NEWLY INCRIMINATED ANOPHELINE VECTORS OF HUMAN MALARIA PARASITES IN JUNIN DEPARTMENT, PERU

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ABSTRACT. Sporozoite data from salivary gland dissections are presented that clearly incriminate *Anopheles trinkae*, *An. pseudopunctipennis*, *An.* sp. near *fluminensis*, *An. oswaldoi*, *An. nuneztovari* and *An. rangeli* as vectors of malaria parasites in the Rio Ene Valley, a hyperendemic malarious area in Junin Department, eastern Peru. *Anopheles trinkae* is considered the most important vector based on dissections, abundance and man-vector contact. Other notes are presented on the relative abundance, bionomics and previous records of these species in Peru and in the study sites.

INTRODUCTION

Hyperendemic malaria was first reported in forested Indian settlements of the eastern slopes of the Andes in Junin Department, southcentral Peru, by Sulzer et al. (1975, 1978). They reported hyperendemic *Plasmodium malariae* (Laveran) and *P. vivax* (Grassi and Feletti) in almost equal proportions, as measured by immunofluorescence and blood smears. Of particular interest was the absence of *P. falciparum* (Welch) in an area before the introduction of control methods. General mosquito studies were also initiated in this area during the same period (Calderon et al. 1974), and *Anopheles pseudopunctipennis* Theobald was considered the probable primary vector of malaria parasites, with *An. oswaldoi* (Peryassu) and *An. rangeli* Gabaldon, Cova Garcia and Lopez projected as secondary vectors.

In 1981 the senior author first visited Mission Cutivireni on the Rio Ene (one of Sulzer's study sites) for a brief period and determined that a new species, *An. trinkae* Faran (1979), appeared to be the most abundant species in the area. At that time, several female *Anopheles* were found positive for malaria sporozoites by dissection. However, because of the difficulty of identifying members of the Oswaldoi Subgroup of *Anopheles* (*Nyssorhynchus*), their identity could not be ascertained without associated immature skins. During a return visit to the area in 1983, the senior author reared progeny adults with associated immature skins from females captured biting humans and established the presence of 4 members of the Oswaldoi Subgroup in the study site: *Anopheles nuneztovari* Gabaldon, *An. oswaldoi*, *An. rangeli* and *An. trinkae*. During that trip oocysts were found in 2 *An. trinkae*-like females.

A longitudinal study of malaria transmission in Mission Cutivireni began in 1985 under the auspices of a National Research Council grant through the BOSTID program (Board on Science and Technology for International Development) to Cayetano Heredia University in Lima. Partial impetus for this project was due to a special Peruvian government project, "Pichis Palcazu," that was established by the Ministry of Agriculture to grant colonists title to land newly opened by roads in the tribal homelands of the Campa (Ashaninga) Indians.

During the initial phase of the project a concerted effort was made to determine the mosquito species in the study site to resolve taxonomic problems and to develop accurate taxonomic keys for the immature and adult *Anopheles*. Personnel from the Walter Reed Biosystematics Unit, Washington, DC, actively participated during that time in collecting and identifying specimens, and preparing the taxonomic keys. These efforts provided the taxonomic basis for mosquito collections, dissections for parasites and enzyme-linked immunosorbent assay (ELISA) studies for malaria sporozoites that were conducted and continued in the study site(s). Voucher specimens from this study have been deposited in the National Museum of Natural History (USNM), Washington, DC. Unfortunately, although Mission Cutivireni was the initial site, politically unstable conditions later in 1985 forced a relocation of the project down river to 2 additional sites, Puerto Ocopa and Puerto Prado, where efforts continued through July 1986.

METHODS

This study was conducted between March 1985 and July 1986. The locations of the study sites are depicted in Fig. 1. Standard World Health Organization procedures (W.H.O. 1975) were used for collecting mosquitoes, determining parity rates and dissecting specimens for *Plasmodium* sporozoites. Female anophelines were collected from human bait during frequent crepuscular collections and during all night collections (1800–0600 hr) made at least once each

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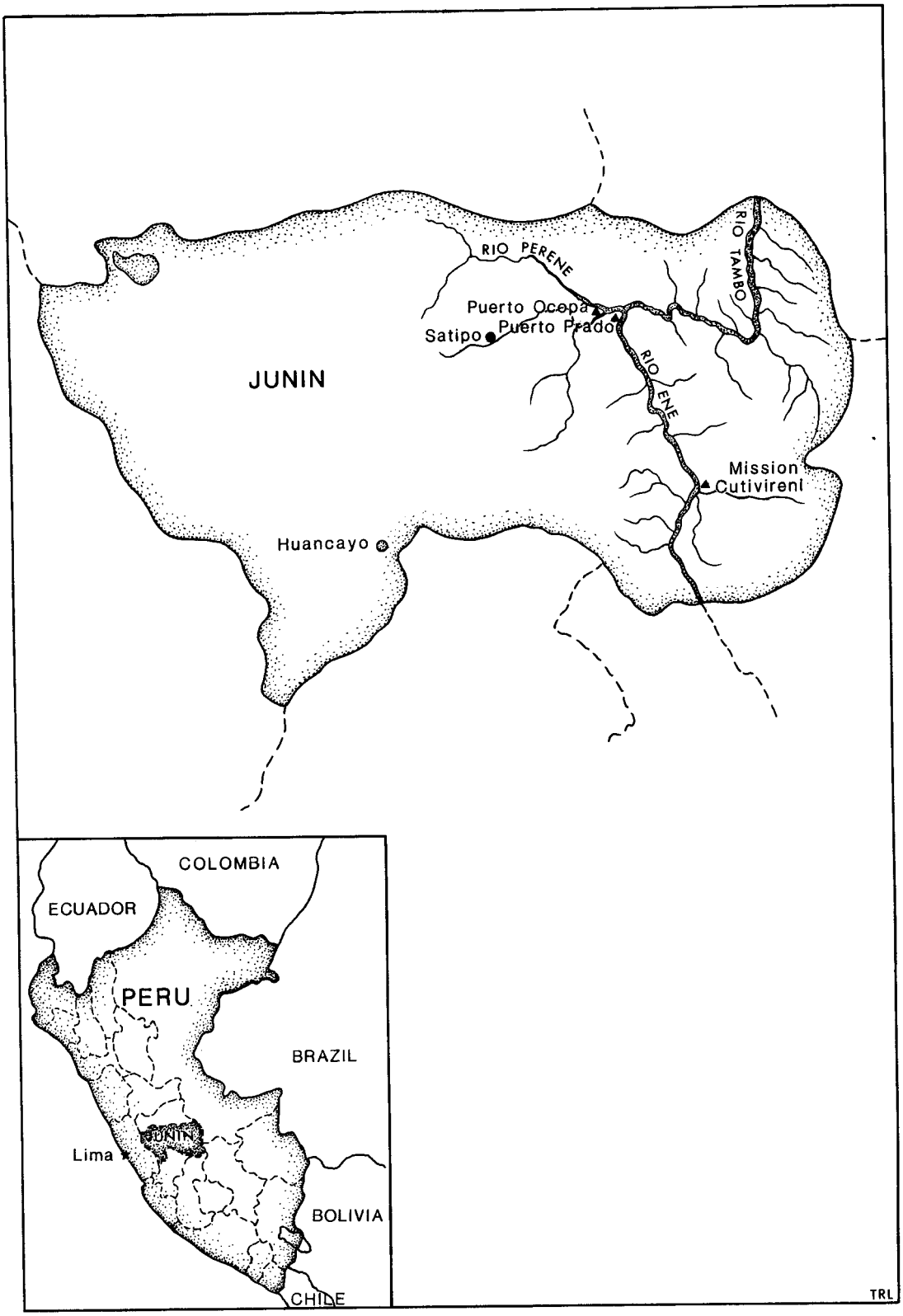

Fig. 1. Map of the malaria vector study sites in the Rio Ene Valley, Satipo Province, Junin Department, Peru.

month inside and outside human habitations (most often without walls). Collections were made using flashlights and were segregated into hourly increments. Females were collected in individual glass vials to facilitate identification, or were collected in large numbers by hand aspirator and placed in gauze-covered cups. Small cotton pads with water were provided to the specimens to keep them alive until they were dissected. All specimens were identified, graded for parity status and dissected (parous and gravid specimens), or discarded (nulliparous) within 12 hr of the time of capture. Dissected mosquitoes were examined for oocysts and sporozoites with primary emphasis on the latter. Technicians were trained to identify sporozoites based on slide preparations provided by the Department of Entomology, Walter Reed Army Institute of Research. The head and thorax of mosquitoes found positive for sporozoites were preserved (dried) and saved for ELISA confirmation to be reported later.

Blood smears were also taken from Indians and colonists from the Rio Ene Valley during the study period for detection and identification of malarial parasites. These slides were processed by a technician from the Navy Medical Research Institute-Detachment (NAMRID), Lima, Peru and technicians from the Ministry of Health, Lima, Peru.

RESULTS

Table 1 provides a summary of the anophelines dissected for parity and sporozoite detection. During the study period 6,956 female mosquitoes were collected, of which 4,784 were dissected for parity status. *Anopheles pseudopunctipennis* had the highest percentage of females in the gravid/parous categories (71%), with *An. nuneztovari* (70%) only slightly less. The gravid/parity percentages for *An. trinkae*, *An. rangeli* and *An. triannulatus* (Neiva and Pinto) were lower, 51, 48 and 44%, respectively. The 3 species found more commonly in the heavily forested study sites, *Chagasia bonneae* Root, *An. oswaldoi* and *An.* sp. nr. *fluminensis* Root, had the lowest gravid/parity percentages, 35, 33 and 32%, respectively. Sporozoites were found in the salivary glands of *An. trinkae*, *An. pseudopunctipennis*, *An.* sp. nr. *fluminensis*, *An. oswaldoi*, *An. nuneztovari* and *An. rangeli*, in declining order of importance. *Anopheles trinkae*, the species with the third highest gravid/parity percentage, had the highest sporozoite infection rate, possibly because of its overwhelming abundance, as measured by man-mosquito contact in all 3 study sites. Although *An. pseudopunctipennis* had the second highest sporozoite infection rate its overall vector importance was considerably reduced by it being abundant in our study sites only during the 3–4 month dry season. The apparent preference of this species for ovipositing in drying-up green algal pools in streams and rivers offers a possible control mechanism through larvicides. Additional data on the bionomics and vector densities will be reported later.

The blood parasite level in 806 Indians and colonists involved in the blood smear survey revealed 331 infections of *P. vivax* and 14 infections of *P. malariae*, including 2 mixed infections, or a 42.8% malaria positive rate.

DISCUSSION

This study establishes that several species of anophelines are involved in the transmission of human malaria parasites on the eastern slopes of the Andes. Previously, only 2 species have been incriminated in Peru as vectors by dissection of sporozoites, i.e., *An. pseudopunctipennis*

Table 1. Anophelines dissected for parity and sporozoite detection in the Rio Ene Valley. Junin Department, Peru, March 1985–July 1986.

Species	Number dissected	Nulliparous		Gravid		Parous		Salivary glands examined	Sporozoite positive	
		No.	%	No.	%	No.	%		No.	%
An.(Ano.) sp. nr. *fluminensis*	410	278	68	64	16	68	16	132	4	1.0
An.(Ano.) pseudopunctipennis	166	49	29	33	20	84	51	117	3	1.8
An.(Nys.) nuneztovari	201	102	30	50	25	49	45	99	1	0.5
An.(Nys.) oswaldoi	161	112	67	24	15	25	18	49	1	0.6
An.(Nys.) rangeli	480	248	52	67	14	165	34	232	2	0.4
An.(Nys.) triannulatus	299	168	56	49	16	82	28	131	0	0.0
An.(Nys.) trinkae	3,010	1,459	49	551	18	1,001	33	1,552	71	2.3
Ch. bonneae	34	22	65	7	21	5	14	12	0	0.0
others*	23	12	52	7	30	4	18	11	0	0.0
Totals	4,784	2,450	51	852	18	1,483	31	2,335	82	1.7

* Members of the Oswaldoi Group of questionable identity (rubbed).

(Peruvian Ministry of Health, unpublished data) and *An. punctimacula* Dyar and Knab (Villalobos and Valderrama 1944). Other species have been incriminated in Peru by the Ministry of Health solely on epidemiological grounds; they are: *An. albimanus* Wiedmann, *An. benarrochi* Gabaldon, Cova Garcia and Lopez, *An. darlingi* Root, *An. oswaldoi* and *An. rangeli*, which have been negative in previous Ministry of Health dissection efforts. The number of species found positive during this study may appear high; however, De Arruda et al. (1986) recently incriminated 5 species (4 by dissection) in a study in Para, Brazil.

These data are the first to incriminate *An. trinkae* as a vector of human malaria parasites, and they clearly establish this species as a primary vector of malaria in the Rio Ene Valley, based on mosquito-man contact. *Anopheles trinkae* was described by Faran (1979) from Puyo, Ecuador, and is now known to extend through Peru into Bolivia, based on specimens in the U.S. National Museum (USNM). The primary role of *An. trinkae* as a malaria vector in the Rio Ene Valley and its abundance in other valleys of eastern Peru, suggests it may also be an important vector on the eastern slopes of the Andes in other countries. We now suspect that a major portion of the *An. rangeli* and *An. benarrochi* from Peru discussed by Elliott (1972) were probably *An. trinkae*. This opinion is based on the apparent dominance of *An. trinkae* over *An. rangeli* in the areas surveyed to date in eastern Peru, and the absence of confirmed specimens of *An. benarrochi* from Peru. *Anopheles trinkae* is the primary species found in transitional-temporary pools in our study sites.

Anopheles pseudopunctipennis, one of the 2 species previously confirmed as a malaria vector in Peru, apparently is not the most significant vector in the Ene Valley. *Anopheles trinkae* apparently is the dominant vector during most of the year (the wet months), while *An. pseudopunctipennis* is the dominant vector during a 3 month period (dry season). Of the 3 sampled study sites, *An. pseudopunctipennis* was most common in Puerto Ocopa. It was found in the Mission Cutivireni area for the first time in 1986, although infrequent Ministry of Health surveys have been conducted in that area since 1972.

Anopheles sp. near *fluminensis* had the third highest sporozoite infection rate. The precise identification of this species is currently pending further taxonomic study. It is either a variant of *An. fluminensis* or a closely related new species. Previously, *An. fluminensis, sensu lato*, has not been considered a vector of human malaria parasites in Peru or elsewhere in South America. Like *An. oswaldoi*, this species is basically a forest species and was most common in the most forested study site, Mission Cutivireni. Additional work is needed on this species to determine if the detected sporozoites represent human or simian malaria parasites. *Anopheles fluminensis, sensu lato*, is a member of the Arribalzagia Series of subgenus *Anopheles* and is closely related to *An. mediopunctatus* (Theobald), which has been incriminated as a vector of simian malaria in several countries (Elliott 1972).

The remaining 3 species found infected with sporozoites in this study were *An. oswaldoi*, *An. nuneztovari* and *An. rangeli*, in declining order of importance. *Anopheles oswaldoi* previously has been considered relatively unimportant in malaria transmission. However, De Arruda et al. (1986) found this species positive for human malaria parasites by ELISA immunoassay in Para, Brazil. Based on our findings (dissections and man-biting density) we feel this species is of secondary importance, at least in eastern Peru. *Anopheles oswaldoi* is a forest-stream species and was captured most frequently in peridomestic collections in Mission Cutivireni. Like *An. trinkae*, it was most abundant during the rainy season.

Another member of the Oswaldoi Subgroup, *An. nuneztovari*, was also of secondary importance in our study area due principally to its low abundance. This species, however, is a late night-endophagic feeder that was most common in man-made temporary oviposition sites in and around Mission Cutivireni. In other areas of South America *An. nuneztovari* is a primary vector of malaria parasites (Elliott 1972), and could be a primary vector where encountered in greater abundance in other areas of eastern Peru.

Anopheles rangeli previously has not been positively incriminated as a human malaria vector, although Forattini (1962: 393) reported that it was suspected as a vector in Ecuador. However, since Forattini, *An. trinkae* has been described and was probably misidentified and responsible for many earlier *An. rangeli* records, including Elliott (1972). In the valleys of eastern Peru surveyed to date, *An. trinkae* is more abundant in man-biting collections than *An. rangeli*. The latter species is, however, fairly abundant, being the second most abundant member of the Oswaldoi Subgroup in the Ene Valley. *Anopheles rangeli* was common in all 3 study sites and most common in the rainy season, but its importance as a vector may have been diminished by alternate hosts (bovids).

The other 2 species examined in our study, *An. triannulatus* and *Chagasia bonneae*, were negative for malaria parasites. De Arruda et al. (1986) incriminated *An. triannulatus* as a vector

in Para, Brazil, by dissection and 2 immunoassay tests. This species was common only in Puerto Prado in our study, and then abundant only for a short period. *Chagasia* species previously have not been incriminated as vectors of human malaria parasites.

Data from the blood smear survey confirms the continued hyperendemicity of malaria in the Ene Valley and the continued absence of *P. falciparum* parasites as previously noted by Sulzer et al. (1975). With the new Peruvian government project, "Pichis Palcazu," which promotes the settlement of malaria endemic areas by translocated mountain colonists with little resistance to malaria, we feel a continued surveillance for *P. falciparum* in these areas is essential.

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