**INTRODUCTION**

*Bartonella bacilliformis* is the etiological agent of a life-threatening illness. Thin blood smear is the most common diagnostic method for acute infection in endemic areas of Peru but remains of limited value because of low sensitivity. The aim of this study was to adapt a *B. bacilliformis*-specific real-time polymerase chain reaction (PCR) assay for use with dried blood spots (DBS) as a sampling method and assess its performance and use for the diagnosis and surveillance of acute *Bartonella* infection. Only two of 65 children (3%) that participated in this study had positive blood smears for *B. bacilliformis*, whereas 16 (including these two) were positive by PCR performed on DBS samples (24.6%). The use of DBS in combination with *B. bacilliformis*-specific PCR could be a useful tool for public health in identifying and monitoring outbreaks of infection and designing control programs to reduce the burden of this life-threatening illness.

**METHODS**

Between November 2011 and July 2012, febrile children (temperature ≥ 37.5°C) ≤ 10 years of age presenting at two outpatient clinics in the Yautan region of Peru were recruited, after written consent of parents or guardians was obtained.
Ethical approval for this study was obtained at the London School of Hygiene and Tropical Medicine and UPCH.

RESULTS

Sixty-five febrile children presenting at two clinics in Yautan province were included in the study. The average age was 4.1 years (range 1–10) and 31 of the 65 children (48%) were male with an average temperature of 38.3°C (37–39.5°C) upon arrival at the clinic. The time between onset of fever and arrival at the clinic varied from 1 to 5 days, with an average of 2.4 days.

Only two of 65 children (3%) that participated in this study had positive blood smears for *B. bacilliformis*, whereas 16 were positive by PCR performed on DBS samples (24.6%) (Figure 1). All samples were extracted and tested twice. The internal PCR positive and negative controls performed as expected in all experiments. To confirm that *B. bacilliformis* was detected, sequencing was performed. The two positive blood smear samples and an additional two PCR positive were successfully sequenced showing 100% match with strain KC583 (NCBI:CP000524).

Even though blood smears are known to have low sensitivity, they are still commonly used in Peru and therefore used as a reference in this study. Using blood smears as a comparator, the sensitivity and specificity of the PCR with DBS samples was 100% (95% confidence interval [CI]: 34–100%) and 78% (95% CI: 66–86%), respectively. Sequence results indicate however that at least two samples negative by blood smears, were in fact positive for *B. bacilliformis*.

DISCUSSION

In certain regions of Peru human bartonellosis is endemic, but outbreaks have been reported in non-endemic regions; the method developed in this study could be a useful surveillance tool for endemic regions and particularly for outbreak investigations. The DBS obviates the need for cold chain transportation requirements and thus greatly simplifies sample collection strategies for surveillance or outbreak investigations in remote settings. The DBS requires a small sample volume and minimal technical expertise to prepare, making this an acceptable and cost-effective method of collecting blood samples.

Although sample size of our study is small, screening with DBS and PCR appears to be a reliable, sensitive, and specific method for the diagnosis of bartonellosis. The two *B. bacilliformis* positive cases that were detected by blood smear were severely ill children with anemia (< 3 g/dL) and had a very high bacterial load in the blood stream (> 100,000 c/mL). The low sensitivity of blood smears found in this study is in line with other studies. Four of the 16 positive samples were sequenced, which were confirmed by gene sequence as *B. bacilliformis*.

The DBS samples can also be used for further analyses, such as monitoring quinolone resistance and, given the recent discovery of *Bartonella rochalimae* causing illness related to Oroya fever, to differentiate between *Bartonella* species. Recent advances in the development of simpler nucleic acid amplification and sequencing assays have enabled these assays to be more widely available in the developing world. The use of DBS samples in combination with pathogen-specific nucleic acid amplification assays such as PCR could be a useful tool for disease control programs in identifying and monitoring disease outbreaks and designing strategies to reduce the burden of bartonellosis.

Received May 8, 2013. Accepted for publication August 5, 2013. Published online September 16, 2013.

Acknowledgments: We thank all study participants and clinicians for their participation in this study. Additionally, the authors thank Cesar Ugarte-Gil, Nelson Solórzano, Nuria Sanchez Clemente, Gisela Henriques, Joyce Holierhoek, and Mathilde Boon for their contribution to the study.

Financial support: This study was funded by the UBS Optimus Foundation.

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