Am. J. Trop. Med. Hyg., 60(3), 1999, pp. 421–429
Copyright © 1999 by The American Society of Tropical Medicine and Hygiene

MALARIA VECTORS IN A TRADITIONAL DRY ZONE VILLAGE IN SRI LANKA

PRIYANIE H. AMERASINGHE, FELIX P. AMERASINGHE, FLEMMING KONRADSEN, KAPILA T. FONSEKA, AND ROBERT A. WIRTZ

Department of Zoology, University of Peradeniya, Peradeniya, Sri Lanka; International Irrigation Management Institute, Colombo, Sri Lanka; Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract. Malaria transmission by anopheline mosquitoes was studied in a traditional tank-irrigation–based rice-producing village in the malaria-endemic low country dry zone of northcentral Sri Lanka during the period August 1994–February 1997. Adult mosquitoes were collected from human and bovid bait catches, bovid-baited trap huts, indoor catches, and pit traps. Mosquito head-thoraces were tested for the presence of *Plasmodium falciparum* and *P. vivax,* and blood-engorged abdomens for the presence of human blood by ELISAs. House surveys were done at two-day intervals to record cases of blood film–confirmed malaria among the villagers. A total of 7,823 female anophelines representing 14 species were collected. Trends in anopheline abundance were significantly correlated with rainfall of the preceding month in *An. annularis*, *An. barbirostris*, *An. subpictus*, *An. vagus,* and *An. varuna,* but were not significant in *An. culicifacies* and *An. peditaeniatus.* Malaria parasite infections were seen in seven mosquito species, with 75% of the positive mosquitoes containing *P. falciparum* and 25% *P. vivax.* Polymorph PV247 was recorded from a vector (i.e., *An. varuna*) for the first time in Sri Lanka. Computations of mean number of infective vector (MIV) rates using abundance, circumsporozoite (CS) protein rate, and human blood index (HBI) showed the highest rate in *An. culicifacies.* A malaria outbreak occurred from October 1994 to January 1995 in which 45.5% of village residents experienced at least a single disease episode. Thereafter, malaria incidence remained low. *Anopheles culicifacies* abundance lagged by one month correlated positively with monthly malaria incidence during the outbreak period, and although this species ranked fifth in terms of abundance, infection was associated with a high MIV rate due to a high CS protein rate and HBI. Abundance trends in other species did not correlate significantly with malaria. It was concluded that *An. culicifacies* was epidemiologically the most important vector in the study area.

Anopheles culicifacies Giles (species B of Green and Miles[1]) was long regarded as the only malaria vector in Sri Lanka following its incrimination in the 1920s.[2,3] However, recent field studies have produced evidence of the involvement of several other anopheline species in malaria transmission, based on ELISAs to detect the circumsporozoite protein (CS) of *Plasmodium vivax* and *P. falciparum,* the only malaria parasite species that are currently known to be present in Sri Lanka.[4–8] This has led to some confusion regarding the true field vector status of these different anopheline species that have been incriminated by ELISA. The overall relative contribution of different local anopheline species to transmission has been quantified,[7] but there have been no studies that link temporal trends in vector population and transmission dynamics to trends in human malaria to clarify which species are of real significance in initiating and maintaining transmission. One of the factors that complicates such an approach is the highly unstable nature of malaria in Sri Lanka, where the case load has fluctuated from 150,000 to 400,000 cases per year during the present decade (Anti-Malaria Campaign of Sri Lanka, unpublished data), and foci of the disease shift spatially virtually from year to year.

Studies on epidemiologic entomology relating to malaria in Sri Lanka have been concentrated in rural habitats associated with modern irrigation development projects such as the Mahaweli Project,[4,5,8] or in village habitats in the southern part of the island.[6,7] Vector bionomics and transmission parameters have not been studied in traditional tank-based villages that still form a substantial small-scale irrigation network in the rice-producing low country dry zone of Sri Lanka. Recently, a multidisciplinary study on the ecology and socioeconomic impact of malaria was initiated in such an area located within the Huruluwewa watershed in the North-central Province of the island. Studies relating to the economic costs of malaria and the population dynamics of immature stages of anopheline mosquitoes have already been published.[9–11] The present paper seeks to clarify the role of vector anophelines in malaria transmission in a typical village within the watershed.

MATERIALS AND METHODS

Study area. The study was done in the village of Mahameegaswewa situated in the 41,950 hectares watershed area of the Huruluwewa Reservoir (capacity = 7,500 hectares) in northcentral Sri Lanka. Details of the layout and climate of the study area have been presented previously.[9] Briefly, the village (area = 163 hectares) consisted of 58 homes, a primary school, Buddhist temple, rice fields, and stabilized chenas (i.e., cleared scrub-forest areas where nonirrigated subsidiary crops were cultivated). Irrigation water for rice cultivation was obtained from two small reservoirs (known locally as tanks) located within the village area. The Yan Oya stream defined the western boundary of the village. In total, 309 people occupied the village at the start of the study. Mahameegaswewa is one of several purana (ancient) villages located within the degraded forest of the watershed, each with its own cascade of small tanks, which are considered to be remnants of a tank-based rice irrigation system dating back 1,000–2,000 years.

The study area was situated within the low country dry zone of Sri Lanka, with an annual rainfall of 600–1,000 mm. The patterns of rainfall recorded at Mahameegaswewa village, and temperature and humidity recorded at the nearest meteorologic station at Mahailluppallama (30 km distance) during the study period are presented in Figure 1.

Scientific and ethical review of the research protocol was carried out within the International Irrigation Management Institute. Malaria prophylaxis and mosquito-proof tents for

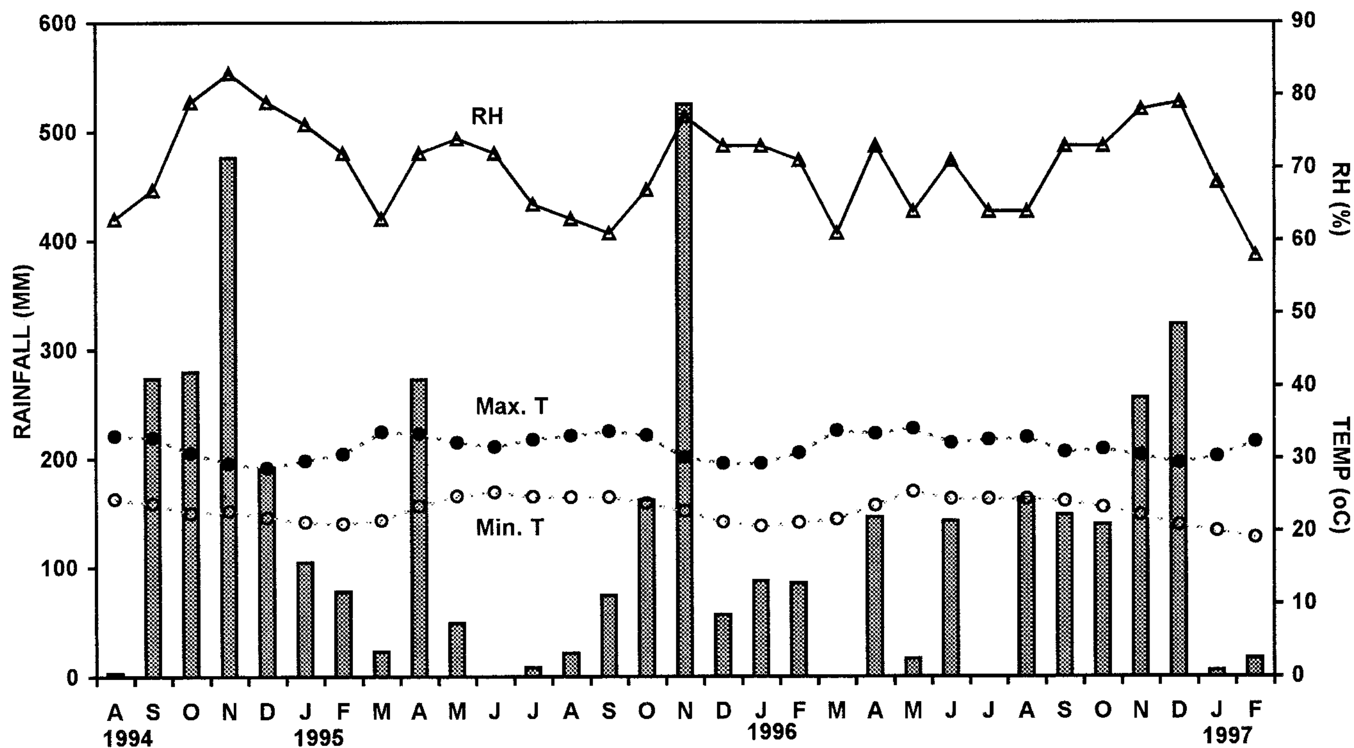

FIGURE 1. Climatic conditions in the study area during the period August 1994–February 1997. Rainfall (**bars**) was measured in the village of Mahameegaswewa, Sri Lanka. Temperature (TEMP) (**broken lines**) and relative humidity (RH) (**solid line**) values were obtained from the nearest weather station at Mahailluppallama, 30 km away. Max. = maximum; Min. = minimum; T = temperature.

sleeping were provided to all field personnel. Village residents were not used in mosquito catches. Informed consent was obtained from project staff who participated in mosquito bait collections.

Mosquito collections. Adult mosquitoes were collected at two-week intervals from August 1994 to February 1997 using the following five methodologies. 1) Outdoor human bait catches (HBC) were made using a two-person team aspirating landing/biting mosquitoes from 6:00 PM to midnight working in relays of 2-hr duration. 2) Outdoor cattle bait catches (CBC) were made using a two-person team aspirating landing/biting mosquitoes from a tethered cow from 6:00 PM to midnight working in relays of 2-hr duration. 3) Cattle-baited trap hut (TRH) catches were made using a cattle bait tethered within each of 2–3 huts (3 × 3 × 3 m) constructed from coconut thatch, leaving a gap of 6 cm around the bottom for the entry of mosquitoes. The bait animal was placed in the trap hut at 6:00 PM and trapped mosquitoes were collected by aspiration at 5:30 AM the next morning. Care was taken to ensure that trap huts were not treated with insecticide during the study period. 4) Indoor resting catches (IRC) were made using two-person teams aspirating mosquitoes for 15 min from 15 randomly selected dwelling houses between 6:00 AM and 10:00 AM. 5) Pit trap catches (PTC) were made in six pits (1.5 × 1.5 × 1.5 m) dug at selected sites within the village area and covered over by woven coconut thatch leaving a 5-cm gap for mosquito entry. Mosquitoes were aspirated from these traps at 6:00 AM and 6:00 PM on each collection day. Details of the methodologies used have been published previously.[4,5,12] Both HBC and CBC were restricted to the first half of the night due to hazards associated with the study area being located close to a civil war zone within Sri Lanka. However, this limitation would not have significantly biased anopheline catches, since previous studies in Sri Lanka have shown that 65–85% of the host-seeking females of the species collected herein bite before midnight.[13,14]

Collected anopheline mosquitoes were anesthetized using chloroform and identified to species in the field using taxonomic keys.[15] All intact females were air-dried, transported to the laboratory at the University of Peradeniya, and rechecked for taxonomic identification. Head-thoraces were separated from abdomens and both were stored separately for immunologic assays.

Immunologic tests. Monoclonal antibody–based ELISAs were used to detect CS proteins of *P. vivax* and *P. falciparum.*[16] Test details have been previously reported.[4] Positivity on initial and confirmatory testing was determined on the basis of a clear visual signal (green coloration with 2,2'-azino-bis-3-ethylbenz-thiazoline-6-sulfonic acid [ABTS] substrate) together with a minimum optical density value that was at least three times greater than the mean negative control value. Quantitative results are presented as a CS protein positive rate, calculated as the proportion of head-thoraces tested that were positive for CS protein.

Human and bovid blood in the abdomens of mosquitoes collected by IRC and TRH catches was detected using a sandwich ELISA based on that of Chow and others.[17] The assay was developed for a panel of seven hosts (human, bovine, cat, dog, pig, chicken, and rat) with homologous and heterologous controls to obtain a species-specific assay. For both assays, a heavy and light chain–specific polyvalent an-

TABLE 1
Anopheline mosquitoes collected by different methods at Mahameegaswewa

Collection effort*	HBC 635	CBC 694	IRC 450	TRH 354	PTC 351	Total (%)
An. aconitus	3	1	0	34	0	38 (0.49)
An. annularis	7	101	0	4	0	112 (1.43)
An. barbirostris	1	120	0	24	1	146 (1.87)
An. culicifacies	4	3	28	364	0	399 (5.10)
An. jamesii	7	419	0	93	1	520 (6.65)
An. maculatus	2	0	0	0	0	2 (0.03)
An. nigerrimus	1	53	0	22	0	76 (0.97)
An. pallidus	0	11	0	7	0	18 (0.23)
An. peditaeniatus	6	400	0	95	0	501 (6.40)
An. pseudojamesii	1	0	0	1	0	2 (0.03)
An. subpictus	0	96	76	127	1	300 (3.83)
An. tessellatus	36	194	2	110	1	343 (4.38)
An. vagus	13	4,516	0	162	2	4,693 (59.99)
An. varuna	6	158	2	420	0	586 (7.49)
Unidentified (damaged)	0	78	0	9	0	87 (1.11)
Total	87	6,150	108	1,472	6	7,823 (100)

* HBC = human bait catch; CBC = cattle bait catch; IRC = indoor resting catch; TRH = cattle-baited trap huts; PT = pit trap catch. Values are Human-hour for HBC, CBC, and IRC; trap-nights for TRH; and no. of trap collections for PTC.

tibody was used as the capture antibody and a heavy chain–specific polyvalent antibody was used as the conjugate (reagents obtained from Kirkegaard and Perry, Gaithersburg, MD). A full panel of controls was used in each assay to ensure the specificity of the result. Casein/bovine serum albumin was used as the blocking buffer for the human blood assay and all non-bovid controls, and only casein was used for the bovid assay. Positivity on initial and confirmatory testing was determined on the basis of a clear visual signal (green coloration with ABTS substrate) together with a minimum optical density value ≥ 3 times the mean of the negative and heterologous control values. Quantitative results are presented as a human blood index (HBI) calculated as the proportion of abdomens tested that were positive for human blood protein.

Human malaria. From September 1994 to February 1997, all households in the village of Mahameegaswewa were visited every other day by one of two trained assistants from the study area. A malaria case was recorded if the patient or a close family member reported an episode of malaria, which had been confirmed positive for malaria parasites by Giemsa-stained thick blood film examination at a government hospital, western-type private health facility, or at the village level malaria treatment facility introduced to the area in May 1996. The information collected by the assistants was cross checked twice or three times per month by the investigators.[11] To minimize the reporting of relapses as fresh cases, patient records at the three nearest hospitals (at Kekirawa, Habarana, and Yakalla towns), as well as at the village level treatment center, were cross-checked. A recurrence of infection within two weeks of the original infection was considered to be a relapse, and not recorded as a new case. To minimize any bias caused by transmission outside the village, we also omitted cases where a person had spent a night outside the village during the previous two weeks.

Data analysis. Anopheline temporal abundance trends are presented as monthly mean numbers of females per trap-night or human-night, depending on the collection method at which each species was predominant. Because only small numbers within individual anopheline species were collected from HBC, a direct measure of the number of bites per human per night could not be obtained. Thus, vectorial capacity (VC) and entomologic inoculation rate (EIR) based on the number of bites per human per night were not estimated. Trends in mean monthly anopheline abundance showed a significant positive correlation among HBC, CBC, and TRH catches (Spearman's correlation coefficient; $P < 0.05$), while IRC showed a significant positive correlation with TRH catches and a nonsignificant positive correlation with HBC and CBC. Thus, we combined data from these four collection methods and calculated an approximation of EIR by multiplying mean species abundance per collection-night over the entire study period by the overall species-specific CS protein rate to estimate the mean number of infective vectors per collection-night. This value was multiplied by the species-specific HBI to estimate the mean number of infective vectors (MIV) that would have inoculated humans per collection night. For comparisons with the monthly pattern of human malaria, MIV computations were based on monthly values for species abundance per collection night, the species-specific CS protein rate, and the species-specific HBI. Thus, only those species that were both sporozoite and human blood positive within a particular month's collection were considered to have contributed to transmission in that month.

RESULTS

Mosquitoes. A total of 7,823 female anopheline mosquitoes representing 14 species were collected (Table 1). Direct CBC and TRH catches were the most productive collection methods. Only trivial numbers were collected from pit traps. *Anopheles tessellatus* Theobald, *An. vagus* Dönitz, *An. subpictus* Grassi, and *An. varuna* Iyengar predominated at HBC, CBC, IRC, and TRH catches, respectively. Overall, *An. vagus* was overwhelmingly the most numerous, followed by *An. varuna, An. jamesii* Theobald, *An. peditaeniatus* Leicester, and *An. culicifacies* (Table 1).

Temporal trends in anopheline abundance (Figure 2B and

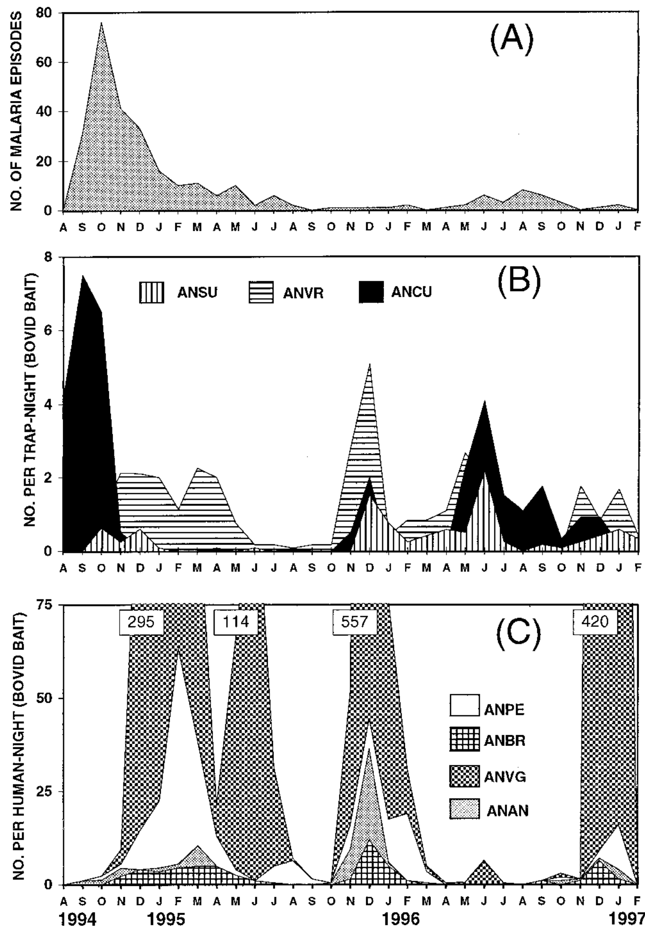

FIGURE 2. Human malaria in relation to relative abundance of malaria-infected anopheline species in Mahameegaswewa, Sri Lanka during the study period. **A,** monthly human malaria incidence; **B,** anopheline species most abundant at bovid-baited trap hut collections; **C,** anopheline species most abundant at direct bovid-bait catches. Numbers within **C** are peak abundance levels for *Anopheles vagus.* ANSU = *An. subpictus;* ANVR = *An. varuna;* ANCU = *An. culicifacies;* ANPE = *An. peditaeniatus;* ANBR = *An. barbirostris;* ANVG = *An. vagus;* ANAN = *An. annularis.*

C) were not correlated with rainfall, temperature, and humidity. However, when monthly rainfall data were lagged by one month with respect to monthly mosquito abundance data (to make allowances for rain-related breeding habitats to generate adult mosquitoes), significant positive correlations were seen for *An. annularis* van der Wulp (Spearman's r = 0.48, $P = 0.007$), *An. barbirostris* van der Wulp (r = 0.49, $P = 0.006$), *An. subpictus* (r = 0.41, $P = 0.03$), *An. vagus* (r = 0.41, $P = 0.03$) and *An. varuna* (r = 0.50, $P = 0.004$). Correlations were not significant in the case of *An. culicifacies* (r = 0.12, $P = 0.54$) and *An. peditaeniatus* (r = 0.32, $P = 0.09$).

Sporozoite rates and human blood indices in mosquitoes. Head-thoraces of 506 of the 7,823 female anophelines collected were not tested due to specimen damage or loss on storage. Twenty-five (0.3%) of 7,317 tested head-thoraces were positive for malaria parasite CS protein (Table 2). Most (19 of 25, 76.0%) of the positive mosquitoes contained *P. falciparum* and the rest (6 of 25, 24.0%) contained *P. vivax.* Seven species were infected, with the highest sporozoite rates in *An. barbirostris, An. culicifacies,* and *An. annularis.* Despite *An. vagus* being the species with the most ELISA-positive samples, its CS protein rate was significantly lower than that of *An. barbirostris* ($\chi^2 = 5.4$, degrees of freedom [df] = 1, $P = 0.02$), *An. culicifacies* ($\chi^2 = 7.9$, df = 1, $P = 0.005$), *An. peditaeniatus* ($\chi^2 = 5.4$, df = 1, $P = 0.02$), and *An. varuna* ($\chi^2 = 5.2$, df = 1, $P = 0.02$). All other comparisons of the CS protein rate between species were not significant. Both parasite species (though not in the same individual mosquito) were carried by *An. culicifacies, An. subpictus, An. vagus,* and *An. varuna,* and only *P. falciparum* was carried by *An. annularis, An. barbirostris,* and *An. peditaeniatus.* It is noteworthy that all three *P. vivax*-positive *An. varuna* were of the VK-247 polymorph, while the other *P. vivax*-positive mosquitoes (An. *culicifacies, An. subpictus,* and *An. vagus*) were of the PV-210 type.

Abdomens of 374 of 1,564 female anophelines collected from IRC and TRH catches were discarded due to their being unfed or fed but damaged after collection. Thirty-eight (3.2%) of 1,190 abdomens in seven of 11 tested species were

TABLE 2.
Malaria infections and human blood index in anopheline mosquitoes as detected by ELISA*

	Circumsporozoite protein				Blood meal assay		
	No. tested	No. positive for PV	No. positive for PF	CSP rate†	No. tested	HBI‡	MIV × 1,000§
An. aconitus	27	–	–	–	28	–	–
An. annularis	103	–	1	0.010	4	–	–
An. barbirostris	132	–	2	0.015	20	–	–
An. culicifacies	378	1	3	0.011	242	0.095	0.772
An. jamesii	477	–	–	–	70	–	–
An. nigerrimus	64	–	–	–	19	0.052	–
An. peditaeniatus	479	–	4	0.008	79	0.025	0.198
An. subpictus	252	1	1	0.008	189	0.016	0.076
An. tessellatus	309	–	–	–	100	0.050	–
An. vagus	4,592	1	7	0.002	76	0.013	0.229
An. varuna	491	3	1	0.008	363	0.008	0.074
Other spp.	13	–	–	–	–	–	–
Total	7,317	6	19	0.003	1,190	0.032	

* PV = *Plasmodium vivax*; PF = *P. falciparum.*
† Circumsporozoite infection rate calculated as the proportion CS protein positive among those tested from all field collection methods.
‡ Human blood index calculated as the proportion human blood positive among those tested from indoor resting catches and cattle-baited trap huts only.
§ Mean number of infective vectors per collection night computed as outlined in the Materials and Methods.

human blood-positive (Table 2). The highest HBI was seen in *An. culicifacies* and the lowest in *An. varuna.* The HBI of *An. culicifacies* was significantly higher than that of *An. peditaeniatus* (P = 0.041, by Fisher's one-tailed exact test), *An subpictus* (P = 0.002, by Fisher's one-tailed exact test), *An. vagus* (P = 0.015, by Fisher's one-tailed exact test), *An. varuna* (χ^2 = 22.1, df = 1, $P < 0.0001$). The HBI of *An. tessellatus* was significantly higher than that of *An. varuna* (P = 0.016, by Fisher's one-tailed exact test). All other comparisons of HBI between species were not significant. Concurrent bovid blood was detected in 14 of 38 human-positive samples overall (36.8%), involving *An. culicifacies* (4 of 23 human-positive samples), *An. nigerrimus* (1 of 1 human-positive samples), *An. peditaeniatus* (1 of 2 human-positive samples), *An. subpictus* (2 of 3 human-positive samples), *An. tessellatus* (3 of 5 human-positive samples), and *An. varuna* (3 of 3 human-positive samples). Thus, human-bovid multiple feeding was a common phenomenon among anophelines in the study area.

Despite being ranked fifth in terms of overall abundance, *An. culicifacies* was first in terms of MIV due to the high HBI and CS protein rate (Table 2). The most abundant species, i.e., *An. vagus,* ranked second, *An. peditaeniatus* third, *An. subpictus* fourth, and *An. varuna* fifth, respectively, in terms of MIV.

Human malaria and vector dynamics. Trends in malaria incidence in the study population (Figure 2A) are shown in relation to the abundance patterns of anopheline species implicated in malaria parasite carriage (Figure 2B and C). A malaria outbreak occurred during the period October 1994–January 1995, in which 134 (45.5%) of 294 village residents experienced at least a single disease episode. Thereafter, the malaria incidence remained at a relatively low level, with a moderate seasonal increase during the latter part of 1996.

During the 12-month period when outbreak malaria occurred (i.e., September 1994–August 1995), monthly malaria incidence and *An. culicifacies* abundance lagged by one month (to allow time for transmission and human infection to develop) and showed a significant positive correlation (Spearman's r = 0.89, $P < 0.001$). A similar analysis done for the 12-month period March 1996–February 1997 when malaria transmission was again evident in the study area also showed a positive correlation with *An. culicifacies* (r = 0.78, P = 0.003). Corresponding analyses with other *Anopheles* species did not produce any significant positive correlations. The trends in malaria and anopheline species abundance over the entire duration of the study period were not significantly correlated primarily due to the virtual absence of the disease during seasonal anopheline abundance peaks between September 1995 and February 1996 (Figure 2). Monthly malaria incidence during the study period was not significantly correlated with monthly rainfall (Spearman's r; $P > 0.05$) either when compared directly or after lagging malaria figures by 1–2 months in relation to rainfall.

The inoculation potential varied sharply within the study period: when monthly MIVs of individual species were summed, the total MIV × 1,000 value was 35.6 for the period September 1994–August 1995 (244 malaria cases), compared with 9.4 for the period September 1995–August 1996 (26 malaria cases). Figure 3A shows the species temporal distribution of sporozoite ELISA-positive specimens in tested mosquitoes, and Fig. 3B shows the corresponding monthly MIV and malaria incidence values. A high MIV was associated with *An. culicifacies* during the onset of the malaria outbreak in October 1994 (Figure 3B). Lower MIVs associated with low levels of human malaria were seen during months when *An. peditaeniatus, An. subpictus,* and *An. varuna* were involved in transmission. Other malaria-infected species containing human blood were not detected (*An. annularis* and *An. barbirostris*) or did not feed on humans in the same months in which they were malaria-infected (*An. vagus*); thus, the monthly MIVs were zero.

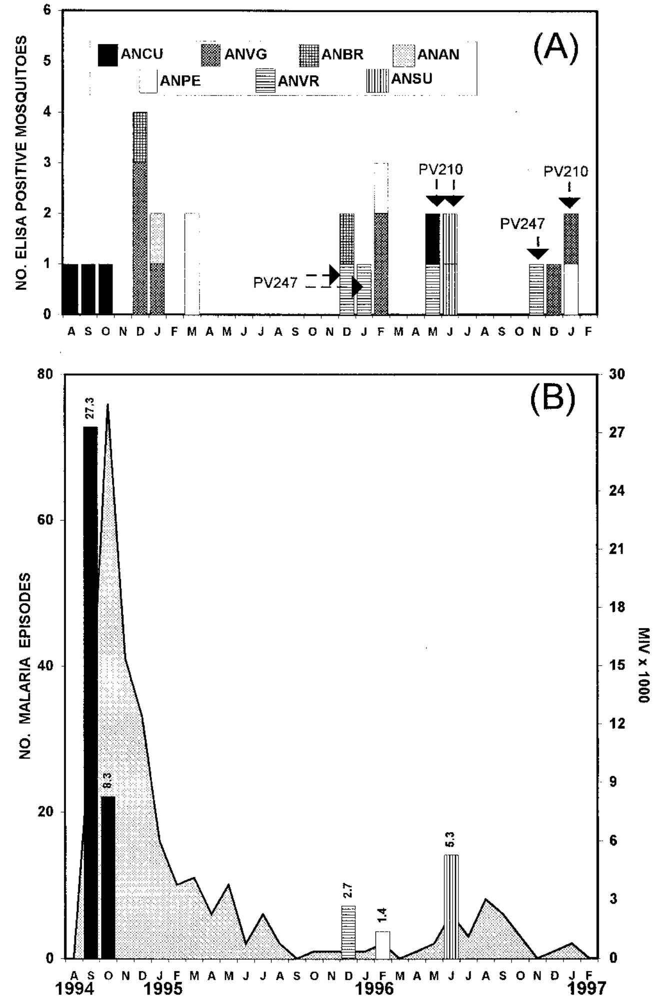

FIGURE 3. Temporal pattern of **A,** ELISA-based sporozoite detections in anopheline mosquitoes and **B,** malaria incidence in Mahameegaswewa, Sri Lanka in relation to species-specific rates of mean infective vectors per collection night (MIV). Abbreviations of the species names are as given in Figure 2.

DISCUSSION

Because of the short recall period (1–2 days) and the heavy emphasis on blood film examination at government clinics, the survey methodology used to monitor human malaria in the study area provided accurate data on disease incidence. However, it did not provide a means to assess the relative prevalence of *P. vivax* and *P. falciparum* since peo-

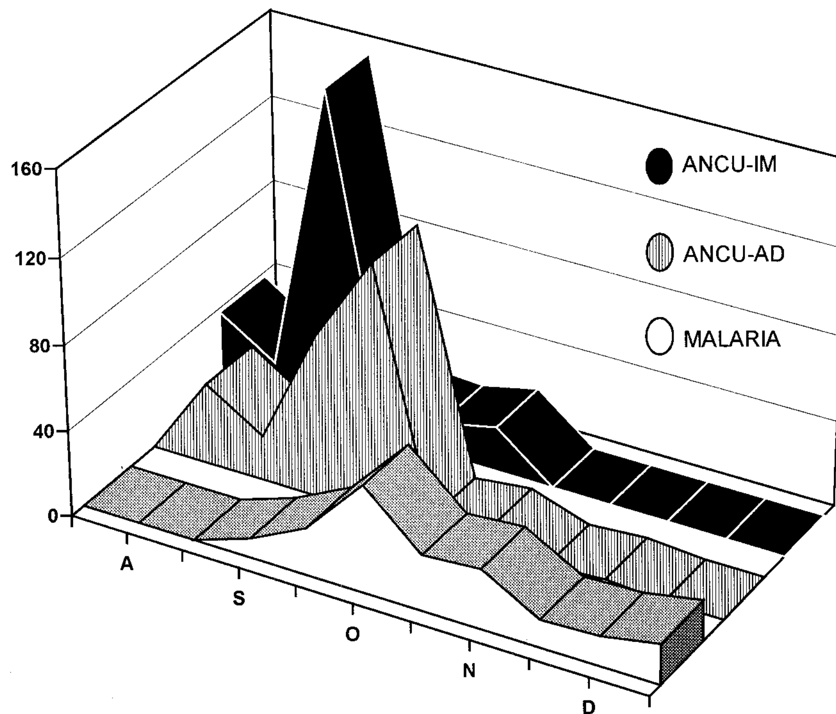

FIGURE 4. Relationship between *Anopheles culicifacies* immature form abundance in the Yan-Oya stream in Sri Lanka (ANCU-IM, expressed as mean number of immature forms per dip per sample × 1,000), adult abundance at bovid-baited trap huts (ANCU-AD, expressed as mean number of females per trap-night × 10), and malaria incidence (number of cases) during the period July–December 1994 when a malaria outbreak occurred. (Note: data for immature form abundance were taken from Amerasinghe and others[9]).

ple were often uncertain of the parasite species identification made by blood film examination at treatment centers they visited. An indication of relative parasite prevalence is available from two mass blood surveys carried out at the study village in September and December 1994, which showed that 26 (74.3%) of 35 detected malaria infections were due to *P. falciparum* and nine (25.7%) of 35 to *P. vivax* (Amerasinghe PH, unpublished data). Matching this high human incidence, only *P. falciparum* was detected from mosquitoes during the August 1994–November 1995 period of the present study (n = 11 positive mosquitoes). Subsequently, the incidence of *P. vivax* incidence increased: sporozoite detections in mosquitoes showed 42.9% to be *P. vivax* and 57.1% *P. falciparum* (n = 14 positive mosquitoes) between December 1995 and February 1997. Data from a village clinic established in May 1996 showed a similar trend of increasing incidence of *P.vivax* in humans in the study village, with 55.0% *P. vivax* and 45.0% *P. falciparum* (n = 20 cases) from May 1996 to February 1997 (Amerasinghe PH, unpublished data).

The unstable nature of malaria in Sri Lanka was clearly demonstrated in the village of Mahameegaswewa during this study, with an outbreak occurring in late 1994–early 1995, and substantially lower disease incidence for two years thereafter. This was reflected in a sharp decrease in MIV values during this period. Such temporal fluctuations in vector inoculation rates have occurred at the village level in malaria hyperendemic regions in Africa,[18] but since the overall inoculation rate is roughly 10^3 times greater than in Sri Lanka, a high incidence of the disease occurs nonetheless. The major malaria vector control measure used in Sri Lanka is indoor house spraying conducted by the Anti-Malaria Campaign, generally on a trimester schedule. While it is noteworthy that this regular spray regimen did not prevent the malaria outbreak in late 1994 in our study area, the subsequent sharp decrease in disease incidence could have been due to the switch from the decade-long usage of malathion (last used in July 1994 in our study village) to newer insecticides such as sumithion (from late 1994 to early 1996) and fenitrothion (from mid 1996 onwards). A contributing factor could have been the reduced availability during 1995–1997 of breeding habitats for *An. culicifacies,* the species with the highest MIV in the study area, due to the water flow dynamics in one of its main breeding habitats, namely, the Yan Oya stream (Amerasinghe FP, unpublished data).

Overall, the pattern of malaria was most closely associated with the population dynamics of *An. culicifacies.* Adult abundance trends in this species were unrelated to rainfall since the major breeding habitats in the study area were stream bed and tank bed pools that were available before the onset of the monsoon rains.[9] The relationship between this species and malaria was of a classical nature during the outbreak period in 1994, when *An. culicifacies* immature form dynamics in the nearby Yan Oya stream (reported previously by Amerasinghe and others[9]) were combined with adult dynamics and malaria at Mahameegaswewa village (Figure 4). The succession of peaks corresponding to immature form abundance in the nearby stream, adult abundance and malaria in the village, together with the high MIV values (Table 2, and Figure 3B) associated with this species leave no doubts as to its epidemiologic importance. A similar conclusion was reached by Dewit and others at a site in

the intermediate zone of Sri Lanka, based on adult population dynamics and vectorial capacity determinations.[19]

Six other anopheline species were implicated in malaria parasite transmission in our study area. With the exception of *An. peditaeniatus,* abundance trends in the these species were related to rainfall since they bred mainly in rain-generated habitats.[9] *Anopheles peditaeniatus* bred in habitats generated by both rainfall and irrigation water,[9] and thus its abundance trends did not correlate significantly with rainfall. All of these species except *An. peditaeniatus* have been implicated in malaria parasite carriage in previous studies in Sri Lanka.[4,7,8] *Anopheles peditaeniatus* is reported infected with *P. falciparum* for the first time locally, following a similar report from Thailand.[20] Sporozoites of the VK247 polymorph of *P. vivax* carried by *An. varuna* also are reported for the first time in Sri Lanka. However, this is not the only species capable of carrying the parasite strain, since *An. subpictus* has also been shown to be infected elsewhere on the island (Amerasinghe PH, unpublished data). Serologic evidence for VK247 infection in humans in Sri Lanka has also been reported.[21] It is evident that previous ELISA studies[4–8] that used only the PV-210 polymorph would have underestimated the CS protein rates of *P. vivax* in Sri Lankan anophelines and thereby the VC and EIR estimates based on these rates.

While some or all of these species may have contributed to malaria transmission, it is clear from the present study that the initiation of noteworthy levels of transmission was associated primarily with *An. culicifacies.* This was especially evident in August–October 1994 when this species was the only anopheline found in significant numbers in the study area (Figure 2). In 1996, an increase in malaria was also observed when this species became abundant in May–July 1996. In both instances, the species was infected with malaria parasites, as determined by ELISA. The other six infected anopheline species could be considered to assist in maintenance transmission, especially of *P. falciparum,* in which recrudescence through parasite dormancy in humans is not known to occur. *Anopheles subpictus* already has been shown to be a secondary vector of malaria in Sri Lanka, capable of carrying both parasite species.[5,7] This species, *An. peditaeniatus,* and *An. varuna* were implicated in the relatively low level of malaria transmission that occurred during late 1995–mid 1996 in the present study (Figure 3B). Seasonally highly abundant, outdoor dusk biting species such as *An. vagus* and *An. peditaeniatus,* in particular, could have an impact on the maintenance of *P. falciparum* at the substantial levels (20–30% of the infections) seen in Sri Lanka over the past decade. However, there is at present no published evidence of any of these species causing outbreak malaria in Sri Lanka in the absence of *An. culicifacies.* Using a complex mathematical formulation, Mendis and others[7] have evaluated the relative contribution of different anopheline species to malaria transmission in southern Sri Lanka, but their study did not take detailed temporal patterns of abundance or transmission potential into account, and thus does not provide a clear insight into the species that are responsible for initiating transmission. Transmission indices computed on ELISA-based sporozoite determinations assume that positive head-thoraces are indicative of salivary gland infections, and thus that the CS protein rate reflect infectivity. However, this may not always be the case since CS proteins in the thorax may be detected even when sporozoite infections abort before reaching the salivary glands.[22] Thus ELISA-based indices could overestimate true infectivity and may also incriminate species that are, in fact, not transmitters in the field. This could well be a factor in Sri Lanka, where in a situation of unstable malaria and low transmission rates, ELISA studies have incriminated a large number of anopheline species.[4–8] As the present study shows, parasite infection in many of these species could not be clearly linked to human malaria. The increasing ELISA-based evidence of multispecies involvement in transmission[4–8] has resulted in some confusion among local vector control personnel regarding target species and control strategies. The findings of the present study that both the initiation of significant levels of transmission and high transmission potential to humans were associated primarily with *An. culicifacies* indicates that the primary target should still be this species, though other vectors may enhance or maintain transmission depending on local abundance trends.

It is well established that south Asian anophelines are mainly zoophagic in their feeding habits.[23] In the present study, 625 human-hours produced only 87 human biting anophelines. Only four *An. culicifacies* were collected at these catches, although this species was the most anthropophagic anopheline in the area with 9.5% of engorged female abdomens containing human blood. This contrasts with a previous report of 35% human feeding in a sample of ~1,000 *An. culicifacies* using a gel diffusion test (Abhayawardena TA, Anti-Malaria Campaign of Sri Lanka, unpublished data). The HBI of *An. culicifacies* s. l. varies temporally and spatially in India, and this is partly attributed to seasonal changes in sibling species population dynamics.[24] This is unlikely in Sri Lanka, where only sibling species B of *An. culicifacies* occurs.[25] However, HBIs are likely to be biased by collection methodology: those based mainly on catches from human dwellings generally have higher human blood rates than samples from bovid-baited catches.[24] Unfortunately, there seems to be no satisfactory unbiased methods of collecting engorged female anophelines in large numbers. Pit traps (present study), light traps,[26] and outdoor resting catches by D-vac type suction aspirators (Amerasinghe FP, unpublished data) have proven to be unproductive in Sri Lanka. The effect of trap bias is to some extent offset by the evidence of multi-host feeding within the same gonotrophic cycle seen in the present as well as previous studies,[27,28] which increases the chances of detecting human feeding even in catches biased in favor of other hosts. Engorged females of all tested anopheline species were overwhelmingly (< 80%) bovid-fed, and several had mixed bovid-human blood. However, natural zooprophylaxis did not seem to occur: an investigation of malaria risk factors in our study village showed that the distance of nocturnally resting bovids from human-occupied houses was not a significant modulator of human malaria.[29]

In a situation where a low level of human feeding occurs, calculating the traditional transmission indices such as the EIR using the number of bites per human per night will not provide reliable estimates due to the low numbers at direct human bait catches. An EIR may also be based on indirect estimation where the human biting rate is derived from the

numbers of freshly blood-fed anophelines in a bedroom divided by the number of humans in the room, the assumption being that the mosquitoes have fed only on the humans.[30] This approach was used by Amerasinghe and others[5] to compute an EIR for indoor resting *An. subpictus* in Sri Lanka, except that the HBI of the indoor resting sample was determined to have a more accurate estimate of human feeding. Extending the indirect estimation approach in the present study where both endophilic and exophilic potential vectors were present, we pooled the mosquitoes across four collection methods, and computed an all-methods females per collection night statistic, which was then multiplied by the ELISA-based HBI and CS protein rate. The pooled methodologies provided a large sample size, which allowed detailed analyses that were not possible with the low numbers present at direct human biting collections. The MIV index used here is, in a sense, a modified EIR, but is not based on the number of bites per human per night, which is not possible to estimate from pooled methodologies. The MIV provided a useful statistic to compare the transmission potential of different anopheline species, and to compare trends in the infectivity rate with trends in malaria in this study. The disadvantage of the present index is that it cannot be used to directly compare the results of different studies unless identical mosquito collection methodologies are used.

Acknowledgments: We thank S. Lionelrathne and S. P. Ranasinghe for field and laboratory assistance, and W. van der Hoek (International Irrigation Management Institute, Colombo, Sri Lanka) for useful suggestions in improving an earlier draft of the manuscript.

Financial support: This work was supported by the Government of Japan and the Danish International Development Agency by grants to the International Irrigation Management Institute.

Authors' addresses: Priyanie H. Amerasinghe and Felix P. Amerasinghe, Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka. Flemming Konradsen and Kapila T. Fonseka, International Irrigation Management Institute, PO Box 2057, Colombo, Sri Lanka. Robert A. Wirtz, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Mailstop F12, 4770 Buford Highway, Atlanta, GA 30341–3724.

Reprint requests: Felix P. Amerasinghe, Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka.

REFERENCES

1. Green CA, Miles SJ, 1980. Chromosomal evidence for sibling species of the malaria vector *Anopheles culicifacies* Giles. *J Trop Med Hyg 83:* 75–78.
2. Carter HF, Jacocks WP, 1929. Observations on the transmission of malaria by anopheline mosquitoes in Ceylon. *Ceylon J Sci (D) 2:* 67–86.
3. Carter HF, 1930. Further observations on the transmission of malaria by anopheline mosquitoes in Ceylon. *Ceylon J Sci (D) 2:* 159–176.
4. Amerasinghe FP, Amerasinghe PH, Peiris JSM, Wirtz RA, 1991. Anopheline ecology and malaria infection during the irrigation development of an area of the Mahaweli Project, Sri Lanka. *Am J Trop Med Hyg 45:* 226–235.
5. Amerasinghe PH, Amerasinghe FP, Wirtz RA, Indrajith NG, Somapala W, Pereira LR, Rathnayake AMS, 1992. Malaria transmission by *Anopheles subpictus* (Diptera: Culicidae) in a new irrigation project in Sri Lanka. *J Med Entomol 29:* 577–581.
6. Mendis C, Gamage-Mendis AC, de Zoysa APK, Abhayawardena TA, Carter R, Herath PRJ, Mendis KN, 1990. Characteristics of malaria transmission in Kataragama, Sri Lanka: a focus for immuno-epidemiological studies. *Am J Trop Med Hyg 42:* 298–308.
7. Mendis C, Herath PRJ, Rajakaruna J, Weerasinghe S, Gamage-Mendis AC, Mendis KN, de Zoysa APK, 1992. Method to estimate relative transmission efficiencies of *Anopheles* species (Diptera: Culicidae) in human malaria transmission. *J Med Entomol 29:* 188–196.
8. Ramasamy R, de Alwis R, Wijesundere A, Ramasamy MS, 1992. Malaria transmission at a new irrigation project in Sri Lanka: the emergence of *Anopheles annularis* as a major vector. *Am J Trop Med Hyg 47:* 547–553.
9. Amerasinghe FP, Konradsen F, Amerasinghe PH, Fonseka KT, 1997. Anopheline (Diptera: Culicidae) breeding in a traditional tank-based village ecosystem in north central Sri Lanka. *J Med Entomol 34:* 290–297.
10. Konradsen F, van der Hoek W, Amerasinghe PH, Amerasinghe FP, Fonseka KT, 1997. Household responses to malaria and their costs: a study from rural Sri Lanka. *Trans R Soc Trop Med Hyg 91:* 127–130.
11. Konradsen F, van der Hoek W, Amerasinghe PH, Amerasinghe FP, 1997. Measuring the economic cost of malaria to households in Sri Lanka. Am J Trop Med Hyg 56: 656–660.
12. Service MW. 1993. *Mosquito Ecology. Field Sampling Methods.* Second edition. London: Chapman & Hall.
13. Amerasinghe FP, Indrajith NG, 1995. Nocturnal biting rhythms of mosquitoes (Diptera: Culicidae) in Sri Lanka. *Trop Zool 8:* 43–53.
14. Ramasamy MS, Kulasekera R, Srikrishnaraj KA, Ramasamy R, 1994. Population dynamics of anthropophilic mosquitoes during the northeast monsoon season in the malaria epidemic zone of Sri Lanka. *Med Vet Entomol 8:* 265–274.
15. Amerasinghe FP, 1990. A guide to the identification of the anopheline mosquitoes (Diptera: Culicidae) of Sri Lanka. I. Adult females. *Ceylon J Sci (Bio Sci) 21:* 1–16.
16. Wirtz RA, Burkot TR, Graves PM, Andre RG, 1987. Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. *J Med Entomol 24:* 433–437.
17. Chow E, Wirtz RA, Scott TW, 1993. Identification of blood meals in *Aedes aegypti* by antibody sandwich enzyme-linked immunosorbent assay. *J Am Mosq Control Assoc 9:* 196–205.
18. Fontenille D, Lochouarn L, Diagne N, Sokhna C, Lemasson J, Diatta M, Konate L, Faye F, Rogier C, Trape J, 1997. High annual and seasonal variations in malaria transmission by anophelines and vector species composition in Dielmo, a holoendemic area in Senegal. *Am J Trop Med Hyg 56:* 247–253.
19. Dewit I, Coosemans M, Srikrishnaraj K, Wery M, 1994. Population dynamics of anophelines in a malathion treated village in the intermediate zone of Sri Lanka. *Ann Soc Belge Med Trop 74:* 93–103.
20. Gingrich JB, Weatherhead A, Sattabongkot J, Pilakasiri C, Wirtz RA, 1990. Hyperendemic malaria in a Thai village: dependence of year-round transmission on focal and seasonally circumscribed mosquito (Diptera: Culicidae) habitats. *J Med Entomol 27:* 1016–1026.
21. Wirtz RA, Rosenberg R, Sattabangkot J, Webster HK, 1990. Prevalence of antibody to heterologous circumsporozoite protein of *Plasmodium vivax* in Thailand. *Lancet 336:* 593–595.
22. Beier JC, Perkins PV, Koros JK, Onyango FK, Gargan TP, Wirtz RA, Koech DK, Roberts CR, 1990. Malaria sporozoite detection by dissection and ELISA to assess infectivity of Afrotropical *Anopheles* (Diptera: Culicidae). *J Med Entomol 27:* 377–384.
23. Ramachandra Rao T, 1984. *The Anophelines of India.* Revised edition. New Delhi: Malaria Research Centre (Indian Council of Medical Research).
24. Collins RT, Sharma GK, Dhal KB, 1987. *Gel Diffusion Determinations on Feeding Behaviour Patterns of Anophelines in Orissa State, India.* Geneva: World Health organization, WHO/VBC/87.951.
25. Abhayawardena TA, Dilrukshi RKC, Wijesuriya SRE, 1996. Cytotaxonomical examination for sibling species in the taxon

Anopheles culicifacies Giles in Sri Lanka. *Indian J Malariol 33:* 74–80.
26. Amerasinghe FP, Munasingha NB, 1988. A predevelopment mosquito survey in the Mahaweli development project area, Sri Lanka: adults. *J Med Entomol 25:* 276–285.
27. Beier JC, 1996. Frequent blood-feeding and restrictive sugar feeding behaviour enhance the malaria vector potential of *Anopheles gambiae* s.l. and *An. funestus* (Diptera: Culicidae) in western Kenya. *J Med Entomol 33:* 613–618.
28. Wekesa JW, Yuval B, Washino RK, 1997. Multiple blood feeding by *Anopheles freeborni* and *Culex tarsalis* (Diptera: Culicidae): spatial and temporal variation. *J Med Entomol 34:* 219–225.
29. Van der Hoek W, Konradsen F, Dijkstra D, Amerasinghe FP, Amerasinghe PH, 1998. Risk factors for malaria: a microepidemiological study in a village in Sri Lanka. *Trans R Soc Trop Med Hyg 92:* 265–269.
30. Gilles HM, 1993. Epidemiology of malaria. Gilles HM, Warrell DA, eds. *Bruce-Chwatt's Essential Malariology.* Third Edition. London: Edward Arnold, 124–163.