

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

## Public Health

journal homepage: [www.elsevier.com/puhe](http://www.elsevier.com/puhe)

## A review of dengue as an emerging disease in Pakistan

S.B. Rasheed<sup>a,b,\*</sup>, R.K. Butlin<sup>a</sup>, M. Boots<sup>c</sup>

<sup>a</sup> Animal and Plant Sciences, University of Sheffield, Sheffield, UK

<sup>b</sup> Department of Zoology, University of Peshawar, Peshawar, Pakistan

<sup>c</sup> Department of Biosciences, University of Exeter, Penryn, UK

### ARTICLE INFO

#### Article history:

Received 20 December 2011

Received in revised form  
28 May 2012

Accepted 22 September 2012

Available online 6 December 2012

#### Keywords:

Pakistan

Dengue vector

Dengue epidemics

Deaths due to dengue

### SUMMARY

The presence of dengue virus has been detected using neutralization and haemagglutination inhibition antibodies in local populations in Pakistan since the 1960s. However, the first epidemic was not reported until 1994. This was followed by some cases in 1995, but the disease was confined to the port city of Karachi. Since 2006, dengue epidemics have occurred every year and the range has extended to most cities in Pakistan. Dengue now affects thousands of people and has caused hundreds of deaths. It has become a major health problem in Pakistan, and it is likely to become an even greater health problem in the coming years. This review gives an insight into the dengue situation from the early 1960s to the most recent epidemics in Pakistan, and also describes the primary vector of this disease (*Aedes aegypti*) in Pakistan. As such, it provides the first comprehensive review of the emergence of this important public health problem.

© 2012 The Royal Institute of Public Health. Published by Elsevier Ltd. All rights reserved.

### Background

Dengue is an acute febrile disease caused by a single-stranded RNA virus with four closely related but antigenically different serotypes: DEN-1, DEN-2, DEN-3 and DEN-4.<sup>1</sup> It has become a major health problem in both the tropics and subtropics, and is currently considered to be the most important human arboviral disease.<sup>2</sup> According to the World Health Organization, approximately 2.5 billion people (approximately half of the world's population) are currently at risk of dengue. Every year, approximately 50 million people are estimated to suffer from dengue virus, with approximately 55,000 individuals requiring hospitalization, many of whom are children.<sup>3</sup> At least 2.5% of these individuals die, but the death rate may be twice as high as this.<sup>4</sup> Mosquitoes of the genus *Aedes* are the vectors of dengue in both rural and urban areas. *Aedes aegypti* is the most important mosquito vector of arboviruses because it is highly anthropophilic and prefers habitats in close

proximity to humans.<sup>5,6</sup> This article will review the dengue situation in Pakistan where it is both an emerging disease and an increasing health problem.

Pakistan, a country characterized by its geographical and climatic diversity, is located in the north-west of South Asia. It is situated between latitudes 23.45° and 36.75° north and longitudes 61° and 75.5° east. Administratively, it consists of Punjab, Sindh, Khyber Pakhtunkhwa (KP), Balouchistan, Gilgit-Baltistan and Azad Jammu and Kashmir. Due to its subtropical location and climatic suitability for vectors, Pakistan is faced with problems associated with many vector-borne diseases including malaria, leishmaniasis, Crimean-Congo haemorrhagic fever, dengue haemorrhagic fever, West Nile virus, Japanese encephalitis and scrub typhus.<sup>7,8</sup> Malaria and some other vector-borne diseases have been reported for more than half a century in Pakistan, but dengue fever is fairly recent. It has extended its range during the last few years, resulting in morbidity in the thousands and

\* Corresponding author. Department of Zoology, University of Peshawar, Jamrud Road, Peshawar 25120, Pakistan. Tel.: +92 91 9216754. E-mail address: [basitrasheed@hotmail.com](mailto:basitrasheed@hotmail.com) (S.B. Rasheed).

hundreds of deaths due to complications of dengue haemorrhagic fever and dengue shock syndrome.

---

## Dengue vectors

Reports of Dengue vectors in the region predate the creation of Pakistan. In 1934, Barraud collected *A. aegypti*, the primary vector of dengue, from Peshawar, Dera Ismail Khan, Lahore, Larkana and Karachi.<sup>9</sup> In 1949, Qutubuddin reported *A. aegypti* from Kohat-Hangu valley in northern Pakistan.<sup>10</sup> The distribution of *A. aegypti* decreased significantly after 1950 as a by-product of a malaria vector eradication programme.<sup>11</sup> Dengue returned in two episodes in Pakistan: one in the south in the 1980s and one in the north in 1993. In the southern metropolis of Karachi, *A. aegypti* was reported in a survey conducted in 1983 to determine the species of mosquitoes acting as vectors of different diseases.<sup>12</sup> However, at that time, the distribution of *A. aegypti* was limited to the port city of Karachi, and it was not collected from the neighbouring district of Thatta.

The second re-emergence of *A. aegypti* occurred in Landi Kotal, a border town of Khyber Agency located 40 km from Peshawar and 10 km from the Afghan border. In 1993, there were reports of a high level of unusual diurnal human-biting mosquitoes in this area. A survey was conducted by a team of entomologists from Peshawar University led by Dr Suleman. On surveying the area, *A. aegypti* mosquitoes were collected in adult and immature forms. The mosquito was breeding in tyres stored in warehouses,<sup>11</sup> and this was the first time that the spread of this mosquito was associated with the tyre trade in Pakistan. In 2000, all five districts of Karachi were resurveyed for the presence of *A. aegypti*, and it was collected from all districts, indicating an increase in its distribution.<sup>13</sup> In another survey of the district of Karachi in 2010, the presence of the vector mosquito was confirmed from all 18 towns of Karachi.<sup>14</sup> It is therefore clear that despite the eradication of this vector mosquito due to the malaria control programme in the 1950s,<sup>11</sup> *A. aegypti* has re-established itself in Pakistan associated with the tyre trade. It is important to examine the link between the tyre trade and the spread of the vector in more detail. This question will be examined elsewhere using genetic markers.

---

## Dengue disease

It is possible that dengue virus may have been endemic in Pakistan, and due to a lack of surveillance and diagnosis, no outbreaks were reported until 1994. However, according to a sero-epidemiological study conducted in the 1960s and the 1980s, substantial proportions of the apparently healthy population in Rawalpindi and Peshawar,<sup>15,16</sup> parts of Punjab<sup>17,18</sup> and Karachi<sup>19</sup> tested positive for neutralization and haemagglutination inhibition antibodies for dengue virus. In another study conducted between 1983 and 1985 on the prevalence of West Nile virus, Japanese encephalitis and dengue in the city of Karachi, 65% of the total population aged between 6 and 65 years showed positive results for haemagglutination inhibition (HI) antibodies against one or more of the three flaviviruses. Individuals aged 6–20 years showed an

increase in the HI sero-antibody positivity rate for dengue type 2 between July and October.<sup>7</sup> The first outbreak of dengue in Pakistan started in August 1994 and continued through November 1994 in Karachi, leading to morbidity in the thousands. Epidemiological data for this epidemic were not recorded but the number of patients seen by physicians was in the thousands. There is no proper estimate of the number of fatal cases, but at least two deaths occurred in different hospitals in Karachi.<sup>20</sup>

A few independent small studies were conducted to confirm this outbreak as dengue. In most of the investigations, the presence of dengue type 1 and dengue type 2 was confirmed by detecting immunoglobulin M (IgM) using an enzyme-linked immunosorbent assay (ELISA). These investigations were made on adults as well as children.<sup>20–23</sup> In one of the studies, Chan *et al.* used semi-nested polymerase chain reaction (PCR) to detect dengue virus in the sera of patients, and also isolated dengue type 1 and type 2 from these sera.<sup>20</sup> This was the only study in which a molecular technique was used and the virus was isolated from the sera of infected patients. As such, there is limited understanding of the extent and nature of this first epidemic.

Dengue cases continued to occur in 1995, but this time in a different city. A contractor building a powerhouse in Hub, 60 km west of Karachi, reported pyrexia of unknown origin in most of its employees.<sup>8</sup> IgM and IgG antibodies for all four serotypes of dengue virus were determined using ELISA. Again, IgM antibodies for serotypes 1 and 2 were detected in this investigation, suggesting that the disease persisted in southern Pakistan for 2 consecutive years. In this outbreak, patients were not only local but also expatriates hired by the company, which suggests that the disease was transmitting locally. No entomological work was undertaken alongside the sero-epidemiological studies, but the employees were complaining of mosquito bites in the daytime. Following destruction of all the possible mosquito habitats in the construction sites of the power plants, the disease did not occur again in that area.<sup>24</sup>

One reason for the 1994 and 1995 outbreaks may have been the introduction of another serotype of dengue virus to the population. Only serotype 2 was confirmed by Sugamata *et al.* in 1987,<sup>7</sup> but serotype 1 was also detected in the patients in the 1990s. The presence of multiple serotypes in a population results in increased risk of antibody-dependent enhancement; a phenomenon in which the heterotypic antibodies from a previous dengue serotype infection result in promotion of viral replication within the mononuclear leukocytes during secondary infection of a different serotype.<sup>25</sup> It was originally suggested by S.B. Halstead in the 1970s that dengue haemorrhagic fever is more likely to occur in patients who have previously been exposed to dengue infection.<sup>26</sup> The 1994 and 1995 outbreaks of dengue resulted in illness in thousands of people, but no serious steps were taken to prevent future epidemics. In particular, no rules were implemented to quarantine people coming from different dengue endemic areas, and the tyre trade was not monitored.

After a break of 10 years, another serotype (serotype 3) of dengue virus was reported in Pakistan in 2005. During the intervening period, there were no major outbreaks of dengue, although cases were still present. According to the National Institute of Health Islamabad, approximately 395 cases of

dengue were confirmed in their laboratory in 2005, all of which were from Karachi. Initially, a local hospital in Karachi investigated some cases with symptoms of dengue fever by conducting ELISA and reverse transcription PCR (RT PCR). In total, 106 patients were included in the study, nine of whom died. Forty-two of the suspected cases tested positive for anti-dengue virus IgM antibody. Of the nine deaths, six patients had both dengue IgM and IgG according to ELISA, and three patients only had dengue IgM. RT PCR confirmed the dengue status of three patients, and a further step was taken and the PCR product was sequenced to determine the serotype and the phylogenetic relationships of the virus. The analysis showed it to be serotype 3, which had significant similarity with an Indian strain of dengue serotype 3 isolated from Delhi.<sup>27</sup> No evidence was given regarding the movement of this virus across the border, but it is most likely to have been via infected humans.

From 2006, the disease extended its range from the south to the north of Pakistan, causing epidemics that have affected thousands of people and caused hundreds of deaths over the last 5 years (Table 1). In 2006, 5800 suspected cases of dengue were reported across Pakistan, with 3000 confirmed cases and 52 deaths.<sup>28</sup> The epidemic started in May and continued until November, after which it started to decline. The maximum number of cases was reported between August and October, when the country received heavy rain due to the monsoon. Karachi was the most affected city, with more than 4500 suspected cases (1500 of which were confirmed by IgM and IgG antibodies) and 50 deaths.<sup>29</sup> Dengue cases were also reported from northern Pakistan. Approximately 800 cases were reported from Punjab, and 31 cases with one death were reported from the north-western province of KP during 2006. In Punjab, Lahore and Rawalpindi were the most affected cities, with more than 400 cases in each city. The capital city of Islamabad was also hit by this epidemic, resulting in 480 cases and one death. This was the first epidemic of dengue in Pakistan to infect such a large number of people over a large proportion of the country. No strategy was present at the time for either the control of dengue or the treatment of patients. Cases continued to occur until November, after which time the number of affected patients decreased because of the colder weather not favouring the mosquito.

In 2007, dengue hit southern and northern Pakistan again, but with lower intensity than in the previous year. In total,

3342 suspected cases of dengue were reported in different hospitals: 1208 cases were confirmed by ELISA and there were 22 deaths. Dengue cases were reported throughout the year, with the maximum number of cases reported in November followed by October. Karachi had the highest number of cases (2900 suspected and 950 confirmed, and all 22 deaths reported this year), followed by Lahore (258 cases). Cases were also reported from the interior of the Punjab, but there were only one or two cases in each city, which were likely to be imported cases. Attock located in the north of Punjab was new to the disease in epidemic form, but it was restricted to two villages: Basal and Thatta Khalil. According to hospital records, approximately 80% of the population of these two villages suffered from dengue-like symptoms in August and September. Later, dengue was confirmed in the patients using ELISA. Since then, no cases have been reported from this area.

In 2008, 3280 cases with 30 deaths were reported from all over Pakistan. The balance shifted towards the north, with 1450 confirmed cases and 20 deaths reported from Punjab. In Karachi, 1470 cases were reported, 585 of which were confirmed by ELISA, with six deaths. Two hundred and ninety cases and 11 deaths were reported from Rawalpindi/Islamabad, and 70 cases (30 of which were confirmed) and four deaths were reported from KP. In 2008, Dengue affected the capital city of Punjab more severely than Karachi. Lahore witnessed 1358 confirmed cases of dengue fever with nine deaths. No cases were reported from January to March, and then only eight cases were reported from April to August. The number of patients continued to rise from September, reaching a maximum number in November, whereupon there was a sudden decrease from 995 in November to 39 in December. The number of patients suffering from dengue halved in 2009 compared with previous years, with only 1650 patients (950 cases confirmed by ELISA) and 16 deaths throughout Pakistan. Only 112 cases with two deaths were reported from January to August. In September, when the monsoon rains started, providing good breeding conditions for vector mosquitoes, the number of cases rose to more than 1500 with approximately 800 confirmed cases. In total, 14 people died during this period, seven of whom were from Haripur, a district of KP, and seven were from Karachi.

In 2010, a number of areas were affected by the worst flood of the decade, which not only resulted directly in the loss of lives and property, but also increased the breeding

**Table 1 – Confirmed cases and deaths from 2006 to 2011 in the affected areas of Pakistan.**

Year	Khyber Pakhtunkhwa		Sindh				Punjab			
	Cases	Deaths	All parts		Karachi		All parts		Lahore	
			Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
2006 <sup>a</sup>	31	1	1500	50	1500	50	800	1	400	0
2007 <sup>a</sup>	0	0	950	22	950	20	258	0	258	0
2008 <sup>a</sup>	30	4	585	6	585	6	1450	20	1358	9
2009 <sup>a</sup>	100	7	550	7	550	7	300	2	300	2
2010 <sup>b</sup>	0	0	5000	35	4500	16	4000	3	4000	3
2011 <sup>b</sup>	296	8	952	18	755	15	21,314	337	17,493	290

a Data collected from National Institute of Health Islamabad.

b Data collected from provincial health departments.

opportunities for vector mosquitoes. As a consequence, according to the press releases of the health departments, the number of dengue cases reported in 2010 was the highest recorded. In total, more than 9000 cases were reported across Pakistan. Approximately 5000 cases and 35 deaths were reported from Sindh; 16 deaths were from Karachi. More than 4000 cases were reported from Punjab, with only three deaths. In the last week of October, 1500 cases were reported, followed by 1400 cases in the first week in November. As in previous years, Karachi and Lahore were the most affected areas, and Karachi had the highest number of patients suffering from dengue, with 16 deaths in total.

2011 proved to be even worst with regard to dengue in Pakistan, with dengue found in almost every city of the three affected provinces. In 2011, according to the health

departments, 22,562 confirmed cases of dengue with 363 deaths were reported in Pakistan. Unofficial reports claimed more than 35,000 confirmed dengue cases and approximately 420 deaths in the country. The epidemic started in August and continued until November (Fig. 1). Punjab was the most affected province of Pakistan, with more than 21,300 cases and 337 deaths reported. Cases were reported from almost every city of Punjab (Table 2). Lahore, the capital city of Punjab, topped the list with 17,493 cases and 290 deaths. Other cities of Punjab that reported deaths due to dengue were Rawalpindi, Chakwal, Faisalabad, Dera Ghazi Khan and Chichawatni. In 2011, dengue mainly affected the areas of Punjab, especially Lahore, but cases were also reported from the provinces of Sindh and KP. Sindh witnessed 952 cases with 18 deaths; 755 of these cases and 15 deaths were reported from

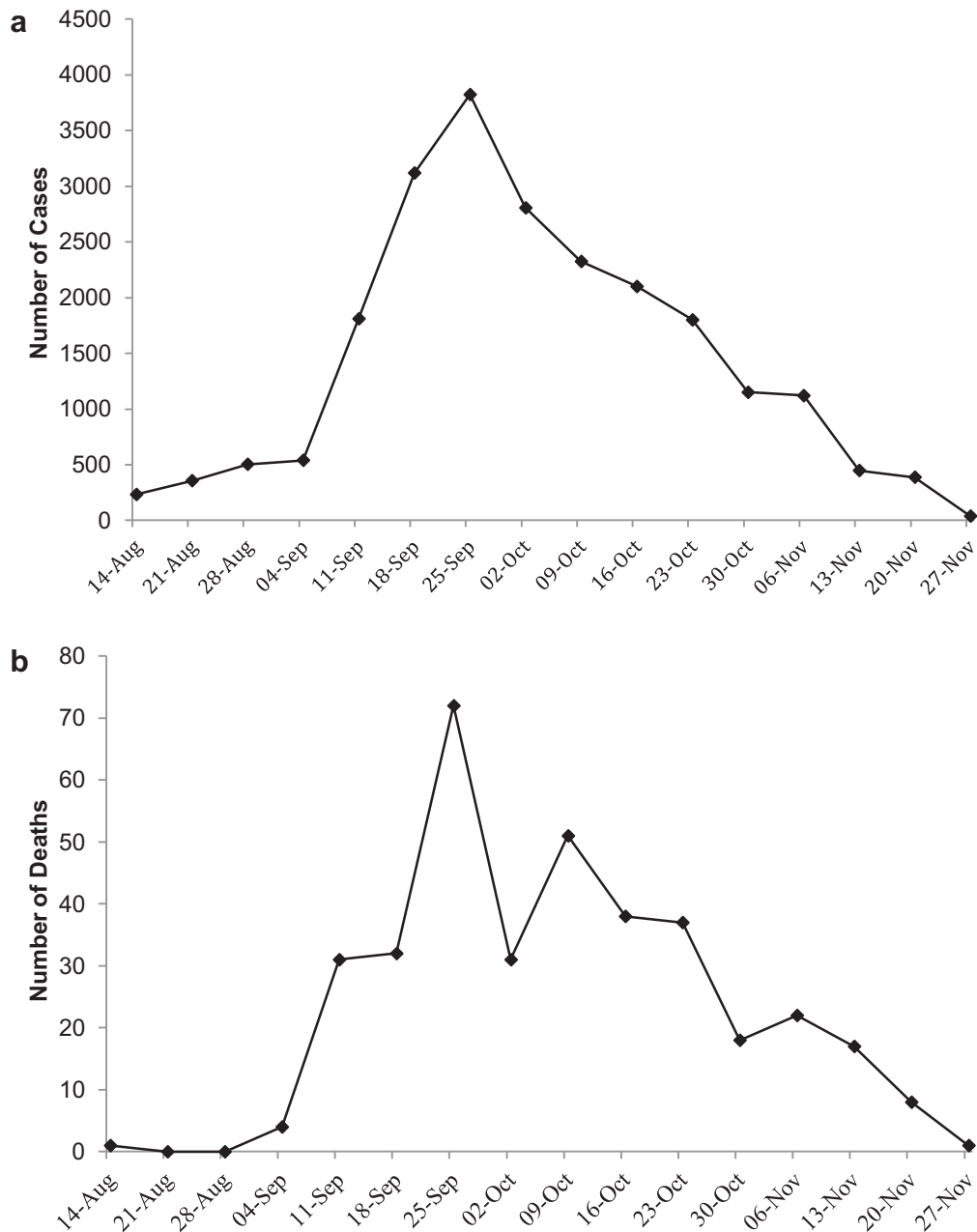


Fig. 1 – Number of weekly cases (a) and deaths (b) due to dengue from 9 August to 28 November 2011.

**Table 2 – Number of cases of dengue reported from different cities of Punjab during the 2011 dengue epidemic in Pakistan.**

City	No. of cases	City	No. of cases
Lahore	17,493	Dera Ghazi Khan	38
Faisalabad	1149	Narowal	36
Multan	538	Jhang	36
Bahawalpur	305	Sahiwal	33
Sargodha	232	Muzaffar Garh	30
Rawalpindin	181	Bhakkar	24
Sheikhupura	156	Chakwal	23
Okara	118	Nankana Sb	23
Rahim Yar Khan	109	Toba Tek Singh	20
Gujranwala	107	Miawali	16
Taxilla	90	Gujrat	15
Vehari	80	Hafizabad	14
Jhelum	71	Mandi Bahawadin	11
Pakpatan	69	Chiniot	11
Sialkot	58	Lodhran	8
Layyah	55	Attock	7
Khanewal	52	Rajanpur	7
Bahawalnagar	51	Khushab	5
Kasur	43	Total	21,314

Data downloaded from [http://dengue.punjab.gov.pk/?q=system/files/Summary\\_13\\_Oct.pdf](http://dengue.punjab.gov.pk/?q=system/files/Summary_13_Oct.pdf) and then updated by health department reports.

Karachi alone. In Sindh, cases were reported from Larkana, Dadu, Ghotki, Hyderabad, Jamshoro, Khairpur, Newshehro feroz, Nawab Shah, Sanghar, Skkhar, Tando Muhammad Khan, Tando Allah Yar, Rajan Pur and Umer Kot. Compared with the Punjab, very few cases were reported from KP; reported cases were from Peshawar, Newshehra, Haripur, Mansehra, Bannu, Mardan, Battagram, Laki Marwat, Khyber Agency, Mingora and Abbottabad. Only 296 confirmed cases were reported from KP, 92 of which reported a history of travel to Punjab. These patients were probably infected in Punjab, travelled to KP with the virus inside their body, and subsequently showed signs and symptoms in KP. The number of cases decreased at the end of November due to cool weather, as *A. aegypti* larvae cannot survive continual exposure at temperatures below 10 °C and adults do not survive well at temperatures below 5 °C.<sup>11</sup>

### Why dengue became common in Pakistan

Prior to 2006, dengue was not common in Pakistan. It was mainly restricted to the southern part of the country, particularly the port city of Karachi. Indeed, no cases were reported from other parts of Pakistan until 2005. The spread of dengue to other parts of Pakistan could be explained by the movement of people carrying the virus from dengue-affected areas to non-dengue-affected areas, or by vertical transmission of dengue in mosquitoes. One interesting example is the villages of Basal and Thatta Khalil in the district of Attock, located on the border between the provinces of Punjab and KP. These two villages are located in a small valley-like location in Attock. The nearest village is approximately 5 km away. In 2007, pyrexia of unknown origin with symptoms consistent with dengue fever

affected approximately 80% of the total population of these villages. The area was visited, and on interview, people reported being bitten by mosquitoes during the day, indicating *Aedes*. According to locals, the disease started when a couple from Karachi came to the village to attend the funeral of a relative who were suffering from a fever consistent with dengue (personal communication). The vector mosquito was already present in the area, and it is likely that the couple acted as a reservoir for dengue, thus spreading it in the local population. The disease did not spread to other areas of the district because these two villages were somewhat isolated due to the hilly nature of the area. A similar case was also found in Rawalpindi when a dengue-infected medical student travelled from Lahore and suffered from dengue in Rawalpindi.

In 2011, most of the patients in KP arrived from Punjab before becoming symptomatic. This movement of infected people is likely to be the main cause of such a rapid spread of Dengue in Pakistan in less than a decade. The movement of dengue-infected people combined with the presence of the vector in the dengue-free areas is likely to be the cause of dengue cases in almost every city of the three affected provinces of Pakistan. No cases have been reported from the province of Baluchistan. As *A. aegypti* is anthropophilic, and Baluchistan has a low population density and a dry climate, this vector mosquito has never been reported from Baluchistan, which could explain the absence of dengue in this province. Another hypothesis is that dengue virus is imported into Pakistan and then distributed within Pakistan through tyre trading. Tyres from dengue-endemic countries may carry desiccated eggs which hatch during the rainy season. This hypothesis needs to be tested by checking the prevalence of dengue virus in adults as well as immature stages of mosquitoes collected from tyres all over Pakistan.

### Control of dengue

Most of the disease control effort has been made to treat dengue-infected people as soon as possible, and therefore to reduce the number of deaths. However, no or very little effort has been made to stop or reduce the number of infected cases through vector breeding control. In all of the affected cities, only thermal fumigation with deltamethrine is carried out to kill adult mosquitoes. Better control may be achieved through three main routes: chemical control, biological control and education. In terms of chemical control, a combination of larvicide and fumigation with adult knockdown effects has the potential to reduce the mosquito population. Chemical control is quick and efficient, but its disadvantages include resistance to insecticides in mosquitoes.<sup>30</sup> However, it works well in the case of *A. aegypti* when there is no rain. In terms of biological control, small fish such as *Gambusia affinis* and *Tilapia* could be released in water tanks, which could help to reduce mosquitoes by eating the larvae that are produced in the tanks. Biological control is slow but could prove to be a good method to control mosquito population growth. Finally, species of mosquito that breed in man-made containers are often aimed to reduce the number of larval habitats (control by source reduction). Such a strategy will not be successful without the involvement of the local community. People could be

encouraged not to store water in pots outside or inside houses, and not to allow water to accumulate in discarded tin cans, vehicles, tyres, small containers, plant pots, bottles etc. Using electronic and print media to tell people how to eradicate mosquito breeding points, and how to protect daily-use items such as drums, buckets, water tanks and other small containers from becoming mosquito factories can help to control the vector and thus control the disease.

## Conclusions

As described in this review, dengue has become a major health problem in Pakistan. Once relatively rare, it has moved from localized infrequent epidemics in a few cities to a wide-spread problem with large numbers of cases. There is a critical need for large-scale entomological surveys in addition to epidemiological surveys, and an urgent need for a vector control programme. Without these, now that it is established, dengue is likely to become a much greater health problem in the coming years.

## Acknowledgements

The authors are grateful to Dr Jaleel Kamran of Epidemic Investigation Cell, National Institute of Health, Islamabad; Dr Khalid Ranjha and Mr Muhammad Asif Mehmood of Provincial Health Department Punjab; Dr Aslam Baloch of Provincial Health Department Sindh; and Dr Zaheen of Provincial Health Office KP for providing the data for compiling this review.

## Ethical approval

None sought.

## Funding

Higher Education Commission Pakistan and University of Peshawar Pakistan.

## Competing interests

None declared.

## REFERENCES

- Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev* 1998;11:480–96.
- Arunachalam N, Tewari SC, Thenmozhi V, Rajendran R, Paramasivan R, Manavalan R, et al. Natural vertical transmission of dengue viruses by *Aedes aegypti* in Chennai, Tamil Nadu, India. *Indian J Med Res* 2008;127:395–7.
- World Health Organization. *Prevention and control of dengue and dengue hemorrhagic fever-comprehensive guidelines*. New Delhi: WHO Regional Office for South-East Asia; 1999.
- World Health Organization. *Dengue and dengue haemorrhagic fever*. Geneva: WHO; 2008.
- Wang E, Ni H, Xu R, Barrett AD, Watowich SJ, Gubler DJ, et al. Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. *J Virol* 2000;74:3227–34.
- Twiddy SS, Farrar JJ, Chau NV, Wills B, Gould EA, Gritsun T, et al. Phylogenetic relationships and differential selection pressures among genotypes of dengue-2 virus. *Virology* 2002;298:63–72.
- Sugamata M, Ahmed A, Kono R, Takasu T. *Seroepidemiological studies of infections with Japanese encephalitis, West Nile and dengue viruses in Karachi-Pakistan in the year 1985: a neuroviro-patho-epidemiological study*. Karachi encephalitis survey team secretarial, 2nd official report. Karachi: Karachi Encephalitis Survey Team Secretariat; 1987. p. 22–26.
- Anonymous. *Country Report Pakistan. Vector-borne diseases in Pakistan*. In: *Inter-country workshop on developing a regional strategy for integrated vector management for malaria and other vector-borne diseases*. Islamabad: Directorate of Malaria Control, Government of Pakistan; 21–23 January 2003. Khartoum, Sudan.
- Barraud PJ. *The fauna of British India, including Ceylon and Burma. Diptera. Family Culicidae. Tribe Megarhinini and Culicinae*. London: Taylor and Francis; 1934.
- Qutubuddin M. The mosquito fauna of Kohat-Hangu valley, West Pakistan. *Mosq News* 1960;20:355–61.
- Suleman M, Arshad M, Khan K. Yellow fever mosquito (Diptera: Culicidae) introduced into Landi Kotal, Pakistan, by tire importation. *J Med Entomol* 1996;33:689–93.
- Kamimura K, Takasu T, Ahmed A. A survey of mosquitoes in Karachi area, Pakistan. *J Pak Med Assoc* 1986;36:182–8.
- Tariq RM, Zafar SMN. Why the population of dengue vector mosquitoes is increasing day-by-day in Karachi and other areas of Sindh, Pakistan? *Pak J Entomol* 2000;15:7–10.
- Tariq RM, Ahmed I, Qadri SS. Population dynamics and mechanical control of dengue vector mosquitoes *Aedes aegypti* and *Aedes unilineatus* in seven towns of Karachi. *Pak J Entomol* 2010;25:21–6.
- Burney MI. A report on the role of arthropod-borne viruses in human diseases in Rawalpindi and Peshawar areas. I. *Pak J Med Res* 1966;5:215–25.
- Burney MI, Munir AH. Role of arthropod-borne viruses in human diseases in Rawalpindi and Peshawar areas. II. Isolation of West Nile virus from human blood and culicine mosquitoes in Rawalpindi area. *Pak J Med Res* 1966;5: 271–84.
- Hayes CG, Baqar S, Ahmed T, Chowdhry MA, Reisen WK. West Nile virus in Pakistan 1. Sero-epidemiological studies in Punjab province. *Trans R Soc Tropic Med Hyg* 1982;76:431–6.
- Hayes CC, Burney MI. Arboviruses of public health importance in Pakistan. *J Pak Med Assoc* 1981;31:16–26.
- Sugamata M, Khono R, Ahmed A, Takasu T. Seroepidemiological research on viral encephalitis in Karachi, Pakistan. Preliminary report. *J Pak Med Assoc* 1986;36:177–82.
- Chan YC, Salahuddin NI, Khan J, Tan HC, Seah CL, Li J, et al. Dengue hemorrhagic fever outbreak in Karachi, Pakistan. *Trans R Soc Tropic Med Hyg* 1994;1995(89):619–20.
- Akram DS, Igarashi A, Takasu T. Dengue virus infection among children with undifferentiated fever in Karachi. *Indian J Pediatr* 1998;65:735–40.
- Ansari JK, Siddiq M, Hussain T, Baig I, Tariq WUZ. Outbreak of dengue hemorrhagic fever in Karachi. *Pak Armed Forces Med J* 2001;51:94–8.
- Qureshi JA, Notta NJ, Salahuddin N, Zaman V, Khan JA. An epidemic of dengue fever in Karachi. Associated clinical manifestations. *J Pak Med Assoc* 1997;47:178–81.
- Paul RE, Patel AY, Mirza S, Fisher-Hoch SP, Luby SP. Expansion of epidemic dengue viral infection to Pakistan. *Int J Infect Dis* 1998;2:197–201.
- Cummings DAT, Schwartz IB, Billings L, Schaw LB, Burke DS. Dynamic effects of antibody-dependent enhancement on the fitness of viruses. *Proc Natl Acad Sci U S A* 2005;102:15259–64.

26. Halstead SB. In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. *J Infect Dis* 1979;**140**:527–33.
27. Jamil B, Hasan R, Zafar A, Bewley K, Chamberlain J, Mioulet V, et al. Dengue virus serotype 3, Karachi, Pakistan. *Emerg Infect Dis* 2007;**13**:182–3.
28. Tang JW, Khanani MR, Zubairi AM, Lam WY, Lai F, Hashmi K, et al. A wide spectrum of dengue IgM and PCR positivity post-onset of illness found in a large dengue 3 outbreak in Pakistan. *J Med Virol* 2008;**80**:2113–21.
29. Khan E, Siddiqui J, Shakoor S, Mehraj V, Jamil B, Hasan R. Dengue outbreak in Karachi, Pakistan, 2006: experience at a tertiary care center. *Trans R Soc Tropic Med Hyg* 2007;**101**:1114–9.
30. Kaunter I, Robinson MJ, Kuhnle U. Dengue virus infection: epidemiology, pathogenesis, clinical presentation, diagnosis and prevention. *J Pediatr* 1997;**131**:516–24.