ROLE OF DOMESTIC ANIMALS AS HOSTS FOR BLOOD-SEEKING FEMALES OF *PSOROPHORA COLUMBIAE* AND OTHER MOSQUITO SPECIES IN TEXAS RICELANDS[1, 2]

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ABSTRACT. Female mosquito collections from stable traps, baited with domestic animals, CO_2 and light, set at a rice-farm site and a bayou floodplain site in Brazoria Co., TX during 1973 included specimens representing 5 mosquito genera. *Psorophora columbiae* was by far the most predominant species in the collections made at both study sites and, therefore, was the species receiving the most attention during the course of this study.

The horse-baited stable trap was generally more attractive to female *Ps. columbiae* than were traps with other baits. The calf-baited trap was the next most attractive to females of this species and out competed the horse-baited stable trap for *Ps. columbiae* females at the rice-farm site. Subsequent assessment of blood-engorged mosquito specimens collected from resting habitats at the same sites during 1974 indicated that cattle and to some degree, horses, served as the primary sources of blood meals for the *Ps. columbiae* specimens. Data on the bloodmeal sources for specimens of other mosquito species collected in this part of the study are included as additional information.

Approximately 1.5 million acres (0.6 million hectares) of land in the coastal praires of northeast Texas are devoted to rice production. About 1/3 of this land is planted in rice each year. The remainder has alternate crops such as soybeans or sorghum, or, until recently, remained fallow and often was used as pasture for cattle. The rice growing region of Texas (the Texas rice belt) produces about 3/4 million cattle annually with most of these animals being beef cattle (Texas Crop and Livestock Reporting Service 1977).

Rice fields and associated pastures represent a significant source of mosquito-breeding sites in Texas with the dark rice field mosquito, *Psorophora columbiae* (Dyar and Knab), being predominant (Olson and Newton 1973, Meek and Olson 1976, 1977). The large populations of *Ps. columbiae* frequently emanating from these riceland habitats are considered by mosquito control districts in the Texas rice belt to be one of the most important recurring sources of human and animal annoyance associated with mosquito attack along the Gulf Coast. Also, *Ps. columbiae* was incriminated as an important vector of Venezuelan equine encephalitis (VEE) virus during an outbreak of this disease in northern Mexico and Texas in 1971 (Sudia and Newhouse 1971, Sudia et al. 1971, Olson and Grimes 1974).

The public health and veterinary importance of riceland populations of *Ps. columbiae* along the Texas Gulf Coast has prompted the Texas Agricultural Experiment Station to develop an intensified research program concerning the biology and control of this species. One aspect of the program has centered on the role of domestic animals, particularly cattle, in the ecology of *Ps. columbiae* populations breeding in Texas ricelands. In this regard, Meek and Olson (1977) have already shown that cattle, by means

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[2] Mention of a commercial or proprietary product does not constitute an endorsement by TAES or USDA.

of their hoof prints, create an important source of oviposition sites for riceland populations of *Ps. columbiae.* These authors estimated overwintering egg populations of this species occurring in cattle hoofprints in Texas riceland pastures could account for 0.7–3.2 million mosquitoes/ha in the spring if all the progeny were to survive.

Cattle and other domestic animals also are an important source of blood meals for female *Ps. columbiae* populations occurring in Texas ricelands. Previous studies by Horsfall (1942) and Steelman and associates (Steelman 1971, Steelman et al. 1973) indicate that considerable interaction occurs between *Ps. columbiae* and cattle in Arkansas and Louisiana. However, prior to this study, no information existed on the relative importance of cattle and other livestock as hosts for this mosquito species in Texas. The objectives of our study, therefore, were 1) to examine the relative attractiveness of domestic animal species for blood-seeking *Ps. columbiae* in Texas riceland habitats, and 2) to determine the degree to which this mosquito actually feeds on cattle and other domestic animals.

METHODS

Field studies were centered in a rice growing region near Danbury, Brazoria Co., TX from 5 May 1973 to 15 September 1974. The study sites represented the 2 major types of habitat available to *Ps. columbiae* and other mosquitoes in the upland rice-growing areas of Texas. One site represented habitats occurring on a rice farm to include those present in rice fields and fallow fields used intermittently as pastures for livestock as previously described by Meek and Olson (1976, 1977). Estimates concerning the composition of domestic mammal populations on or near (within 2.0 km) the rice farm site were as follows: cattle (65.0%), horses (7.8%), dogs (6.4%), humans (13.0%), goats (3.9%) and pigs (3.9%).

The other site was characteristic of the habitats associated with floodplains along bayous and other waterways juxtaposed to the riceland areas. This site consisted of a field bordered on 3 sides by a bayou, a seepage stream and a country road. On the remaining open side and at a distance of about 135 m from the center of the study pasture was a farmhouse and barnyard area with pigs, chickens, dogs and horses all of which had free access to the field and adjacent areas. Cattle were pastured in nearby fields not contiguous to the study site but within easy flight range (2.0 km) of the mosquito populations in the area. The field at the study site was plowed occasionally and reinvaded by short grasses and forbs while the bayou next to the field was immediately bordered by a thick stand of oak (*Quercus* spp.) mixed with dense shrubs, climbing vines and small trees of various species. Cattle again represented the predominant domestic animal species in the vicinity of the floodplain site and comprised 55.6% of the total estimated mammal population observed in the area. The other animals included in this estimate were horses (13.9%), dogs (13.9%), pigs (8.3%) and humans (8.3%).

Data concerning the relative attractiveness of various domestic mammal species for female *Ps. columbiae* involved the use of a modified version of animal-baited stable traps such as those described by Magoon (1935) and Roberts (1965). Our traps were designed to prevent trapped mosquitoes from feeding on bait animals since the extremely large mosquito populations might adversely affect the health of the bait animals. Each trap consisted of a screened-in animal stanchion surrounded by 4 collecting boxes (Fig. 1). Each collection box (0.91 × 1.82 × 0.31 m) consisted of a plywood (1.9 cm) frame covered by 20 mesh Lumite Saran® screening. Two louvres were fitted into each box at 7.3 cm and 48 cm above ground level on the side of the box facing away from the animal stanchion to allow for mosquito entry into the box. The opening of the louvres through which mosquitoes entered was 2.5 cm in width and ran the full length of each box. The

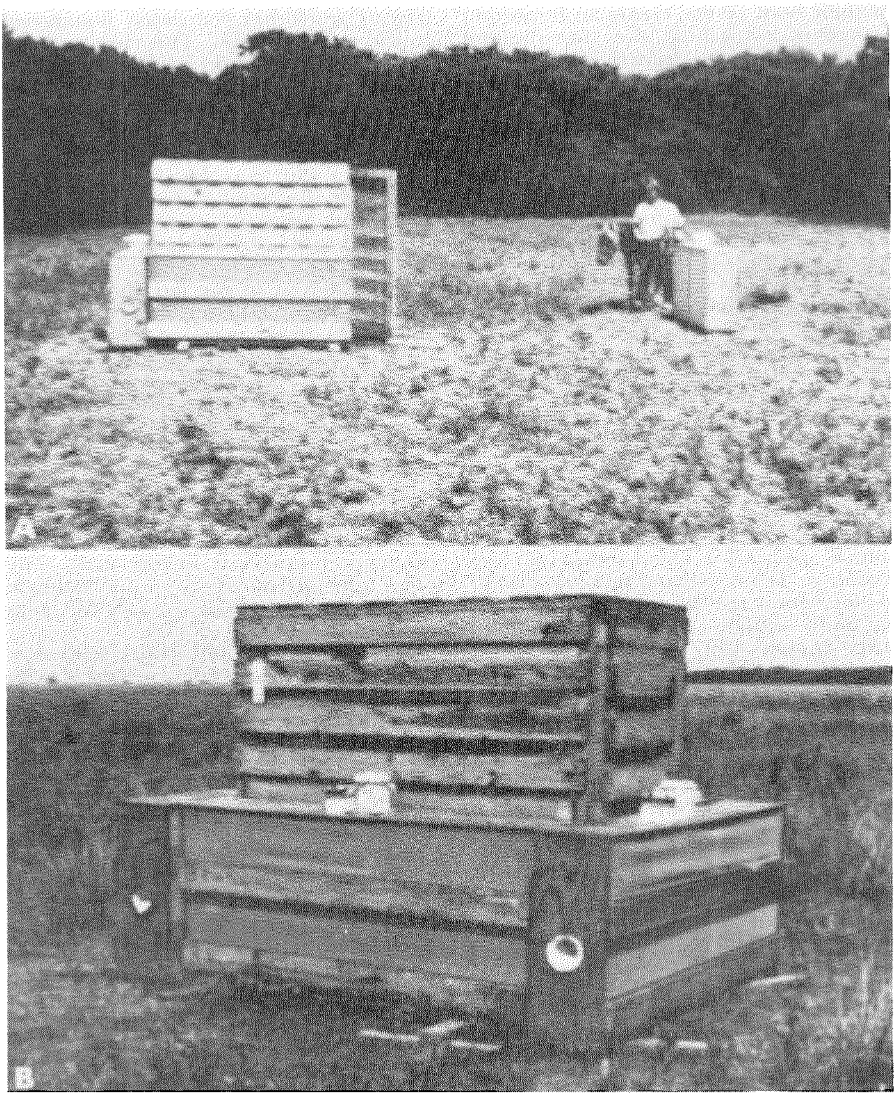

Fig. 1. Stable trap assembly: (A) Stable trap with end trap box removed in order to place bait animal in the trap stanchion: (B) Stable trap with the 4 trap boxes in place (louvres closed).

louvres were equipped with door panels which were closed after each trapping period. Trapped mosquitoes were removed through stocking-fitted arm holes on the ends and top of each box using either vacuum cleaner aspirators or a light trap device in the manner described by Rade.[3]

A pair of stable traps (15 m apart) was placed at each of the 2 study sites. Both traps at the same site were activated simultaneously when mosquito sampling was done. One trap was always baited with a horse since, at the time (1973), there was concern about the degree of interaction between mosquitoes and horses and the potential for spread of epidemic strains of VEE virus from horses to other domestic animals. The other trap was either not baited or baited with a calf, pig, dog, sheep, light, or CO_2 selected at random from a list of potential baits used in the study. Due to operational limitations, one animal in each category was used throughout this particular study. The CO_2 source was a pressurized tank of this gas which was placed into the stanchion of the alternate trap. The CO_2 was released at the rate of 3 liters/min—a rate reported by Roberts (1972) to be quite attractive to mosquitoes and other biting flies. The source of light was a bank of 4 flashlight bulbs similar to the ones used in CDC miniature light traps (Sudia and Chamberland 1962). The bulbs were individually powered by 6-volt lantern batteries placed into the center of the trap stanchion.

A trapping effort at a given site extended over a 7-day period (referred to hereafter as a trap week). On each date of a given trap week, the pair of traps (one baited with a horse and the other with no bait or baited with the alternate bait scheduled for the date) were activated for a 2-hour period extending from 1 to 3 hr after official sunset. At the end of the 2-hr period, the louvre doors on the trap boxes were closed and the trapped mosquitoes were removed; placed into containers labeled as to date, time, bait and site; placed in freeze boxes containing dry ice and stored in this manner until they could be returned to the mosquito research laboratory at Texas A&M University for sorting and identification. Each alternate bait was used once over a given trap week and each site was sampled twice with all bait combinations during 5 June–30 August 1973.

Insight as to the degree to which mosquitoes actually feed on various domestic animal species in the riceland areas was gained by collecting blood-engorged mosquitoes from the 2 field sites and determining the sources of their bloodmeals using the capillary tube precipitin test as modified by Tempelis and Lofy (1963). Since engorged mosquitoes are less likely to respond to common trap inducements such as light, CO_2 and animal baits (Jobbins et al. 1961), other methods of collecting engorged specimens had to be employed. The primary collecting method in this case involved a d-Vac® gasoline-powered backpack vacuum aspirator which was used to periodically vacuum blood-engorged mosquitoes from resting sites located in dense, shaded grassy areas within pastures (fallow rice fields) and in thick stands of low vegetation along the edge of woodlines bordering the bayous and other waterways occurring at the 2 field sites. A car-top trap, similar to the one described by Steelman et al. (1968), was also used in initial sampling efforts; but, collections made with this method contained a low percentage of engorged mosquitoes and were eventually discontinued.

Blood-engorged mosquitoes were collected from 5 May 1973 through 15 September 1974. Mosquito samples collected at the field sites were labeled as to date, site, method, and exact location of collection and were immediately placed on dry ice to prevent further bloodmeal degradation in engorged specimens. The samples were held in this manner until transported back to the mosquito re-

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[3] Rade, B. D. 1974. Relative attractiveness of certain domestic animals for mosquitoes in the Central Texas Coastal Zone. Master's thesis, Texas A&M Univ., College Station, TX. 68 p.

search laboratory where they were transferred to a freezer for storage.

All mosquitoes collected at the study sites were counted and identified to species on chill tables (Sudia et al. 1965) using the keys of Stojanovich (1964) and Carpenter and LaCasse (1955). Maintenance of the mosquito specimens under the semi-frozen conditions provided by the chill tables preserved the quality of the specimens, facilitating their identification and preventing degradation of bloodmeals in the engorged specimens.

Reference antisera used to identify the mosquito bloodmeal sources were obtained from the Department of Entomology, Walter Reed Army Institute of Research, Washington, DC. These antisera were produced in chickens in a manner similar to that described by Campbell et al. (1970). Source identification of the mosquito bloodmeals involved preparation of the bloodmeal (or antigen) of individual mosquitoes using the techniques of Tempelis and Lofy (1963), and layering this antigen over a series of reference antisera capillary tubes. Clarification of the reactants prior to their placement into the tubes was accomplished with a Swinney® hypodermic filter apparatus. The reactions between the bloodmeal preparation and the antisera were allowed to proceed at room temperature (25°C). Positive reactions, evidenced by a slight cloudiness (ideally a ring) at the interface of the 2 reactants, generally occurred within 2 hr under these conditions.

In each case, a bloodmeal was first tested against a broad-spectrum anti-avian and anti-mammalian serum. The samples showing reactivity with the anti-mammalian serum were subsequently tested against antisera specific for dogs, pigs, goats, horses, cattle and humans. Control tubes containing saline solutions layered over the various reference antisera were included in each series of tests. In cases where an antiserum remained cloudy in spite of filtering, these controls proved vital in determining positive test readings.

RESULTS AND DISCUSSION

The combined stable-trap collections of female mosquitoes from the bayou floodplain site and the rice-farm site included a total of 11,570 and 11,448 specimens, respectively. Specimens representing 5 genera were present in these collections. *Aedes sollicitans* (Walker), *Ae. taeniorhynchus* (Wied.), *Ae. vexans* Meigen, *Anopheles crucians* Wied., *An. quadrimaculatus* Say, *Culex quinquefasciatus* Say, *Cx. salinarius* Coq., *Cx. (Melanoconion)* spp., *Coquillettidia perturbans* (Walker), *Psorophora ciliata* (Fabr.), *Ps. columbiae*, as well as some unidentified *Culex* species, were all represented in stable trap collections from the floodplain site. Representatives of these same species were also present in the rice-farm collections except for *Cx. (Melanoconion)* spp. and *Cx. quinquefasciatus*. *Psorophora columbiae* was the predominant species in both instances and represented 67.0 and 85.6% of the total female mosquitoes collected in stable traps during the 3-month period in 1973 at the floodplain site and the rice-farm site, respectively. *Culex salinarius* (12.8%), *An. quadrimaculatus* (5.9%), and *Ae. taeniorhynchus* (3.8%) were the next most common species in the mosquito collections from the floodplain site, while *An. quadrimaculatus* (6.4%) and *An. crucians* (4.5%) were the next most common species in collections from the rice farm.

As shown in Table 1, the horse-baited stable trap was more attractive to female *Ps. columbiae* than were competing traps containing other baits. The only exception was in the case of the calf-baited trap at the rice-farm site. This trap collected 1.4 times more *Ps. columbiae* per night than did the horse-baited trap. The calf-baited trap also was much more competitive with the horse-baited trap at the floodplain site than were traps containing other baits; although, in this case, the horse-baited trap did capture more *Ps. columbiae* females (Table 1). In summary, the stable trap results indicate that the horse and calf were the most attractive

Table 1. The relative attractiveness of a stable trap containing various baits as opposed to another stable trap baited with a horse for female populations of *Psorophora columbiae* occurring in a riceland habitat in Brazoria County, Texas, 6 June–30 August 1973.

	Ps. columbiae females collected at			
	Bayou floodplain site		Rice farm site	
Bait combination in stable traps	Total specimens caught (Alternate bait/Horse-bait)	Alternate bait:Horse collection ratio[a]	Horse specimens caught (Alternate bait/Horse-bait)	Alternate bait:Horse collection ratio[a]
Calif/Horse	420/635	0.66	620/440	1.41
Pig/Horse	50/480	0.10	48/687	0.07
Dog/Horse	167/913	0.18	140/932	0.15
Sheep/Horse	6/386	0.02	3/220	0.01
Co_2/Horse	385/3138	0.12	806/1195	0.67
Light/Horse	15/483	0.03	108/2040	0.05
No Bait/Horse	0/683	0.00	0/2562	0.00

[a] Difference between the attractiveness of the trap baited with various baits and the horse-baited trap was significantly different to the 0.01 confidence level in each instance as determined by the formula

$$Z = \frac{A - 0.50}{\sqrt{\frac{0.5 \times 0.5}{B}}}$$

where A = % of the total specimens collected in both traps on a given trap night represented by the specimens collected in the horse-baited trap and B = total specimens collected.

Table 2. Precipitin test results for blood-fed mosquito specimens collected at the bayou floodplain site in Brazoria County, Texas during 5 May 1973 to 15 September 1974.

	Number of specimens	% of a given species determined to have fed upon[a]								
		Av	UIM	Bov	Eq	Hum	Can	Cap	Por	UIH
Aedes sollicitans	21	0.0	4.8	62.0	14.3	4.7	4.7	0.0	0.0	9.5
Ae. taeniorhynchus	3	0.0	0.0	66.7	33.3	0.0	0.0	0.0	0.0	0.0
Anopheles crucians-bradleyi	154	0.0	0.0	64.3	28.6	0.0	0.0	0.0	2.6	4.5
An. pseudopunctipennis	18	0.0	0.0	83.4	5.5	0.0	0.0	0.0	0.0	11.1
An. quadrimaculatus	37	0.0	0.0	83.8	8.1	5.4	0.0	0.0	0.0	2.7
Culex salinarius	1	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Psorophora ciliata	12	0.0	0.0	52.4	25.0	0.0	8.3	0.0	0.0	8.3
Ps. columbiae	91	0.0	0.0	82.4	11.0	2.2	2.2	0.0	2.2	0.0
TOTAL	337	0.0	0.3	72.1	19.3	1.5	1.2	0.0	1.8	3.8

[a] Av–Avian, UIM–Unidentified mammal, Bov–Bovine, Eq–Equine, Hum–Human, Can–Canine, Cap–Caprine, por–Porcine, UIH–Unidentified host.

Table 3. Precipitin test results for blood-fed mosquito specimens collected at the rice farm site in Brazoria County, Texas during 5 May 1973 to 15 September 1974.

	Number of specimens	% of a given species determined to have fed upon[a]								
		Av	UIM	Bov	Eq	Hum	Can	Cap	Por	UIH
Aedes sollicitans	19	0.0	0.0	73.7	10.5	0.0	0.0	0.0	0.0	15.8
Ae. taeniorhynchus	3	0.0	0.0	66.7	0.0	0.0	0.0	0.0	0.0	33.3
Culex salinarius	1	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Psorophora ciliata	7	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Ps. columbiae	344	0.0	3.5	83.7	7.3	0.9	1.1	0.9	0.6	2.0
TOTAL	374	0.0	3.2	83.4	7.2	0.8	1.1	0.8	0.5	2.9

[a] Av–Avian, UIM–Unidentified mammal, Bov–Bovine, Eq–Equine, Hum–Human, Can–Canine, Cap–Caprine, por–Porcine, UIH–Unidentified host.

mammalian baits used and that these 2 domestic animal species are potentially important bloodmeal sources for *Ps. columbiae*. The same trends were noted for the other mosquito species collected in the stable traps; however, collections of these other species were too small to go beyond this general statement.

D-Vac and car-top trap collections of female mosquitoes made at the 2 study sites during 1973 and 1974 yielded a total of 2036 specimens (blood-engorged and unfed specimens combined) from the floodplain site and 2969 specimens from the rice farm site. *Psorophora columbiae* was again the predominant species and comprised 53.8% and 55.2% of the specimens collected at the floodplain and rice farm sites, respectively. A total of 711 blood-engorged mosquitoes were collected from the 2 sites of which 435 (61.2%) were *Ps. columbiae*. Precipitin test results indicated that cattle, and to some degree horses, served as the primary sources of the blood-meals for each of the species collected (Tables 2 and 3). No evidence of any of the specimens having fed on more than one kind of host was detected during this study. In the case of *Ps. columbiae* over 80% of the blood-engorged specimens from each site were positive for bovine sera. *Psorophora columbiae* females testing positive for horse sera ranged between 11.0% and 7.3% of the total specimens collected from the floodplain and rice farm sites, respectively (Tables 2 and 3).

Application of the forage ratio technique of Hess et al. (1968) to data for *Ps. columbiae* gave further evidence of the role that domestic animals play as bloodmeal sources for this species in Texas riceland habitats. The forage ratio for *Ps. columbiae* on cattle ranged between 1.3 and 1.4 while those for other potential sources of blood all were less than 1.0 (Table 4). A forage ratio greater than 1.0 indicates a tendency toward selective preference on the part of the mosquito for a given host species.

The results of this study coupled with those previously reported by Meek and Olson (1976, 1977) indicate that cattle are a very important component in ecology of *Ps. columbiae* populations breeding in the Texas riceland agroecosystem. Cattle appear to serve as the most common source of bloodmeals for this mosquito; they apparently serve to attract female mosquitoes into potential oviposition sites and they create a significant source of oviposition sites by means of their hoofprints (Meek and Olson 1977). Recognition of the high level of interaction between cattle and riceland populations of *Ps. colum-*

Table 4. Domestic mammal forage ratios for blood-fed specimens of *Psorophora columbiae* collected from 2 study sites in Brazoria County, Texas, 5 May 1973–15 September 1974.

Host	Percent of total bloodmeals tested (A)	Percent of total estimated domestic population (B)	Forage ratio (A/B)
	Bayou floodplain site		
Bovine	82.4	55.6	1.4
Equine	11.0	13.9	0.8
Human	2.2	8.3	0.3
Canine	2.2	13.9	0.2
Caprine	0.0	0.0	—
Porcine	2.2	8.3	0.3
	Rice farm site		
Bovine	83.7	65.0	1.3
Equine	7.3	7.8	0.9
Human	0.9	13.0	0.1
Canine	1.2	6.4	0.2
Caprine	0.9	3.9	0.2
Porcine	0.6	3.9	0.2

biae may prove useful in developing strategies for more effective survey and control of this mosquito species. Cattle might be used as indicators of where the highest numbers of mosquito eggs are most likely being deposited in riceland areas. Also, cattle have the potential of serving both as the attracting agent and the killing agent for riceland populations of this mosquito species. This would be possible if cattle could be treated topically with a safe but effective adulticide which promotes a high level of mortality within mosquito populations coming in contact with the treated animals for short periods of time to feed.

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