PARASITES OF MOSQUITOES IN SOUTHWESTERN WYOMING AND NORTHERN UTAH [1]

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Extensive world-wide reports have been published on mosquito parasites. These reports include viruses, rickettsia, bacteria, spirochaeta, fungi, protozoa, rotatoria, trematoda, nematoda and acarina. Of these, the fungi and protozoa have received the most intensive study because of their high prevalence and demonstrated pathogenicity to mosquitoes.

The literature pertaining to mosquito parasites is extensive in regard to both larvae and adults. Excellent bibliographies on the subject are contained in the publications of Howard *et al.*, (1912), Speer (1927), Christophers (1952, 1960), Jenkins (1964), and Steinhaus (1963).

The present study was conducted during the mosquito active season of 1965 and 1966 and involved several objectives. The first of these was to survey the mosquito fauna of a reasonably large geographic region to determine the kinds, distribution and prevalence of the parasites present. The area studied extended from the Salt Lake Valley along the Great Salt Lake and Utah Lake at about 4200 feet elevation, through the mountain areas of northern Utah to the high plains of Wyoming and Utah north of the Uinta Mountains. The area is one of considerable habitat diversity. The Salt Lake Valley constitutes the northern end of the Bonneville Basin and the collection sites used were in the counties bordering the Great Salt Lake where the habitat ranged from the marshes along the lake through agricultural and suburban and urban developments. Collection sites in the mountains extended as high as 9000 feet in the aspen-fir and spruce-fir habitats where the species of mosquitoes present were of the single-brooded *Aedes* type, as were those of the high plains north of the Uinta Mountains.

A second objective was to compare mosquito-inhabiting parasites from areas that had been under intensive control for some years with areas in which no control was present.

Mosquito samples were collected from a number of sites in each of the counties in the study area. Records were compiled and reported as one set of data per county in Table 1. Some major differences existed in the mosquito fauna throughout the area. *Aedes dorsalis* (Meigen), *Culex pipiens* (Linnaeus), and *C. tarsalis* Coquillett, and *Culiseta inornata* (Williston), all multi-brooded species, were the species most commonly collected and dissected. Larvae of these species were found to occur in a variety of temporary to permanent pools created through irrigation practices and in sites of natural or man-made origin. The species present in the high plains of Uinta County, Wyoming, and Daggett County, Utah, were mostly single-brooded species and consisted of *Aedes communis* (De Geer), *A. increpitus* Dyar, *A. pullatus* (Coquillett), *A. cinereus* Meigen, *A. fitchii* (Felt and Young), and *Culiseta impatiens* (Walker).

Living larvae of the third and fourth instars were examined under a dissecting microscope, identified to species, and checked for parasites. A proportion of the larvae found infected with microsporidians was fixed in Bouin's or Kahle's fluid

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for later sectioning. Smears were made of swollen white or grayish tissue, fixed in methyl alcohol, and stained with Giemsa. A sample of the larvae infected with mermithid nematodes was dissected as soon as possible, or in some cases where immediate attention was not possible, was placed in Kahle's fixative. Nematodes were prepared for study by the dehydrated glycerine method of Cobb or cleared in chlorolactophenol of Amann for detailed study.

From all collections, samples of the mosquito larvae were placed in containers for transportation to the laboratory. Attempts were then made to rear them through to the adult stage in an effort to study pathogenicity of parasitic organisms to the host, and to study life cycles. Mermithid specimens which emerged from hosts were reared in the laboratory in water and wet soil from the collection site. Water level was maintained by adding distilled water.

RESULTS. Mosquito larvae, totaling 9,094 specimens belonging to eighteen species, were examined for parasites. Results of dissection are tabulated in Table 1 by mosquito species, number examined, and locality from which collected. The species examined were *Aedes campestris* Dyar and Knab, *A. cataphylla* Dyar, *A. cinereus, A. communis, A. dorsalis, A. fitchii, A. implicatus* Vockeroth, *A. increpitus, A. niphadopsis* Dyar and Knab, *A. pullatus, A. schizopinax* Dyar, *A. sierrensis* Ludlow, *A. sticticus* (Meigen), *Anopheles freeborni* Aitken, *Culex pipiens, C. tarsalis, Culiseta inornata* and *C. impatiens.*

Two groups of pathogenic endoparasites were recovered. In addition, several species of organisms were found attached externally, one of which was observed to be parasitic while the others appeared to be cases of phoresy. Endoparasites consisted of several species of *Thelohania* (Nosematidae, Sporozoa) and an apparently new genus and species of nematode, *Reesimermis nielseni* (Nematoda:Mermithidae) which is being described in a separate paper. Data are presented in Tables 1 and 2 regarding parasite species, host range, and geographic localities from which collected.

Occurrence of microsporidians infecting mosquito larvae populations was found to be common, but infection rates were usually low, ranging from zero to 12.3 percent. Four species were found to be infected with microsporidians during the present study, with infected specimens being taken in the Salt Lake Valley and in Lone Tree, Wyoming.

Attempts were made to assess the effect of mosquito control on the incidence of larval parasitism. The infection rate with microsporidians was found to be slightly higher in *A. dorsalis* (3.1 percent, 23/739) and *C. inornata* (0.67, 4/597) taken from non-controlled areas than from controlled areas where the former showed 2.7 percent, 31/1161, and the latter species 0.3 percent, 3/965. On the other hand, infection rates in *C. tarsalis* from localities which have had long-standing control were much higher (12.1 percent, 21/174) than in non-controlled areas (2.2 percent, 3/137). There is a question as to the degree of significance that can be attached to these results because of the irregular distribution of the parasites in the breeding pools of a region; however, some recognition should be taken of the results based on the fact that the *Thelohania* inhabiting each of the above hosts appear to be separate species and are strongly host-specific. As none of these have been determined to species, they have been designatd as species A, B, C, and D.

(1). *Thelohania* sp. A, in *C. tarsalis,* had macro-spores measuring 11 x 6.5 μ and microspores 6–7 x 4–5 μ and averaging 6.5 x 4.5 μ The capsule held 8 spores and had rather thick walls. The capsule membrane proved to be very thin and easy to break so that each spore in the capsule could easily be separated. The spores were found to be densely distributed in the fatty bodies of the thorax and abdominal segment of hosts.

(2). *Thelohania* sp. B, parasitizing *C.*

TABLE 1.—Distribution of mosquito parasites according to county. (Mermithidae found only in Uinta County, Wyoming).

Location	Degree of Control*	MOSQUITO SPECIES																	
		Aedes campestris	*Aedes cataphylla*	*Aedes cinereus*	*Aedes communis*	*Aedes dorsalis*	*Aedes fitchii*	*Aedes implicatus*	*Aedes increpitus*	*Aedes niphadopsis*	*Aedes pullatus*	*Aedes schizopinax*	*Aedes sierrensis*	*Aedes sticticus*	*Culex pipiens*	*Culex tarsalis*	*Culiseta impatiens*	*Culiseta inornata*	*Anopheles freeborni*
MICROSPORIDIA:																			
Salt Lake County, Utah																			
No. mosquitoes examined	C	..	..	..	..	757	..	..	..	..	..	..	..	..	522	170	..	943	..
No. with Microsporidia		..	..	..	..	24	..	..	..	..	..	..	..	..	0	21	..	3	..
% with Microsporidia		..	..	..	..	3.18	..	..	..	..	..	..	..	..	0	12.3	..	0.3	..
Weber County, Utah	C	..	..	..	..	404	..	..	..	..	..	..	..	..	..	4	..	22	..
No. mosquitoes examined	NC	..	..	19	..	..	9	..	15	..	..	46	61	16	..	..	..	..	..
No. with Microsporidia		..	..	0	..	7	0	..	0	..	..	0	0	0	..	0	..	0	..
% with Microsporidia		..	..	0	..	1.7	0	..	0	..	..	0	0	0	..	0	..	0	..
Summit County, Utah																			
No. mosquitoes examined	NC	..	150	..	8	..	39	145	121	5	172	..	..	..	..	40	..	88	..
No. with Microsporidia		..	0	..	0	..	0	0	0	0	0	..	..	..	..	0	..	0	..
Tooele County, Utah																			
No. mosquitoes examined	NC	5	..	..	..	108	..	..	..	252	..	..	..	..	..	45	..	188	9
No. with Microsporidia		0	..	..	..	0	..	..	..	0	..	..	..	..	..	2	..	1	0
% with Microsporidia		0	..	..	..	0	..	..	..	0	..	..	..	..	..	4.4	..	0.5	0
Utah County, Utah																			
No. mosquitoes examined	NC	..	..	..	..	631	..	..	..	..	..	..	..	..	24	52	..	321	5
No. with Microsporidia		..	..	..	..	23	..	..	..	..	..	..	..	..	0	1	..	3	0
% with Microsporidia		..	..	..	..	3.6	..	..	..	..	..	..	..	..	0	1.9	..	0.9	0
Daggett County, Utah																			
No. mosquitoes examined	NC	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	89	..	..
No. with Microsporidia		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	0	..	..
Uinta County, Wyo.-Lone Tree																			
No. mosquitoes examined	NC	..	..	15	204	..	4	..	1748	..	1197	..	..	..	..	..	441	..	..
No. with Microsporidia		..	..	0	0	..	0	..	0	..	0	..	..	..	..	..	9	..	..
% with Microsporidia		..	..	0	0	..	0	..	0	..	0	..	..	..	..	..	2.04	..	..
TOTAL MOSQ. EXAMINED (9,094)		5	150	34	212	1900	52	145	1884	257	1369	46	61	16	546	311	530	1562	14

Table 1.—Continued

Location	Degree of Control*	MOSQUITO SPECIES																
		Aedes campestris	*Aedes cataphylla*	*Aedes cinereus*	*Aedes communis*	*Aedes dorsalis*	*Aedes fitchii*	*Aedes implicatus*	*Aedes increpitus*	*Aedes niphadopsis*	*Aedes pullatus*	*Aedes schizopinax*	*Aedes sierrensis*	*Aedes sticticus*	*Culex pipiens*	*Culex tarsalis*	*Culiseta impatiens*	*Culiseta inornata*	*Anopheles freeborni*
MERMITHIDAE:																			
Uinta County, Wyo.-Lone Tree	NC	..	..	15	204	..	4	..	1748	..	1197	..	..	..	..	..	441	..	..
No. mosquitoes examined		..	..	8	35	..	2	..	920	..	642	..	..	..	..	..	2	..	..
No. with Mermithidae		..	..	53.3	17.2	..	50.0	..	52.6	..	53.6	..	..	..	..	..	0.45	..	..
% with Mermithidae																			

* C = Controlled Area.
NC = Non-controlled Area.

TABLE 2.—Comparison of mosquito parasite prevalence between mosquito controlled and non-controlled areas.

Location	MOSQUITO SPECIES																	
	Aedes campestris	*Aedes cataphylla*	*Aedes cinereus*	*Aedes communis*	*Aedes dorsalis*	*Aedes fitchii*	*Aedes implicatus*	*Aedes increpitus*	*Aedes niphadopsis*	*Aedes pullatus*	*Aedes schizopinax*	*Aedes sierrensis*	*Aedes sticticus*	*Culex pipiens*	*Culex tarsalis*	*Culiseta impatiens*	*Culiseta inornata*	*Anopheles freeborni*
CONTROLLED AREAS:																		
No. mosq. examined	..	..	..	..	1161	..	..	..	..	..	..	..	..	522	174	..	965	..
No. with Mermithidae	..	..	..	..	0	..	..	..	..	..	..	..	..	0	0	..	0	..
% with Mermithidae	..	..	..	..	0	..	..	..	..	..	..	..	..	0	0	..	0	..
No. with Microsporidia	..	..	..	..	31	..	..	..	..	..	..	..	..	0	21	..	3	..
% with Microsporidia	..	..	..	..	2.7	..	..	..	..	..	..	..	..	0	12.1	..	0.3	..
NON-CONTROLLED AREAS:																		
No. mosq. examined	5	150	34	212	739	52	145	1884	257	1369	46	61	16	24	137	530	597	14
No. with Mermithidae	0	0	8	35	0	2	0	920	0	642	0	0	0	0	0	2	0	0
% with Mermithidae	0	0	23.5	16.5	0	3.8	0	48.8	0	46.9	0	0	0	0	0	0.4	0	0
No. with Microsporidia	0	0	0	0	23	0	0	0	0	0	0	0	0	0	3	9	4	0
% with Microsporidia	0	0	0	0	3.1	0	0	0	0	0	0	0	0	0	2.2	1.7	0.67	0
TOTAL Mos. Exam'd (9094)	5	150	34	212	1900	52	145	1884	257	1369	46	61	16	546	311	530	1562	14

inornata, showed spores that measured 4–5 x 3–4 μ with an average of 4.8 x 3.6 μ. The capsule membrane of this form seemed quite rigid and strong so that each spore was hard to separate from the compact 8-spore capsule. The spore walls appeared to be rather thin.

(3). *Thelohania* sp. C, from *A. dorsalis,* had spores measuring 4–6 x 3.5–5 μ with an average of 5.6 x 4.6 μ in 4 percent formalin samples, and 6.7 x 4.7 μ, average 6.0–7.2 x 4.4–5.1 μ, in fresh samples. The spore wall seemed very thin and it was not easy to isolate the spores from the 8-membered capsules. The nucleus in the spore was distinct and spherical. The spores were found mostly in fatty bodies located in the thorax of the hosts and were rather sparse in the abdominal segments.

(4.) *Thelohania sp.* D, in *C. impatiens,* had an average size of 6.6 x 4.4 μ (6.0–7.2 x 4.0–4.8 μ) in fresh material. The capsule membrane holding the eight spores was irregular in shape, very thin and easily ruptured.

A group of aquatic, ecto-parasitic red mite larvae, *Arrenurus* sp. (Arrenuridae, Acarina), was also found adhering to the body surfaces of many pupae, and on the abdomens of adult *A. pullatus, A. increpitus,* and *C. impatiens.* The size of the mite larvae averaged about 0.30 mm in length and 0.21 mm in width, and the hypostome teeth were stout, sharp, strong and slightly curved. The segments of the pedipalps were greatly reduced and were difficult to count. The pedipalps were further modified into a lip-like structure to aid the mites in holding their position when they penetrate the intersegmental areas of the abdomen of adult mosquitoes to suck body fluids. In appearance, the mites were red on the dorsal side of the body only and were collected exclusively from mountain *Aedes* mosquitoes during June and July. The prevalence ranged from 24.3 percent in the pupal stage to 13.8 percent in adult mosquitoes (Table 3).

Three ecto-symbionts, a species each of *Vorticella* and *Epistylis* (Peritrichida, Ciliata) and one green algae of the genus *Characium* were found on the anal gills, bases of antennae, segments of thorax, and abdomen of the larvae. *Vorticella* sp. was very common and abundant and showed no particular host species preference. It was conjectured that an abundance of these organisms might cause some hindrance to the normal behavior of the larvae, but actual injury was not evident.

DISCUSSION. To determine if a significant difference exists in the type and degree of parasitism of mosquitoes between areas controlled and those uncontrolled with pesticides, additional data should be procured as it is difficult to draw a comprehensive and definite conclusion from the amount of data presented in Tables 1 and 2. The reasons for the inconclusiveness are: (1) Each parasite species has its own ecological requirements and host specificity; for example, infections with mermithids and microsporidians appear to be completely different in this respect; (2) No strictly defined separation of uncontrolled and controlled areas was possible. Some areas being regarded as controlled had not been treated for long periods of time but were considered as in the controlled group because of the long residual of some of the insecticides previously used. In addition, one does not have a record of the insecticide applications that have been made for agricultural or range pests which could produce an effect in areas that have not been under mosquito control per se, and tests for insecticide residuals in soil and water were not made. (3) No experimental work was done to determine the relationship of different insecticides and the existence of healthy larvae and larvae infected with parasites.

However, infections with microsporidians among *A. dorsalis* and *C. inornata* showed a slightly higher incidence in uncontrolled than in controlled pools, and the distribution was more widespread in uncontrolled localities. The explanation of

TABLE 3.—Prevalence of red mites on mosquitoes in Lone Tree, Uinta County, Wyoming.

	MOSQUITO PUPAE			ADULT MOSQUITOES											
				Aedes increpitus			*Aedes pullatus*			*Culiseta impatiens*			TOTAL		
Date	Total Number Exam'd	No. with mites	% with mites	Total No. Exam'd	No. with mites	% with mites	Total No. Exam'd	No. with mites	% with mites	Total No. Exam'd	No. with mites	% with mites	No. Exam'd	No. with mites	% with mites
5-29-66	15	0	0	..	..	..	..	..	..	..	..	..	..	..	..
6-22-66	14	5	35.7	..	..	..	..	..	..	..	..	..	..	..	..
7-13-66	32	17	53.1	..	..	..	..	..	..	..	..	..	..	..	..
7-27-66	50	5	10.0	..	..	..	..	..	..	..	..	..	..	..	..
TOTAL PUPAE	111	27	24.3	..	..	..	..	..	..	..	..	..	..	..	..
6-19-66	..	..	..	20	1	5.0	22	1	4.5	0	0	0	42	2	4.8
6-28-66	..	..	..	23	2	8.7	69	10	14.5	1	0	0	93	12	12.9
7-13-66	..	..	..	0	0	0	11	4	36.4	6	3	50.	17	7	41.2
TOTAL ADULTS	..	..	..	43	3	7.0	102	15	14.7	7	3	43.	152	21	13.8

this situation may be that the insecticide dosage that kills the mosquito host may also affect the survival and infective ability of the microsporidians. On the other hand, insecticides may suppress the development of microsporidia and diminish the usual sources of infection in the locality, although these parasites are known to have extremely resistant spores, a short life-cycle, and have ovarian transmission in the host to maintain the parasite species. In contrast, the incidence obtained of the microsporidian in *C. tarsalis* was higher in controlled areas. Further work on host species susceptibility or host habitat action on insecticides was not attempted. At present no suitable hypothesis can be advanced to explain the data obtained.

As a result of the study, it appears evident that the seasonal occurrence of the mermithid nematode is more specific and its distribution much more restricted than that of microsporidians. The mermithid worms seem to require more exact ecological conditions and require a host that can maintain them at high population levels in the earlier summer.

Both microsporidian and mermithid infections appeared to make larval mosquitoes sluggish and inactive and were observed to be highly fatal to infected larvae.

It has been established previously that in cases of multiple parasitism, a heavy infection with one species is usually not accompanied by a similar heavy infection with a second species. However, during the study of the nematode infection in the *Aedes* species at Lone Tree, Wyoming, the habitat in which the infection was present apparently established good cultural conditions for propagation of both the nematode and the microsporidian so that some cases of heavy infection with both were collected. The specific environmental conditions in this case are pastures that are rich in cattle manure and decayed vegetation to support free-living stages and which are flooded periodically so that the soil does not become dry.

It is also interesting to note that some species of aquatic red mite larvae, with three pairs of legs, were found as ectoparasites on the bodies of some pupae and adults, but not larvae, of *A. increpitus, A. pullatus,* and *C. impatiens.* The mite larvae were attached mostly on both sides of the appendage pouches of pupae. When the dorsal side of the thorax of the pupa was split, upon adult emergence, the mite larvae rapidly migrated from the pupal skin to the young adult mosquito where they chose a suitable anchorage in the intersegmental soft area. Here they inserted their capitula into the host's body and sucked the body fluids. These mite-infested mosquitoes developed a swollen abdomen after 1–2 days of feeding on the hosts, and it is probable that the mites damaged the host when abundant, but the degree of pathogenicity and the natural history of the mite were not determined.

SUMMARY. The parasitism of the mosquito species present in six counties in northwestern Utah and one in southwestern Wyoming was investigated to determine kinds, effects on the host, and distribution. A comparison was also made between areas in which mosquito control was, or had been, in effect recently and those with no control.

Larvae, totaling 9,094 specimens comprising four genera and eighteen species, were examined.

Endoparasites belonging to two phyla were present as represented by four undetermined species of microsporidians (*Thelohania*) and a new mermithid nematode species, *Reesimermis nielseni,* n. sp. Ectoparasitic red mite larvae, *Arrenurus* sp., were also removed from pupae and adults of three mosquito species.

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REPELLENT TESTS AGAINST *ANOPHELES ALBIMANUS* WIEDEMANN IN THE PANAMA CANAL ZONE [1]

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The progessive steps required to safely develop insect repellents have been discussed in detail by Smith (1958) and Gilbert *et al.* (1957). As described in these references, exhaustive laboratory evaluations of candidate repellents are followed by tests against hematophagous insects in selected areas of the world. During the last twenty-five years field tests have been conducted against *Aedes* and *Culiseta* spp. (Altman and Smith 1955, Gilbert 1957) stable flies (Travis and Smith 1951), Simuliidae (Travis *et al* 1951) and other important groups. Because of the difficulty in finding suitable populations of *Anopheles* spp. few field tests have been conducted against this important genus.

During the latter part of 1967 exceptionally heavy populations of *Anopheles albimanus* Wiedemann were present at Frijoles, Panama Canal Zone. The mosquitoes were uniformly distributed for approximately 10 miles along the shore line of Gatun Lake where the tests were made. The population was heaviest near the lake, but large numbers were also present in the jungle at distances greater than one mile from the lake. The mosquitoes were breeding in dense mats of aquatic vegetation (primarily *Elodea* sp., *Naias marina* and *Ceratophyllum demersum*) along the margin of the lake and in the numerous ponds in the area. There was little diurnal activity in the unshaded areas, but *A. albimanus* fed throughout the day in densely shaded jungle areas. Intense biting began at twilight and continued for several hours, with some biting throughout the night.

This heavy *Anopheles* population proved to be optimal for testing repellents and was in low malaria risk area.

MATERIALS TESTED. Tests were made with five repellents and one mixture of repellents obtained from the USDA, ARS Entomology Research Division Laboratory in Gainesville, Florida. The names of the repellents, formula of the mixture, and the USDA code numbers are shown below. Reference to the materials is made by these code numbers throughout the paper.

USDA No.	Repellent
22542	*N,N*-Diethyl-*m*-toluamide (deet)
2706	2,2,4-trimethyl-1,3-pentanediol
262	dimethyl phthalate
14913	N,N-diethylbenzenesulfonamide
375	ethyl hexanediol
M-2020	dimethyl phthalate (40%) ethyl hexanediol (30%) dimethyl carbate (30%)

TESTING METHODS. The repellents were tested at full strength and as ethanol dilutions. Repellents were applied to the forearms and legs of the test subjects, 1 milliliter to the forearms from the wrist to the elbow and 1½ milliliter to the legs from the ankle to the knee. Two of the

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