Short Report: Dried Blood Spots for qPCR Diagnosis of Acute *Bartonella bacilliformis* Infection

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**Abstract.** *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.

**INTRODUCTION**

*Bartonella bacilliformis* is the etiological agent of a life-threatening bacterial illness “Carrion’s disease” or human bartonellosis, which occurs in the inter-Andean regions of Peru, Ecuador, and Colombia, with sporadic cases in Bolivia and Chile; this neglected infectious disease is thought to be transmitted by the sand-fly *Lutzomyia verrucarum*. Climate change influences the geographical distribution and is associated with outbreaks, as seen with El Niño. Infection is biphasic with acute infection, causing severe anemia and fever, known as Oroya fever, and a chronic infection causing skin manifestations (*verruga peruana*). Relatively little is known about the epidemiological situation other than that incidence appears to be both geographically and temporally focal. One study, dating back from 2002, indicated an incidence of 12.7/100 person years in a Peruvian mountain valley community, with highest rates in children <5 years of age.

Thin blood smear is the most common diagnostic method for Oroya fever in endemic areas of Peru but remains of limited value caused by low sensitivity. Diagnosis is therefore usually clinical. Although sensitive serological assays for the detection of chronic infection have been developed, no suitable diagnostic methods currently exist for the detection of acute *B. bacilliformis* infection. The DNA-based detection methods currently available require restriction fragment length polymorphism analysis or reverse line blotting to differentiate *Bartonella* species, which are labor intensive, slow, and complex procedures.

The aim of this study was to adapt a *B. bacilliformis*-specific real-time polymerase chain reaction (PCR) for use with dried blood spots (DBS) and assess its performance and use for the diagnosis and surveillance of acute *Bartonella* infection.

**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.

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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 KC853 (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 gm/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.

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