HOST-BLOOD SOURCES AND MULTIPLE-FEEDING HABITS OF MOSQUITOES IN KANSAS [1, 2]

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INTRODUCTION. Knowledge of mosquito feeding habits aids epidemiological studies of mosquito-borne diseases and control operations. Several methods have been employed in attempts to establish sources of mosquito blood-meals in nature. Serological techniques to identify blood meals in adult mosquitoes collected from their natural habitats provide a method with minimum bias to assess feeding habits. Although some difficulty may be encountered in collecting representative samples of certain species, a rather accurate picture of hosts of natural mosquito populations in a given locality can be secured.

Relatively little is known concerning host-blood sources of most Kansas mosquitoes. A study of several species, particularly in Riley County, was undertaken to expand knowledge on this subject. In addition to information concerning host-blood sources, attempts were made to secure data on multiple-feeding habits, host specificity, seasonal feeding patterns and potential vector ability.

MATERIALS AND METHODS. Standard New Jersey light traps baited with dry ice were used to collect adult mosquitoes. Location of the traps appeared to be critical for a representative sample, so particular care was exercised in choice of trap sites. Traps were placed in a variety of habitats that contained both a high mosquito population and the widest possible choice of potential hosts. All light trap sites were within a five-mile radius of Manhattan, except for one collection made near a salt marsh in Stafford County, Kansas. Collections were made each night from mid-May through mid-October, 1963. Mosquitoes showing even slight evidence of a blood meal were pinned and stored in low humidity at room temperature until tested. No specimen was stored longer than six months.

Antisera were prepared against beef, sheep, human, horse, dog, hog, guinea pig, rabbit and chicken sera. All antisera were prepared in rabbits except for anti-rabbit serum which was prepared in chickens. Only antisera that gave a homologous interfacial-test titer of 64,000+ were used. Antisera that cross-reacted with other test sera were rendered specific by absorption techniques (Weitz, 1952).

All blood-engorged mosquitoes were extracted for 6–16 hours at 5 degrees C. in 1 ml of .85 percent buffered physiological saline of pH 7 (Evans, 1922). Extracts were placed in serological tubes (3 mm inside diameter) and equivalent amounts (0.1 ml.) of undiluted test antisera were layered beneath the extracts. A "ring" of precipitate at the interface between extract and antiserum indicated a positive precipitin reaction. Tests were read at 20-minute intervals for one hour. Negative (antisera + saline), positive (antisera + homologous sera) and cross-reaction (antisera + heterologous sera) controls were performed with each bottle of antisera before and during use.

Preliminary screening of each extract was carried out with group antisera (Table 1). The sequence of precipitin tests performed on each blood-meal extract is illustrated in Figure 1. Amounts of spe-

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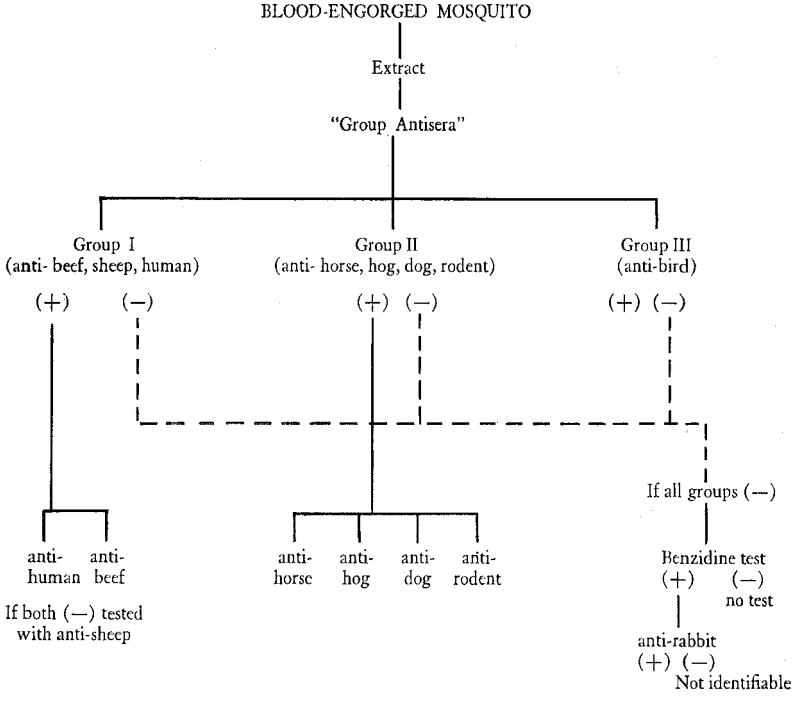
TABLE 1.—Composition and reactivity ranges of group antisera.

Contents	*Reacted strongly (1:64,000) with sera of*
Group I —equal parts undiluted human and beef or sheep antisera	human, beef, sheep (assumed goat and deer)
Group II —equal parts undiluted horse, dog, hog and guinea pig antisera	horse, dog, hog, guinea pig, mouse (assumed all rodents and coyote)
Group III—undiluted chicken antisera	chicken, duck, quail, starling, sparrow, pigeon (assumed all birds)

FIG. 1.—The Sequence of Precipitin Tests Performed on Engorged Mosquitoes



cific beef and sheep antisera available were insufficient to include both in the specific tests. Extracts giving a positive test with group I and negative tests with beef and human antisera were then tested for sheep blood. The testing order for beef and sheep occasionally was reversed, depending on amounts of test antisera available. Unabsorbed beef and sheep antisera were nonspecific for both beef and sheep sera so it was necessary to add only one or the other to group I mixtures. Extracts showing a negative reaction with all group antisera were subjected to the benzidine test for

blood (Hawk *et al.*, 1954). Those giving a positive benzidine reading were tested with anti-rabbit serum, which was not available in sufficient amounts to include in all group tests. When tests for rabbit blood were negative, the blood meals were considered not identifiable (NI) in this system.

Multiple-blood meals involving rabbit blood could not be detected in this system and similarly multiple-feeds involving sheep or beef could not always be detected and those including both sheep and beef could never be detected.

RESULTS AND DISCUSSION. Little difficulty was experienced in obtaining moderate numbers of blood-fed mosquitoes with light traps. Approximately 2–5 percent of all female mosquitoes in most collections were blood-fed.

Approximately 8 percent of all extracts tested were NI. Most NI extracts showed no color and it was assumed that digestion had proceeded too far to detect blood-proteins with the precipitin test. However, it is possible that some NI extracts contained reptile, amphibian or some other blood not represented in this testing procedure.

A wide variety of mammalian hosts were attacked by the five species of *Aedes* mosquitoes tested (Table 2). Beef was the most common host for all species except in the small sample of *Aedes trivittatus*. Except for possibly *Aedes dorsalis*, bird feedings appear to occur rarely.

Beef was also the main host for all species of *Culex* tested although samples of three species were limited to only one or two specimens (Table 2). *Culex tarsalis* fed on a wide range of mammalian hosts in addition to birds. Avian hosts were rare for *Culex salinarius* and absent in single-feedings of the *Culex pipiens* complex. Four multiple-fed *C. pipiens* contained bird blood. No attempt was made to separate *C. pipiens* from *C. quinquefasciatus* or their intermediates, all of

TABLE 2.—Host-blood sources of mosquitoes as determined by precipitin tests.

Species	Total Blood-meals Identified	Total Single Feedings	Number Singly Fed on Each Host
Aedes dorsalis	31	28	B(18) F(5) S(2) Rb(2) H(1)
A. nigromaculis	342	298	B(163) S(80) Rb(35) Hu(9) H(5) R(5) F(1)
A. sollicitans	50	45	B(40) S(4) Rb(1)
A. trivittatus	9	7	S(2) Hu(2) Rb(2) B(1)
A. vexans	1,417	1,154	B(723) Hu(192) S(142) Rb(46) P(29) P(29) H(14) F(7) D(2)
Anopheles punctipennis	4	2	B(1) Hu(1)
Culex erraticus	1	1	B(1)
C. pipiens	30	24	B(18) Hu(5) P(1)
C. restuans	2	2	B(1) F(1)
C. salinarius	79	50	B(22) S(11) P(7) D(6) Rb(2) F(2)
C. tarsalis	204	160	B(73) F(34) S(27) Hu(8) Rb(6) P(4) D(3) R(3) H(2)
C. territans	1	1	B(1)
Culiseta inornata	93	81	B(56) S(15) Hu(4) Rb(3) F(2) H(1)
Psorophora ciliata	68	26	S(10) D(7) B(4) Hu(3) F(2)
P. confinnis	280	180	S(59) B(46) D(20) Rb(15) Hu(13) R(12) H(7) F(5) P(3)
P. cyanescens	38	29	B(13) S(8) F(3) P(2) R(2) D(1)
P. discolor	118	80	B(28) S(15) D(10) F(8) Hu(7) P(5) Rb(5) H(2)
P. signipennis	6		
Total	2,773	6	B(3) S(3)

B—(beef), S—(sheep), Hu—(human), H—(horse), P—(hog), D—(dog), —(rodent), Rb—(rabbit), F—(bird).

which are thought to occur in this area of Kansas.

Beef or sheep were most often attacked by the *Psorophora* mosquitoes tested. Bird feedings occurred in samples of four species but represented a small proportion of all feedings. Beef was the main host for *Culiseta inornata,* which also fed rarely on avian blood.

Little information concerning the identification of mosquito bloodmeals in North America is available for comparative purposes. In comparing results of precipitin-test studies, it must be borne in mind that the collection method and hosts available as well as other factors may greatly influence the results obtained.

Reeves and Hammon (1944) found no bird feedings in *A. dorsalis* blood-meals tested from Yakima valley in Washington. In Canadian studies, Shemanchuk *et al.* (1963) found 5 percent and Rempel *et al.* (1946) 13 percent of the *A. dorsalis* tested to have avian blood. Rempel *et al.* (1946) and Shemanchuk *et al.* (1963) reported approximately 20 percent avian feeding in samples of *A. vexans,* and 10 percent in *A. nigromaculis. Aedes sollicitans* collected from the coastal islands off Virginia had fed mainly on larger mammals with only two bird-feedings (Thompson *et al.,* 1963). Reeves *et al.* (1944, 1963) reported 86 percent of all *C. tarsalis* blood-meals tested from California and 46 percent from Yakima valley contained avian blood. In earlier studies in the Yakima valley (Bang and Reeves, 1942) only 6 percent bird feedings were found and in 88 *C. tarsalis* tested from Alberta, 21 percent had avian blood (Shemanchuk *et al.,* 1963).

Willingness to bite humans reportedly differs greatly among members of the *C. pipiens* complex and Reeves and Hammon (1944) found a high incidence of bird-feedings in *C. pipiens* tested. Results similar to our findings have been reported for *P. confinnis* and *P. discolor* in Arkansas (Whitehead, 1951). Reeves and Hammon (1944), Washino *et al.* (1962) and Shemanchuk *et al.* (1963) found *C. inornata* to have fed mainly on large mammals.

Very little host specificity for any of the species tested was evidenced by our results. All species preferred mammalian blood. The larger and more common mammals in this area (beef and sheep) were most readily attacked. Among species with at least 25 blood-meal identifications, *Aedes vexans* (13.6%) and *Culex pipiens* (16.7%) had the most human feedings. Horses were present but never abundant in the collection area; few horse feedings were found. Among other mammals (hog, dog, rabbit and rodent), rabbit feedings were most common except in the *Psorophora* species and *Culex salinarius* where dog or hog feedings were most common. Swine did not appear very attractive in view of their abundance near most collecting sites.

Census of domestic hosts within a two-mile radius of each collecting site indicated a wide range of potential hosts in all areas. Birds (primarily chickens) were the most abundant near most collecting sites. Beef, hogs, sheep, humans and dogs, generally in that order, were also abundant within one-half mile of most collecting sites. Only minor differences in hosts attacked by a given species could be seen among samples from different locations. Species collected at a location with hutches of domestic rabbits near by usually showed a higher incidence of rabbit feedings. Similar differences could be noted between sites where numbers of other available hosts differed widely.

Host animal populations on which the majority of the feedings occurred do not undergo marked seasonal changes in numbers or availability in this area during the mosquito season. No evidence of significant seasonal changes in feeding habits could be demonstrated.

The most striking observation was the high incidence of multiple-feeding found in several species (Table 3). The data represent minimum multiple-feeding since all combinations of multiple-hosts could not be detected and multiple-feeding on hosts of the same species or the same host cannot be assessed by this method. Most

TABLE 3.—Multiple feeding patterns.

Species	Percent of Total Feeds That Were Mult.	No. of Mult. Feeds	2 Feeds	3 Feeds	4–5 Feeds	Hosts Involved in Multiple Feeding							
						B	S	Hu	H	P	D	R	F
Aedes dorsalis	9.7	3	1	2	..	3	..	2	..	1	..	1	1
A. nigromaculis	12.9	44	41	1	2	22	20	7	10	3	..	6	25
A. sollicitans	10.0	5	5	..	..	2	3	4	..	..	..	..	1
A. trivittatus	22.2	2	1	1	..	..	2	1	..	..	..	..	2
A. vexans	18.5	263	194	43	25*	179	39	163	56	51	36	24	82
Anopheles punctipennis	50.0	2	2	..	..	1	1	..	..	..	..	..	2
Culex pipiens	20.0	6	6	..	..	4	..	..	2	2	..	..	4
C. salinarius	36.7	29	22	7	..	19	7	1	..	12	17	6	3
C. tarsalis	21.5	44	36	7	1	20	13	7	11	5	12	4	25
Culiseta inornata	12.9	12	10	1	1	8	3	5	1	2	1	1	7
Psorophora ciliata	61.8	42	33	7	2	12	15	21	7	11	11	6	12
P. confinnis	35.7	100	86	14	..	21	61	25	25	15	19	12	36
P. cyanescens	23.7	9	9	..	..	2	6	..	2	2	3	1	2
P. discolor	32.8	38	32	4	2	14	15	10	6	3	7	2	27

B—(beef), S—(sheep), Hu—(human), H—(horse), P—(hog), D—(dog), R—(rodent), F—(bird).

* One contained 6 different host bloods.

multiple-feeds involved two hosts, although one instance of as many as six different host-bloods was encountered in *A. vexans*. In general the main hosts involved in single-feedings were also involved in multiple-feeds. Note the higher number of feedings on avian blood in multiple blood-meals of *A. vexans* and *A. nigromaculis* as compared to the single-feedings. The same was true for *C. tarsalis, C. inornata, P. ciliata, P. confinnis* and *P. discolor*.

This phenomenon is unexplained; however, similar increases can be found in some of the smaller mammalian hosts. Shemanchuk *et al.* (1963) noted a similar increase in rodent feedings from multiple-fed mosquitoes. They offered a possible explanation on the basis that smaller animals can scratch almost any part of their bodies thereby increasing the chances of interrupted-feeding. In our studies, *Culex* and *Psorophora* species showed a higher incidence of multiple-feeding than *Aedes* and *Culiseta* species. *Psorophora ciliata,* the largest North American mosquito, displayed the highest incidence of multiple-feeding (61.8%). The size and/or severity of the bite of this species might promote interrupted-feeding.

Multiple-feeding in other precipitin-test studies has, for the most part, been ignored. Blood meals reacting with more than one host have, for statistical purposes either been omitted, treated as half-values or only the first or strongest reaction has been considered. Rempel *et al.* (1946) tested blood meals from five species of *Aedes* in Canada (including *A. vexans, A. nigromaculis* and *A. dorsalis*) and reported approximately one in three to have had more than one host-blood. They also demonstrated in the laboratory that all bloods from mosquitoes fed on three different hosts within a four-day period could be readily identified by the precipitin test (Riddell *et al.*, 1947). Reeves *et al.* (1963) found less than 1 percent of the *C. tarsalis* tested from Kern County, California, to have multiple feeds. In contrast, Bang and Reeves (1942) reported 28 double-feedings of 65 *C. tarsalis* tested from Yakima valley in Washington. However, in their study only the strongest reaction was considered. Downe (1960) and Shemanchuk *et al.* (1963) reported multiple-feedings ranging from less than 1 percent to 6 percent in studies with several Canadian species. Multiple-feeding habits of mosquitoes, as well as single-feedings, seem to vary considerably in different localities. Multiple-feeding might well provide a topic for investigations of impetus for epidemiological consideration.

SUMMARY. 1. Eighteen species of blood-engorged mosquitoes collected in light traps near Manhattan, Kansas, were subjected to precipitin tests to determine host-blood sources.

2. A wide range of domestic hosts was present near most collecting sites, with birds (chickens), beef, hogs, sheep, humans and dogs most abundant, in that order.

3. Little evidence of host specificity could be demonstrated for any species tested. All species seemed to prefer mammalian blood. The larger and more abundant mammals (beef and sheep) were most often attacked. Small numbers of most species tested had fed on avian hosts; *Culex tarsalis* and *Aedes dorsalis* showed the highest incidence of bird-feeding.

4. No evidence of seasonal changes in feeding patterns was observed.

5. A high incidence of multiple-feeding, from 9.7 to 61.8 percent, was observed. In some instances, hosts seldom attacked in single-feedings were much more abundant in multiple-feedings.

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RELATIONSHIP OF MOSQUITO LIGHT TRAP COLLECTION DATA TO LARVAL SURVEY DATA IN SALT LAKE COUNTY [1]

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The value and need for making measurements of mosquito populations is generally recognized by mosquito control workers. It is logical to assume that in order to determine the effectiveness of a control program and guide a mosquito abatement operation in both current and long-range planning, it is necessary to carry out a continuous program of measuring the mosquito population.

As early as 1922, Headlee suggested that an "index of potential annoyance" be established and he pointed out that efforts should be made to develop a mechanical substitute for the human collector. Peters, 1956, stated, "The subject 'mosquito measurements' is probably as under-appreciated, I would say, as anything we are doing in mosquito control."

A number of methods are used in making mosquito population studies. Those commonly used by mosquito abatement districts consist of the operation of light traps, biting or body counts, resting station collections, and larval collections. In selecting the method or methods to be used in determining changes in mosquito populations, several factors should be considered. These include the species of mosquito involved, the information desired, and the time that can be devoted to obtaining the desired information.

Light traps have been operated continuously in the Salt Lake City Mosquito Abatement District since 1933 and in the South Salt Lake County Mosquito Abatement District since its organization in 1953. A detailed mosquito larval survey

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