the United States Department of Agriculture, Agriculture Research Service, authorized by Public Law 480.

References

Altman, R. M. and Smith, C. N. 1955. Investigations of repellents for protection against mosquitoes in Alaska, 1953. J. Econ. Ent. 48:67–72.

Anonymous, 1967. Materials evaluated as insecticides, repellents and chemosterilants at Orlando and Gainesville, Fla. 152–64.

Gilbert, I. H. 1955. New mosquito repellents. J. Econ. Ent. 48:741–743.

Gilbert, I. H. 1955. Evaluation of repellents against mosquitoes in Panama. The Florida Ent. 38:153–163.

Gilbert, I. H. 1957. Evaluation of repellents against mosquitoes and deer flies in Oregon. J. Econ. Ent. 50:46–48.

Gilbert, I. H., Gouck, H. K. and Smith, C. N. 1957. New insect repellents. Soap and Chem. Specialities 33(5):115–117, 129–133.

Kempthorne, O. 1952. The design and analysis of experiments. John Wiley & Sons, Inc. 631 pp.

King, W. V., Compiler, 1954. Chemicals evaluated as insecticides and repellents at Orlando, Fla. U.S. Dept. Agr. Handb. 69, 397 pp.

Smith, C. N. 1965. Insect repellents—Their present usefulness and future development. Proc. 12th Inter. Congress Ent. 507–509.

ARBOVIRUS ISOLATIONS FROM MOSQUITOES COLLECTED AT CALLAO, UTAH 1966 AND 1967

ROBERT E. ELBEL, GEORGE T. CRANE, LAWRENCE E. STIPE, GEORGE B. VAN NOSDOL AND KEITH L. SMART

Ecology and Epidemiology Division, Deseret Test Center, Dugway, Utah 84022

Crane *et al.* (1970) reported arbovirus isolations from mosquitoes collected in Central Utah. Further isolations are reported here from mosquitoes collected in and near Callao, Utah, a small farming community east of the Deep Creek Mountains in western Utah. Mosquitoes were collected in 1966 by light traps and livestock-baited stable traps at eight sites (Olson *et al.*, 1968); in 1967 light traps alone were used at five of the 1966 sites. Light-trapped mosquitoes and arbovirus isolates are compared from the five sites used both years. Light traps were operated from May to October each year, 5 nights a week in 1966 and 3 nights a week in 1967. Collections were made in 1966 by military personnel of the Dugway Ecology and Epidemiology Division and in 1967 by the David C. Bagley family of Callao.

At each site two to four light traps were located at least 100 feet apart where vegetation was heaviest. Traps were placed at the same location each year, but not all locations were used both years. The elevation of all sites was about 4,300 feet. At Callao three sites were on the Bagley farm separated by ½ to 1 mile. At Redden (=Redding) Springs (Horne, 1945), two sites were about 1 mile apart. South Redden Springs is 7 air miles from Callao. Two trap locations at Callao North Marsh were on the border of a sedge (*Carex* spp.) marsh and a field of greasewood (*Sarcobatus vermiculatus*) and saltgrass (*Distichlis stricta*). The marsh was lined with wild rose (*Rosa* spp.) and willows (*Salix* spp.). Adjacent to one trap, was a stream channel covered with cattail (*Typha angustifolia*). Four trap locations at Callao West Field were alongside irrigation ditches lined with white poplar (*Populus alba*), rabbitbrush (*Chrysothamnus* spp.), clematis (*Clematis* spp.), wild rose, mixed grasses, and greasewood. An irrigated alfalfa field was adjacent to three traps. At Callao Center Meadow, four trap locations were along a fence separating irrigated meadows of mixed grasses.

The fence was lined with Russian olive (*Elaeagnus angustifolia*), wild rose, and willows. Spring-fed seepage containing sedge (*Carex* spp.) bordered one trap. At South Redden Springs, rushes (*Juncus* spp.) of the marsh ended in a wild rose-willow thicket having 3 sides borderd by greasewood-saltgrass fields; three trap locations were on the periphery of the thicket. At North Redden Springs, rushes (*Juncus* spp.) and sedges (*Scirpus* spp.) dominated the marsh; three trap locations were between the marsh and a field of saltgrass, and pickleweed (*Allenrolfea occidentalis*). Two wild rose thickets supported two traps.

METHODS. Methods were similar to those described by Crane *et al.* (1970), and by Sudia and Chamberlain (1967). Using CDC Miniature Light Traps with dry ice supplement, mosquitoes were collected alive, sealed in vials, frozen on dry ice, and transported to the Dugway laboratory; on a CDC chill table, mosquitoes were pooled by species by sight for each week of collection and site; all identifications were verified under a stereomicroscope on a chill table (Elbel, 1968).

Maximum pool size was 50 for *Culiseta inornata* and 100 for other species. Supernatant fluid from the suspension of each mosquito pool was injected intracerebrally into suckling mice. When death or sickness occurred in two successive passages, a specimen was considered positive, and assayed on primary chick-embryo cell cultures under agar overlay. Isolates were identified by neutralization in suckling mice using antisera prepared against the viruses of Cache Valley, Turlock, Hart Park, Trivittatus, Modoc, California encephalitis, St. Louis encephalitis, Western equine encephalomyelitis, and Venezuelan equine encephalitis. The California encephalitis group antiserum was prepared against a local strain, 30521, antigenically related to BFS 283 strain of California encephalitis virus (Crane *et al.*, 1970). Most of the other antisera, including Cache Valley, were prepared against viruses obtained from Dr. Preston Holden, National Communicable Disease Center (NCDC), Fort Collins, Colorado. The Callao isolates were obtained before the reference Cache Valley virus was brought into the Dugway laboratory.

RESULTS. *Culiseta inornata, Aedes dorsalis, Anopheles freeborni, Culex tarsalis,* and *Culex erythrothorax* constituted 99 percent of the total 75,748 mosquitoes collected by light traps from Callao and Redden Springs during 1966 and 1967. The numbers of these species collected each year from each area and averages per trap night are shown in Table 1. Arbovirus isolates and averages per trap night for each of the totals of *C. inornata, A. dorsalis, A. freeborni,* and *C. tarsalis* are shown in Figure 1. This figure was prepared following suggestions from Dr. D. B. Francy, NCDC, Fort Collins, Colorado. From Callao a total of 7,947 specimens and 689 trap nights in 1966 gave an average per trap night of 12, but 25,084 specimens and 457 trap nights in 1967 gave an average per trap night of 55. From Redden Springs a total of 30,697 specimens and 517 trap nights in 1966 gave an average per trap night of 59, and 11,113 specimens from 230 trap nights in 1967 gave an average per trap night of 48. Most of the remaining mosquitoes each year were *Aedes vexans* and *A. nigromaculis.*

Arbovirus isolates were either California encephalitis group (CEG) or Cache Valley (CV). The failure of these isolates to produce plaques on chick-embryo cell cultures indicated the absence of Western equine encephalomyelitis (WEE) (Crane *et al.*, 1970). Isolations and infection ratios in mosquitoes collected by light traps from Callao and Redden Springs are shown in Table 2. Only 3 isolates were obtained from 38,776 mosquitoes in 1966, but there were 44 isolates from 36,972 mosquitoes in 1967. At Callao 25 of 26 isolations of CEG were from 1967 mosquitoes; and at Redden Springs 18 of 19 CEG and 1 of 2 CV isolations were from 1967 mosquitoes (Fig. 1). Table 3 shows that 4 CEG and 2 CV isolates were obtained in 1966 from *A. free-*

TABLE 1.—Female mosquitoes collected by light traps at Callao and Redden Springs, Utah, 1966 and 1967.

Location and date	Total trap nights	Mosquitoes and averages per trap night (TNA)										
		Culiseta inornata		*Aedes dorsalis*		*Anopheles freeborni*		*Culex tarsalis*		*Culex erythrothorax*		
		No.	TNA	No.	TNA	No.	TNA	No.	TNA	No.	TNA	
Callao 1966												
May	73	24	0	2021	28	10	0	188	3	0	..	
June	152	898	6	1334	9	28	0	1112	7	9	0	
July	148	46	0	40	0	21	0	525	4	17	0	
August	145	102	1	60	0	46	0	1120	8	19	0	
September	171	113	1	115	1	13	0	49	0	37	0	
Totals	689	1183	2	3570	5	118	0	2994	4	82	0	= 7947
Callao 1967												
May	109	140	1	468	4	3	0	11	0	8	0	
June	103	91	1	2796	27	4	0	130	1	0	..	
July	85	656	8	6123	72	288	3	3960	47	45	0	
August	100	932	9	3667	37	449	4	4031	40	234	2	
September	60	327	5	405	7	22	0	198	3	96	2	
Totals	457	2146	5	13459	30	766	2	8330	18	383	1	= 25084
Redden Springs 1966												
May	54	65	1	20	0	490	9	1058	20	36	1	
June	11	912	8	143	1	3113	27	2855	25	13	0	
July	118	381	3	212	2	2460	21	3883	33	392	3	
August	107	1055	10	94	1	1979	18	7179	67	1118	10	
September	123	706	6	813	7	267	2	534	4	919	7	
Totals	517	3119	6	1282	2	8309	16	15509	30	2478	5	= 30697
Redden Springs 1967												
May	50	183	4	30	1	91	2	28	1	81	2	
June	43	145	3	339	8	222	5	75	2	15	0	
July	40	634	16	290	7	1079	27	616	15	36	1	
August	56	1546	28	1255	22	351	6	1890	34	517	9	
September	41	820	20	210	5	92	2	368	9	200	5	
Totals	230	3328	14	2124	9	1835	8	2977	13	849	4	= 11113

borni collected in livestock-baited stable traps at South Redden Springs. Another 1966 CV isolate was from 149 specimens of *C. inornata* collected in light traps in September at a Callao site not used in 1967.

Typing of CEG isolates was not attempted beyond group specificity (Crane *et al.*, 1970). Reisolations in suckling mice were achieved on both CV isolates shown in Table 2. Dr. C. H. Calisher, NCDC, Atlanta, Georgia, confirmed the four 1966 CV identifications in complement fixation tests.

Table 4 shows the Callao weather data and the normals for western Utah for May to October (Climatological Data for Utah, 1966, 1967). The weather station is on the Bagley farm closest to the Callao Center Meadow.

DISCUSSION. Table 4 shows that in 1967 total precipitation was considerably above normal each month except July and August, and average temperature was slightly below normal each month except August and September; but in 1966 total precipitation was considerably below normal each month except September, and average temperature was slightly above normal each month. Graham *et al.* (1960) showed that increases in *C. tarsalis* population and in WEE in horses in Utah were associated

TABLE 2.—Isolations of California encephalitis group (CEG) and Cache Valley (CV) viruses from mosquitoes collected by light traps at Callao and Redden Springs, Utah, 1966 and 1967.

Location and date	Virus	Isolates (and mosquito infection ratios)			
		Culiseta inornata	*Aedes dorsalis*	*Anopheles freeborni*	*Culex tarsalis*
Callao 1966					
September	CEG	1 (1:113)			
Total	CEG	1			
Callao 1967					
July	CEG		12 (1:510)*		
August	CEG	5 (1:186)*	5 (1:734)	1 (1:449)*	
September	CEG	1 (1:327)	1 (1:405)		
Totals	CEG	6	18	1	
Redden Springs 1966					
September	CEG		1 (1:813)*		
September	CV			1 (1:267)	
Totals	CEG		1		
	CV			1	
Redden Springs 1967					
June	CEG				1 (1:75)
July	CEG	1 (1:634)	1 (1:290)		
August	CEG	10 (1:155)*			
September	CEG	5 (1:164)			
September	CV	1 (1:820)			
Totals	CEG	16	1		1
	CV	1			

* Mosquitoes at population peak as shown by averages per trap night in Table 1.

with above normal precipitation in May or June and an unusually dry July and August. Similarly, at Greeley, Colorado, Hess *et al.* (1963) found that unusually warm springs were exceptionally dry; and unusually cool springs were generally associated with excessive rainfall, a condition favorable for WEE transmission rates in avian sentinels. Thus, we should expect more mosquitoes and arboviruses in 1967. Figure 1 shows this to be so only for CEG, *C. inornata,* and *A. dorsalis.*

The following information is based on the data in Table 1. At Callao over 3 times more mosquitoes were collected in 1967 using 232 fewer trap nights than in

TABLE 3.—Isolations of California encephalitis group (CEG) and Cache Valley (CV) viruses from *Anopheles freeborni* collected in livestock-baited stable traps at South Redden Springs, Utah, 1966.

Month	Total trap nights	Number of specimens	Averages per trap night	Virus	Isolates	Infection ratios
May	8	2667	333	CEG	2	1:1333
June	10	9887	989	CEG	2	1:4943
July	10	10432	1043	0	0	.....
August	8	3452	431	CV	2	1:1726
September	10	194	19	0	0	.....
Totals	46	26632	578	CEG	4	
				CV	2	

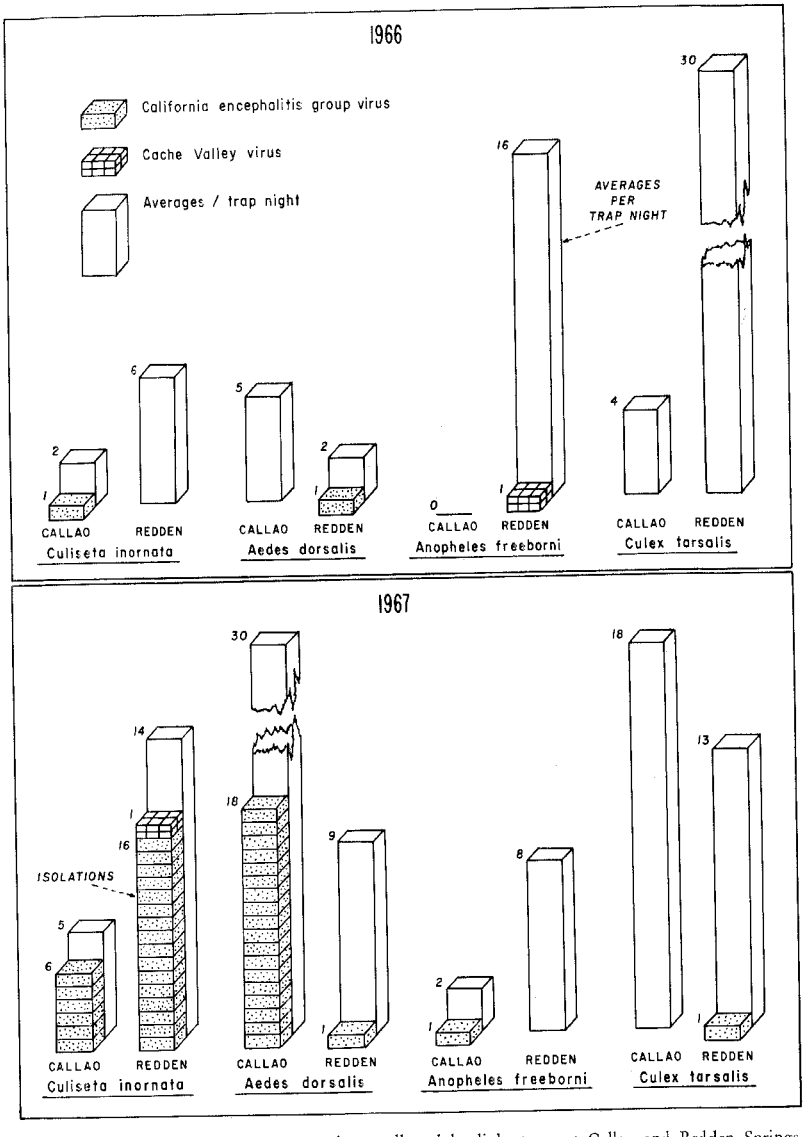

FIG. 1.—Arbovirus isolations from mosquitoes collected by light traps at Callao and Redden Springs, Utah, 1966 and 1967.

TABLE 4.—Climatological data for Callao, Utah, 1966 and 1967.

	Total precipitation (inches)			Average temperature (degrees F)		
	Callao		Western Utah Normals	Callao		Western Utah Normals
Month	1966	1967		1966	1967	
May	0.22	1.22	0.93	61.1	55.7	56.4
June	0.28	2.82	0.60	66.0	61.1	65.0
July	0.19	0.64	0.71	76.8	73.5	74.0
August	0.28	0.21	0.73	72.2	72.3	72.1
September	0.84	0.89	0.49	65.2	63.5	62.8
Averages	0.36	1.16	0.69	68.3	65.2	66.1

1966; the average per trap night was about 5 times greater in 1967 than in 1966. At Redden Springs nearly 3 times more mosquitoes were collected in 1966 using 287 more trap nights than in 1967; the average per trap night was about the same both years, and similar to the 1967 value for Callao. Apparently the larger body of water at Redden Springs limited the effects of the dry 1966 conditions on the mosquitoes. More specimens were obtained in 1967 than in 1966 for each mosquito species at Callao but only for *C. inornata* and *A. dorsalis* at Redden Springs. Since more specimens of *C. tarsalis* and *C. erythrothorax* were collected at Redden Springs in 1966 than in 1967, the larval habitat may have been more favorable for these species as a result of a more stable water level in the dry 1966. Both species were more abundant each year at North Redden Springs, possibly due to the presence of more water since traps were on the periphery of the marsh. More *A. freeborni* were collected from Redden Springs in 1966 than in 1967, but the majority of these specimens were from South Redden Springs which attracted more *A. freeborni* each year than any other site. Since the larval habitat should have been essentially the same at either site, South Redden Springs with the marsh only on 1 side and greasewood fields on 3 sides may have supported more cattle which attracted more *A. freeborni*. Most specimens of *C. inornata* were collected each year from Redden Springs, but most of the *A. dorsalis* each year were from Callao (Fig. 1).

The requirements of the larvae would seem to be satisfied best for *C. inornata* by the Redden Springs marsh and for *A. dorsalis* by the irrigated areas of Callao. Nielsen and Rees (1961) stated that *C. inornata* larvae preferred permanent or semi-permanent, brackish or polluted, water, and *A. dorsalis* larvae preferred pools formed from irrigation waste water. For Callao, *C. inornata* and *A. dorsalis* had peak populations in May and June of 1966 but in July and August of 1967. For Redden Springs these species had peaks in August or September both years. Crane *et al.* (1970) found that most of the arbovirus isolates from Central Utah were from mosquitoes collected at population peaks during August and September, so we should expect about the same proportion of isolates from Redden Springs each year.

Figure 1 shows that the majority of the arbovirus isolations were from 1967 collections of *C. inornata* at Redden Springs and of *A. dorsalis* at Callao. Table 2 shows that the 26 Callao isolates were all CEG as were 19 of the 21 Redden Springs isolates. From *C. inornata* CEG isolates numbered 7 from Callao and 16 from Redden Springs. The Callao isolates were all from August-September collections, and

all but one were from 1967 mosquitoes. *C. inornata* was at a population peak for five isolations from Callao in August. The Redden Springs CEG isolates from *C. inornata* were from 1967 collections, 15 from August and September and 1 from July, but the infection ratio was so low in July as to suggest that *C. inornata* was a better vector during August and September. *C. inornata* was at a population peak for 10 isolations from Redden Springs in August. From *A. dorsalis* CEG isolates numbered 18 from Callao and 2 from Redden Springs. The Callao isolates were from 1967 collections, 6 from August and September and 12 from July when *A. dorsalis* was at a population peak. The Redden Springs isolates were one in July 1967 and one in September 1966 when *A. dorsalis* was at a population peak; this is the only 1966 isolate from a mosquito at population peak. The Callao CEG isolate from *A. freeborni* was collected in August 1967 when the mosquito was at a population peak. The Redden Springs CEG isolate from *C. tarsalis,* collected in June 1967, was the earliest isolation from light-trapped mosquitoes. However, four CEG isolations from *A. freeborni* collected in livestock-baited stable traps at South Redden Springs in 1966 were in May and June (Table 3). Most of the Callao isolations were obtained from *A. dorsalis,* but most of the Redden Springs isolations were from *C. inornata.* However, for both areas *C. inornata* is considered to be the better vector of CEG because of the larger number of high infection ratios. Similarly, Crane *et al.* (1970) found *C. inornata* to be the better vector of CEG in central Utah; their isolations were from *C. inornata* and *C. erythrothorax* during August and September, and from *A. dorsalis* and *C. tarsalis* during June and July.

Table 2 shows that both isolations of CV were from Redden Springs in September; one from *A. freeborni* in 1966 gave an infection ratio of 1:267, and the other from *C. inornata* in 1967 gave an infection ratio of 1:820. The CV isolate in 1966 from light-trapped *C. inornata* in September from a Callao site not used in 1967 gave an infection ratio of 1:149, but two CV isolates from *A. freeborni* collected in livestock-baited stable traps at South Redden Springs in August 1966 gave an infection ratio of 1:1726 (Table 3). Thus, the infection ratios for CV in *A. freeborni* and in *C. inornata,* are not sufficient to determine the better vector. Holden and Hess (1959) isolated and described CV from one pool of *C. inornata* collected in Cache Valley, Utah; they obtained positive serum neutralization from the serum of four horses. Possibly, *C. inornata* and *A. freeborni* are the main vectors of CV in Utah, but more collecting is necessary to substantiate this and to determine the better vector.

SUMMARY. Mosquitoes, collected by light traps in western Utah at irrigated areas of Callao and at Redden Springs marsh, were pooled by species, assayed for arboviruses, and compared as to numbers of specimens and arbovirus isolations. May and June of 1966 were warm and dry, but these months in 1967 were cool and wet. More specimens were collected in 1967 than in 1966 of *Culiseta inornata, Aedes dorsalis, Anopheles freeborni,* and *Culex tarsalis* at Callao but only of *C. inornata* and *A. dorsalis* at Redden Springs. Arbovirus isolates were two California encephalitis group (CEG) and one Cache Valley (CV) from 38,776 mosquitoes in 1966 and 43 CEG and 1 CV from 36,972 mosquitoes in 1967. At Callao 18 of 26 CEG isolates were from *A. dorsalis* and at Redden Springs 16 of 19 CEG isolates were from *C. inornata.* One CV isolate each was from *A. freeborni* and from *C. inornata;* both were from Redden Springs. Additional 1966 isolates from traps or sites not used in 1967 were four CEG and two CV from *A. freeborni* collected in livestock-baited stable traps and one CV from light-trapped *C. inornata.* The larger number of high infection ratios indicated that *C. inornata* was the best vector of CEG.

ACKNOWLEDGMENTS. We express sincere

thanks to: Dr. T. H. Work, School of Public Health, University of California at Los Angeles, who suggested Callao for arbovirus studies; D. C. Bagley, Callao, Utah, and Dugway Ecology and Epidemiology Division staff members, T. R. Williams, D. R. Thompson, C. R. Kyte, for collecting and processing mosquitoes; E. R. Kern for technical support in the virus laboratory; J. A. Menge for determining plant dominants; and D. C. Kelly, Dugway Editorial Office, for preparing Figure 1.

Research was conducted according to the principles enunciated in the *Guide for Laboratory Animal Facilities and Care*, prepared by the National Academy of Sciences—National Research Council.

References

Climatological Data for Utah. 1966. Annual Summary. U.S. Dep. Com. 68:199–210.

Climatological Data for Utah. 1967. Annual Summary U.S. Dep. Com. 69:201–213.

Crane, G. T., Elbel, R. E., Klimstra, D. E. and Smart, K. L. 1970. Arbovirus isolations from mosquitoes collected in Central Utah in 1967. Amer. J. Trop. Med. and Hyg. 19:540–543.

Elbel, R. E. 1968. Sight identification key for mosquitoes of the Great Salt Lake Basin. Mosq. News 28:169–171.

Graham, J. E., Bradley, I. E. and Collett, G. C. 1960. Some factors influencing larval populations of *Culex tarsalis* and Western equine encephalitis in Utah. Mosq. News 20:100–103.

Hess, A. D., Cherubin, C. E. and LaMotte, L. C. 1963. Relation of temperature to activity of Western and St. Louis encephalitis viruses. Amer. J. Trop. Med. and Hyg. 12:657–667.

Holden, P. and Hess, A. D. 1959. Cache Valley virus, a previously undescribed mosquito-borne agent. Sci. 130:1187.

Horne, F. D. Bean. 1945. Autobiography of George W. Bean and his family record. Utah Printing Co. 377 pp., Salt Lake City.

Nielsen, L. T. and Rees, D. M. 1961. An identification guide to the mosquitoes of Utah. Univ. Utah Biol. Ser. 12:1–58.

Olson, J. K., Elbel, R. E. and Smart, K. L. 1968. Mosquito collections by CDC Miniature Light Traps and livestock-baited stable traps at Callao, Utah. Mosq. News 28:512–516.

Sudia, W. D. and Chamberlain, R. W. 1967. Collection and processing of medically important arthropods for arbovirus isolation. U.S. Pub. Health Ser., Nat. Commun. Dis. Cen. 29 pp.

SAMPLING OF OUTDOOR RESTING POPULATIONS OF *ANOPHELES CULICIFACIES* AND *ANOPHELES FLUVIATILIS* IN GUJARAT STATE, INDIA [1]

A. M. SHALABY

Zoology Department, Faculty of Science, University of Alexandria, Alexandria, Egypt

INTRODUCTION. The resting habits of the malaria vectors are among the important indices in the assessment of the epidemiological capacity of the vector population. In another paper (Shalaby, in press), the outdoor resting anophelines collected from artificial pit shelters dug at four localities in Panchmahals district of Gujarat State in India, have been investigated. Twelve species of *Anopheles* were detected. They were: *Anopheles culicifacies* Giles, *A. tesselatus* Theob., *A. theobaldi* Giles, *A. fluviatilis* James, *A. barbirostris* v.d.w., *A. stephensi* Liston, *A. pallidus* Theob., *A. annularis* Wulp, *A. maculatus* Theob., *A. splendidus* Koidzumi, *A. jamesi* Theob. and *A. subpictus* Grassi. Of the 12 species, *A. culicifacies*, a major vector of malaria in Panchmahals district, ranked first in abundance and *A. fluviatilis*, an additional vector and the sole vector in some parts of Gujarat State,

[1] This paper is based on work carried out during the period that the author was a staff member of the World Health Organization.