ROLE OF WASTEWATER IRRIGATION IN MOSQUITO BREEDING IN SOUTH PUNJAB, PAKISTAN

Muhammad Mukhtar[1], Nathaly Herrel[1], Felix P Amerasinghe[2], Jeroen Ensink[1], Wim van der Hoek[2] and Flemming Konradsen[2,3]

[1]International Water Management Institute, Lahore, Pakistan; [2]International Water Management Institute, Colombo, Sri Lanka; [3]Department of International Health, University of Copenhagen, Denmark

Abstract. Mosquito breeding within the wastewater irrigation system around the town of Haroonabad in the southern Punjab, Pakistan, was studied from July to September 2000 as part of a wider study of the costs and benefits of wastewater use in agriculture. The objective of this study was to assess the vector-borne human disease risks associated with mosquito species utilizing wastewater for breeding. Mosquito larvae were collected on a fortnightly basis from components of the wastewater disposal system and irrigated sites. In total, 133 samples were collected, about equally divided between agricultural sites and the wastewater disposal system. Overall, 17.3% of the samples were positive for *Anopheles*, 12.0% for *Culex* and 15.0% for *Aedes*. Four anopheline species, viz, *Anopheles stephensi* (84.3% of total anophelines), *An. subpictus* (11.8%), *An. culicifacies* (2.0%) and *An. pulcherrimus* (0.2%) were present, as were two species of *Culex*, viz, *Cx. quinquefasciatus* (66.5% of culicines) and *Cx. tritaeniorhynchus* (20.1%). *Aedes* were not identified to species level. The occurrence of different species was linked to particular habitats and habitat characteristics such as physical water condition, chemical water quality and the presence of fauna and flora. Anophelines and *Aedes* mosquitos were mainly collected during the month of July, while *Culex* were collected in September. The prevalence of established vectors of human diseases such as *An. stephensi* (malaria), *Cx. tritaeniorhynchus* (West Nile fever, Japanese encephalitis) and *Cx. quinquefasciatus* (Bancroftian filariasis, West Nile fever) in the wastewater system indicated that such habitats could contribute to vector-borne disease risks for human communities that are dependent upon wastewater use for their livelihoods. Wastewater disposal and irrigation systems provide a perennial source of water for vector mosquitos in semi-arid countries like Pakistan. Vector mosquitos exploit these sites if alternative breeding sites with better biological, physical, and chemical conditions are not abundant.

INTRODUCTION

The use of urban wastewater in agriculture has become a widespread practice (WHO, 1989). Wastewater provides a continuous supply of water with high nutrient content. Thus, it serves as a reliable source of water and fertilizer and is one way to meet the growing demand for food under conditions of increasing water scarcity. In developing countries, including the breadbaskets of China and India, approximately 80% of urban wastewater is used for irrigation (Cooper, 1991). Unfortunately, much of this is untreated or inadequately treated. Urban wastewater consists of sewage and industrial wastes that pose biological and chemical health risks for the irrigators and communities in prolonged contact with the untreated wastewater, and for consumers of crops irrigated with wastewater.

Pakistan has a semi-arid to arid climate and is facing severe water-shortage in the context of a rapidly expanding human population. This increases the scope for wastewater irrigation, which is already practised in almost all cities. Treatment systems have been constructed in

Correspondence: Muhammad Mukhtar, International Water Management Institute (IWMI), 12 Km Multan Road, Chowk Thokar Niaz Baig, Lahore 53700, Pakistan.
Tel: +92-42-5410051/3; Fax: +92-42-5410054
E-mail: m.mukhtar@cgiar.org

some cities of Pakistan, but due to financial constraints most of them are non-functional (Aftab, 1999). Recently, periurban wastewater irrigation around the city of Haroonabad in South Punjab, Pakistan, was studied with the objective to describe the advantages and disadvantages of wastewater use in order to come to a comprehensive cost-benefit analysis. The work reported herein was a part of that project, and pertains to the potential of wastewater use systems to produce disease-transmitting mosquitos. Negative health impacts could threaten the sustainability of urban and periurban agriculture, but have not been adequately described. To the author's knowledge, there have been no previous published studies on mosquitos breeding in wastewater-irrigated sites in Pakistan.

MATERIALS AND METHODS

Study area

The study was carried out in an agricultural area irrigated with untreated urban wastewater around the city of Haroonabad in the Bahawalnagar district of Punjab Province, Pakistan. The city (population 63,000) is situated at the edge of the Cholistan Desert (72.08′E, 29.9′N) at an altitude of 90 m above mean sea level. The mean annual maximum and minimum temperatures of the area are 46±2ºC and 9±2ºC respectively and the average annual precipitation is 160 mm. The water table ranged from 1.5-2.5 m below the soil surface, but ground water was not used for irrigation due to its high salinity. The total area receiving wastewater was 127 ha. The main crops were vegetables, such as round gourds, cauliflower, brinjal (aubergine) and tomatoes. Other field crops, such as cotton, wheat, sugarcane and maize were also grown.

Wastewater system

The wastewater disposal system in Haroonabad consisted of a primary disposal site northeast of the city where wastewater was pumped to fields through field watercourses. A secondary pumping well was located at the end of one field watercourse, 1.2 km from the main pumping site. Here, a mobile pump was installed to shift water to other field watercourses, which were slightly elevated. An old disposal system that was constructed in 1960 but that had been non-functional since 1979, was located in the same area (Fig 1). The components of this system included grid chambers, sieve chamber and collecting chambers. In the original system, wastewater from Haroonabad City was channeled to the grid chamber to remove large solid wastes, then to the sieve chamber to remove finer wastes, and finally to the collecting chambers from which water was pumped onto fields for irrigation. Even at present, sometimes wastewater is pumped into the collecting chambers, particularly in the rainy season when there is excess water standing on fields and when there is also a large flow of wastewater from the city. Thus the water in the collecting chambers consisted of a mixture of ground water, rainwater and wastewater. The grid chamber, too, regularly received wastewater by surface run-off from surrounding fields, but this water was not used for agriculture because of its high salinity.

Larval collections

Mosquito larvae were collected on a fortnightly basis from July to September 2000, between 8.00 hr and midday, from 10 selected sites within the study area. This period coincided with the monsoon season, which is generally the peak breeding time for mosquitos. For larval collection, all water bodies were divided into two main categories, *ie*, (A) agricultural sites, comprising of field water courses, irrigated fields, pumping wells and drainage ponds; and (B) the old and non functional wastewater disposal system, including the grid chamber, sieve chamber and collecting chambers. Sometimes, water was not present in the field watercourse or the irrigated fields during the harvesting period, in August. The drainage pond was sampled only once because it was dry during most of the study period. The components of the old disposal system were sampled regularly. The surface of each sampled site was estimated in m^2. The samples

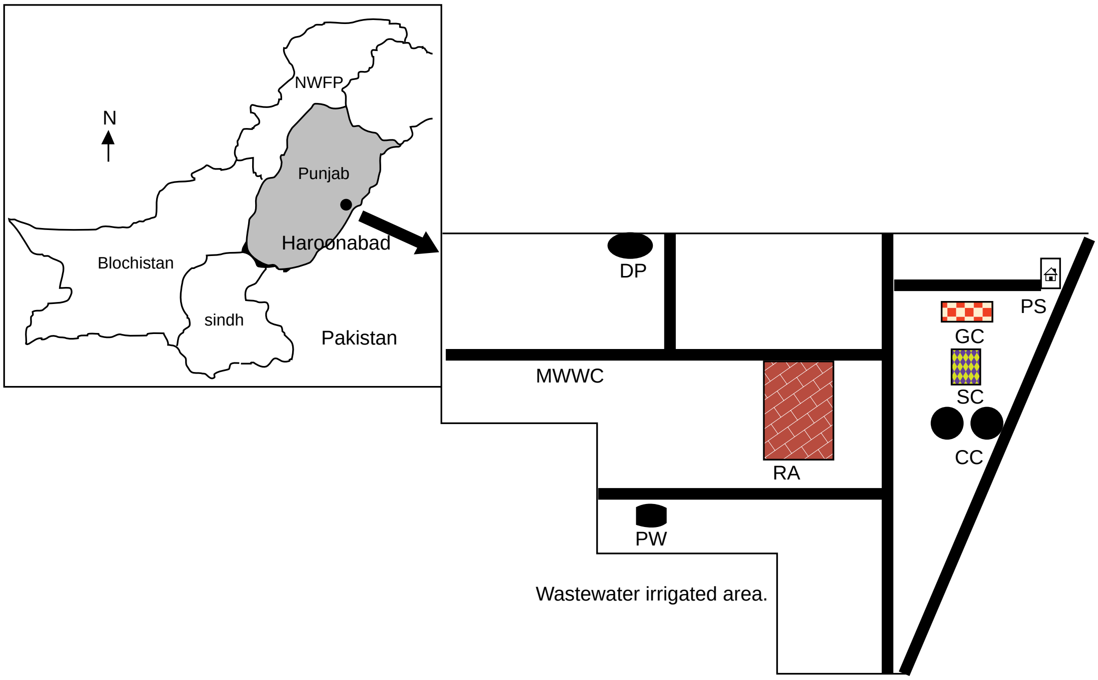

Fig 1–CC = Collecting chamber; SC = Seiver chamber; GC = Grid chamber; PW = Pumping well; DP = Drainage pond; PS = Pumping station; MWWC = Main wastewater channels; RA = Residential area.

were collected by using a standard 350 ml aluminum dipper and dipping at the rate of six dips per m^2 of surface area for smaller habitats. For larger ones, a "sample" representing 30 dips was taken within a 5m^2 area (*ie* equivalent to 6 dips per m^2). For sites with a surface area between 5-10 m^2, one sample was collected, while for 11-20 m^2, two samples were taken, and so on (Herrel *et al*, 2001). The collected larvae were preserved in vials containing 70% isopropyl for later identification. The 3rd and 4th instar larvae were identified using the keys of Amerasinghe *et al* (2001), Glick (1992), Harbach (1988), Rao (1984) and Reuben *et al* (1994) for the identification of *Anopheles* and *Culex* immature stages. Representative samples were reared in the laboratory to the adult stage for the confirmation of species. Other mosquito groups were only identified up to the generic level. Early instars and damaged larvae were counted but not identified.

Habitat characteristics, such as vegetation and fauna, were noted on each sampling occasion. Fauna were sub-divided into predators and non-predators, with the former category comprising fish, water bugs (*Diplonychus* sp, Hemiptera: Notonectidae), water beetles and water beetle larvae (*Dytiscus* sp, Coleoptera: Dytiscidae), damselfly larvae (*Agrion* sp, Odonata: Agrionidae), dragonfly larvae (*Pantala* sp, Odonata: Libellulidae), water scorpions (*Nepa* sp, Hemiptera: Nepidae), water boatmen (*Corixa* sp, Hemiptera: Corixidae) and backswimmers (*Notonecta* sp, Hemiptera: Notonectidae). The group of non-predators included Chirinomid larvae, mayfly larvae, water fleas, snails and worms. The substratum was classified as either soil or cement. Water was recorded as flowing or standing, and its physical condition assessed by eye as colored/foul-smelling and colorless/foul-smelling. Light conditions at sampling sites were recorded as exposed, partially shaded or shaded. The chemical water quality parameters measured were dissolved oxygen (DO, in mg/l, Hach DO 175 Meter), electro-conductivity (EC, in mS/cm, Hach EC 20 Meter) and pH (Hanna Instruments). *In-situ* measurements were not possible in the case of collecting chambers and the sieve chamber, and readings were made from a freshly collected water sample in the dipper itself.

All water bodies in the study area were classified into the following categories:

(1) Field watercourse: earthen watercourse that delivers wastewater to fields. Flowing water. This category also includes pools in the watercourse.

(2) Irrigated field: field that has been deliberately inundated with wastewater for cultivation purposes. Includes field pools created after a wastewater irrigation turn. Standing water.

(3) Drainage pond: pond to which excess wastewater is diverted and which also receives ordinary irrigation canal water. Surface area: > 10,000 m^2. Water stands for a relatively long time and is relatively clean. Standing water.

(4) Grid chamber: deep, non-functional disposal structure where wastewater and groundwater accumulate. Covered by thick emergent vegetation. Surface area: 5 m^2. Standing water.

(5) Seiver chamber: deep and rectangular concrete basin with grid, part of the non-functional disposal works. Surface area: 2 m^2. Standing water, with heavy algal cover.

(6) Collecting chambers: two round concrete basins with perpendicular walls, parts of the non-functional disposal works. Mixture of ground, rain and wastewater. Surface area: 50 m^2. Standing water, with heavy algal cover and floating debris.

(7) Pumping well: brick structure and plastered walls where water is collected to be pumped to another watercourse. Thick grasses and floating debris. Surface area: 1.5 m^2. Standing water.

RESULTS

Out of 133 collected samples, 53.4% (n = 77) were from agricultural sites and 46.6% (n = 56) were from components of the wastewater disposal system. Most of the samples were taken from irrigated fields and collecting chambers, *ie*, 39.1% (n = 52) and 31.6% (n = 42) respectively, while 9.8% (n = 13) of samples came from field watercourses. This sampling effort represents the availability of water at different sites. Overall, 17.3% (n = 23) of samples were positive for *Anopheles*, 12.0% (n = 16) for *Culex* and 15.0% (n = 20) for *Aedes* mosquitos. Details of species collected in different habitats are provided in Table 1. Of the 1,338 anopheline larvae examined, 92.0% (n = 1,222) were found in the collecting chambers, followed by the pumping well, from where 6.1% (n = 81) were collected. *Anopheles stephensi* was the dominant species, comprising 84.3% (n = 1,120) of the total anophelines; *An. subpictus* comprised 11.8% (n = 156), *An. culicifacies* 2.0% (n = 27), and *An. pulcherrimus* 0.2% (n = 2). The main breeding sites for all *Anopheles* species were the collecting chambers, from where 96.9% of *An. stephensi*, 69.2% of *An. subpictus*, and 94.1 % of *An. culicifacies* were collected. The pumping well-produced 1.6% of *An. stephensi* and 25.6% of *An. subpictus*. *Culex* larvae occurred predominantly in the pumping wells (96.8%, n = 2,857) and at low levels in the collecting chambers (2.0%, n = 59) and grid chambers (1.1%, n = 32). *Culex quinquefasciatus* was the dominant species, comprising 66.5% (n = 1,964) of the total *Culex* larvae collected. The other identified species present was *Cx. tritaeniorhynchus*, which comprised 20.1% (n = 594). In the case of *Aedes*, 79.2% (n = 909) of a total of 1,148 immatures was collected from the grid chamber, 13.2% (n = 152) from the pumping well and 7.1% from the collecting chambers (Table 1).

The present study was of short duration, only three months (July-September 2000), but different mosquito groups showed high population densities during different months. Of the total 1,338 anophelines, 88.0% (1,178) were collected during the month of July. Most of the *Aedes* (65.6%, n = 764) and *Culex* (75.4%, n = 2,227) were collected during the months of July and September, respectively. Similarly,

Table 1
Numbers of mosquitos collected in different components of the wastewater system.

	Agricultural sites				Wastewater disposal system			Total
	FWC	IF	DP	PW	CC	SC	GC	
No. of samples	13	52	6	6	42	7	7	133
Percentage	9.8	39.1	4.5	4.5	31.6	5.3	5.3	100.0
An. stephensi	2	0	0	18	1,086	13	1	1,120
An. subpictus	0	0	0	40	108	4	4	156
An. culicifacies	0	0	0	0	16	11	0	27
An. pulcherrimus	0	0	0	0	2	0	0	2
Unidentified	0	0	0	23	10	0	0	33
Anopheles sub total	2	0	0	81	1,222	28	5	1,338
Cx. tritaeniorhynchus	0	1	0	508	55	1	29	594
Cx. quinquefasciatus	0	2	0	1,955	4	0	3	1,964
Unidentified	0	0	0	394	0	0	0	394
Culex sub total	0	3	0	2,857	59	1	32	2,952
Aedes spp	4	2	0	152	81	0	909	1,148
Grand total	6	5	0	3,323	2,665	29	946	5,438

CC = Collecting chamber; DP = Drainage pond; FWC = Field watercourse; GC = Grid chamber; IF = Irrigated field; PW = Pumping well; SC = Seiver chamber.

Table 2
Abundance of wastewater-breeding mosquito larvae by month.

Species	Months		
	July	August	September
An. stephensi	0.74±2.21 (n=1,078)	0.06±0.23 (n=23)	0.03±0.09 (n=19)
An. subpictus	0.05±0.07 (n=71)	0.00±0.00 (n=0)	0.13±0.62 (n=80)
An. culicifacies	0.02±0.15 (n=26)	0.01±0.06 (n=01)	0.00±0.00 (n=00)
Cx. tritaeniorhynchus	0.87±4.34 (n=449)	0.31±1.42 (n=111)	0.09±0.55 (n=34)
Cx. quinquefasciatus	0.01±0.05 (n=10)	0.16±0.69 (n=56)	4.79±31.76 (n=1,898)
Aedes spp	0.82±2.54 (n=764)	0.29±1.12 (n=259)	0.34±1.54 (n=125)

Note: The values are mean per dip ± SD, with the number of identified immatures in parentheses.
Unidentified first and second instar larvae and damaged immatures were omitted from computation.

different species showed high population densities in different months: for instance, 92.3% (n = 1,078) of *An. stephensi* and 96.6% (n = 1,898) of *Cx. quinquefasciatus,* were collected during the months of July and September, respectively (Table 2).

Biophysical conditions in breeding habitats are summarized in Table 3. Vegetation was present in almost all habitats, but the presence of predators and non-predators fluctuated widely. Water conditions, too, varied widely between habitats, as did dissolved oxygen (DO) and electrical conductivity (EC). However, pH was more uniform, fluctuating within the alkaline range. Most anophelines occurred in collecting chambers, which were characterized by standing water with high proportions of predators, non-predators, colorless/foul-smelling water, and relatively high levels of DO and EC. In contrast, most of the *Culex* spp were collected

Table 3
Selected physical, chemical and biological characteristics of habitats sampled.

Habitat	Vegetation	Fauna		Water condition		Water quality		
		Predators	Non-predators	Colorless & foul	Colored & foul	DO	EC	pH
Irrigated field	100	4	83	15	85	0.9	5.0	7.8
Field water course	100	8	69	8	92	0.9	4.2	7.8
Drainage pond	100	0	0	0	100	1.2	12.3	8.2
Pumping well	100	50	100	17	83	0.7	2.1	7.3
Collecting chamber	100	98	79	86	14	5.8	14.3	8.7
Siever chamber	86	100	100	14	86	4.9	8.9	8.1
Grid chamber	100	100	100	43	57	0.2	21.3	8.9

Values for vegetation, fauna and water condition are percentages of occurrence; DO = Dissolved oxygen (mg/l); EC = Electrical conductivity (mS/cm); values for DO, EC, and pH are means.

from the pumping well. The key characteristics of this habitat were standing water with a mainly colored and foul-smelling condition, lower proportions of predators, and low DO and EC. Most of the *Aedes* larvae were collected from the grid chamber. This standing water habitat was characterized by the presence of vegetation, predators and non-predators in all samples, a roughly even split between colored and colorless foul water, and the lowest DO and highest EC values recorded in the study (Table 3).

DISCUSSION

The study showed that *Anopheles*, *Culex* and *Aedes* mosquitos bred in various components of the wastewater irrigation system at Haroonabad. Species within each of these three mosquito genera were found to be associated with specific breeding habitat locations and environmental characteristics. Anophelines were attracted to the collecting chambers, whereas *Culex* and *Aedes* were attracted to the pumping well and grid chamber, respectively. The collecting chambers and grid chamber were part of a non-functional old wastewater disposal system, while the pumping well was a functional part of the wastewater irrigation system. Irrigated fields were always mosquito-negative. This was probably due to the percolation of water (within a day) through the porous soil, which resulted in the rapid elimination of potential mosquito breeding sites. Field watercourses were also negative, probably due to the continuous flow of water that would wash away eggs and immature stages.

The results of this study provided some interesting contrasts with previous findings, indicating that *An. culicifacies*, *An. stephensi*, *An. subpictus* and *An. pulcherrimus*, collectively, preferred clear or turbid non-foul breeding water (Herrel *et al*, 2001; Reisen *et al*, 1981; Talibi and Qureshi, 1956; Ansari and Nasir 1955; Ansari and Shah, 1950). Under the arid conditions of the study area, it was clear that foul water habitats were exploited by these species. However, only one species, *An. stephensi*, appeared to breed prolifically in these foul water habitats, with *An. subpictus* a distant second. The other two species occurred in trivial numbers and the wastewater system was apparently a relatively minor component of their overall breeding habitat palette. Previously, Krishnan (1961) found *An. stephensi* associated with polluted water habitats in the winter season, when there was low rainfall and less irrigation practices in the Punjab. In contrast, our study was carried out during the hot summer months. It seems that the species can exploit foul water habitats under both environmental extremes. *Anopheles stephensi* has been considered a malaria vector in parts of Asia, including the Indo-Pakistan subcontinent and the Persian Gulf (Rao, 1984; Manouchehri *et al,* 1976). *An. stephensi* was naturally infested with plasmodia in urban Karachi (Rehman and Muttlib, 1967) and also in rural Punjab (Pervez and Shah, 1989). Recent evidence from Sheikhupura, in northern Punjab, suggests that *An. stephensi* may be a more important vector than previously believed. It was five times more prevalent than *An. culicifacies*, and *P. falciparum* malaria cases peaked after *An. culicifacies* had disappeared, but when *An. stephensi* was present (Rowland *et al*, 2000). Three morphological variants of *An. stephensi* are presently recognized (Sweet and Rao, 1937; Subbarao *et al,* 1987), and it is still not clear which variant(s) are good and poor vectors. It would be interesting to investigate whether breeding in foul and non-foul water is a variant-dependent behavioral trait.

The next most prevalent anopheline, *An. subpictus,* was mainly recorded from foul water in collecting chambers and also from the pumping well. This is consistent with previous research in the Punjab that showed it to breed in foul and polluted water in septic tanks, street pools and street drains in a rural irrigated village ecosystem (Herrel *et al*, 2001). The species also has been recorded from extremely saline, highly silted habitats (Ansari and Nasir, 1955), and is generally considered to be able to tolerate a wide range of physio-chemical conditions, including a high organic matter content (Herrel *et al*, 2001; Reisen *et al*, 1981). *Anopheles subpictus* exists as a complex of four sibling species whose breeding ecologies are largely unknown, apart from the fact that sibling B generally breeds in saline water (Suguna *et al,* 1994). As in the case of *An. stephensi*, it is not known whether foul water breeding in *An. subpictus* is related to sibling species preferences.

Anopheles culicifacies occurred infrequently in the wastewater habitats. Only 27 immatures were collected, and these, too, only from the collecting chamber and seiver chamber. Interestingly, these two habitats had the highest DO values among all the sampled habitats. Previous research in South Asia (Amerasinghe *et al*, 1995; Reisen *et al*, 1981; Russell and Rao, 1942) has shown the breeding of this species to be closely linked to water with a high DO content. The present results confirm this association, even in breeding water that is physically foul-smelling and has generally lower DO levels than unpolluted water.

Culex quinquefasciatus and *Cx. tritaeniorhynchus* occurred primarily in the pumping well, characterized by colored foul water with a low DO content. Both species are pollution-tolerant, and their existence in such habitats is well documented in the literature. For instance, Carlson and Knight (1987) recorded extremely high *Cx. quinquefasciatus* populations in wastewater treatment ponds in Florida. The WHO (1995) reported that stagnant polluted water bodies are also a favored breeding habitat of *Cx. pipiens fatigans* and *Cx. tritaeniorhynchus*. In Pakistan, Reisen *et al* (1981) and Aslamkhan and Salman (1969) reported that *Cx. tritaeniorhynchus* was a dominant species in pools and ponds as they grew older and more polluted in the summer; a similar phenomenon occurred with *Cx. pipiens fatigans* in the winter. *Culex tritaeniorhynchus* is a vector of Japanese encephalitis in South and Southeast Asia and Japan, and also a vector of West Nile virus in Pakistan and India (Peiris and Amerasinghe, 1994). *Culex quinquefasciatus* is well known to be a major vector of lymphatic filariasis throughout the tropics, and also has been incriminated in the transmission of West Nile virus in Pakistan and India (Peiris and Amerasinghe, 1994). Historically, cases of human filariasis have been reported in Pakistan among repatriated refugees from Bangladesh, although transmission to indigenous Punjabis remains to be documented (Aslamkhan and Pervez, 1981). West Nile fever is generally a mild febrile infection, but recent outbreaks of a more severe form of West Nile virus, that have resulted in significant human case fatalities in Europe, Pakistan, the Middle East and the United States (Petersen and Roehrig, 2001), are of concern. Among major zoonotic hosts of the virus are migratory birds that could potentially carry virulent strains into new areas such as Pakistan. Wastewater systems that generate potential vectors, such as *Cx. quinquefasciatus*, and *Cx. tritaeniorhynchus*, and also attract aggregations of birds under the arid climatic conditions of the region, could increase the risks of transmission to humans.

In our study, *Aedes* mosquitos were mainly recorded from the grid chamber and from the pumping well to some extent. Since species identifications were not done, we cannot comment on their potential disease implications. However, there is little doubt that they would constitute a nuisance hazard to humans and livestock around the wastewater system.

One of the limitations of this preliminary study was its short duration, which precluded the recording of seasonal trends in breeding. However we did observe that within the 3-month span of the study, *An. stephensi* and *An. culicifacies* were collected mainly during July, *An. subpictus* in July and September, *Cx. tritaeniorhynchus* in July-August, and *Cx. quinquefasciatus* in September. The *Aedes* spp decreased from July to September. Previous research in Pakistan and the neighboring Indian Punjab show *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus* peak around these same times, whereas for *An. culicifacies,* the peak abundance was during September-December, and for *An. stephensi* no clear seasonal trend was observed (Ansari and Nasir, 1955; Aslamkhan and Salman, 1969; Reisen, 1978; Reisen and Milby, 1986; Reisen *et al*, 1976; 1982). The extent to which wastewater habitats are seasonally utilized by mosquitos is an aspect that needs further investigation. Overall, however, this short study highlighted the fact that the wastewater system in Pakistan generated substantial populations of mosquitos, such as *An. stephensi* (malaria), *Cx. tritaeniorhynchus* (West Nile fever, Japanese encephalitis) and *Cx. quinquefasciatus* (Bancroftian filariasis, West Nile fever), thereby contributing to vector-borne disease risks to human communities dependent upon wastewater use for a livelihood. Amelioration of this mosquito problem could be achieved by covering the smaller non-functional components of the wastewater system so that mosquitos do not have direct access to the water within, flushing the components of the functional system frequently to wash away larvae, and regular removal of floating debris and marginal and emergent vegetation from the open components of the system.

ACKNOWLEDGEMENTS

The authors thank Tariq Nazir, Asim Munawar and Tipu Naveed for their dedicated field assistance; Najaf Ali Khan for data entry and management; and Dr M Aslamkhan, for providing literature and useful discussions. Help from Dr M Yousuf, Head of the Department of Agricultural Entomology, University of Agriculture, Faisalabad, Pakistan, for the identification of non-dipterous insect fauna of wastewater also is highly appreciated. The IWMI studies on irrigation and health in the southern Punjab are supported by the Canadian International Development Agency (CIDA).

REFERENCES

Aftab MP. Existing sewage treatment facilities in Pakistan. Country report. National Engineering Services of Pakistan, Environment and Public Health Engineering Division, 1999.

Amerasinghe FP, Indrajith NG, Ariyasena TG. Physiochemical characteristics of mosquito breeding habitats in an irrigation development area in Sri Lanka. *Ceylon J Sci* 1995; 24: 13-29.

Amerasinghe FP, Mukhtar M, Herrel N. Keys to the anopheline mosquitoes (Diptera: Culicidae) of Pakistan. *J Med Entomol* 2001; 39: 28-35.

Ansari MAR, Shah KS. A guide to the mosquitos of the Punjab. Lahore: Institute of Hygiene and Preventive Medicine, 1950: 45 pp.

Ansari MAR, Nasir AS. A preliminary note on anophelism of Lahore suburbs. *Pakistan J Health* 1955; 4: 212-23.

Aslamkhan M, Pervez SD. Imported filariasis in Pakistan. *Trans R Soc Trop Med Hyg* 1981; 75: 869-71.

Aslamkhan M, Salman C. The bionomics of the mosquitoes of the Changa Manga National Forest West Pakistan. *Pakistan Zool* 1969; 1: 183-205.

Carlson BD, Knight RL. Mosquito production and hydrological capacity of southeast Florida impoundments used for wastewater retention. *J Am Mosq Control Assoc* 1987; 3: 74-83.

Cooper RC. Public health concerns in wastewater reuse. *Water Sci Technol* 1991; 24: 55-65.

Glick JI. Illustrated key to the female *Anopheles* of southwestern Asia and Egypt (Diptera; Culicidae). *Mosq Syst* 1992; 24: 125-53.

Harbach RE. The mosquitoes of the subgenus *Culex* in southwestern Asia and Egypt (Diptera: Culicidae). *Contrib Am Entomol Inst* 1988; 24: 1-240.

Herrel N, Amerasinghe FP, Ensink J, Mukhtar M, van der Hoek W, Konradsen F. Breeding of *Anopheles* mosquitoes in irrigated villages of South Punjab, Pakistan. *Med Vet Entomol* 2001; 15: 236-48.

Krishnan KS. *A. stephensi* Liston, 1901. In: Vectors of malaria in India. Delhi: National Society of India for Malaria and Other Mosquito-Borne Diseases, 1961: 39-58.

Manouchehri AV, Javadian E, Eshghy N, Motabar M. Ecology of *Anopheles stephensi* in southern Iran. *Trop Geogr Med* 1976; 28: 228-32.

Peiris JSM, Amerasinghe FP. West Nile fever. In: Beran GW, Steele JH, eds. Handbook of zoonoses, Section B: Viral. 2nd ed. Boca Raton: CRC Press, 1994: 139-48.

Pervez SD, Shah IH. Role of *Anopheles stephensi* as malaria vector in rural areas of Pakistan. *Pakistan J Health* 1989; 26: 73-84.

Petersen LR, Roehrig JT. West Nile virus: a reemerging global pathogen. *Emerg Infect Dis* 2001; 7: 611-4.

Rao TR. The Anophelines of India. New Delhi: Malaria Research Centre, Indian Council of Medical Research, 1984.

Rehman M, Muttlib A. Determination of malaria transmission in central part of Karachi city and incrimination of *An. stephensi* as the vector. *Pakistan J Health* 1967; 17: 73-84.

Reisen WK. A quantitative mosquito survey of 7 villages in Punjab Province, Pakistan with notes on bionomics, sampling methodology and the effects of insecticides. *Southeast Asian J Trop Med Public Health* 1978; 9: 587-601.

Reisen WK, Aslamkhan M, Basio RG. The effects of climatic patterns and agricultural practices on the population dynamics of *Culex tritaeniorhynchus* in Asia. *Southeast Asian J Trop Med Public Health* 1976; 7: 61-71.

Reisen WK, Siddiqui TF, Aslamkhan M, Malik GM. Larval interspecific associations and physicochemical relationships of the ground-water breeding mosquitoes of Lahore. *Pakistan J Sci Res* 1981; 3: 1-23.

Reisen WK, Mahmood F, Parveen T. Seasonal trends in population size and survivorship of *Anopheles culicifacies*, *An. stephensi* and *An. subpictus* (Diptera: Culicidae) in rural Punjab Province, Pakistan. *J Med Entomol* 1982; 19: 86-97.

Reisen WK, Milby MM. Population dynamics of some Pakistan mosquitoes: changes in adult relative abundance over time and space. *Ann Trop Med Parasitol* 1986; 80: 53-68.

Reuben R, Tewari SC, Hiriyan J, Akiyama J. Illustrated keys to species of *Culex* (*Culex*) associated with Japanese encephalitis in Southeast Asia (Diptera: Culicidae). *Mosq Syst* 1994; 26: 75-96.

Rowland M, Mahmood P, lqbal J, Carneiro I, Chavasse D. Indoor residual spraying with alphacypermethrin controls malaria in Pakistan: a community-randomized trial. *Trop Med Int Health* 2000; 5: 472-81.

Russell PF, Rao TR. On the ecology of larvae of *Anopheles culicifacies* Giles, in borrow pits. *Bull Entomol Res* 1942; 32: 341-61.

Subbarao SK, Vasantha K, Adak T, Sharma VP, Curtis CF. Egg-float ridge number in *Anopheles stephensi*: ecological variation and genetic analysis. *Med Vet Entomol* 1987; 1: 265-71.

Suguna SG, Rathinam KG, Rajavel AR, Dhanda V. Morphological and chromosomal descriptions of new species in the *Anopheles subpictus* complex. *Med Vet Entomol* 1994; 8: 88-94.

Sweet WC, Rao BA. Races of *A. stephensi* Liston. *Indian Med Gaz* 1937; 72: 665-74.

Talibi SA, Qureshi MS. Anopheline mosquitoes of Pakistan, their distribution, ecology and relation to malaria. Karachi: Government of Pakistan Press. *Health Bull* 1956; 4: 1-35.

WHO. Health guidelines for the use of wastewater in agriculture and aquaculture. *WHO Tech Rep Ser* 1989; 778.

WHO. Vector control for malaria and other mosquito-borne diseases. *WHO Tech Rep Ser* 1995; 857.