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Distribution of dengue cases in the state of Oaxaca, Mexico, during the period 2004–2006

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ABSTRACT

Background: Dengue virus infection is an emergent viral disease and the most important transmitted by a vector worldwide. In Mexico it has been an important public health problem since 1995 and Oaxaca is one of the most affected states in the country.
Objective: To determine the geographic distribution of confirmed dengue cases in the state of Oaxaca, Mexico, the serotypes circulating, and the main gender and age groups affected.
Study design: Information about confirmed dengue cases obtained by LESPO during the period 2004–2006 was classified, sorted, and analysed. A RT-PCR technique was used to determine the serotype of the virus in serum samples.
Results: A substantial increment in the number of dengue cases was noticed during the period of this study. The most affected sanitary jurisdiction was located on the coast where the climatic conditions were ideal for vector development and where there is significant migratory activity. The most affected group was the 11–15-year-old group. Dengue haemorrhagic fever was more frequent in men than in women over 16 years old, with a significant difference evaluated by χ^2-test ($p < 0.001$). Four serotypes of the virus were detected in the state and two co-infections with DEN2–3 and DEN3–4 were identified.
Conclusions: The increment in the number of dengue cases in the state of Oaxaca could be explained by several factors such as the presence of the four serotypes of the virus, the migratory phenomenon, the climatic conditions and the socioeconomic level of the population.

1. Background

The four dengue (DEN) virus serotypes are the infectious agents responsible for the most important mosquito-borne viral disease in the world, affecting more than 100 countries including America, Africa and South East Asia.[1–4] Approximately 50–100 million cases are reported annually, of which 250,000–500,000 require hospitalization and 24,000 are fatal.[3,5,6]

DEN virus is a member of the *Flaviviridae* family, *Flavivirus* genus and is transmitted to humans mainly by the mosquito *Aedes aegypti*, which is distributed worldwide between latitudes 35° North and 35° South.[6]

The infection caused by the four serotypes of DEN virus may be asymptomatic or displays symptoms of a mild disease named dengue fever (DF), which is characterized by fever, headache with retro-orbital pain, arthralgia, myalgia, anorexia and occasionally a rash. Secondary infections are more likely to be associated with a more serious illness known as dengue haemorrhagic fever (DHF), which is characterized by bleeding that usually requires hospitalization. DHF can evolve to a dengue shock syndrome (DSS) that might be fatal.[4,5,7]

The number of cases of DEN has increased worldwide since the seventies[1,2,4,8] as a consequence of several factors such as invasion of virulent genotypes of the virus to new geographic areas,[2,9] and increased vector distribution as a result of unplanned and uncontrolled urbanization, migration of the rural population to the cities, inadequate wastewater management, and failure of the vector control programmes.[1,4,5,10,11]

The first DHF outbreak in Mexico occurred in 1995[10,11] and at the present time, DEN is an important public health problem in the country[12] where Oaxaca is one of the most endemic states

Abbreviations: DEN, dengue; DF, dengue fever; DHF, dengue haemorrhagic fever; DSS, dengue shock syndrome; LESPO, Laboratorio Estatal de Salud Pública de Oaxaca (Public Health Laboratory of Oaxaca); PAHO, Pan American Health Organization; WHO, World Health Organization.

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in Mexico. In 2008, 25,040 cases of DF and 6114 of DHF were reported and, among the 25 states affected, Oaxaca was ranked sixth.[13]

2. Objectives

To determine the distribution of DEN cases in the state of Oaxaca, Mexico, the virus serotypes circulating and the main age and gender groups affected during the period 2004–2006.

3. Study design

3.1. Dengue cases

Patients from all six sanitary jurisdictions with probable clinical manifestations of DEN virus infection were selected according to the Panamerican Health Organization (PAHO) guidelines[14] by the staff of the Public Health Laboratory of the state of Oaxaca (Laboratorio Estatal de Salud Pública, LESPO), Mexico. All cases detected during the years 2004, 2005 and 2006 were included. They were classified as DF or DHF according to PAHO criteria.[14]

3.2. DEN virus diagnosis

Blood samples from all patients with suspected clinical manifestations of DEN virus infection collected during the period 2004–2006 in Oaxaca were analysed by LESPO staff using the Dengue IgM Capture ELISA (E-DEN01M Panbio diagnostics)[15] to detect specific anti-DEN IgM antibodies. Only the positive (confirmed) cases were included in our study and they were sorted by gender, age, sanitary jurisdiction and severity (DF or DHF).

3.3. DEN virus serotyping

Serum samples received by LESPO during the year 2005 were used for serotyping. One hundred and nine blood samples that were obtained during the first 7 days of clinical onset and immediately stored in ice were included in this study. They were collected from clinically suspected cases of DEN infection according to PAHO guidelines[14] by LESPO staff with previous authorization from the patient. The blood cells were removed by centrifugation, and the serum was processed for viral RNA extraction. For purification of viral RNA, 200 μl of serum sample was placed in a 1.5 Eppendorf microtube, and 1 ml of TRIZOL® (Invitrogen) was added. The rest of the procedure was performed according to the manufacturer's instructions and the RNA was resuspended in nuclease-free water. Then it was treated with 10 U of RNAse-free DNAse (Roche) for 15 min at room temperature. The enzyme was inactivated at 80 °C for 20 min, and the RNA was precipitated with 2.5 volumes of 100% ethanol (Merck) and 0.1 volume of sodium acetate 3 M (Sigma) for 30 min at −70 °C. It was quantified in a spectrophotometer (Beckman model DU 650). The detection and the serotyping of the virus was performed with the RT-PCR Access kit® (Promega) using the nested RT-PCR method reported by Seah[16] and modified by Günther.[17] Briefly, the RT reaction was performed at 48 °C for 45 min followed by 35 PCR cycles of 94 °C for 30 s, 55 °C for 60 s and 68 °C for 60 s with a final cycle of 68 °C for 10 min using DV1 sense and DV1 anti-sense primers. The positive samples were subjected to 30 additional PCR cycles using specific primers for each DEN virus serotype (DSP1–DSP4) in the same conditions described above. All reactions were run in a PerkinElmer thermocycler (Geneamp PCR System 2400) and analysed by 2% agarose gel electrophoresis stained with ethidium bromide.

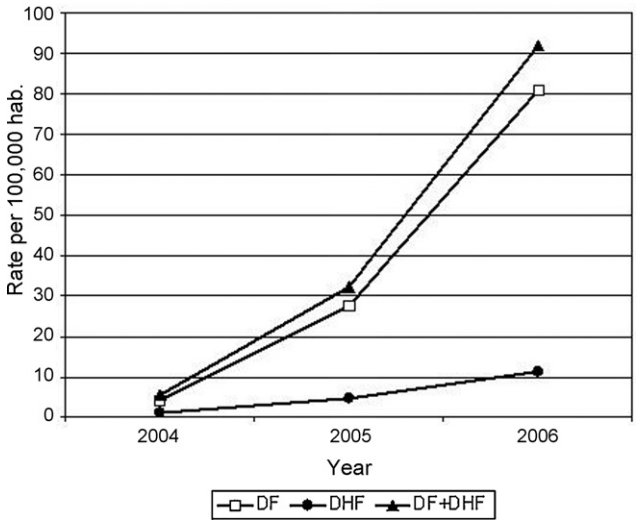

Fig. 1. Total number of DEN confirmed cases reported during the period 2004–2006. Rates per 100,000 habitants. DF, dengue fever; DHF, dengue haemorrhagic fever.

3.4. Statistical analysis

The data of confirmed cases of DEN were sorted by gender, age, and severity. The cases of DF and DHF in men and women from the same age group were organized in a 2×2 contingency table and analysed using the χ^2 (chi square) test and the SAS® software (U.S. Regional Offices, SAS Institute Inc. Headquarters, SAS Campus Drive, Cary, NC 27513-2414, USA), with one degree of freedom. A statistical difference was considered when $p < 0.05$.

4. Results

The information received by LESPO during the years 2004, 2005 and 2006 was collected, classified, and sorted. This period was selected because a significant gradual increment in the total number of cases of DEN in the state was notified compared with previous years.[18]

A substantial increase in the number of confirmed DF cases was observed, from 147 in 2004, to 970 in 2005 and 2836 in 2006, with rates per 100,000 inhabitants of 4.19, 27.66, and 80.87, respectively (Fig. 1). This means an increase of nineteen-fold (1830%) from year 2004 to 2006. For DHF the number of cases was lower but the pattern was similar, increasing from the 43 cases reported in 2004, to 164 in 2005 and 394 in 2006, with rates of 1.23, 4.68, and 11.23, respectively (increment of nine-fold, 813%) (Fig. 1). The state of Oaxaca includes six sanitary jurisdictions: I (Central Valley), II (Tehuantepec), III (Tuxtepec), IV (Pacific Coast), V (Mixteca) and VI (Mountains). During the period 2004–2006 the jurisdictions most affected by DEN virus were II, III, and IV, with rates per 100,000 inhabitants of 161.52, 62.56, and 437.13, respectively (Fig. 2). Sanitary jurisdiction number II occupied first place during the year 2005 with a rate of 95.52, number III during the year 2004 with 21.92, and number IV in 2006 with 437.13 (Fig. 2).

Jurisdictions I, II and IV had increasing numbers of DEN casualties during the period of study. For example, number IV had an increase of almost three hundred-fold (29,840%), number I seventy seven-fold (7586%) and number II eleven-fold (981%) (Fig. 2). Unfortunately, information about jurisdictions V and VI in 2004 was not available but, in general, they were less affected (Fig. 2).

The diagnosis method used by LESPO to confirm DEN cases in the state is the Dengue IgM Capture ELISA assay, which detects the presence of IgM-specific antibodies against DEN virus in the serum but is not able to determine the serotype involved. Therefore we analysed

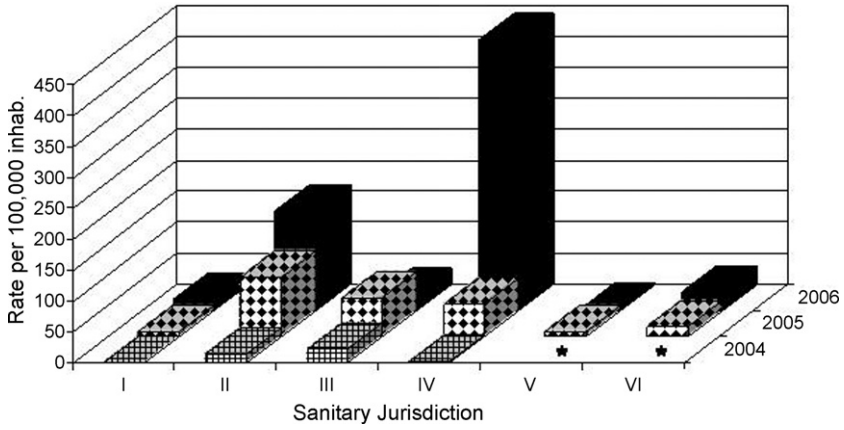

Fig. 2. Distribution of DEN confirmed cases by sanitary jurisdiction in the state of Oaxaca during the period 2004–2006. Rates per 100,000 in habitants by sanitary jurisdiction. Unavailable data are indicated by (*).

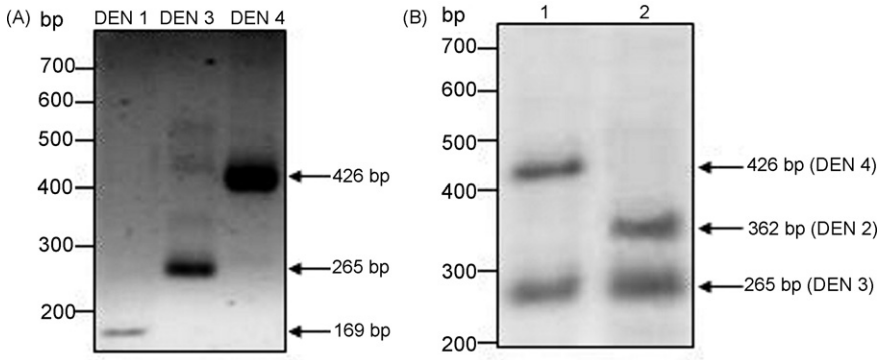

Fig. 3. 2% agarose gel electrophoresis. Viral RNA from serum samples from clinically suspected patients with DEN was purified and used in a RT-PCR/nested PCR reaction as described in study design to determine both the presence of the virus and the serotype. (A) Samples of single serotype-infected patients; the serotype detected is indicated in each line. (B) Samples of two co-infected patients. The arrows indicate the size of the fragments amplified. The molecular markers (λ-ladder 100) are indicated on the left side of each gel.

selected blood samples from 109 patients with suspected clinical manifestations of DEN virus infection during the year 2005 using a nested RT-PCR technique (see study design) (Fig. 3). Sixty-five serum samples (59.6%) were positive and the four serotypes were detected, with DEN1 and DEN4 being the most frequent (Table 1). At least two serotypes were detected in each jurisdiction, except number VI, and all four were present in jurisdiction III. Interestingly, co-infections with two different DEN serotypes were found in two serum samples, where DEN2–3 and DEN3–4 were detected (Fig. 3B and Table 1).

The DHF and DF cases were most frequent in the 0–15-year-old group (Table 2) with the 11–15-year-old group being the most affected (data not shown). Statistical analysis did not reveal any relationship between gender and DF or DHF in children, but in people aged more than 16 years old but less than 50 the incidence of DHF was higher in men (19.6%) than in women (10.8%). This difference was analysed by χ^2-test and was statistically significant ($p < 0.001$) (Table 2).

Table 1
Summary of the results obtained from RT-PCR assays. Serum samples from clinically suspected cases of dengue in Oaxaca, Mexico were collected by LESPO during the year 2005 and then processed to extract de viral RNA which was used in a RT-PCR-nested PCR reaction to detect dengue virus as described in study design.

	Samples analysed	Negative samples	Positive samples				
			Total	DEN1	DEN2	DEN3	DEN4
Number	109	44	65	22*	12	6	25**
Percentage	100%	40.4%	59.6%	20.2%	11%	5.5%	22.9%

* Positive sample for DEN2 and 3.
** Positive sample for DEN3 and 4.

Table 2
Confirmed cases of dengue in Oaxaca during the period 2004–2006 arranged by age, gender and severity.

Age (years)	Gender								Total
	Men				Women				
	DF	DHF	Total	%DHF	DF	DHF	Total	%DHF	
0–15	1063	155	1218	12.7	959	143	1102	13	2320
16–50	641	156*	797	19.6	976	118*	1094	10.8	1891
+50	143	12	155	7.8	171	17	188	9.1	343
Total	1847	323	2170	14.9	2106	278	2384	11.7	4554

DF: dengue fever. DHF: dengue haemorrhagic fever. %DHF: percentage of DHF cases from the total per age group. The number of DHF cases in women and men from the same age group were compared and analysed by χ^2-test.
* Statistical significance ($p < 0.001$).

5. Conclusions

This study analysed the distribution of confirmed DEN cases from the state of Oaxaca, Mexico, determining the virus serotypes that were circulating.

An increase in the number of cases was clearly observed, consistent with the increment in Mexico reported by the WHO[19] and

coincident with the course of DEN worldwide, where it is reappearing in tropical areas with an increase in the number of cases.[7,8,10] Several factors could be involved in this increase in the number of DEN cases in Oaxaca, such as the viral genotype, vector competence, and the socioeconomic level of the population. The Asian–American genotype of DEN2 was detected in the year 2000[20,21] in the state and this was the main causative agent of the outbreak in the state of Yucatan in 2002.[22] The increase in the number of DEN cases in Oaxaca could be related to the circulation of this relatively new genotype although DEN1 and DEN4 were the most frequently found serotypes in our serological study. Related to this, we had access to 109 blood samples from almost all sanitary jurisdictions of Oaxaca in 2005 for DEN virus detection and serotyping. Even though only a small number of samples were analysed, some important conclusions can be made: all four serotypes are circulating and at least two are present in almost all sanitary jurisdictions. They are important factors for developing DHF since secondary infections with DEN virus have been associated with more severe illness.[5,23] In addition, the strain of *A. aegypti* detected in Puerto Escondido, Oaxaca, is among one of the best transmitters of DEN virus.[24] On the other hand, the general socioeconomic level of the population in Oaxaca is low, which is a factor, besides the unplanned urbanization,[1,6] that has been associated with inappropriate management of water disposal,[25] thus facilitating the development of eggs and larvae of *A. aegypti*.[26]

DEN cases in the state of Oaxaca were concentrated in three jurisdictions: II, III and IV. It is well known that climatic factors are important in the transmission of DEN virus in a geographic area[1,11,27] and that the temperature and the rainy season are directly involved in the development of the vector.[22,28–31] This could be the reason why jurisdiction IV (the Pacific coast) was the most affected. On the other hand, it has previously been shown that many of the locations in the state of Veracruz, Mexico, that were affected by DEN virus were located below 600 m above sea level where the climatic conditions are adequate for a high vector density.[11] In our study, the jurisdictions that were least affected by DEN virus were I, V, and VI, probably because almost all of their territory is at 1600 m or more above sea level, an altitude not frequented by the *A. aegypti* mosquito in Mexico.[32,33] Finally, another factor that may explain the high number of cases reported in jurisdictions II and IV is migratory activity. These jurisdictions are close to the state of Chiapas which is on the border with Central America (Guatemala) and they are subject to significant migratory movement that facilitates the spread of DEN virus. Furthermore, Huatulco, Salina Cruz, Juchitán, Puerto Escondido and Pinotepa Nacional, where the most cases were reported, are located in both jurisdictions and they are important urban and/or tourist centres with high migration activity that may facilitate the importing and exporting of DEN virus.[11]

In our study the most affected part of the population was that between 11 and 15 years old, which is consistent with previous reports.[3,22,28] Interestingly, in groups aged more than 16 years old, the DHF cases were more frequent in men than in women, as reported in India,[39,31] Saudi Arabia,[34] Pakistan,[35] and Mexico (state of Yucatán).[22,36] The difference was statistically significant ($p < 0.001$). At present we do not know the cause of this phenomenon but several factors could be involved, such as a better immune response and/or lower increase in capillary permeability in women,[3] the HLA haplotype in the Mexican population,[37] and mutations in the FcγIIA receptor.[4] Population activities may also be involved since the men of Oaxaca travel between locations more often than women and as a result they may be exposed to more than one DEN virus serotype.[3] On the other hand, men undertake more outdoor activities than women,[38–40] and thus they are in contact with the vector more frequently. In addition, it has recently been reported that pupal production[41] and the number of adults[42] is higher outdoors than inside houses.

Co-infection with two DEN virus serotypes has been reported since 1985, DEN2–3 being the most frequent combination detected[20,29,43–51] and this phenomenon is facilitated by the circulation of different DEN serotypes in a single population. Indonesia and India have reported the highest number of cases of co-infections, which is coincident with their high morbidity from DEN. In this study, one of the cases came from jurisdiction III, one of the most affected regions in Oaxaca and where the four serotypes are circulating. Unfortunately there is not enough information to establish a definitive relationship between the severity of the infection and the concomitant presence of the two serotypes.[20,45,49] In this study, of the two cases detected, one developed DF, and the other DHF, and more studies should be performed to evaluate the relevance of these findings.

Several epidemiological studies have been performed in Mexico related with dengue virus but they have generally focused on the relationship between the presence of the virus and a specific factor like climate conditions, socioeconomic level of the population, vector densities, etc.[25,27,30,33] In this study we analysed the epidemiology of DEN virus in a specific state of Mexico in a more global way, considering all the possible factors that may be involved. This information will be useful to establish and/or modify control programmes in the state and provides important data about the epidemiology of DEN virus in the country.

Conflicts of interest

No conflicts of interest have been identified.

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