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STUDIES ON THE MOSQUITOES OF NORTH ARCOT DISTRICT, MADRAS STATE, INDIA

Part 4: Host preferences as shown by precipitin tests[1]

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Abstract: Stomach blood samples of common mosquito species were tested by precipitin test with antisera prepared in rabbits and chickens. The *Culex vishnui* group of species had fed mainly on cattle, with relatively few feedings on humans and pigs. *Culex tritaeniorhynchus* showed the smallest proportion of feedings on non-bovids. *Aedes aegypti* and *Culex pipiens fatigans* from houses had fed predominantly on man.

In earlier papers (Reuben 1971a, b) studies of mosquitoes entering baited traps, and captured feeding on bait at villages in South India were described. These studies showed that certain mosquito species would feed on various hosts, and gave an indication of host preferences under specified conditions of trapping, or capture when feeding on the various hosts. The most reliable way of assessing the feeding habits of mosquitoes under natural conditions is by the identification of blood meals of specimens collected from daytime resting places. The results of such studies are presented here. A few specimens from baited traps were also tested, and the results have been included.

METHODS

Collection of mosquitoes: Mosquitoes were collected between 1962 and 1965, and were stored for from a few weeks to 2 years before being tested.

Culex pipiens fatigans and *Aedes aegypti* were collected in houses in Vellore town. About 20 other species were collected in villages near Vellore or from the semirural outskirts of the town. The outdoor shelters sampled were located in fields, banana plants, and low herbage, all within .8 km (1/2 mile) of human habitation. Only *Culex* (*Lophoceraomyia*) sp. came from a forested area far from human habitation. The villages around which mosquitoes were collected were all very similar, containing people, cattle, a few sheep and goats, chickens and dogs. Pigs were not present in all the villages, but many mosquitoes (particularly the *Culex vishnui* group, comprising *C. vishnui*, *C. pseudovishnui* and *C. tritaeniorhynchus*) were collected outdoors from a number of villages which were selected because pigs were present. Other villages from which specimens were collected were chosen at random.

Mosquitoes were collected in the mornings with flashlight and suction-tube. They were brought alive to the laboratory before 1100 hr. At first they were kept on ice in the field, but later this practice was abandoned; it was sufficient if mosquitoes with cherry red to dark red blood in their stomachs were picked out for blood analysis as soon as they were brought to the laboratory. After identification the mosquitoes were either stored whole in sealed vials at −20 °C, or their stomachs were dissected out and smeared on filter paper and stored at 5 °C.

Preparation of antisera: The methods of Tempelis & Lofy (1963) were followed for the preparation of antisera. Broadly-reacting antisera were produced in rabbits immunized against pooled bird, mammalian and amphibian-reptilian sera. In addition, satisfactory screening antisera were produced in white Leghorn cockerels immunized against pooled mammalian and amphibian-reptilian sera. Individual antisera against human, cattle, sheep, goat, pig, dog, mongoose and rat sera were prepared in cockerels. Later, when a number of amphibian-reptilian feedings were identified, chickens were immunized individually against various amphibian and reptilian sera.

The immunization schedule followed was that of Tempelis & Lofy (1963). The pool used to sensitize rabbits against bird serum contained sera from domestic chickens, geese, cormorants (*Phalacrocorax niger*), herons (*Ardea cinerea*), egrets (*Egretta garzetta*), quails (*Turnix tanki*), mynas (*Sturnus pagodarum* and *Acridotheres tristis*), crows (*Corvus splendens*), babblers (*Turdoides affinis*), and sparrows (*Passer domesticus* and *Petronia xanthocollis*). The mammalian serum pool contained sera from man, cattle, sheep, pigs, dogs, mongoose (*Herpestes*

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edwardsi), rats (*Rattus rattus rufescens*) and shrews (*Suncus murinus*). The amphibian-reptilian serum pool contained sera from frogs (*Rana crassa*) and the common garden lizard (*Calotes versicolor*). Immune sera against the mammalian, amphibian and reptilian species were prepared separately also. Sera from *Suncus murinus* and *Rattus rattus rufescens* were pooled in an attempt to produce a combined small mammal antiserum. The resulting immune serum reacted in high titer to *R. r. rufescens* serum, but not to that of *S. murinus*. In effect, therefore, no shrew immune serum was prepared.

Antisera prepared in chickens were brought to a NaCl concentration of 8% before use following Tempelis & Lofy (1963). All antisera prepared were tested in tubes or by the gel diffusion method. Both methods gave similar results. One of the mammalian screening antisera prepared in a rabbit failed to react at a dilution of 1:10,000 with sera which were used to immunize the rabbit. This was rejected. All other antisera reacted with homologous sera at titers ranging from 1:20,000 to 1:400,000.

As pointed out by Tempelis & Lofy (1963), the antisera prepared in chickens against sera from various mammalian species were family specific. Cattle, sheep and goats belong to the family Bovidae and are referred to collectively as bovids throughout this paper. Antisera for these species showed cross reactivity at dilutions of 1:10,000 or more. There was also cross reactivity between the 2 species of the Carnivora for which antisera were prepared, dog and mongoose. Other antisera prepared were specific to their homologous antigens.

Testing of blood meals: Whole mosquitoes were placed in 1 ml of 0.85% phosphate buffered NaCl, pH 7.2 and their abdomens crushed with glass rods. They were then centrifuged at about 2000 rpm for 20 minutes and the supernatant fluid removed to another test tube ready for testing. Mosquito stomach contents on filter paper were allowed to soak in 0.5 ml of phosphate buffered NaCl at 5°C overnight, following Weitz (1956). The filter papers were removed and the fibers removed by centrifugation.

Plain capillary glass tubes, 2 mm OD, were used. The testing procedure has been described in full by Tempelis & Lofy (1963). At first, the blood meals were screened with the broadly-reacting bird and mammal antisera, and then if positive

TABLE 1. Results of precipitin tests on stomach bloods of 4 common mosquito species from outdoor shelters in North Arcot District, India.

Species	Period	No. of specimens	Source of blood meal: Amphibian/reptilian A	R	A/R†	Mammalian: Bird	Bov.	Man	Dog	Pig	Rat	Neg.	Test not completed
	Jan.-Mar.	102	1	2	3	4	83	5	1	0	0	3	0
	Apr.-June	208	0	5	0	22	171	6	0	1	0	1	2
Culex vishnui	Jul.-Sept.	79*	0	0	0	0	76	3	0	0	0	1	0
	Oct.-Dec.	140	0	0	2	1	128	3	0	0	0	4	2
	Total	529*	1	7	5	27	458	17	1	1	0	9	4
	%	100.0	0.2	1.3	1.0	5.1	87.1	3.2	0.2	0.2	0	1.7	–
	Jan.-Mar.	256	0	1	2	9	237	1	0	1	0	1	4
	Apr.-June	114	0	0	0	28	83	2	0	0	0	0	1
Culex pseudovishnui	Jul.-Sept.	19	0	0	0	0	19	0	0	0	0	0	0
	Oct.-Dec.	117	0	1	0	22	90	1	0	0	1	1	1
	Total	506	0	2	2	59	429	4	0	1	1	2	6
	%	100.0	0	0.4	0.4	11.8	85.8	0.8	0	0.2	0.2	0.4	–
	Jan.-Mar.	71*	0	0	0	1	69	1	0	0	0	1	0
	Apr.-June	107	0	0	0	0	103	0	0	0	0	0	4
Culex tritaeniorhynchus	Jul.-Sept.	221	0	0	0	1	215	0	0	0	0	3	2
	Oct.-Dec.	119*****	0	0	0	5	118	0	0	0	0	0	1
	Total	518******	0	0	0	7	505	1	0	0	0	4	7
	%	100.1	0	0	0	1.4	97.7	0.2	0	0	0	0.8	–
	Jan.-Mar.	82	0	0	0	43	33	3	0	2	1	0	0
	Apr.-June	19**	0	0	0	12	4	3	0	2	0	0	0
Culex bitaeniorhynchus	Jul.-Sept.	3	0	0	0	0	2	0	0	0	0	1	0
	Oct.-Dec.	24	0	0	0	19	5	0	0	0	0	0	0
	Total	128**	0	0	0	74	44	6	0	4	1	1	0
	%	100.0	0	0	0	56.9	33.8	4.6	0	3.1	0.8	0.8	–

Percentages have been calculated only for those specimens which were completely tested. Double feeds have been treated as 2 separate feeds for this purpose.

* = each asterisk represents a double feed.

† = not tested with specific antisera.

for mammal, with individual mammalian antisera. When it became apparent that the great majority of specimens tested were positive for bovid blood, the testing procedure was altered to test first for bird and bovid blood and, only if negative for these, with the other antisera prepared. By this method some multiple feedings may have been missed.

Mosquitoes known to have fed in the laboratory on amphibians, reptiles, birds and the various species of mammals were used to test the antisera before the precipitin test was applied to wild-caught mosquitoes.

RESULTS

TABLE 1 shows the results of precipitin tests on stomach contents of the 4 mosquito species most common in hand collections in outdoor shelters. *C. vishnui*, *Culex pseudovishnui* and *Culex tritaeniorhynchus* were predominantly feeders on bovids, with *C. tritaeniorhynchus* showing the highest proportion of bovid feeds (97.7%). Of *C. pseudovishnui* tested, 11.8% had fed on birds, while corresponding percentages for *C. vishnui* and *C. tritaeniorhynchus* were 5.1% and 1.4%, respectively. Of *C. vishnui* specimens, 3.2% were positive for human blood as compared to 0.8% for *C. pseudovishnui* and 0.2% for *C. tritaeniorhynchus*. Of *C. vishnui* and *C. pseudovishnui* tested, 0.2% reacted with pig antiserum, but no feeding on pigs was recorded for *C. tritaeniorhynchus*. Six bird-bovid double feeds were recorded for *C. tritaeniorhynchus*. *Culex bitaeniorhynchus* was predominantly a feeder on birds. It can be seen in TABLE 1 that for all 4 species there were no major shifts in feeding pattern between the 3-month periods. However, numbers of blood smears collected in different quarters were not equal, nor were collections made systematically in the same places, so small seasonal changes could have been obscured. Less than 2% of the specimens tested failed to react.

The results of precipitin tests on species which were present in smaller numbers in the collections (44 specimens, or fewer, tested) were as follows: 15 of 16 *Anopheles hyrcanus* had fed on bovid blood. 16 *Aedes pseudomediofasciatus*, from betel fields, had fed only on man. This species is a voracious day biter in the betel fields, and some of these specimens may have fed on the field assistants collecting mosquitoes, and subsequently have been captured when resting. Of 11 *Mansonia uniformis*, 10 had fed on bovid, and one each on man and bird blood (one double feed). Twenty-one of 26 *Culex* (*L.*) *fuscanus* were positive for bird and one for bovid antiserum and 4 were negative. Thirty-eight *Culex* (*Mochthogenes*) sp. collected from mudholes in 2 localities reacted with the combined amphibian-reptilian antiserum. Twelve were tested against specific amphibian and reptilian antisera. Of these, 2 were positive for reptilian and 10 for amphibian antiserum. Seven of 10 *Culex* (*Lophoceraomyia*) sp. (probably *minutissimus*), from a forest area, had fed on birds, and one was positive for the combined amphibian-reptilian antiserum. Of 19 *Culex fuscocephalus*, 18 had fed on bovids, and one on a pig. Of 44 *Culex gelidus* tested, 32 were positive for bovid, one for human, 2 for pig, 6 for bird and 2 for reptilian antiserum, and one was negative. Three of 4 *C. p. fatigans* collected in sugarcane fields were positive for human and the fourth for bird antiserum. Positives against bird antiserum were recorded for *Aediomyia venustipes*, *Aedes albopictus*, *Culex mimulus* and *Culex sitiens*, and against bovids for *Aedes lineatopennis*, *C. sitiens* and *Culex whitmorei*. Two human feeds were recorded for *Aedes albopictus*.

Some specimens from baited traps and from inside buildings were tested. One of 4 *C. vishnui* collected from human dwellings had fed on man, and the others on cattle. Four *C. pseudovishnui* and 2 *C. tritaeniorhynchus*, also from human dwellings, had fed on cattle. Six *C. pseudovishnui* and 10 *C. tritaeniorhynchus* from dwellings shared by people and cattle had fed on cattle. All specimens from cattle sheds (8 *C. vishnui*, 17 *C. pseudovishnui* and 23 *C. tritaeniorhynchus*) were positive for bovid blood. When blooded specimens from pig-baited traps were tested, 4 out of 4 *C. vishnui*, 25 out of 27 *C. pseudovishnui* and 6 out of 7 *C. tritaeniorhynchus* had fed on pigs. The remainder were positive for bovid blood. A few *C. vishnui* and *C. pseudovishnui* from mongoose-baited traps had apparently fed on the bait animal though it was not possible to state this more definitely because of the cross reactivity between antisera prepared against sera from carnivores, discussed in the Methods section. Of 30 *C. p. fatigans* from urban houses, 21 had fed on man and 9 on birds. Of 112 *Ae. aegypti* from the same source, 88 were positive for human blood, 6 had fed on birds, 2 on bovids, one on a rat and 16 were negative to all available antisera (one double feed).

DISCUSSION

Trapping and hand collecting from bait showed that the *C. vishnui* group of species was strongly attracted to cattle, with *C. tritaeniorhynchus* the most strongly attracted (Reuben 1971a). Precipitin tests

have confirmed these findings. *C. pseudovishnui*, a species which was frequently taken in bird-baited traps, showed a higher proportion of feeding on birds than did *C. vishnui*. *C. tritaeniorhynchus*, a species not attracted to birds in traps, nevertheless showed a small proportion feeding on birds. No attempt was made to identify the feedings on birds for these, since birds have been shown to be relatively unimportant in the epidemiology of Japanese encephalitis virus in this area (Carey et al. 1969). A relatively high proportion feeding on humans was recorded for *C. vishnui*, but only one of 518 *C. tritaeniorhynchus* tested had fed on man.

No feedings on pigs were identified for *C. tritaeniorhynchus*. This is surprising in view of the evidence for a *C. tritaeniorhynchus*-pig cycle for Japanese encephalitis virus in nature (Carey et al. 1969). In our studies, there is evidence that this species does feed on pigs, as shown by the positive reactions for pig blood obtained for specimens from pig-baited traps, and mosquitoes have been collected actually biting pigs (Reuben 1971b). The failure to demonstrate feeding on pigs in *C. tritaeniorhynchus* from outdoor shelters could be because the percentage of the population feeding on pigs in nature is so low that more than 500 tests would have to be performed before there was a reasonable chance of detecting it.

Blood meals have been identified from other parts of Asia for some of the species discussed in this paper. Macdonald et al. (1967) reported feeds from pigs and chickens for *C. tritaeniorhynchus* and *C. gelidus* resting near these 2 hosts in Sarawak. *C. annulus* (synonymous with *C. vishnui*, Reuben 1969) and *C. pseudovishnui* resting near poultry houses had fed on chickens. The results of Colless (1959) from Singapore were in general very similar to the results obtained in South India, with the difference that the proportion feeding on pigs in Singapore was higher for *C. pseudovishnui*, *C. tritaeniorhynchus* and *C. sitiens*. Pennington & Phelps (1968), working in Okinawa, concluded that *C. tritaeniorhynchus* preferred cattle to pigs, although 60% of the blood meals identified were from pigs. Few feedings on chickens were recorded and very few on humans. In general, a higher proportion of feedings on pigs and a correspondingly lower proportion of bovid feedings have been reported from other parts of Asia. The reason for this difference is the large number of pigs in the places where the authors cited above have worked, and the large number of cattle in North Arcot District, India. The 1961–1962 Livestock Census for North Arcot gives the following figures: 1,386,692 cattle and buffaloes, 803,070 sheep, 236,706 goats, 732 horses, 6,559 donkeys, 65,513 pigs and 735,884 fowls and ducks. The preponderance of bovids is reflected in the large number of blood meals from these animals recorded in the present study.

C. bitaeniorhynchus, as was expected from trapping studies, had fed mainly on birds, with the next largest number of feedings on bovids. More surprising was the finding that *C.* (*L.*) *fuscanus*, a relatively common mosquito, fed mainly on birds. This species has only occasionally been taken in bird-baited traps. A possible explanation is that its large size discourages it from entering traps designed for the entry and retention of mosquitoes of moderate size. *Culex* (*Mochthogenes*) sp. was found to be feeding on amphibians and reptiles. Rao & Rajagopalan (1957) observed specimens of *C.* (*Moch.*) *malayi* actually feeding on a frog.

Very few specimens tested were negative to all the antisera prepared. *A. aegypti* was the only species for which an appreciable number of tests were negative, 14.3%. It would be worthwhile to prepare antisera to other animals to find out on what this important species is feeding. Among the feedings identified for this species were some on birds and bovids. Hens sitting on eggs are often kept indoors in Vellore houses, and cows and goats are commonly kept in sheds close to houses, or even under the same roof as humans.

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