Short Communication

Improvement of leptospirosis surveillance in remote Pacific islands using serum spotted on filter paper

Didier Musso a, *, Claudine Roche a, Maria Marfel b, Martin Bel c, Eric J. Nilles d, Van-Mai Cao-Lormeau a

a Institut Louis Malardé, Tahiti, French Polynesia
b Yap State Hospital, Yap State, Federated States of Micronesia
c Waab Health Center, Yap State, Federated States of Micronesia
d World Health Organization, Fiji

A R T I C L E   I N F O

Article history:
Received 8 October 2013
Received in revised form 19 November 2013
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Leptospirosis
Dengue
Neglected disease
PCR
Filter paper

S U M M A R Y

Objectives: Leptospirosis is a serious neglected disease in the Pacific. Because sensitive and specific laboratory tests are largely unavailable, the burden of disease and epidemiological data are often unreliable and do not allow informed disease prioritization and efficient control. We report the use of serum spotted on filter paper to improve the surveillance of leptospirosis in remote and resource-limited settings.

Methods: A total of 172 acute-phase serum samples collected from patients with suspected dengue at Yap State Hospital, Federated States of Micronesia, were spotted on filter paper and sent by regular mail to the Institut Louis Malardé, French Polynesia. Real-time PCR protocols for dengue and leptospirosis confirmation were performed on all specimens.

Results: A total of five leptospirosis infections were detected amongst the patients with suspected dengue.

Conclusions: This study confirms the use of filter paper as a convenient tool to improve leptospirosis surveillance capacity in remote areas. New surveillance strategies, notably based on the regular use of this type of tool, are essential to more adequately describe the epidemiology and burden of neglected diseases.

© 2013 The Authors. Published by Elsevier Ltd on behalf of International Society for Infectious Diseases. Open access under CC BY-NC-ND license.

1. Introduction

Leptospirosis is a severe neglected disease in much of the tropics, including the Pacific region. The epidemiology and disease burden in most endemic countries is poorly understood, largely due to the absence of the advanced laboratory facilities that are required for the diagnosis of acute-phase leptospirosis. Leptospirosis is often clinically indistinguishable from several other acute febrile illnesses, and laboratory confirmation is essential. Novel protocols adapted to remote and resource-poor regions are necessary to improve the surveillance of leptospirosis and other neglected diseases. In the present study, we evaluated the use of serum spotted on filter paper for confirmation of acute-phase leptospirosis in a remote Pacific setting.

2. Methods

One hundred seventy-two serum samples were collected for diagnosis purposes during a dengue virus serotype 2 (DENV-2) outbreak, from February through October 2012, from patients presenting to Yap State Hospital, Federated States of Micronesia. These patients had symptoms for less than 5 days. During the study period, the diagnosis of an acute febrile illness in Yap relied on physical examination and basic laboratory tests, including blood count, erythrocyte sedimentation rate, urine, blood, and stool culture, and dengue rapid diagnostic tests (Dengue Duo, Standard Diagnostics Inc., Korea; detecting dengue virus-specific non-structural 1 antigen (NS1Ag) and anti-dengue immunoglobulin M and G class antibodies). Leptospirosis diagnostic testing was not available.
Approximately 250 μl of serum sample was spotted onto filter paper (Sorobuvard, LDA22; Ploufragan, France), which was then dried at room temperature, placed into an individual Ziploc plastic bag (Dutsch, France), and stored at 4 °C until shipment by standard mail at ambient temperatures to the Institut Louis Malardé, French Polynesia.

In French Polynesia, the serum spotted on the filter paper was submitted to nucleic acid extraction using the easyMAG extraction system (bioMérieux). DENV amplification was performed using a TaqMan multiplex real-time reverse transcription PCR (RT-PCR), and *Leptospira* amplification was done using a TaqMan real-time PCR targeting the lipL32 gene, as previously reported, using a CFX96 Bio-Rad thermocycler.\(^5\) Two negative controls (non-spotted filter paper and sterile water) and two positive controls (*Leptospira* and DENV) were included in each run.

This study was a non-interventional study conducted on serum initially collected for the purpose of diagnosis of an acute febrile illness, on the medical instructions of a physician.

### 3. Results

Delivery of the spotted serum filter paper by standard mail to French Polynesia took approximately 4 weeks. A DENV-2 infection was confirmed by real-time RT-PCR in 15 sera: 12 had previously tested NS1Ag-positive and three had tested NS1Ag-negative by rapid diagnosis tests in Yap. *Leptospira* was detected by real-time PCR in five sera. No co-infections were diagnosed. Amplification was negative for all negative controls and positive for all positive controls. Clinical features and laboratory results for the five leptospirosis-positive patients are reported in Table 1.

### 4. Discussion

Oceania exhibits a significant burden of leptospirosis and, with Southeast Asia, appears to have the highest incidence of this disease.\(^6\) In addition, due to the lack of laboratory capacity, the disease burden of leptospirosis in most of the Pacific, like much of the tropics, is underestimated and the epidemiology of the disease is largely unknown.\(^7\) Access to accurate laboratory tests in addition to rigorous investigational studies are necessary to evaluate the burden of the disease. The confirmation of leptospirosis is difficult in most leptospirosis-endemic countries, and a sensitive and specific diagnostic tool for confirmation of acute-phase disease is urgently needed. Reference laboratories can offer support for the diagnosis of leptospirosis, including PCR testing on acute-phase samples; however, the shipment of specimens in freezing conditions is expensive, subject to restrictive regulations, and rarely possible from remote areas. Serum spotted on filter paper is not subjected to dangerous goods regulation and represents a cheap and convenient tool for sample transportation.\(^8\)

We report the successful detection of five leptospirosis patients by performing PCR on serum spotted on filter paper collected from patients with suspected dengue. Of these patients, only two received a first-line leptospirosis antibiotic treatment. Thanks to this retrospective case confirmation, it was possible to alert the local health care workers in Yap to the risk of unrecognized leptospirosis infection during dengue outbreaks, as reported previously.\(^9\)

Because diagnostic testing of leptospirosis was not available in Yap, it was not possible to evaluate the sensitivity of our protocol by comparison to culture.

Many Pacific island countries have limited resources to definitively differentiate undifferentiated acute febrile illnesses, and as a result there is a need for an efficient regional surveillance system targeting endemic and epidemic pathogens.\(^10\) The surveillance protocol we have described allows remote and resource-limited settings to collect, store, and ship samples for advanced molecular investigations. It may also contribute to improve locally-used diagnosis algorithms based on clinical presentation and available laboratory tools. We confirm that both viral and bacterial diagnosis and identification could be performed from a single sample using serum dried on filter paper. This protocol is a potentially powerful tool to enhance the surveillance capacity of leptospirosis and other infectious diseases in the Pacific and in other remote or resource-poor regions. Novel diagnostic strategies such as this protocol are essential to more adequately describe the epidemiology and burden of leptospirosis and potentially other neglected diseases.

**Ethical approval:** The study was approved by the Ethics Committee of French Polynesia (reference number 61/CEPF).

**Conflict of interest:** All authors disclose no financial or personal relationships with other people or organizations that could have inappropriately influenced this work.

### References


