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## Stability of *Coxiella burnetii* in stored human blood

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### Abstract

**Background**—*Coxiella burnetii*, an obligate intracellular organism, is the causative agent of the zoonotic disease Q fever. The seroprevalence rate for Q fever in the United States is 3.1%, suggesting a high number of infections each year. However, less than 200 cases of Q fever are reported to the CDC annually. This discrepancy is likely the result of underutilized diagnostics and a high percentage (>50%) of asymptomatic infections. The detection of *C. burnetii* in patient blood during the first 2–3 weeks of infection raises the possibility that the organism could be present in donated human blood. The purpose of this study was to determine if extracellular *C. burnetii* would be stable in blood under normal storage conditions.

**Study Design and Methods**—Donated human blood was separated into whole blood, leukoreduced whole blood, packed RBCs, and plasma. Each component was spiked with purified, extracellular *C. burnetii* strain Nine Mile Phase 1, and the viability and infectivity of the organisms were tested weekly.

**Results**—*C. burnetii* did not decrease in viability or the ability to infect cells after storage in any of the blood products, even after six weeks of storage at 1–6°C.

**Conclusions**—Extracellular *C. burnetii* can survive and remain infectious in donated blood products.

### Introduction

*Coxiella burnetii* is a gram-negative bacterium that causes the zoonotic disease Q fever. It is considered a category B agent of bioterrorism due to its low infectious dose, transmission by inhalation, and environmental stability<sup>1</sup>. Humans typically acquire Q fever after inhalation of contaminated aerosols that are derived from the waste products of infected animals. The most common reservoirs related to human infection are sheep and goats<sup>2</sup>. The presentation of Q fever in its acute form can include fever, headache, malaise, and myalgia<sup>3</sup>. An atypical pneumonia is often seen. A small percentage (2–5%) of *C. burnetii* infections result in a chronic disease that is difficult to treat and can present as a life-threatening endocarditis.

Q fever has been a nationally notifiable disease in the US since 1999, with reported cases never exceeding 200 in any given year. However, a study conducted using samples from the

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2003–2004 survey cycle of the National Health and Nutrition Examination Survey found that seroprevalence in the United States was 3.1%, suggesting that many cases of Q fever go unreported<sup>4</sup>. Reasons for under-reporting of Q fever probably include the non-specific nature of its symptoms, difficulties in diagnosis, and the possibility that more than 50% of infections may be asymptomatic in humans<sup>3</sup>.

During acute Q fever, *C. burnetii* can be detected by PCR in blood and serum up to 17 days after the onset of symptoms<sup>5</sup>, but the presence of *C. burnetii* DNA in the blood typically declines after the emergence of the antibody response. In patients with chronic Q fever it is also possible to detect *C. burnetii* in blood months after the initial infection<sup>6</sup>. In a screen of blood donors from the Netherlands during a large outbreak of Q fever in 2009, 3/1004 (0.3%) blood donors tested positive for *C. burnetii* DNA by PCR and showed an antibody response, but none of these donors reported Q fever symptoms<sup>7</sup>. Thus, it is possible for asymptomatic, *C. burnetii*-infected donors to introduce *C. burnetii* into the blood supply. However, transmission of *C. burnetii* by transfusion has not been documented.

The length of time that *C. burnetii* can remain viable and infectious in stored blood is not known. *C. burnetii* has the ability to form a spore-like “small cell variant” (SCV) when it is not replicating, and this SCV form of *C. burnetii* has been reported to be very stable in a variety of environments<sup>8</sup>. However, very little is known about the survival of *C. burnetii* in human blood under normal storage conditions. In this study, human blood and blood products were spiked with purified *C. burnetii*, and the survival of the bacteria under normal storage conditions was monitored.

## Materials and Methods

Five hundred mls of blood was obtained from each of 3 healthy human donors. Blood was drawn into a leukotrap WB system CPDA-1 double blood bag unit (Pall Medical, Covina, CA). An aliquot of whole blood was removed from each unit prior to leukoreduction. The blood was then leukoreduced using the leukotrap filter of the WB system (Pall Medical, Covina, CA). After an aliquot of leukoreduced blood was taken, the leukoreduced blood was separated into packed RBCs and plasma according to the manufacturer’s instructions. Blood was obtained in accordance with the ethical standards of the institutional review boards of Emory University and the Centers for Disease Control and Prevention.

Aliquots (40 mls) of whole blood, leukoreduced whole blood, packed RBCs, and plasma were placed in 50 ml conical tubes and spiked with approximately  $1.6 \times 10^6$  genome equivalents of purified *C. burnetii* strain Nine Mile Phase 1 (RSA493). Aliquots of 600  $\mu$ l were taken from each sample immediately after spiking, and then the tubes were stored at 1–6°C. At days 7, 14, 22, 28, 35, and 42 the samples were gently mixed and additional 600  $\mu$ l aliquots were taken.

For each time point, 10  $\mu$ l of the aliquot was plated on a 100 mm plate containing ACCM-2 in 1% agarose and overlaid with ACCM-2 in 0.25% agarose. ACCM-2 was made according to published instructions<sup>9</sup>. The plates were incubated at 37°C in a tri-gas incubator (Thermo Scientific Heracell 150i, Waltham, MA), at 2.5% O<sub>2</sub>, 5% CO<sub>2</sub>, and

92.5% N<sub>2</sub>. Colonies were counted 14 days after plating. Also for each time point, 200 µl of the aliquot was introduced into a T-25 flask containing a 70% confluent monolayer of rabbit kidney (RK-13) cells. At 7 and 14 days after inoculation, 200 µl of the culture media was removed, DNA isolated using a QIAmp DNA mini kit tissue protocol (Qiagen, Valencia, CA), and quantitative PCR targeting IS1111 was performed as described previously<sup>10</sup>.

## Results

Whole blood from three random donors was separated into leukoreduced blood, RBCs, and plasma. Aliquots (40 mls) of each type of blood product were stored at 1–6°C, spiked with *C. burnetii* strain Nine Mile Phase 1, and samples were taken weekly for analysis. Although *C. burnetii* is an obligate intracellular bacterium, recent work has shown that *C. burnetii* can be grown on plates using a unique semisolid agarose medium and a microaerobic environment<sup>9</sup>. The colony forming units (CFU) determined by use of this growth system was employed to monitor viability of *C. burnetii* in the stored blood products. After separation of the components and adding *C. burnetii*, three of the products (whole blood, leukoreduced blood, and plasma) contained similar numbers of viable *C. burnetii* (Figure 1A, 1B, and 1C). The number of viable organisms in RBCs was reduced compared to the other products (Figure 1D). This difference was statistically significant when compared to each of the other three blood products ( $p < 0.022$ ).

The number of viable *C. burnetii* in whole blood or in specific fractionated blood products did not significantly decline during the 42 days that these products were stored and evaluated (Figure 1). The only significant change was an apparent increase in the number of viable *C. burnetii* in plasma by day 14. There was a statistically significant difference between the number of CFU at day 14 compared to days 0 and 7 ( $p < 0.03$ ). From day 14 through day 42, the number of viable *C. burnetii* in plasma did not significantly increase or decrease. The reason for higher numbers of bacteria in plasma at days 14–42 is not known.

To determine if the *C. burnetii* in the stored blood products remained infectious, aliquots were placed on RK13 (rabbit kidney) cells and the growth of the organisms in culture was monitored by quantitative PCR. As shown in Figure 2, all of the samples were infectious in culture and demonstrated robust growth between the two sampling points (7 and 14 days after inoculation of the cultures). The most robust growth was seen in the plasma samples, most likely due to higher numbers of bacteria introduced into the cultures.

## Discussion

This study demonstrates that *C. burnetii* can retain significant viability and infectivity when kept in whole blood and blood products at 1–6°C. There was no decline in viability during 42 days of storage. Although plasma can be stored frozen for longer periods of time, blood products are not typically stored past 42 days at 1–6°C. For the CPDA-1 anticoagulant used in this study, the recommendation is to use the whole blood or leukoreduced blood product within 35 days, but a 42 day shelf life can be achieved with some additional additives. Therefore, testing out to 42 days of storage shows that *C. burnetii* present in a donated blood product stored at 1–6°C could maintain its ability to infect a recipient for the duration of the

product's shelf life. This suggests that blood product transfusion represents a possible means of transmitting Q fever.

For this study, *C. burnetii* was added to the blood products after separation, and was therefore present in the extracellular portion of each component. In human infections, *C. burnetii* primarily infects cells of the monocyte lineage, and can only replicate when inside a parasitophorous vacuole. However, there is evidence that a significant portion of *C. burnetii* is present in the extracellular compartment of human blood during infection. Early in infection, *C. burnetii* can readily be detected in human serum by PCR<sup>5</sup>, and in mouse experiments we have found similar numbers of *C. burnetii* genomes present in murine serum and whole blood during acute infection (unpublished observations). *C. burnetii* found in both whole blood and serum during acute infection is viable. Leukoreduction of infected blood may remove most or all of the intracellular *C. burnetii*, but the evidence suggests that significant numbers of organisms will remain after leukoreduction. It is difficult to test this idea directly because of the low availability of sufficient quantities of blood from acute Q fever patients. Nevertheless, the results described here demonstrate that any *C. burnetii* found in stored blood components will easily survive normal storage conditions.

*C. burnetii* exists in two morphological forms: the large cell variant (LCV) and the small cell variant (SCV). The LCV form is the replicative form and will predominate in the parasitophorous vacuole. The SCV form is considered to be spore-like, undergoes little or no replication, and has the ability to survive for extended periods under relatively harsh conditions. Extracellular *C. burnetii* will transition to the SCV after leaving the vacuole. It is therefore expected that the SCV form of *C. burnetii* will predominate in leukoreduced blood, plasma, or RBCs. *C. burnetii* in whole blood likely be a mixture of LCV and SCV and could convert to the SCV form during storage. Thus, the extended survival attributes of the SCV would be quite relevant for survival of *C. burnetii* in blood products.

Although the stability of *C. burnetii* was very similar among the different blood products, there were distinct differences in the growth and yield of organisms among the products. Immediately after spiking, the number of CFU in plasma was similar to whole blood and leukoreduced blood, but the number of CFU in plasma increased at day 7 and day 14. The possibility exists that there was some cell-free growth of *C. burnetii* in plasma, however it is more likely that whole blood contains inhibitors of *C. burnetii* colony formation that were removed in the plasma. These inhibitors may be present in RBCs, as fewer CFU were recovered from RBCs at all time points compared to the other products. Interestingly, the inhibition of growth imparted by RBCs was even observed after RBC-stored *C. burnetii* was inoculated onto RK13 cultures (Figure 2). The source of this inhibition warrants further study.

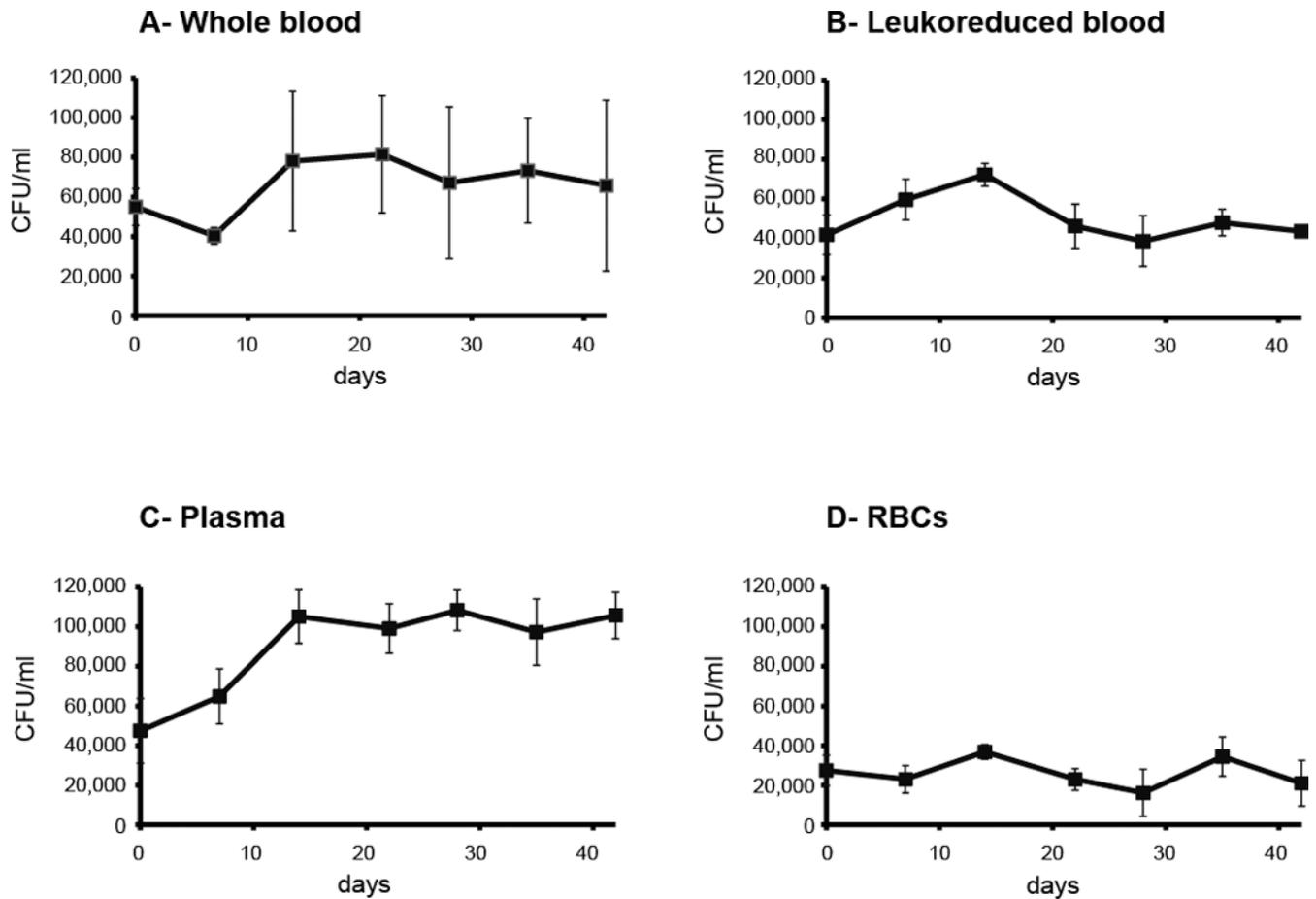
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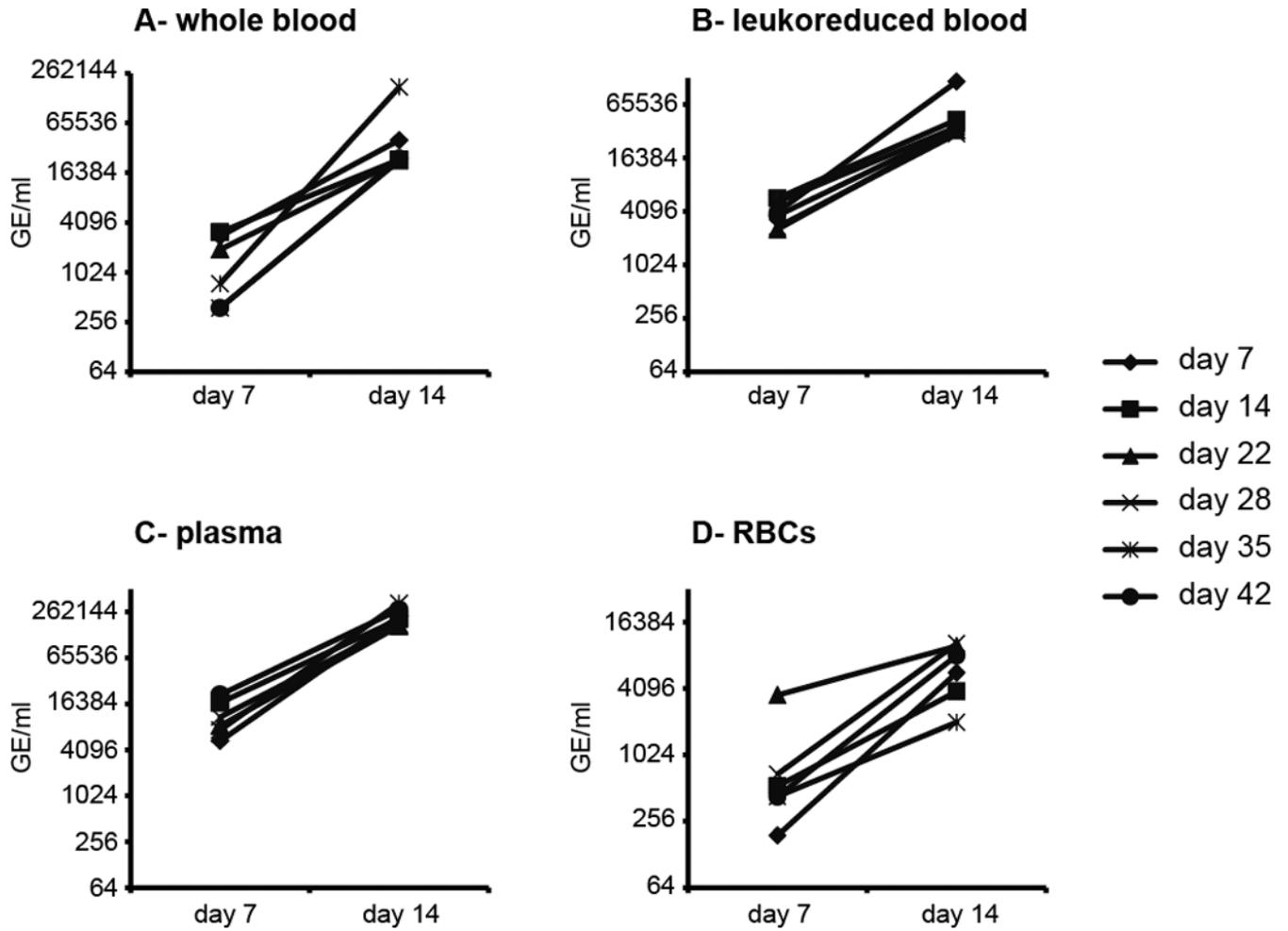
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**Figure 1.**

Viability of *C. burnetii* in whole blood and blood products. Donated human blood was separated into leukoreduced blood, plasma, and RBCs; spiked with viable *C. burnetii*, and then stored at 1–6°C. Aliquots were taken at the indicated times and the number of colony forming units (CFU) in the stored blood or blood products determined by counting colony growth on ACCM-2 agar plates.



**Figure 2.**

*C. burnetii* stored in whole blood and blood products remains infectious. Donated human blood was separated into leukoreduced blood, plasma, and RBCs; spiked with viable *C. burnetii*, and then stored at 1–6°C. Aliquots were taken at the indicated times and used to inoculate cultures of RK13 rabbit kidney cells. At 7 and 14 days after inoculation an aliquot of the culture was analyzed by quantitative PCR targeting the *C. burnetii* IS1111 transposase. A higher number of genome equivalents (GE) at day 14 indicates infection of the RK13 cells and growth in culture.