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Adult anopheline ecology and malaria transmission in irrigated areas of South Punjab, Pakistan

N. HERREL, F. P. AMERASINGHE*, J. ENSINK, M. MUKHTAR, W. VAN DER HOEK* and F. KONRADSEN†,*
International Water Management Institute, Lahore, Pakistan, *IWMI, Colombo, Sri Lanka and †Department of International Health, University of Copenhagen, Denmark

Abstract. Surface irrigation in the Punjab province of Pakistan has been carried out on a large scale since the development of the Indus Basin Irrigation System in the late 19th century. The objective of our study was to understand how the population dynamics of adult anopheline mosquitoes (Diptera: Culicidae) could be related to malaria transmission in rural areas with intensive irrigation and a history of malaria epidemics. In this paper we present our observations from three villages located along an irrigation canal in South Punjab. The study was carried out from 1 April 1999 to 31 March 2000. Mosquitoes were collected from bedrooms using the pyrethroid spraycatch method and from vegetation and animal sheds using backpack aspirators. Overall, *Anopheles subpictus* Grassi *sensu lato* predominated (55.6%), followed by *An. stephensi* Liston s.*l.* (41.4%), *An. culicifacies* Giles s.*l.* (2.0%), *An. pulcherrimus* Theobald (1.0%) and *An. peditaeniatus* Leicester (0.1%). Most mosquitoes (98.8%) were collected from indoor resting-sites whereas collections from potential resting-sites outdoors accounted for only 1.2% of total anopheline densities, confirming the endophilic behaviour of anophelines in Pakistan. *Anopheles stephensi*, *An. culicifacies* and *An. subpictus* populations peaked in August, September and October, respectively. High temperatures and low rainfall negatively affected seasonal abundance in our area. There were interesting differences in anopheline fauna between villages, with *An. culicifacies* occurring almost exclusively in the village at the head of the irrigation canal, where waterlogged and irrigated fields prevailed. Monthly house-to-house fever surveys showed that malaria transmission remained low with an overall slide positivity rate of 2.4% and all cases were due to infection with *Plasmodium vivax*. The most plausible explanation for low transmission in our study area seems to be the low density of Pakistan's primary malaria vector, *An. culicifacies*. The role of other species such as *An. stephensi* is not clear. Our observations indicate that, in South Punjab, irrigation-related sites support the breeding of anopheline mosquitoes, including the vectors of malaria. As our study was carried out during a year with exceptionally hot and dry climatic conditions, densities and longevity of mosquitoes would probably be higher in other years and could result in more significant malaria transmission than we observed. To assess the overall importance of irrigation-related sites in the epidemiology of malaria in the Punjab, more studies are needed to compare irrigated and non-irrigated areas.

Key words. *Anopheles culicifacies*, *An. pulcherrimus*, *An. stephensi*, *An. subpictus*, environmental management, irrigation, malaria, mosquito population dynamics, Pakistan, Punjab.

Correspondence: Dr Felix P. Amerasinghe, IWMI: Water, Health and Environment Theme, P. O. Box 2075, Colombo, Sri Lanka. E-mail: f.amerasinghe@cgiar.org

Introduction

The plains of the Punjab are low-lying, wet and often inundated. Malaria in the Punjab is seasonal and unstable, with epidemics recurring at approximately 8-year intervals (Christophers, 1911; Yacob & Swaroop, 1945, 1946). Heavy rainfall in the monsoon season has been implicated as a possible explanation for the variation in malaria transmission from year to year (Yacob & Swaroop, 1945, 1946). The periodicity of malaria epidemics has more recently been linked to cyclical climate patterns, notably the El Niño phenomenon (Bouma & van der Kaay, 1996). The last great epidemic in the Pakistani Punjab dates back to 1972 (de Zulueta *et al.*, 1980). The situation nowadays is much improved and malaria has a lower public health significance (Donnelly *et al.*, 1997a). However there still are substantial variations in the epidemiology of malaria between different districts of the Punjab (Donnelly *et al.*, 1997a). Some areas apparently have stable and endemic – albeit seasonal – malaria which has been linked to the presence of perennial water bodies close to villages where *Anopheles culicifacies* breeds perennially (Khaliq *et al.*, 1985; Mahmood & Macdonald, 1985; Strickland *et al.*, 1987). Such villages are more malarious than nearby similar, but dryer, areas (Strickland *et al.*, 1987).

With more than 70% of the Punjab under irrigation since the development of the Indus Basin Irrigation System (IBIS) in the 1860s, Pakistan has become the most intensively irrigated country in the world. In the arid Punjab Province particularly, agriculture would be virtually impossible without irrigation. World-wide, the role of irrigation in malaria transmission has received much attention and studies have yielded a complex picture (Ijumba & Lindsay, 2001). In areas with stable malaria transmission where populations have developed immunity to malaria, the introduction of irrigation does not significantly worsen transmission (Ijumba & Lindsay, 2001). However, in areas where malaria is unstable, the increased densities of vectors resulting from irrigation can lead to increased incidence of malaria. Our investigation was designed to understand better whether irrigation-related water bodies within and around villages in Punjab did indeed support anopheline breeding and whether this translated into high vector densities and significant malaria transmission. This is all the more relevant as there is conclusive evidence in more recently constructed schemes in neighbouring India (Singh *et al.*, 1997, 1999; Tyagi, 1998, 2002) for increased malaria transmission as a direct result of the introduction of canal-irrigation.

Our investigations were launched with the objective of developing community-based environmental management methods to reduce anopheline breeding and control malaria. Such methods could be potentially very useful in the context of Pakistan where malaria control has been plagued by numerous difficulties such as: (1) very limited vector control activities due to shortage of insecticides; (2) development of insecticide resistance in vector mosquitoes; (3) development of resistance to chloroquine in the malaria parasites. In the present paper we report on the adult anopheline population dynamics and malaria epidemiology of villages near irrigated areas in south Punjab, Pakistan. Our studies on larval ecology, which were conducted simultaneously, showed that anophelines were breeding in habitats directly or indirectly linked to the irrigation system (Herrel *et al.*, 2001).

Materials and methods

Study area

The study was carried out from 1 April 1999 to 31 March 2000 within the irrigation command area of Hakra 6R, one of the largest irrigation distributaries within the IBIS. The study area was located near Haroonabad (29°37′ N, 73°08′ E) in Bahawalnagar District, ~300 km SW of Lahore. The development of the IBIS during the colonial era profoundly changed the ecology of the area, which was previously a sparsely populated desert unfit for agricultural activity.

Human migration to villages of the Hakra command area has resulted in a current population of around 160 000 inhabitants. Agriculture is the dominant activity with crops such as sugarcane, cotton, wheat and fodder. Rice remains a minor crop because soils tend to be unsuitable and the irrigation system is not flexible enough to allow periodic flooding of ricefields. This crop is therefore limited to villages in the head of the system where water availability tends to be higher.

Groundwater tables are 0.5–2.0 m below soil surface in the head and middle villages and at a depth of more than 10 m in the tail-end of the irrigation distributary. These levels remained stable throughout the study period. The area is arid with <200 mm annual rainfall and is situated in the Cholistan portion of the Thar Desert, which stretches into the neighbouring state of Rajasthan in India. Average rainfall per village for the study period was particularly low (132 mm) and was far exceeded by evaporation rates because the entire region (including Pakistan, Afghanistan and India) experienced a severe drought. A more detailed description of the study area, site map and climate data were given by Herrel *et al.* (2001).

Village selection

Of 94 villages in the Hakra 6R command area, three were selected for this study. The villages 149/6R (population: 900), 111/6R (population: 1530) and 438/6R (population: 970) were chosen to represent the head, middle and tail of the irrigation canal to reflect a range of different environments, from severely waterlogged to desert conditions. Villages were otherwise similar in their layout and general characteristics with no levelling, no paved roads, no water supply scheme nor any drainage systems.

Household collections

Household and bedroom selection. Mosquitoes were collected from 20 pre-selected bedrooms per village on a fortnightly basis. During the study, the overall number of households did not vary significantly nor were there any important population or livestock movements in or out of the villages. Criteria for household selection were their geographical location in the village, their construction and the consent of the family. Households were not randomly selected as it was considered more important to include households with different constructions and which were representative of spatial differences in the village (to reflect proximity or distance to potential mosquito breeding sites).

In each selected household, the family was asked to identify the room most frequently used for sleeping. This room was then sampled for the duration of the study. All selected rooms were described using a questionnaire at the start of the study. Information was gathered about the condition and construction of walls, ceilings, windows, eaves and also whether animals were kept inside the compound overnight. Respondents were also asked whether cooking was practised in the room and whether or not they had electricity and a ceiling fan.

Spray catch collection technique. Villages were sequentially sampled in the order 149/6R, 438/6R and 111/6R on separate days every fortnight between 06.00 and 13.00 hours. The order in which the houses were sprayed was rotated at each visit to eliminate bias associated with the timing of the collections (i.e. exit behaviour of female mosquitoes). We followed the standard knockdown spray-catch procedure (Service, 1993). After removal of all food items, white cloth sheets were laid out to cover the entire floor surface. Windows, shutters and doors were closed and other openings were blocked to prevent the exit of mosquitoes. A commercially available insecticide containing pyrethroids (Mortein®: tetramethrin 0.023% w/w, deltamethrin 0.015% w/w; Reckitt & Coleman of Pakistan Ltd, Karachi) was sprayed for 30–60 s and the room was kept closed for 15 min to ensure maximum knock-down of mosquitoes. All mosquitoes were collected from sheets with a hand-held battery-powered aspirator (Hausherr's Machine Works, Toms River, NJ, U.S.A.). Specimens were killed with chloroform, placed in a Petri dish and stored in an ice-box. Mosquitoes were transported to the laboratory and stored at 7 °C until identified.

At each spraycatch, an adult family member was asked how many people had slept in the selected bedroom on the previous night and whether any mosquito control method had been used.

Animal shed and vegetation collections

Site selection. To include other potential anopheline resting sites, animal sheds and vegetation (within a 1000 m radius of the village) were sampled during the period 1 June 1999 to 31 March 2000. Collections were made fortnightly between 07.00 and 13.00 hours using a Modified CDC Backpack Aspirator (Model 1412, John W. Hock Company, Gainesville, FL, U.S.A.). Collections were made from cotton, rice, wheat, fodder and sugarcane fields as well as trees/bushes/shrubs, reeds, and vegetation along earthen irrigation channels. Two animal sheds were also sampled at each visit. Other sites encountered by the field team were also sampled on an ad hoc basis. Maps of the crops present around each of the villages were made every 2 months to ensure that all potential resting sites would be included in the sampling.

Backpack collection technique. Aspirations were standardized on time by carrying out 15-min collections from each site. Farmers were asked whether particular fields had recently been sprayed with pesticides and, if so, these were not sampled. Collections from fields were done by moving methodically through crops and sampling from the base to the tips of plants. The presence of standing water, crop height and the time of sampling was noted. In animal sheds, wall surfaces, ceilings and feeding troughs were sampled. Care was taken not to sample the same sites on consecutive field visits. Specimens were stored in containers in the field and brought back to the laboratory for sorting and identification.

Mosquito identification

Based on the checklists of Pakistan *Anopheles* contained in Aslamkhan (1971) and Glick (1992), an updated taxonomic key for the identification of female anophelines developed by Amerasinghe *et al*. (2002) was used. *Anopheles* females were identified to species level using a dissecting microscope and classified according to trophic status (unfed/fed/semigravid/gravid).

Malaria surveys

We performed active case detection surveys on one day per month in each village from 1 April 1999 to 30 November 2000. Prior to the surveys, households were numbered and a map was completed showing their location. Villagers were informed in advance of the fever survey dates. Two teams consisting of one government Communicable Disease Control (CDC) Supervisor and one IWMI research staff member conducted the house-to-house visits. The regular duties of CDC staff include visiting villages, preparing bloodfilms from suspected malaria patients and dispensing presumptive chloroquine treatment. Survey teams were accompanied by one local and/or one expatriate physician on most visits. A local physician was responsible for examining all suspected malaria patients and issuing prescriptions. Each team was allocated one half of the village. Family heads were asked whether anyone in their household was suffering from fever at the time of the survey or had a history of fever. A standard bloodfilm (Gilles, 1993) was taken from all patients with this case definition. Thick bloodfilms were stained with Giemsa stain and examined under 100× magnification (Gilles, 1993). Slides were declared negative when 100 fields were found with no malaria parasites. Slides were first examined by the District Microscopist at our laboratory and later re-confirmed by the National Institute of Malaria Research and Training in

Lahore. All patients were informed of their slide result the following day and *Plasmodium vivax*-positive patients were given free treatment with chloroquine (3 days) and primaquine (5 days) in accordance with the official treatment protocol of the Pakistan Government.

One contraindication for the use of Primaquine is the glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency. As compared with some other parts of the world, this deficiency is more common in Pakistan and affects 3.0–6.9% of the population (Fleming, 1996). It is mostly a concern among the Pathan ethnic group where, in the North-west Frontier Province, it has been shown to affect 7.0% of Pathan individuals (Bouma *et al.*, 1995). In villages near the Lahore area, the deficiency is present in around 1.5% of the population (Bollinger & Zafar-Latif, 1985). To minimize the risk of haemolysis in areas with a high proportion of G6PD–deficient patients, a 5-day course is often given instead of the WHO recommended 14-day course (Shah *et al.*, 1997). As we did not know the prevalence of this deficiency in our study area, we adopted the 5-day approach as a precaution.

Results

Bedroom characteristics

The layout of homesteads in the study area was typical of the wider situation in rural Punjab. People typically lived in compounds with high mud walls surrounding an inner courtyard. The selected bedrooms from each village were generally similar; the majority being relatively small in size (area $< 19\,m^2$) (Table 1). Bedrooms typically contained wooden sleeping cots, kitchenware and large storage chests. Village 111/6R was comparatively wealthier and this was reflected in bedroom constructions, a majority (60.0%) of which were classified as 'good' with walls frequently made of bricks and cement as opposed to mud in the other villages. All households in 111/6R were electrified and most could afford ceiling fans. Most bedrooms in all three villages had unscreened windows but eaves were typically closed. In village 149/6R it was common for animals to be kept within the household compound overnight, a practice that was not as widespread in the other villages. A livestock survey indicated that the most common animals in the villages were goats, buffaloes, cattle and sheep.

Mosquito control activities

On collection days, households were asked whether or not any anti-mosquito measures had been taken the previous night. Our questionnaires listed measures such as mosquito coils, repellents, fans, bednets, etc. Only 8.0% (98/1275) of all households visited reported having used a 'mosquito control' measure and, of those, most reported having used a ceiling fan. Furthermore, the great majority of people slept outdoors during the months August to October when night-time temperatures were high. Our observations in the villages also confirmed that no anti-mosquito measures were being taken whether collectively or at household level. During the course of this study no government mosquito control operations were carried out in the study villages.

Adult anopheline population dynamics

Overall, collections were dominated by *An. subpictus* (55.6%) and *An. stephensi* (41.4%) followed by *An. culicifacies* (2.0%), *An. pulcherrimus* (1.0%) and *An. peditaeniatus* (0.1%) (Table 2). Most specimens were collected inside bedrooms and animal sheds, whereas outdoor sites were not very productive, accounting for only 1.2% of all mosquitoes collected. As collections from vegetation and animal sheds were both carried out with backpack aspirators, some comparisons of indoor vs. outdoor resting anopheline densities were possible. Across all villages, densities per man-hour within animal sheds compared with vegetation were much higher for *An. subpictus*, *An. stephensi* and *An. culicifacies*. Densities of *An. pulcherrimus* were higher on vegetation than in animal sheds. Alhough only a few *An. peditaeniatus* were collected, all were from outdoor resting sites.

There were important contrasts in the anopheline fauna collected from the different villages. Overall, the majority of specimens were collected from villages 149/6R (46.9%) and 438/6R (38.0%), whereas collections from the middle village (111/6R) accounted only for 15.1% of the total anophelines. On the whole, densities per bedroom were lower in this village than in the others (Table 2) and, on average, fewer than 20 females were collected from each individual bedroom (data not shown). This is most probably a reflection of the better construction of houses in this village (Table 1), which was in general wealthier than the other two. Average anopheline densities for most individual bedrooms from villages 149/6R and 438/6R were lower than 20. However, in both these villages, a few bedrooms were extremely productive with averages ranging from 25 to 130 females per room. None of the characteristics from Table 1 could clearly explain these differences.

Anopheles stephensi and *An. subpictus* were collected in substantial numbers in all three villages. However, in village 149/6R, *An. stephensi* was the predominant species (19.2 females per bedroom) and in village 438/6R *An. subpictus* dominated (19.5 females per bedroom). For *An. stephensi*, 84.2% of all collected females came from village 149/6R, and 2.2% from village 438/6R. For *An. subpictus*, the trend was the opposite: 16.6% in village 149/6R and 66.4% in village 438/6R. The major vector of malaria in Pakistan, *An. culicifacies*, was present in significant numbers only in the head village 149/6R. This was also the village with the highest species richness, as *An. pulcherrimus* and *An. peditaeniatus* were also present, albeit at low densities.

In terms of seasonal abundance, data from bedrooms (Fig. 1) correlated quite well with data from animal sheds

Table 1. Characteristics of study villages and selected bedrooms

Characteristics	Villages 149/6R	111/6R	438/6R
Villages			
Location on Hakra 6R	Head	Middle	Tail
Population	900	1530	970
Total number of households	128	206	130
Number of bedrooms selected[a]	21	25	24
Total bedrooms sampled	426	423	426
Total animal sheds sampled	35	37	39
Total vegetation sites sampled	104	108	106
Bedrooms			
Area (%)			
1–19 m^2	81.0	52	50
20–39 m^2	19.0	48	42
>40 m^2	0	0	8
Overall construction[b]			
'Good'	33.0	60	29
'Medium'	57.0	32	54
'Poor'	10.0	8	17
Wall (%)			
Bricks	14.0	28	4
Cement	38.0	60	17
Mud	48.0	12	67
Other	0	0	12
Ceiling (%)			
Bricks	48.0	36	46
Straw	43.0	64	46
Other	9.0	0	8
Open eaves (%)	5.0	24	17
Screened windows[c] (%)	24.0	16	17
Electricity (%)	95.0	100	0
Ceiling fan (%)	52.0	76	0
Animals in compound overnight (%)	90.0	48	29
Animal shed in compound (%)	81.0	24	8
Cooking within bedroom (%)	95.0	96	4

[a]Twenty bedrooms per village were selected at the start of the study. However, during the course of the study, new bedrooms had to be selected to replace houses which were abandoned as well as a few households that withdrew from the study.
[b]Overall construction of each bedroom was rated as 'poor', 'medium' or 'good' based on a qualitative assessment made by our research team at the start of the study. This classification took into account construction materials, condition of walls and ceilings, overall bedroom appearance, etc. For example, bedrooms rated as 'poor' mostly had mud walls with numerous cracks, open eaves and poorly closing doors and shutters. In contrast, 'good' rooms were those having cement walls with no cracks, closed eaves, well-fitting doors and shutters.
[c]Percentage excludes bedrooms with no windows.

(Fig. 2). Most mosquitoes were collected in the August–October period, which corresponded to a period with no rainfall, high potential evaporation rates (150–300 mm/month) and with minimum and maximum temperatures of 25 °C to 35 °C, respectively (Herrel *et al.*, 2001). Populations of the three main species began to build up from June onwards, with *An. stephensi* densities peaking in August (77.5 females per bedroom), *An. culicifacies* in September (3.7 females per bedroom) and *An. subpictus* in October (116.4 females per bedroom) (Fig. 1). As observed by previous researchers in the Punjab (e.g. Ansari & Nasir, 1955), *An. subpictus* adults disappeared from January to July and began to reappear from August onwards.

For the three main species, we noted that females remained gonotrophically active all year-round with unfed, bloodfed, semigravid and gravid females collected in each month (data not shown).

Malaria prevalence surveys

Results of our active case detection surveys showed that malaria transmission remained low throughout the year with only 2.4% of slides testing positive for malaria parasites (Table 3). Cases occurred in all villages and throughout the study period. With such a low number of cases it was

Table 2. Abundance of female anophelines collected from bedrooms[a], animal sheds[b] and vegetation[b] in the study villages: Average per sample and total collected

	Number of samples[c]	Species									
		An. culicifacies		*An. stephensi*		*An. subpictus*		*An. pulcherrimus*		*An. peditaeniatus*	
		Average	Total	Average	Total	Average	Total	Average	Total	Average	Total
Village 149/6R											
Bedrooms	426	1.0	434	19.2	8175	5.8	2475	0.3	143	0	10
Animal sheds	35	2.6	90	53.6	1878	6.2	218	0.1	5	0	0
Vegetation	104	0	3	1.2	128	0	1	1.2	122	0	4
Village 111/6R											
Bedrooms	423	0	9	2.3	986	3.8	1617	0	4	0	0
Animal sheds	37	0.3	11	17.5	647	28.8	1066	0	0	0	0
Vegetation	108	0	0	0	2	0.75	81	0	0	0	1
Village 438/6R											
Bedrooms	426	0	21	0.6	239	19.5	8304	0	4	0	0
Animal sheds	39	0.4	17	0.8	32	63.4	2471	0	0	0	0
Vegetation	106	0	0	0	0	0	0	0	1	0	0

[a]Collection method: pyrethroid spraycatch.
[b]Collection method: backpack aspirator catch.
[c] 'sample' refers to a spraycatch in a bedroom or a 15-min aspirator collection from an animal shed or vegetation.

neither meaningful to compare prevalences between villages nor to discuss seasonal aspects of transmission. Surveillance data available from government health facilities indicated a considerably lower figure; the official reported slide positivity rate (SPR) for 1999 was 0.4% for the district as a whole (Table 4). Overall, as compared with neighbouring districts, malaria transmission in Bahawalnagar district seemed to be much lower (Table 4). However, there was a clear downward trend for the period 1997–1999, with both SPR and annual parasite incidence (API) halving in the two adjoining districts. National malaria statistics show a long-term downward trend indicating that from 1973 to 1994, SPR has dropped from 14.1% to 3.9% and API from 13.8% to 0.8% (Munir *et al.*, 1994). Although the *P. falciparum* : *P. vivax* ratio has more than tripled in Bahawalnagar district over the past 3 years (Table 4) and is rising in the Punjab as a whole, *P. vivax* still accounts for most malaria cases. This is confirmed by our data, as all cases were due to *P. vivax* infection.

Health systems infrastructure

In Pakistan, passive case detection (PCD) activities are the responsibility of Basic Health Units (BHUs). Of 11 such primary health care units visited in our study area, only three had a medical doctor assigned to them, indicating severe understaffing. Although most BHU registers indicated that bloodfilms were taken from clinical malaria cases, none reported any slide-confirmed malaria cases for the period 1997–1999. As microscopists were not present at BHU level, bloodfilms were routinely sent to other government facilities for examination. According to the official procedure, slide results are sent back to the BHU. In practice, results do not seem to be fed back or, if they are, this is often too late to be of any therapeutic use for the management of the patient. When they do not receive a result, staff at the BHU assume a negative slide. Chloroquine tablets were available at all BHUs in the study area.

Communicable Disease Control Supervisors (CDCS) were responsible for ACD activities, which include taking bloodfilms from suspected malaria patients and administering presumptive treatment with chloroquine. Slides were sent for examination but, in practice, they did not come back with results. More than half of all the CDCS positions in the Haroonabad area were vacant.

Apart from government health facilities, many other health providers operated in the area such as: traditional herbal practitioners (*hakims*), religious practitioners (*maulana* or *pirs*), homeopaths (some government-licensed), western-style medical doctors and pharmacies/drugstores. Our survey of pharmacies in the Hakra 6R area (data not shown) revealed that numerous anti-malarials (amodiaquine, artemether, chloroquine, mefloquine, sulphadoxine-pyrimethamine) were freely available over the counter without a prescription. Previous researchers have shown that a majority of malaria patients (87%) seek treatment at private health facilities; reasons given for this choice were the more effective antimalarials available at private clinics and the poor service and lack of drugs at government-operated facilities (Donnelly *et al.*, 1997b). These observations support the hypothesis that government health facilities receive a very small proportion of malaria patients, as they resort primarily to private practitioners for care including self-medication from pharmacies.

Discussion

In the Punjab, where irrigation is practised on a large scale, it is generally believed that irrigation structures offer ideal

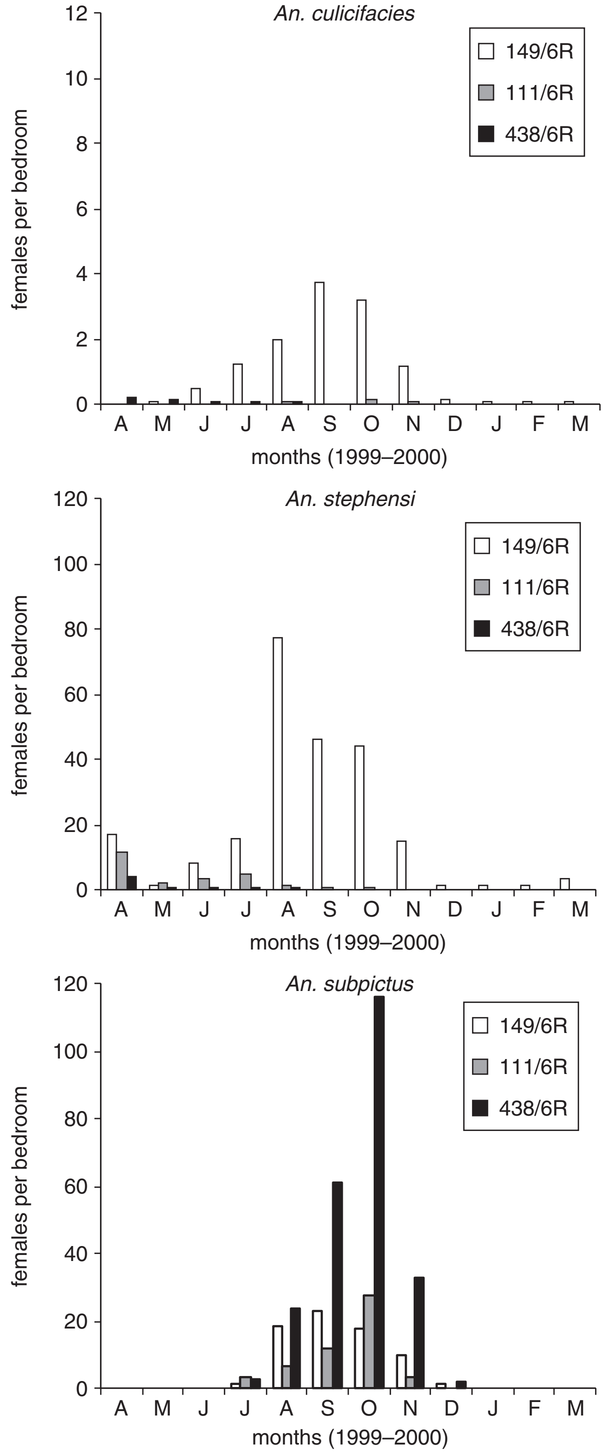

Fig. 1. Indoor-resting mosquito abundance as determined by spray-catches from selected bedrooms in study villages.

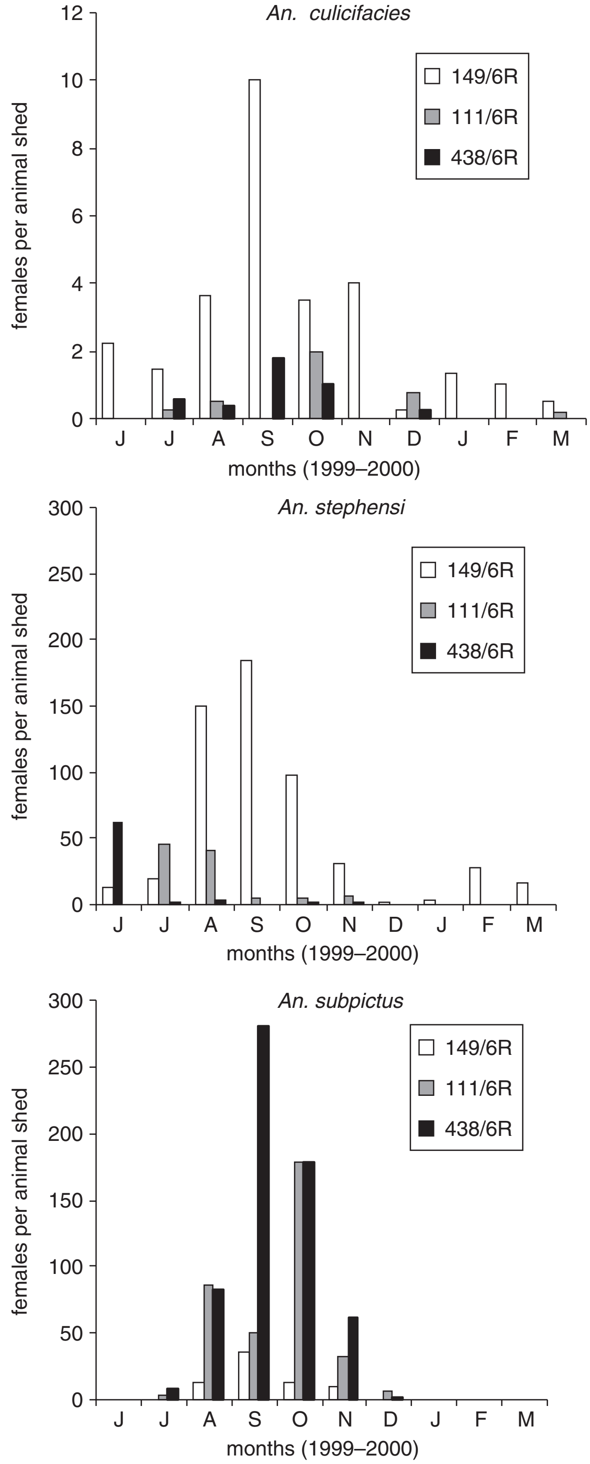

Fig. 2. Mosquito abundance as determined by backpack aspirator collections from selected animal sheds in study villages.

habitats for the proliferation of anophelines, including vectors of malaria. In South Punjab where rainfall is low, it appears that most breeding sites are, in one way or another, linked to the irrigation system. Because in such arid areas anophelines appear to be restricted to irrigation-related sites, there could be interesting opportunities to control mosquitoes through water management. The recent introduction of irrigation just across the border from our

Table 3. Active malaria case detection surveys in study villages

Months (1999)	Number of positive slides 149/6R	111/6R	438/6R	Total slides	Overall SPR[b]
April	0	1	0	50	2.0
May	0	0	1[a]	58	1.7
June	0	1	0	44	2.3
July	0	0	0	37	0
August	0	0	1[a]	46	2.2
September	0	1	0	38	2.6
October	1	0	0	72	1.4
November	2	0	1[a]	29	10.3
Total	3	3	3	374	2.4[c]

[a]All three malaria-positive slides from village 438/6R were from the same individual who, despite treatment, was found to be infected with *P. vivax* in three separate surveys.
[b] Slide Positivity Rate (%) = (number of positive slides) × 100/(total slides collected).
[c]All cases were due to *P. vivax* infections. Age range: 2–50 years; 7 males; 2 females.

study area (in Rajasthan, India) has worsened the malaria scenario in that area (Tyagi & Chaudhary, 1997; Tyagi, 1998, 2002). This prompted us to look into the situation for the Pakistani Punjab, where surface irrigation has been in practice since the late 1800s but where the possible linkages with malaria have not been studied. Our study area falls within the area historically described as prone to severe epidemics (Gill, 1921).

The anopheline fauna on which we report is consistent with previous findings for Pakistan (Aslamkhan, 1971; Reisen & Milby, 1986; Glick, 1992). As reported by other authors, we found that *An. culicifacies*, *An. stephensi* and *An. subpictus* were endophilic in their resting habits, whereas *An. pulcherrimus* was slightly more exophilic (Reisen, 1978; Reisen & Milby, 1986). As in the present study, *An. stephensi* and *An. culicifacies* females are apparently gonotrophically active all year round (Reisen *et al.*, 1986). In terms of overall relative abundance our results agree well with what has previously been reported for the Punjab, as *An. culicifacies*, *An. stephensi* and *An. subpictus* tend to dominate collections (Ansari & Nasir, 1955; Reisen, 1978; Mahmood *et al.*, 1984; Reisen & Milby, 1986).

As observed elsewhere in the Punjab (Reisen & Milby, 1986), temperature extremes markedly affected the seasonal abundance of all species in our study villages as densities of both larvae (Herrel *et al.*, 2001) and adults decreased in the hot summer season (April–June) and the colder winter season (December–March). Unlike the bimodal pattern of abundance typically reported for *An. culicifacies* and *An. stephensi* (Ansari & Nasir, 1955; Reisen, 1978; Reisen & Milby, 1986), we only observed one, clear peak in the August to October period. The typical early peak in spring (March–April) was absent, most probably due to the high temperatures combined with low relative humidity. These seasonal trends in adult abundance follow the patterns of larval occurrence as only 14–16% of samples were positive for anopheline larvae in the March–April period in contrast with 42–51% for the August–October season (Herrel *et al.*, 2001).

Our data on *An. subpictus* adults agree well with those of previous researchers, who have described this species as unimodal-monsoon and remark that adults disappear from January to June (Ansari & Nasir, 1955; Reisen, 1978; Reisen *et al.*, 1982; Mahmood & Macdonald, 1985; Reisen & Milby, 1986). Because both adults and larvae are absent in this period, it is generally believed that *An. subpictus* becomes locally extinct and is then annually reintroduced during the monsoon (Ansari & Nasir, 1955; Reisen, 1978; Reisen *et al.*, 1982). However, we found larvae throughout the year, which appears to indicate that the species is, in fact, able to overwinter in our study area (Herrel *et al.*, 2001).

We found that transmission of *P. vivax* malaria occurred throughout the period April–November at a low intensity,

Table 4. Malaria surveillance data for the three districts within Bahawalpur Division, South Punjab: Slide Positivity Rate (SPR), Percentage *P. falciparum* cases (%Pf) and Annual Parasite Incidence (API)[a]

District	SPR (%Pf) 1997	SPR (%Pf) 1998	SPR (%Pf) 1999	API 1997	API 1998	API 1999
Bahawalnagar	0.3 (8.6)	0.4 (21.5)	0.4 (28.7)	0.1	0.2	0.2
Rahim Yar Khan	3.6 (24.0)	1.8 (29.9)	1.2 (30.1)	1.9	0.9	0.6
Bahawalpur	4.2 (35.3)	2.8 (36.1)	2.4 (14.9)	1.0	0.8	0.4

[a] Data supplied by the District Health Office Bahawalnagar.

in different age groups and across the three study villages. However, the overall number of cases was low and our surveys were compromised by the fact that we did not distinguish between relapses and new infections. Although the *P. falciparum* : *P. vivax* ratio is increasing for the Punjab as a whole (WHO, 1996; Shah *et al.*, 1997) and in our district, *P. vivax* is still the main species in our area. The level of malaria transmission we recorded agrees with Birley (1990), who observed that in South Punjab, below the 254 mm rainfall isohyet (which includes our study area), malaria transmission is much reduced. We did not observe the classic seasonal pattern of malaria transmission usually experienced in the Punjab, i.e. peak transmission during the monsoon season, August to mid-October and a secondary increase occasionally during late spring, April to mid-May). This transmission pattern is usually paralleled by similar seasonal abundance patterns of the primary vector species, *An. culicifacies* (Reisen & Mahmood, 1979).

The apparently low level of malaria transmission that we observed in this area could be due to mosquito-related factors such as: (1) relatively low densities of the primary malaria vector; (2) reduced breeding sites due to dry climatic conditions; and (3) reduced vector longevity due to low relative humidity. On the other hand, factors related to the human population could also be relevant, such as levels of acquired immunity and levels of self-protection including self medication.

It is likely that one reason for the low prevalence of malaria in our study area is simply the low densities we recorded for *An. culicifacies*, the primary malaria vector in rural Punjab (Reisen & Boreham, 1982; Mahmood *et al.*, 1984; Reisen *et al.*, 1986; Pervez & Shah, 1988). Of the five sibling species known to comprise the *An. culicifacies* complex, species A and B both occur in rural Punjab, with the field and laboratory evidence pointing to species A as the most likely primary vector of malaria (Mahmood *et al.*, 1984; Mahmood & Macdonald, 1985; Joshi *et al.*, 1988). We do not know which sibling species of *An. culicifacies* are present in our study area.

Of the three ecological variants of *An. stephensi* (Sweet & Rao, 1937; Subbarao *et al.*, 1987), the urban 'type' form is an efficient vector, whereas the rural 'mysorensis' form is a poor vector (Subbarao *et al.*, 1987; Subbarao *et al.*, 1988). The role of these different ecological variants in the epidemiology of malaria in Pakistan has not been explored and there remains considerable debate over the importance of *An. stephensi* in rural areas. The species has been implicated in urban transmission within Karachi city (Rahman & Muttalib, 1967) and its vector status there has been linked to chromosomal polymorphisms, which influence behaviour (Nalin *et al.*, 1985). In rural Pakistan it has traditionally been considered a poor vector (Reisen & Mahmood, 1980; Mahmood *et al.*, 1984; Mahmood & Macdonald, 1985; Subbarao *et al.*, 1988). However, Pervez & Shah (1988) have detected Plasmodia in rural *An. stephensi* and argue that it is also an important vector. More recent evidence from rice-growing areas in the Northern Punjab indicate peak malaria transmission in October when *An. stephensi* was abundant yet *An. culicifacies* had almost entirely disappeared (Rowland *et al.*, 2000). In other parts of Pakistan such as the North-west Frontier Province, *An. stephensi* is also the predominant species, with sporozoite rates similar to *An. culicifacies* (Rowland *et al.*, 1997). In a more recently constructed irrigation system in Rajasthan, just across the border from the Pakistani Punjab, *An. stephensi* and *An. culicifacies* are primary vectors (Tyagi & Chaudhary, 1997). *Anopheles stephensi* s.l. was abundant in our collections. However, we are not aware which ecological variant(s) are present in our study area and we are not able to conclude what importance this species may have had in malaria transmission as we lack data on survivorship, parous rates, sporozoite rates, etc. Other than *An. culicifacies* and *An. stephensi*, it is generally accepted that there are no other important vectors of malaria in the Punjab (Reisen & Boreham, 1979).

Anophelines in Pakistan are notoriously zoophilic, making it difficult to interpret the role of different vectors in the ecology of malaria (Reisen & Mahmood, 1979). Investigations in the Punjab showed overwhelming preference of mosquitoes for bovine hosts (Reisen & Boreham, 1976, 1982; Reisen & Mahmood, 1979; Mahmood & Macdonald, 1985). Overall, however, *An. culicifacies* has the greatest host range among the anophelines (Reisen & Boreham, 1976) and, despite its low anthropophily, it is the species most often caught in human landing catches and the species most often found positive for human blood (up to 3%) (Reisen & Boreham, 1982; Mahmood & Macdonald, 1985).

The harsh climatic conditions in our study area certainly influenced our results. Excessive rainfall during the summer monsoon, and related higher relative humidity, have been recognized as the most important factors in the genesis of epidemics in the plains of the Punjab (Christophers, 1911; Gill, 1923; Yacob & Swaroop, 1945, 1946; Bouma & van der Kaay, 1996). These conditions were absent in our study area and this probably partly explains the apparently low level of malaria transmission. The year of our study was marked by a severe drought, which afflicted the entire region of Pakistan, Afghanistan and India. This resulted in higher than normal temperatures, low relative humidity and very little rainfall. Previous authors have commented that, under hot conditions (especially in the pre-monsoon season), most breeding sites will dry up and breeding is restricted to permanent drains and canals until the advent of monsoon rains in June or July (Mahmood & Macdonald, 1985). Due to low rainfall, vector breeding in South Punjab is confined to irrigation structures (de Zulueta *et al.*, 1980). Mosquito breeding may therefore have been limited to the irrigation system and irrigation-related sites because of the low availability of other sites. In addition, the extremely dry conditions probably shortened the lifespan of the vectors, thus reducing vectorial capacity. Mosquito survivorship of 7 and 8 days is required for the transmission of *P. vivax* and *P. falciparum*, respectively (at temperatures of $28 \pm 2\,^{\circ}C$ and relative humidity of $70 \pm 10\%$), and these were probably not achieved in our study area.

Although malaria is classically considered to be unstable in the Punjab, some early publications mention areas where malaria was endemic and stable (Macdonald & Majid, 1931; Schüffner, 1931; Hicks & Majid, 1937; Strickland *et al.*, 1987). These sites were characterized by proximity to a body of water or irrigated land which increased anopheline breeding sites. A series of reports from the Kasur district of Punjab (about 60 km south of Lahore) provide evidence for locally endemic and stable malaria transmission in villages close to a drain where *An. culicifacies* breeding occurred (Strickland *et al.*, 1987). Children had higher parasite prevalence rates and malaria-positive adults were oligo- or asymptomatic. These findings were attributed to high levels of acquired immunity (Hadi *et al.*, 1985; Khaliq *et al.*, 1985; Zafar-Latif *et al.*, 1985; Strickland *et al.*, 1987, 1988). Strickland *et al.* (1987) hypothesized that because many villages in the Punjab are close to perennial sources of water suitable for *An. culicifacies* breeding, the stable pattern of malaria transmission they observed could be true for other areas as well. For our specific area, the low vector densities together with the low number of cases reported through the official health system and our own surveys, indicate a low level of malaria transmission. Such low transmission levels would not be sufficient to induce the development of immunity and it is therefore unlikely that the low number of cases we recorded was due to a high proportion of asymptomatic (semi-immune) individuals.

The intensity of malaria transmission in a community is also related to human behavioural factors. In our study area, exposure of persons to mosquito bites was probably high because: (1) the overwhelming majority of villagers slept outdoors due to the hot and dry climate conditions during the period August–October; (2) very few families reported using any type of mosquito control method and, in particular, no-one owned a bednet. Discussions with health officials throughout the Punjab and our own observations show that this is typical for the rest of the Province too.

It seems likely that a substantial proportion of human malaria cases are being missed by the government surveillance system. Our data only capture the malaria transmission seasons for one year and our observations are limited to three villages. However, if these data are indeed representative of the situation for the entire district, then actual malaria prevalence is five times higher than the official statistics. This proportion of missed cases corresponds to previous estimates of under-reporting in the Punjab (Donnelly *et al.*, 1997b). Studies on treatment-seeking behaviour have shown that the majority of malaria patients do not resort to government health facilities and instead opt for care in the private sector (Donnelly *et al.*, 1997a,b). Our observations in the field confirm that there are numerous private practitioners who operate in addition to the government facilities. Furthermore, a substantial proportion of *P. falciparum*- and *P. vivax*-infected persons are asymptomatic (Hadi *et al.*, 1985; Khaliq *et al.*, 1985; Prybylski *et al.*, 1999). Because the current ACD and PCD surveillance systems are both based on symptoms, these cases would be missed (Hadi *et al.*, 1985; Khaliq *et al.*, 1985; Fox *et al.*, 1987).

In our study area of South Punjab where rainfall was extremely low in the year under study we provide evidence for the breeding of many anopheline species including *An. culicifacies*, the primary vector of malaria in Pakistan, and the suspected additional vector *An. stephensi*. Our larval studies have shown that irrigation-related sites such as waterlogged and irrigated fields, fishponds and drinking water tanks constitute important breeding sites for the main vector species. Densities of the known malaria vector remained low and we also recorded few human malaria cases. It is plausible that with the return of regular climate patterns (greater rainfall, higher humidity) breeding opportunities would be greater and mosquito populations would increase. Malaria might also then be a more severe public health problem than at present. The situation is particularly worrying in view of the severe staff and funding shortages which hamper the National Malaria Control Program. Only limited vector control activities are carried out and the surveillance system is poor.

Based on one year's data for three villages only, it is difficult to extrapolate our results to the whole Punjab, as it is well known that malaria transmission fluctuates widely from year to year and from one district to the next. However the villages we studied are quite typical for the Punjab and, although malaria transmission remained low, we found important differences in the anopheline species present in the villages depending on their ecological characteristics. Of particular importance was the finding that *An. culicifacies* was restricted to the head village. Follow-up studies should assess to what extent irrigation and associated problems such as waterlogging, salinization and rising groundwater tables are having an impact on malaria transmission. Further studies should aim to compare irrigated vs. non-irrigated areas to assess how relevant irrigation is in the epidemiology of malaria in Pakistan.

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