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Malaria vectors in the changing environment of the southern Punjab, Pakistan

Eveline Klinkenberg[a,b,*], **Flemming Konradsen**[b,c], **Nathaly Herrel**[a], **Muhammad Mukhtar**[a], **Wim van der Hoek**[b], **Felix P. Amerasinghe**[b]

[a] *International Water Management Institute, 12 Km. Multan Road. Thokar Niaz Baig, Lahore, Pakistan*
[b] *International Water Management Institute, P.O. Box 2075, Colombo, Sri Lanka*
[c] *Department of International Health, Institute of Public Health, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark*

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Summary The Pakistani Punjab experienced several devastating malaria epidemics during the twentieth century. Since the 1980s, however, malaria has been at a low ebb, while in other areas of Pakistan and neighbouring India malaria is on the increase. This raises the question of whether transmission in the Pakistani Punjab may have been influenced by a change in vector species abundance or composition, possibly induced by environmental changes. To investigate this question, routinely-collected government entomological data for the period 1970 to 1999 for the district of Bahawalnagar, in the Indus Basin irrigation system in the southern Punjab, was analysed. Our findings suggest that *Anopheles stephensi* has increased in prevalence and became more common than *A. culicifacies* during the 1980s. This shift in species dominance may be due to the large-scale ecological changes that have taken place in the Punjab, where irrigation-induced waterlogging of soil with related salinization has created an environment favourable for the more salt-tolerant *A. stephensi*. Some biotypes of *A. stephensi* are suspected of being less efficient vectors and, therefore, the shift in species dominance might have played a role in the reduced transmission in the Punjab, although further research is needed to investigate the effect of other transmission-influencing factors.

1. Introduction

Historically, the Punjab was one of the most malarious areas in South Asia and the scene of some of the worst epidemics on record, the most devastating occurring in 1908 with over 300 000 deaths in an estimated population of 20 million (Zulueta et al., 1980). In 1947 the Punjab was divided between the new states of Pakistan and India. Pakistan launched a nationwide eradication campaign under the auspices of the WHO in 1961. Malaria was reduced from

*Corresponding author. Present address: IWMI-Ghana, PMB CT 112, Cantonments, Accra, Ghana. Tel.: +233-21-784753; fax: +233-21-784752.
E-mail addresses: e.klinkenberg@cgiar.org (E. Klinkenberg), f.konradsen@pubhealth.ku.dk (F. Konradsen), n_herrel@hotmail.com (N. Herrel), m.mukhtar@cgiar.org (M. Mukhtar), w.van-der-hoek@cgiar.org (W. van der Hoek), f.amerasinghe@cgiar.org (F.P. Amerasinghe).

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7 million cases in 1961 to 9500 in 1967 and the percentage of blood slides positive for malaria (slide positivity rate, SPR) dropped from 15% to less than 0.01% (Zulueta et al., 1980). There was a resurgence of disease after 1967 and an epidemic occurred in 1972—73 with an SPR of 20% for the Punjab province in both years (Zulueta et al., 1980). The increase in malaria was attributed to a combination of factors, including administrative and budgetary constraints within the government health services, the development of pesticide resistance within the vector population and the spread of malaria to urban areas (Zulueta et al., 1980). A five year Malaria Control Program based on large-scale indoor residual spraying was launched in 1975, but ran into operational problems related to insecticide distribution and by 1979, spraying was focused on high transmission areas identified through active case detection surveys (Reisen, 1986). In more recent years no large-scale vector control activities have been undertaken in the Pakistani Punjab.

In an analysis of national malaria statistics Munir et al. (1994) showed a long-term downward trend indicating that from 1973 to 1994, SPR for the whole of Pakistan dropped from 14.1% to 3.9% and annual parasite incidence from 13.8 to 0.8 per 1000 population. However, it is possible that this decline may also have been influenced by the inclusion of more urban inhabitants in recent years, exaggerating the sharp downward trend. Rowland et al. (2002a) stated that, based upon an estimation of annual SPR at district level over time, in the 1980s and 1990s malaria in the Punjab remained at approximately the same level with an SPR around 2%, while there was a gradual increase in the other provinces. The low malaria incidence in the Punjab cannot be fully attributed to an effective control system as recent years have seen reductions in funds allocated for malaria control activities in the Punjab and the development of insecticide resistance in mosquito vector species. Currently, malaria control mainly consists of treatment of patients largely by the private health sector (Donnelly et al., 1997; Rowland et al., 2002a).

Over the twentieth century, the Punjab underwent major changes in land use through the development of the Indus Basin Irrigation System, currently the world's largest contiguous irrigated area. The irrigation-induced rise in groundwater table, waterlogging, and accumulation of salts in the soil have become major environmental problems. By the late 1950s, 25% of the then 9 million hectares of agricultural land in the Punjab was affected by waterlogging and salinity (Ahmad and Chaudry, 1988). In the 1960s the Salinity Control and Reclamation Projects (SCARP) started to reclaim land by installing tubewells in high water table areas to decrease the groundwater table and where possible to use the pumped water to increase agricultural production. In the 1990s the World Bank estimated that despite the SCARP activity 22% of the total area was still waterlogged (water table within ∼150 cm of the surface during at least part of the year) and 23% was salt affected (salinity levels sufficiently high to impede plant growth) (World Bank, 1992).

We were interested in assessing if there had been a change in malaria vector species abundance or composition over the years, possibly due to the irrigation-related environmental changes that took place in the Punjab. Two species are known vectors for malaria in Pakistan. *Anopheles culicifacies* is considered to be the main vector in rural Pakistan while *A. stephensi* is considered to be a secondary vector in rural areas and at least partly responsible for malaria transmission in urban areas (Carmichael, 1972; Reisen and Boreham, 1979, 1982). This paper aims to assess vector species abundance and composition from 1970 to 1999 by analysing entomological surveillance data for Bahawalnagar district in the Indus Basin Irrigation System in the southern Punjab, Pakistan. The paper then discusses the entomological changes that have taken place in relation to the environmental modifications of the area and the levels of malaria transmission in the district under study.

2. Materials and methods

2.1. Study area

Bahawalnagar district is located between 20°51′ to 30°22′ N and 72°17′ to 73°58′ E. The district covers an area of 8878 km^2 with a population of 2 million in 1998. The district is subdivided into five administrative units called *tehsils*. The region has an arid climate with large temperature differences between summer and winter. Meteorological data for the district over the period 1970 to 1995 show an average minimum temperature ranging from 3.0 °C in December—January to 31.0 °C in May—June, an average maximum temperature ranging from 19.2 °C in December—January to 44.4 °C in May—June, an average monthly relative humidity ranging from 17% to 77%, and an average annual rainfall of 196 mm (Pakistan Meteorological Department, unpublished data). Seventy percent of the district is covered with irrigated land on which cotton and wheat are the main crops.

2.2. Data collection

Data on groundwater and salinity were collected from the SCARP Monitoring Organization and the International Waterlogging and Salinity Research Institute in Lahore, Pakistan. Salinity data (Electrical conductivity [EC] values) are average values from piezometers installed throughout the Bahawalnagar district. Ground water data are recorded as percentage of the district surface area that has a water table of 150 cm or less from the soil surface.

Malaria surveillance in the government system was based on active case detection and passive case detection. Active case detection was carried out by Communicable Disease Control (CDC) officers, who were each assigned to about four villages. They were instructed to visit their assigned villages on at least a monthly basis and carry out house-to-house visits and take blood slides of every patient with fever or a history of fever. Blood slides were sent to the nearest microscopy facility and a single dose of presumptive treatment was given to each patient based only on clinical symptoms. A review of the results of blood film examinations available for the Bahawalnagar district for the period 1980 to 1999, based upon both active and passive case detection, indicates a very low SPR (Figure 1, Department of Health Bahawalnagar District, unpublished data) but no trend in the number of cases could be established over time. Data could not be obtained from before 1980. The annual SPR ranged from 0.02% to 2.45% with only one year above 2%. With the exception of one year, the SPR was well below the SPR for all of Pakistan for the period 1980 to 1994 (Munir et al., 1994).

Entomological data were collected from existing data sheets available at the District Health Office in Bahawalnagar. Information on changes in methods of collection, collection effort, spraying activities, staff turnover etc. that had taken place over time, was obtained through key informant interviews with knowledgeable entomologists who had served in the district, most of them now retired from government service. Data were available for the period 1970—1999 although some years and months were missing. No data could be obtained for the period before 1970. The district had an entomological team that visited villages and collected mosquitoes from houses and animal sheds. The selection of villages for entomological surveys was based on the SPR as calculated from passive and active case detection methods. Collections were done in villages where malaria control was carried out by residual insecticide spraying as well as in villages where no spraying took place. Collections were made from living rooms, which were generally the rooms where people slept, storerooms and animal sheds. Animal sheds were generally part of the house where animals were kept overnight. It was only after 1985 that the type of rooms was specified on the forms and, therefore, all rooms were analysed together. In more than 95% of the cases, the collection method was the pyrethroid space spray catch method. Hand captures were only carried out for susceptibility tests but this was not always clearly marked on the data sheets and, therefore, all surveys were analysed together. Pyrethroid spray collections took place according to a national protocol. This included room-spraying after closing of all windows and doors and removal of food items. Fifteen minutes after spraying, the mosquitoes were collected from white sheets laid out in the room. Identification of mosquitoes was done in the field and specimens brought back to

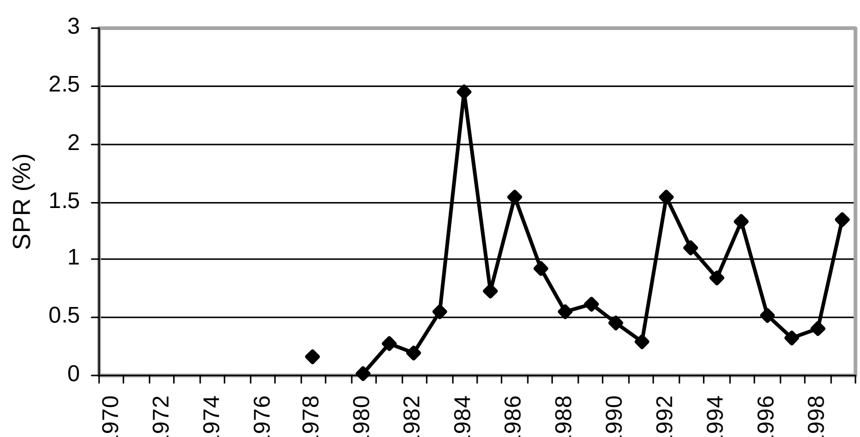

Figure 1 Slide positivity rate (SPR) for the District of Bahawalnagar, Pakistan, as recorded by the District Health authorities. Data include passively (clinics) and actively (surveys) detected cases. Data from before 1978 could not be obtained.

the District Health Office for labelling and preservation for a period of one year, after which the collections were discarded.

2.3. Data analysis

For the present paper, two measures were used in the analysis: the percentage of rooms checked that were positive for a certain mosquito species, and the total number of female mosquitoes of a species that were collected divided by the total number of rooms positive for that species (the female positive room density). The outcome of interest was the change over time in abundance of *A. stephensi* relative to *A. culicifacies*. A measure of this is the percentage of rooms positive for *A. stephensi* minus the percentage of rooms positive for *A. culicifacies*. This parameter was calculated from annual values for each *tehsil*. The relative importance of temporal or spatial variation on the species composition was tested with analysis of variance using a general linear model. In the model, the difference between the percentage of rooms positive for *A. stephensi* and *A. culicifacies* was used as the dependent variable, calendar year as the covariate and *tehsil* as the fixed factor.

3. Results

In the Bahawalnagar district, the groundwater levels have been rising from the early 1960s and the available data indicate that periodic floods have aggravated the problem. The areas affected by severe waterlogging in the Bahawalnagar district have fluctuated over the study period but waterlogging has remained a very serious problem with up to 64% of the area having a depth to groundwater of 150 cm or less (Table 1). Measurements of average salinity of the groundwater indicate an increased level from the early 1970s with especially high concentrations for the period from the late 1970s to the mid- 1980s (Table 1).

The entomological data set consisted of a total of 3200 records of surveys from 1160 different localities over the five *tehsils* of the district for the period 1970—1999. A total of 15 717 rooms were surveyed for mosquitoes, in which 41 150 *A. culicifacies* and 97 580 *A. stephensi* were collected. On average 629 (range 10 to 1573) rooms and 135 (range 2 to 260) localities were surveyed each year. On average 3903 (range 0 to 28 312) *A. culicifacies* and 5589 (range 9 to 34 839) *A. stephensi* were collected each year. Unfortunately, data from before 1970 could not be retrieved because many records were damaged (apparently by termites). Data for the epidemic year 1972 was missing. Missing data could also not be found at the Provincial Health Office in Lahore.

Table 1 Percentage of area of Bahawalnagar district with depth to groundwater of 150 cm or less and average salinity value of groundwater from 1970 to 1999

Year	GWT% <150 cm	EC (ds/m)
1970	50.6	2.08
1971	55.1	1.97
1972	62.1	1.01
1973	64.6	1.28
1974	58.8	2.65
1975	49.3	*
1976	*	*
1977	*	*
1978	47.2	4.03
1979	40.7	3.65
1980	*	4.56
1981	47.3	4.59
1982	47.9	4.98
1983	58.4	4.88
1984	49.5	4.99
1985	39.3	3.98
1986	46.3	4.00
1987	36.9	3.25
1988	42.1	3.77
1989	49.1	3.99
1990	46.1	3.65
1991	42.4	2.35
1992	47.8	2.10
1993	46.4	3.48
1994	36.7	3.17
1995	61.1	3.90
1996	64.0	3.88
1997	53.3	*
1998	40.9	*
1999	15.4	3.24

Salinity is measured as electrical conductivity (EC), expressed in deci-siemens per meter (dS/m). EC values are average values from piezometers installed all over Bahawalnagar district. GWT: groundwater table is the percentage of the district with a water table 150 cm or less from the soil surface. Information obtained from the SCARP Monitoring Organization, Lahore, Pakistan, and the International Waterlogging and Salinity Research Institute, Lahore, Pakistan.
* No data.

The entomological records focused on *A. culicifacies* and *A. stephensi*. The occurrence of other anophelines, notably *A. subpictus*, *A. annularis* and *A. pulcherimus* was not recorded systematically. Figure 2 presents the changes over time for *A. stephensi* in relation to *A. culicifacies* for the Bahawalnagar District, with Figure 2A showing the percentage of rooms positive for each species, and Figure 2B the average female vector density per positive room for each species. The figure shows

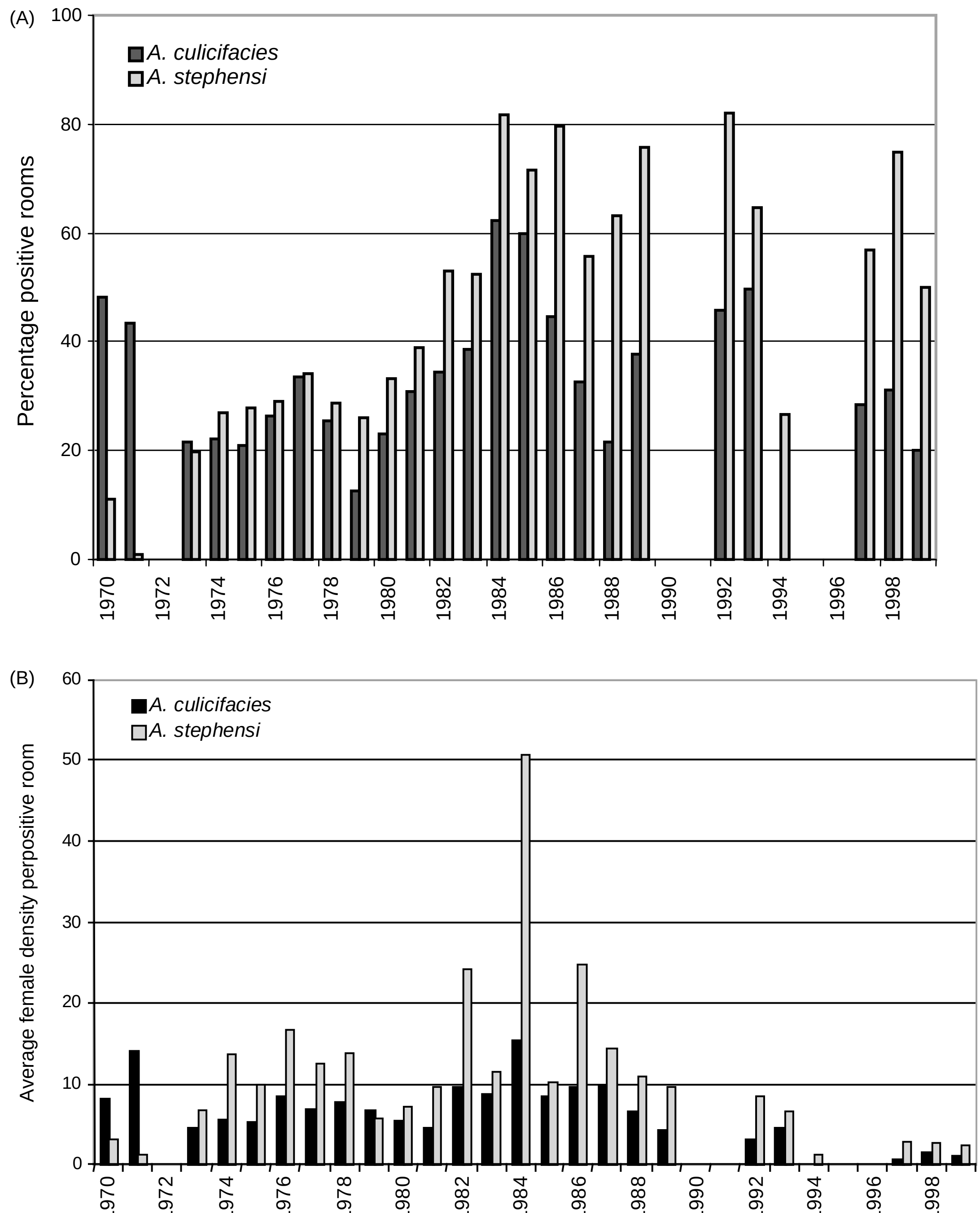

Figure 2 (A) Annual percentage of positive rooms of total number of rooms sprayed for *Anopheles stephensi* and *A. culicifacies* over time for the Bahawalnagar District, Pakistan. (B) Annual average female mosquito density per positive room for *Anopheles stephensi* and *A. culicifacies* over time for the Bahawalnagar District, Pakistan.

large year-to-year fluctuations for both indicators but suggests an increase in the abundance of *A. stephensi* relative to *A. culicifacies*. This increase in the relative abundance of *A. stephensi* seems to progress gradually from the early 1980s until the late 1990s when the percentage of rooms positive for *A. stephensi* was at least twice as high as that for *A. culicifacies*. All five *tehsils* within the Bahawalnagar district showed the same pattern of increasing relative abundance of *A. stephensi* when compared with *A. culicifacies* (data per *tehsil* not shown), although the progression differed in time between the *tehsils*.

In the general linear model analysis, both year and *tehsil* were significant ($P < 0.001$). The explained variation by year (39.9%) was larger than by *tehsil* (12.9%). The regression coefficient for the covariate 'year' had the value 2.219, indicating that with each calendar year, there was about a 2% increase in abundance of *A. stephensi* relative to *A. culicifacies*.

4. Discussion

The entomological data suggest that *A. stephensi* has gradually become more common than *A. culicifacies* in the southern Pakistani Punjab during the 1980s. This might have played a role in maintaining a low malaria incidence observed in the Punjab from 1972 onwards because *A. stephensi* is reported to be a poorer vector than *A. culicifacies* (Mahmood et al., 1984; Mahmood and Macdonald, 1985; Reisen and Mahmood, 1980).The decreased ability or in some areas, incapability, of *A. stephensi* to transmit malaria is ascribed to its shorter lifespan than *A. culicifacies* and therefore inability to live long enough to transmit the malaria parasites, especially *Plasmodium falciparum* (Mahmood et al., 1984). However, a study by Rowland et al. (2000) in the Sheikhupura district of the Punjab reinforces a concern raised earlier by Pervez and Shah (1988) that *A. stephensi* plays a more important role in transmission than previously suspected. In the North West Frontier Province, bordering Afghanistan, *A. stephensi* is clearly the main vector (Hewitt et al., 1996; Rowland et al., 1997). A recent study by Rowland et al. (2002b) confirmed that *A. stephensi* was also the most important malaria vector in Eastern Afghanistan.

Anopheles culicifacies is a species complex with various siblings and *A. stephensi* has various biotypes for which different behaviour and vectorial capacities are reported, and this could play a role in malaria transmission. However, government data analysed in this study did not differentiate between siblings or biotypes. *Anopheles culicifacies* consists of five sibling species of which siblings A and B can be found in the Punjab (Mahmood et al., 1984). Both sibling species are capable of malaria transmission in the laboratory although *A. culicifacies* species A is considered the primary malaria vector under field conditions (Mahmood et al., 1984; Mahmood and Macdonald, 1985). Of the ecological variants of *A. stephensi*, the urban 'type' form is an efficient vector whereas the rural 'mysorensis' form is considered a poor vector (Subbarao et al., 1987, 1988; Sweet and Rao, 1937). 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 *A. stephensi* in rural areas. Molecular research is needed to map the distribution of different sibling species and biotypes and to assess their importance in malaria transmission. This would contribute to a better understanding of the importance of the shift in vector composition that has taken place in the southern Punjab.

The most obvious large-scale environmental change that has taken place in the district under investigation over the past decades, as in most of the Punjab, is the rising groundwater table, with waterlogging, and salinization. How this may have influenced vector composition and malaria transmission is still largely unknown. At first one would expect that the present environment with large-scale irrigation and high water tables would become more suitable for the vectors of malaria. James (1903) when first describing an irrigated area (at Mian Mir) close to Lahore, Punjab, found a clear association between *A. culicifacies* and irrigation water supply especially in canals that have grass and weeds growing along the edges. Christophers (1911) hypothesized that groundwater that is close to the surface may result in larger areas of surface water, which may increase mosquito abundance and thereby increase malaria transmission. However, the potential for mosquito proliferation and the species of mosquito breeding is also influenced by the quality of the water. Under the climatic conditions in Punjab, waterlogging with high evaporation leads to an increase in saline content of water and soil. This would favour vector species that are relatively more salt-tolerant. The information from Bahawalnagar district indicates that the shift seen in species composition coincided with the increase in groundwater salinity.

Both *A. culicifacies* and *A. stephensi* breed in clean water and breeding sites include agricultural drains, small irrigation channels, temporary pools, pits, puddles and paddy fields (Mahmood and Macdonald, 1985). Recent research in the Bahawalnagar district (Herrel et al., 2001) also showed considerable overlap in site preference between the two vectors but the studies found a clear majority of indoor resting *A. stephensi* (41%) compared with *A. culicifacies* (2%). Data from the literature therefore do not give a clear indication as to how environmental changes could have affected the relative abundance of *A. culicifacies* and *A. stephensi*. In neighbouring Rajasthan, India, *A. stephensi* is considered to be the vector in desert areas, maintaining a very low prevalence of malaria. In areas where irrigation has been introduced, such as along the Indira Ghandi Canal, *A. culicifacies* has become more abundant and replaced *A. stephensi*.

The introduction of *A. culicifacies* in desert areas due to irrigation development has been mentioned as the main cause of recent epidemics in the Thar Desert (Tyagi, 2002; Tyagi and Chaudhary, 1997; Tyagi and Verma, 1991). On the Pakistan side of the border, the irrigation systems are much older and decades of irrigation with excess water and poor drainage have led to a rise of the groundwater table, waterlogging, and ensuing salinization. There is, at present, no proof that the long-term changing malaria pattern in the southern Punjab is related to these environmental changes. One hypothesis could be that salinization has created more favourable conditions for the relatively more salt-tolerant *A. stephensi* (Reisen et al., 1981) which has allowed it to become more prevalent than *A. culicifacies* during the period 1970—1999.

Large-scale reclamation projects are being implemented to reverse the process of waterlogging and salinization, mainly by constructing large numbers of tubewells that pump groundwater. The water extracted is either used for irrigation or discarded in drains, depending upon its salinity. More money is now spent on reclaiming waterlogged areas than on constructing new irrigation structures. If successful, these projects would be expected to also affect the vector ecology in the long term. However, this is speculative in the absence of specific studies. While the relation between climate variability and malaria transmission has been studied in detail (Bouma and van der Kaay, 1996), the role of environmental change in the changing malaria pattern of the Punjab remains obscure.

The present study relied entirely on secondary data from the government system. This has obvious limitations. Entomological teams were often fielded in response to malaria outbreaks. However, the routine entomological monitoring was to a large extent based on a number of fixed sampling sites and, after studying the records and interviewing key informants, we were of the opinion that the data were of sufficient quality to show historical trends. The routinely-collected information on human malaria from the area under investigation indicates a very low level of transmission, with an SPR well below the national average throughout the 1980s and 1990s. However, the quality of the information on human malaria did not allow for a more detailed analysis of linkages between the changes in vector composition and the influence on human malaria. No consistent pattern could be found in the way blood slides were collected. Very often the collection of blood slides by CDC officers was in response to suspected outbreaks and the number of slides collected varied greatly between months. It is well known that the majority of malaria patients in the Punjab do not resort to government health facilities and instead opt for care in the private sector (Donnelly et al., 1997). Therefore, official government statistics under-report the real incidence of malaria and this makes it difficult to relate malaria with entomological data. Health-seeking behaviour has changed over time and the malaria case records are therefore not suitable for detecting trends over time. Use of SPR has constraints, because in the 1980s there was a gradual switch from active case detection to passive case detection and passive case detection tends to produce higher SPR (Shah et al., 1997). Thus, in the present study we were not able to directly compare the entomological and parasitological trends.

We conclude that there has been a significant shift in vector species composition in the Southern Punjab over the past 30 years with an increase in the abundance of *A. stephensi* relative to *A. culicifacies*. This generates the hypothesis that this change may have occurred as a result of irrigation-induced environmental changes favouring one vector against the other. However, other influences on malaria transmission also need to be evaluated before a more definite insight can be gained into the changing patterns of malaria transmission in the Punjab and possibly other regions of Pakistan.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

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