Long time survival of *Bartonella bacilliformis* in blood stored at 4 ºC. A risk for blood transfusions

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Dear Sir,

Bartonella bacilliformis is an autochthonous bacterium from rural Andean areas of Peru, Ecuador and Colombia affecting less-favoured populations causing the so-called Carrion's disease. Carrion's disease is a re-emerging illness that has two clinically distinct phases: an acute or haematic phase, known as Oroya Fever characterised by fever and severe anaemia that may be fatal in the absence or delay of antibiotic treatment and an eruptive or tissue phase, known as Peruvian Wart[1]. Additionally, in endemic areas, the presence of asymptomatic individuals has been reported in whom *B. bacilliformis* may be recovered or detected from blood samples[1].

B. bacilliformis is a fastidious low-growth microorganism, requiring blood-enriched media, controlled temperature and specific atmospheric conditions to grow. Additionally the reported infections rates are around 7-20%[1]. These facts limit the utility of bacterial culture as a diagnostic tool.

When Oroya Fever is suspected, the most common diagnostic method in endemic Peruvian areas is the Giemsa stain of a blood smear, in which the blue-coloured extra or intra-erythrocytic bacilli or coco-bacilli can be observed. Unfortunately, in centres lacking adequate training in this diagnostic technique, the sensitivity can be as low as 36%[1]. The limitations of blood culture together with the potential low sensitivity of Giemsa-stained blood smears and the severity of the illness usually results in empiric antibiotic treatments based on the clinical diagnosis and government guidance for treatment of the illness[1].

It has been reported that other members of the genus *Bartonella*, such as *B. henselae* are able to survive more than 35 days in red blood cell units stored at 4 ºC, and it has also been detected in blood donors and suspicious cases of transfusion infections have been described[2]. Meanwhile, *B. bacilliformis,* contagion by direct contact with infected blood or tissues has been reported several times either in experimental or accidental manner, including at least one case of possible post-transfusion acquisition[1,3]. To our knowledge, only one study from 1926 reported the ability of *B. bacilliformis* to survive in blood samples of experimentally infected monkeys kept at 4 ºC for at least 152 days[4]. These results suggest that the risk of *B. bacilliformis* infection by transfusion may be underestimated, especially when no specific test for detection in blood donors is performed in endemic areas.

Thus, the aim of this report was to assess the ability of *B. bacilliformis* to survive long periods of time in infected human blood.

Fifty-five peripheral blood samples of patients with a clinical diagnosis of Carrion's disease and a confirmatory positive Giemsa-stained blood smear were stored at 4 ºC for a minimum of 24 months. Both after collection and the end of this storage time, the samples were cultured on Columbia Agar adding 10% of sheep blood and incubated at 28 ºC for a period of 10 weeks. Every 14 days the plates were visually inspected to detect any bacterial growth.

Recovered microorganisms were identified by molecular tools. Thus, DNA was extracted using a commercial kit (High Pure Template Peparation Kit, Roche Applied Science, Germany) and a fragment of 1503 bp of the *16s RNA* gene was amplified as previously described[5]. The amplified products were sequenced (Macrogen, Seoul, Korea).

The initial culture showed the growth of 11 out of 55 samples (20%) after 2-5 weeks of incubation, requiring an average of 3.9 weeks to obtain positive growth, while after 24-30 months of storage at 4 ºC, 6 out of these 55 (11%) samples, all belonging to the 11 with previous positive cultures (54.5%), showed bacterial growth requiring between 4 and 10 weeks of incubation. Thus 2 out of these 6 cultures were positives after 4 weeks, while

the remaining isolates were positive after 6-10 weeks of growth (Table I) requiring an average time of 6.6 weeks to grow. Thus after storage the average time needed to obtain a positive culture was 71% longer that at the time of collection. These results suggests the possibility that the rate of culture positivity will be increase if incubation time is more than 10 weeks. Finally, none of the samples in which no growth was observed at the time of collection showed any growth after storage at 4 ºC.

In all cases the microorganisms grown were morphologically compatible with *B. bacilliformis* and all were identified as *B. bacilliformis* by analysis of the *16s RNA* gene.

Although an intensive literature search was performed, only clear information of a report of the year 1972 about a transfusional acquisition of *B. bacilliformis* was found. In this report a newborn died by Oroya's Fever after receipt of a blood transfusion from a family member living in a *B. bacilliformis* endemic area. Although vertical transmission has been described in different cases, the mother did not have a previous *Bartonella* infection[3]. In line with this, the present results clearly show the risk of long term survival of *B. bacilliformis* in infected human blood stored at 4 ºC, and therefore the potential risk of a transfusion transmission of this microorganism. This potential risk is enhanced firstly because this microorganism requires a prolonged incubation period (usually more than 21 days), which exceeds the usual period of blood cultivation for detection of bacterial infections[2], and secondly because of the high rates of asymptomatic infections that have been reported in some studies[1]. Thus, it is necessary to reinforce the screening to detect *B. bacilliformis* in blood banks in endemic areas, as well as in those of nearby areas due to the interchange of population with endemic ones.

As a corollary our results open the door to the development of molecular diagnostic tools able to be implemented in regional reference centres which might test blood samples drawn elsewhere and transported under refrigeration conditions. Development of rapid, efficient and inexpensive techniques capable of detecting *B. bacilliformis* in blood samples for use in risk areas is a current need. To this end several different direct blood PCR approaches are currently under investigation by our group (unpublished data). These techniques, might allow the development of a new and useful clinical diagnostic tool and enable screening for the presence of *B. bacilliformis* in banked blood.

In summary, the results demonstrate the ability of *B. bacilliformis* to survive long periods of time in blood samples stored at 4 ºC, suggesting the risk of transfusion-transmitted infections and the need to implement rapid techniques to detect infections in donated blood.

Table I - Microbiological data of the samples.

Sample	Initial growth[1]	Final growth[2]	Storage[3]
1	5 weeks	6 weeks	30 months
2	2 weeks	4 weeks	30 months
8	4 weeks	8 weeks	30 months
14	2 weeks	Negative	30 months
28	4 weeks	Negative	30 months
31	5 weeks	Negative	29 months
32	5 weeks	Negative	29 months
35	5 weeks	8 weeks	29 months
36	4 weeks	Negative	29 months
44	4 weeks	4 weeks	28 months
54	3 weeks	10 weeks	24 months

Legend: 1: time required for growth at collection moment, 2: time required for growth after 4 ºC storage, 3: storage time at 4 ºC.

Acknowledgements

This work has been performed thanks to the support of Optimus Foundation.

Joaquim Ruiz has a Miguel Servet fellowship from the Instituto de Salud Carlos III (CP05/0130). Luis J. del Valle has been supported by AECID (PCI C/033641/10).

The Authors declare no conflicts of interest.

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Arrived: 15 November 2011 - Revision accepted: 28 February 2012
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