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Difficulties in the differential diagnosis of dengue and leptospirosis in Guayaquil

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Abstract

Leptospirosis and dengue fever are infectious diseases that co-occur during rainy seasons and both produce similar clinical signs. The purpose of this study was to assess the relative frequency of leptospirosis and dengue fever during a rainy season and the clinical difficulty to distinguish them. Blood samples from febrile patients in Guayaquil were obtained during the rainy season of 2008 and were analyzed by IgM ELISA for both diseases. Additionally, retrospective data (2003-2007) from febrile patients who attended one of largest public hospitals in Guayaquil were obtained. From 135 febrile patients samples, 15 (11.1%) were positive to leptospirosis; 36 (26,7%) to dengue fever; 3 (2.2%) to both pathogens and 81 (60%) were negative for both. Based on clinical diagnosis, cases were classified 68.1% as dengue fever; 20.7% as leptospirosis; 9.6% as malaria and 1.5% as other. However, 60% of patients clinically diagnosed as dengue had only antibodies against *Leptospira* and 25% patients diagnosed as leptospirosis had antibodies to dengue virus. The hospital archives indicated that 72.8% of patients clinically diagnosed as dengue fever had antibodies to *Leptospira* and not to dengue virus. The results suggest the two diseases are often misidentified which is a serious problem because both diseases require different medical treatment.

Keywords. 5-6 Leptospirosis, Dengue, ELISA, MAT

Resumen

La leptospirosis y dengue son enfermedades que presentan una sintomatología muy similar y ocurren durante las épocas lluviosas. El propósito del presente trabajo fue investigar la frecuencia de leptospirosis en la población de los barrios marginales de Guayaquil y el grado de dificultad que existe para distinguir clínicamente leptospirosis de dengue. Muestras de sangre de pacientes febriles provenientes de los barrios pobres de Guayaquil fueron colectadas durante la estación lluviosa del año 2008. Las muestras se sometieron a análisis de ELISA IgM para dengue y leptospirosis. Adicionalmente se obtuvieron datos retrospectivos (2003-2007) de historias clínicas de pacientes que acudieron a uno de los hospitales públicos más grandes de Guayaquil. De un total de 135 pacientes febriles, 15 (11.1%) fueron positivos a leptospirosis por ELISA, 36 (26.7%) fueron positivos a dengue, 3 (2.2 %) fueron positivos a ambos patógenos y 81 (60%) fueron negativos para los dos. Sin embargo, 60% de los pacientes diagnosticados clínicamente como dengue tuvieron anticuerpos contra *Leptospira* y no para dengue y 25% pacientes diagnosticados como leptospirosis tuvieron anticuerpos contra el virus de dengue y no para *Leptospira*. Adicionalmente, los archivos hospitalarios (2003-2007) indicaron que 72.8% de los pacientes diagnosticados clínicamente como dengue tuvieron anticuerpos contra *Leptospira* y carecían de anticuerpos contra virus de dengue. Los resultados sugieren que ambas enfermedades son frecuentemente identificadas erróneamente lo que representa un problema grave de salud pública pues ambas enfermedades requieren un tratamiento médico completamente distinto.

Palabras Clave. 5-6 Leptospirosis, Dengue, ELISA, MAT

Introduction

Leptospirosis, a zoonosis that occurs throughout the world, especially in tropical climates [1–4] is caused by any of the eleven pathogenic species of the spirochete *Leptospira* [5] and produces symptoms ranging from flu-like to life threatening hemorrhagic syndromes [6, 7].

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Although leptospirosis is classically associated to individuals working in close proximity to domestic and wild animals [8, 9] recent reports show increasing numbers of cases in people exposed to fresh water in urban settings or during recreational activities associated with fresh water [2, 4, 10–12]. In developing countries the disease causes a significant health burden in low-income and rural populations during rainy seasons [2, 6, 12, 13]. Urine from animal reservoirs (dogs, rats, pigs, cattle and wild mammals) contaminates rivers and puddles were humans and other animals get infected through water contact with lacerated skin or mucosa [4, 7]. People inhabiting low income communities are especially vulnerable to this infection due to deficient sewage systems, poor drainage, and large number of animal carriers [2, 6, 12–14].

Dengue viruses are transmitted by mosquitoes of the genus *Aedes*, which are widespread in tropical and subtropical climates [15–17]. There are four distinct serotypes of dengue arbovirus: DENV1, DENV2, DENV3, DENV4 [15, 16]. The incidence of this illness tends to increase during rainy seasons due to the presence of water collections which favor the multiplication of mosquitoes [18, 19]. Similar to leptospirosis, dengue viruses can cause symptoms ranging from the classical self-limiting flu-like disease to a severe, potentially fatal hemorrhagic syndrome known as dengue shock syndrome [17–19]. Dengue is also an emerging disease because of the recent geographic expansion of the vector especially in Western Pacific Regions [16]. Multiple factors have contributed to the recent dissemination of the disease including rapid deficient drainage, population mobility, poor vector control, and more intense El Niño phenomena [16, 20, 21].

Leptospirosis and dengue fever are two diseases that co-occur in rainy seasons in tropical cities and share many symptomatic features and leptospirosis cases are often misdiagnosed as dengue [22–24]. The purpose of this study was to assess the burden of leptospirosis in the slums of Guayaquil and its possible confusion with dengue. Anti-leptospiral and anti-dengue virus IgM antibodies were investigated in blood from febrile patients inhabiting a slum of Guayaquil during rainy season 2008. Additionally we did a retrospective review of the clinical archives of the main infectious diseases public hospital of Guayaquil concerning these two infectious diseases.

Materials and Méthods

Febrile patients

This project was approved by the bioethics committee of Universidad San Francisco de Quito. Febrile patients, residing at the Bastión Popular and Pascuales areas of Guayaquil, were detected by the health brigades of the Ecuadorian Ministry of Health and asked to fill out an informed consent and to donate a drop of blood during rainy season of 2008. Additionally, patients were asked to fill out a survey form in order to identify risk factors associated to leptospirosis and Epi Info 3.4 package was used to calculate the Odds Ratio. Sample exclusion criteria were patients showing diarrheic symptoms, common cold symptoms, and patients younger than 4 years of age. Blood drops [25] were allowed to dry for at least 4 hours, wrapped with waxed paper and stored with silica gel for up to two weeks at room temperature and then stored at -20°C until processing [26, 27].

Serological tests

This procedure was previously utilized for HIV [28] and Dengue [27] surveillance. A 6 mm punch of the blood spot in filter paper was eluted in 150 μl of PBS and 29 μl of the eluted serum was placed in a well containing 71 μl of serum diluent reagent [26]. ELISA kits used for both leptospirosis and dengue were PanBio Pty. Ltd, Australia. Plates were covered and incubated at 37°C for 30 minutes, then washed with wash buffer 6 times, and allowed to dry. Instructions provided by the manufacturer were followed thereafter.

A microagglutination test [29] was performed in leptospiral ELISA positive sera and we used sera elution-correction previously described in order to obtain 1:100 sera dilution.

Retrospective hospital data

Partial data from leptospirosis suspected patients attending to Hospital de Infectología José Daniel Rodríguez Maridueña de Guayaquil during the last 5 years (2003-2008) was obtained and analyzed based on interview field form.

Results

A total of 135 specimens were obtained from febrile patients attending local health centers at Bastión popular and Pascuales, 15 (11.1%) were positive to *Leptospira* and 36 (26.7%) were positive for dengue, 3 (2.2%) were positive for both antigens and 81 (60%) were negative to both by IgM ELISA. Dengue fever was clinically diagnosed in 68.1% of the febrile patients, leptospirosis in 20.7%, malaria 9.6%, and other 1.5% (Table 1). Clinical diagnosis of these patients showed little correspondence to their serologic reactivity; 9 patients (60%) clinically diagnosed as dengue cases showed positive IgM titers to *Leptospira* and no reactivity to dengue. Conversely 25% of patients clinically diagnosed as leptospirosis cases had positive IgM titers to dengue virus and did not show any reactivity to leptospiral test. *Leptospira* ELISA positive sera showed highest MAT titers to serovar Patoc in 7 sera, serovar Panama in 3 sera, serovar Pomona in 3 sera, serovar Icterohaemorragiae in 2 sera, serovar Tarassovi in 2 sera and serovar Autumnalis in 1 serum.

Archival clinical data from the hospital corresponding to the period 2003 to 2007 showed that the number of

Clinical Diagnosis	Number of ELISA Positive Sera			
	Dengue	Leptospirosis	Both	None
Leptospirosis	9	4	1	14
Dengue Fever	23	9	2	58
Malaria	4	2	0	7
Other	0	0	0	2
Total	36	15	3	81

Table 1: Clinical diagnosis compared to serologic results. Numbers correspond to febrile patients from slums in Guayaquil (2008) diagnosed clinically and by ELISA.

suspected dengue cases was 59 and the number of suspected leptospirosis cases was 29. Inconsistencies between clinical diagnosis and laboratory results were also evident in these records; 72.88% of patients clinically diagnosed as dengue showed positive serology to *Leptospira* and no reactivity to dengue virus and 17.2% of patients clinically diagnosed as leptospirosis had positive serology to dengue virus and no reactivity to *Leptospira*, 25.4% of patients clinically diagnosed as dengue had positive sera for both, *Leptospira* and dengue virus, 1 case reported as dengue fever, and 5 reported as leptospirosis were negative for both ELISA tests (Table 2).

Symptoms associated to leptospirosis ELISA positive and dengue ELISA positive sera were very similar (data not shown) and the main risk factors associated (OR > 2.0) to sera positive to leptospirosis were: contact with contaminated drain water at home, flooding water near to home and evidence of rats at home or at work. Risk factor associated to sera positive to dengue ELISA were garbage close to home, proximity to drain water and evidence of rats at home.

Conclusions

The data presented here suggests that dengue and leptospirosis are two very common infectious diseases that co-occur during rainy seasons in poor communities in Guayaquil. Dengue reactivity was more frequent than leptospirosis in sera from febrile patients during the rainy season of 2008. The results also suggested that 68.5% of the febrile cases (classified as dengue or leptospirosis) were misdiagnosed and the discrepancy between the clinical diagnosis and the serology may reflect the similarity of the clinical manifestations of these two diseases. Misdiagnosis of these two diseases is an important public health concern because both diseases require different therapeutic approaches. Clinical complications of leptospirosis can be easily avoided by using antibiotics [6, 9, 30].

Dengue fever and leptospirosis also share some risk factors associated to low income and rapidly growing communities in tropical cities during rainy seasons (accumulation of water and deficient drainage). It is important to take into account that some of these conditions may worsen by global warming [14, 15, 20].

In rainy season of 2008 we found more febrile patients with evidence of dengue than patients with evidence of leptospirosis, however these proportions may vary in different locations and different years; a recent report showed that leptospirosis is the most frequent infection in febrile patients inhabiting towns in the Ecuadorian Amazon region [31] and in the rainy season 1997-1998 a leptospirosis outbreak in Guayaquil produced 11.8% mortality and high hospitalization rates [32].

Clinical Diagnosis	Number of ELISA Positive Sera				
	Dengue	Leptospirosis	Both	None	Total
Dengue Fever	–	43	15	1	59
Leptospirosis	5	4	15	5	29
Total	5	47	30	6	88

Table 2: Clinical diagnosis compared to serologic results in archival clinical data from leptospirosis suspected patients attending Hospital de Infectología José Daniel Rodrigues Maridueña (Guayaquil) 2003-2008

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