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EPIDEMIOLOGY

Medical Examiner Samples: a Source for Dengue Surveillance

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Post-mortem medical examiner samples may be useful for sentinel surveillance of disorders usually detected by antibody determinations on specimens from ill patients or from surveys. We found anti-dengue IgM positivity in 3% (23/780) and anti-dengue IgG positivity in 77% (597/777) of sera obtained at the Puerto Rico medical examiner (Institute of Forensic Sciences) in December 2000, April 2001, and October 2001. This approach may be a useful alternative for estimating the population prevalence of serologic markers for dengue and other infectious diseases.

Key words: Dengue, Forensic science, Medical examiner, Public health surveillance, Sentinel sites, Seroprevalence

C ase reports of laboratory-positive dengue cases in Puerto Rico and elsewhere have identified patients, including some with fatal outcomes, with severe concurrent illnesses (1-3). These results present an obstacle to the elucidation of the primary cause of death and to the evaluation of dengue severity and the quality of dengue treatment in a community. Since anti-dengue IgM antibodies (IgM) can be detected up to 3 months or more after infection, a large proportion of the population after an epidemic may be IgM positive after regaining health (4-5). This consideration is reflected in the current Centers for Disease Control and Prevention (CDC) and World Health Organization definitions for disease surveillance, which indicate a "probable" category for cases with only a positive IgM result and a "confirmed" category for cases with virus identification or four-fold increase in antibody titer in paired specimens (6-7). Anti-dengue IgG antibodies (IgG), in contrast, are a marker of a previous dengue infection at an undetermined time and their prevalence in the very young measures the intensity of recent transmission. Sera from representative samples of human populations are difficult to obtain, because of cost, ethical, and administrative considerations. We studied a surrogate population — a sample of cases submitted to the medical examiner's office — to determine its potential as a sentinel for dengue deaths and its utility to estimate the prevalence of anti-dengue IgM and IgG antibodies in the Puerto Rico population. Sentinel surveillance provides limited but useful data on disease occurrence. Even if the signals are not representative of an entire population, they may provide early warning, or may facilitate the detection of an unusual occurrence (8).

Methods

We obtained sera and anonymous data (age, sex, municipality of residence, manner of death, cause of death, date of death) from post-mortem examinations of Puerto Rico residents that resulted in autopsy or collection of blood for toxicology tests, including fatalities from intentional and natural causes, conducted by the Puerto Rico Institute of Forensic Sciences in December 2000, April 2001, and October 2001. These months were associated with the peaks (December and October) and trough (April) of seasonal dengue incidence in Puerto Rico in that period. Using the same anti-dengue IgM capture ELISA and IgG ELISA methods used as part of the laboratory-based surveillance system, we tested sera from 94% (780 for IgM and 777 for IgG, 782 total persons) of 835 post-mortem examinations in which blood was obtained in adequate quantity (9-10). The statistical significance of frequency differences between groups was tested using the Chi-square calculated by Epi-Info 6.04 (11). Since the data belonged to deceased individuals, the research did not require review by an institution's committee for oversight on human subject participation in research, but confidentiality was nevertheless preserved.

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Results

The decedents tested for anti-dengue antibodies came from 76 of Puerto Rico's 78 municipalities, and 30% (229/774) were residents of the San Juan metropolitan area in comparison with 23% of the population in the 2000 Census. Decedents were more often male (671/782, 86%) and older (median age for males, 37 years, range 18 days to 100 years; for females, 42 years, range 15 days to 87 years) than the general population (median age for males, 31 years, females 34 years). Among the 758 tested decedents with a defined manner of death, females died significantly more often (80%, 83/104) of natural or accidental causes than males (60%, 391/654), significantly less often died from homicide (12%, 12/104, and 31%, 206/654, respectively), but similarly frequently died from suicide (9%, 9/104 and 57/654).

Overall IgM positivity was 3% (23/780, 95% confidence limit 2-4%) and did not vary significantly by month of examination, in contrast with the monthly incidence of laboratory-positive dengue cases detected during these months by surveillance: 3 (December), 1 (April), and 12 (October) per 100,000 persons. IgM positivity among decedents with a defined manner of death was 6% (4/66) for suicides, 4% (11/307) for "accidents," 3% (5/166) for natural causes, and 1% (2/217) for homicides, but the differences are not statistically significant. IgM prevalence did not vary significantly by sex, but was found only in adults (median age 42 years, range 23-86 years), and in residents of 17 (22%) of 78 municipalities. Sixteen (70%) of the 23 IgM positive decedents came from municipalities with high reported dengue incidence (≥ 1.1 /1,000 population) in 2000 or 2001.

Among the 23 IgM positive cases, the manner of death was deemed "accidental" in 11 (48%: 6 with physical trauma, 3 intoxicated with alcohol, opiates or both, one drowning, and one with dehydration and mental disturbances), natural in 5 (22%: cerebrovascular accident, arrhythmia, coronary disease, brain hemorrhage, pneumonia), suicide in 4 (17%: 3 by hanging, one by bullet wound), homicide in 2 (9%: bullet wounds), and 1 (4%) died from head trauma of unknown cause. Compared to the 757 IgM-negatives, the manner of death of the IgM positive cases was more likely to be classified as suicide (17% vs. 8%) and less likely to be classified as homicide (9% vs. 28%) and about as likely to be classified as "accidental" (48% vs. 39%) or natural (22% vs. 21%), but none of the differences are statistically significant. There was no appreciable geographic clustering by manner of death among IgM positives.

Overall IgG positivity was 77% (597/777, 95% CL 74-80%) and did not vary significantly by sex or manner of death. IgG negatives came from 60 municipalities. In only two municipalities were all decedents IgG negative (Aibonito 5/5, Villalba 2/2, both in the central mountains). The seven (54%) IgG positives among 13 infants under one year of age were aged 2 weeks to three months, so IgG positivity was possibly due to the transfer of maternal antibody. IgG positivity by age group of decedent gradually increased from 14% (ages 1-4 years, 1/7) to 25% (5-9 years, 1/4) and then became relatively stable, at 75% (10-14 years, 3/4), 67% (15-19 years, 29/43), 68% (age 20-24, 78/114), and 77-89% in older age groups. IgG seroprevalence estimates for age groups younger than 15 years, because of the small number of decedents, have broad confidence intervals that overlap 50%.

Conclusions

The first objective of this study was to determine the potential of medical examiner samples to provide sentinel data for dengue surveillance. We found a high (3%) prevalence of anti-dengue IgM, indicating that markers for acute dengue infection are not rare in the population under study. We also tried to determine the frequency of finding a positive IgM result in a fatal case that was not referred for dengue diagnosis, to guide our interpretation of results in patients with apparent multiple fatal etiologies. Our sample provided positivity estimates ranging from 1% (among victims of homicide) to 6% (among deaths attributed to suicide). The manner of death least likely to be related to a clinically undocumented acute dengue infection is homicide, but it was still associated with an IgM positivity of 1%. In consequence, since the chance that a single result of anti-dengue IgM positivity unrelated to the cause of death may be close to 1%, other causes must be carefully considered when assigning etiology to suspected dengue fatalities in Puerto Rico.

Two questions arose from these data, and relate to the absence of IgM positive cases in the very young, and the possibly higher prevalence of IgM among suicides. The first may be due to biases in the source of tested decedents: less than 10% were under 20 years of age, and possibly pediatric fatalities are more likely to occur in a hospital and be excluded from the legal requirement of medical examiner testing. The difference in the frequency of suicides among IgM positives (17%, 4/23) compared to IgM negatives (8%, 62/757) would require a sample twice as large as ours to reach statistical significance. The finding is intriguing, given the reports of depression and even suicide following an acute dengue infection (12).

A third objective of our study was to determine the utility of testing medical examiner cases as a surrogate for a population-based serosurvey for IgG. Our results did

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not add to our previous knowledge, because the small number of samples from the younger age groups resulted in estimates with broad margins of error. Age-stratified testing of samples would be a more efficient approach to estimate IgG positivity.

The principal limitations of this pilot study are its small sample size and the biases inherent in the types of cases from which the samples were obtained. Post-mortem samples came from an older and probably less affluent and educated group than the general population, which would bias our results towards overestimation of prevalence. Both limitations may in part explain why IgM positivity was found in all manner of deaths (1-6%) and that there was no significant reduction in overall IgM positivity even in the month that marks the nadir (April) of the dengue "season" in Puerto Rico.

A smaller study in Maryland (414 serum samples from medical examiner cases) demonstrated the high prevalence (33%) of markers to any of four parenterally transmitted viruses (HIV-1, HTLV-I/II, HBV, HCV) and the importance of the use of universal precautions while conducting post mortem examinations (13). Our study demonstrates the high level of IgM prevalence among the decedents studied, and the utility of post-mortem medical examiner samples as a variant of the usual (clinical) sentinel sites, useful for detection or monitoring particular events. These methods, improved by the use of stratified sampling and a larger number of cases, may be useful in deriving estimates of incidence and seroprevalence for dengue and other infectious diseases usually diagnosed by antibody determinations.

Acknowledgments

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