis encephalitis (SLE) virus in 33 *Cx. tarsalis* collected in August 1973 (Table 4) may suggest that Beaver Dam Wash could serve as a pathway for viruses into Utah. SLE virus has not yet been isolated in Utah but was isolated in southern California, southern Arizona, New Mexico, and Mexico (Bown and Work 1973, Hayes et al. 1976, Smith et al. 1969, and Sudia et al. 1975b). These authors found that most of the isolations were SLE from *Cx. tarsalis* but Hayes et al. (1976) found more WEE and Smith et al. (1969) showed more isolations from *Cx. quinquefasciatus*. At Greeley, Colorado SLE virus transmission rates in avian sentinels were favored by unusually warm, dry springs, conditions that caused minimal activity of WEE (Hess et al. 1963). These qualities were present at Beaver Dam in 1973 since total precipitation was below normal and average temperature was above normal each month May through October (Table 5). Thus, the presence of SLE could have been anticipated. Beaver Dam Wash was 885 feet lower than St. George and a few degrees warmer each year May through October than either St. George or the northwest Arizona normals (Table 5). This may explain why WEE was not found at Beaver Dam Wash.

Of the total Beaver Dam Wash insects, only 4% were *Cs. inornata* but 53 *Cs. inornata* collected in October 1973 yielded Jamestown Canyon (JC), a California (CAL) Group virus (Table 4). Similarly, in a year with an unusually warm, dry spring and summer at Callao, Utah, Elbel et al. (1971) obtained only 2 CAL Group viruses from 38,776 mosquitoes compared to 43 CAL Group viruses from 36, 972 mosquitoes in a year with an unusually cool, wet spring. Therefore, more CAL Group viruses should have been found at Beaver Dam Wash in 1972, the only year of the 4-year study with an unusually wet spring (Table 5), but no viruses were isolated. However, average temperature was above normal each month May through October. In 1972 Hayes et al. (1976) isolated CAL Group viruses from southern Arizona and New Mexico and Sudia et al. (1975b) isolated Trivittatus, a CAL Group virus, from Mexico. In 1965 from New Mexico, Sudia et al. (1967) isolated CAL Group viruses which were later typed as LaCrosse and California encephalitis subtypes (Sudia et al. 1971). Since these authors listed JC from Texas, its presence at Beaver Dam Wash was not unusual.

Blood meals of 83 engorged insects indicated possible hosts of the viruses (Table 6). Virus isolations were from *Cx. tarsalis* from Bloomington, Washington Field and Beaver Dam Wash; *C. variipennis* from Middleton; *An. freeborni* and *Cs. inornata* from Beaver Dam Wash (Tables 1, 2, and 4). Feedings by *Cx. tarsalis* were on a horse, a cow, and a bird at Bloomington and at Washington Field but on a rabbit at Beaver Dam Wash. Of 31 rabbit feedings, 3 were by *C. variipennis* at Bloomington, 12 were by *An. franciscanus* including 9 at Middleton and 1 double feeding on rabbit and horse at Bloomington, and 11 were by *An. freeborni* including 7 at Middleton and 3 at Beaver Dam Wash. Of 34 cow feedings, 11 were by *Ae. dorsalis* including 9 at Washington Field, and 8 were by *Cs. inornata* including 6 at Bloomington and 1 at Beaver Dam Wash. Feedings by *Ae. vexans* were on 5 rabbits and 6 cows, mostly at Bloomington.

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Table 6. Precipitin results on freshly engorged insects collected by light traps in southwestern Utah and at Beaver Dam Wash, Arizona, 1972–1975.

Area, Year	Number of host feedings per insect species									Totals
	Anopheles franciscanus	*Anopheles freeborni*	*Aedes dorsalis*	*Aedes vexans*	*Culex tarsalis*	*Culex thriambus*	*Culiseta inornata*	*Culicoides variipennis*	*C. hiero-glyphicus*	
Bloomington										
1972			1H1C							1H1C
1973	1RH		1C	5R3C	1C		1H4C	3R		8R1H9C1RH*
1974	2H	1R			1H1B					1R3H1B
1975							2C1M			2C1M=29
Middleton										
1972		1H							2H	3H
1973	2R									2R
1974	7R	7R3C			1C	1B				14R4C1B=24
Washington Field										
1974	1C		2C		1H1C1B					1H4C1B
1975			7C	3C1M		1M	1C			11C2M=19
Beaver Dam Wash										
1973	1R1C									1R1C
1974		1R			1R	1B				2R1B
1975	1R1C	2R1M					1C			3R2C1M=11
Totals	11R2H 3C1RH =17	11R1H3C 1M =16	1H11C =12	5R6C 1M =12	1R2H 3C2B =8	1M2B 3=	1H8C1M =10	3R =3	2H =2	31R9H34C* 1RH4M4B =83

* Host legend: R=Rabbit, H=Horse, C=Cow, RH=Double feeding on Rabbit and Horse, M=Unidentified mammal, B=Bird.

Arbovirus Reference Unit, New Haven, Connecticut, for verifying the Anopheles A Group isolate and reviewing the manuscript.

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