Borrelia burgdorferi: Survival in Experimentally Infected Human Blood Processed for Transfusion

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The isolation of *Borrelia burgdorferi* from blood raises the possibility of bloodborne transmission of Lyme borreliosis through transfusions. To assess this possibility, the ability of *B. burgdorferi* to survive in human blood processed for transfusion was studied. Human blood was inoculated with *B. burgdorferi* type strain B-31 (ATCC 35210) at 0.2, 20, or 2000 viable cells/ml, processed by conventional blood banking procedures, stored at 4°C, and cultured for *B. burgdorferi* at 12, 23, 36, and 48 days of storage. After processing, most *B. burgdorferi* were found in the packed cell fraction. At inoculum levels of 20 or 2000 viable cells/ml, *B. burgdorferi* survived in processed blood through 48 days of storage at 4°C. *B. burgdorferi* was isolated from packed cells after 36 days of storage at 4°C even when the initial inoculum level was as low as 0.2 cells/ml. The data demonstrate that *B. burgdorferi* can survive the blood processing procedures normally applied to transfused blood in the USA. Since hematogenous spread of the spirochete seems to occur early in the illness, primarily in symptomatic patients, the risk of transfusion-associated Lyme disease may be small. However, the possibility of survival of *B. burgdorferi* under blood banking conditions warrants a heightened awareness of this potential problem.

Lyme borreliosis is a multisystem illness caused by infection with the tick-borne spirochete *Borrelia burgdorferi*. The disorder often begins with a distinctive skin lesion and may be followed by neurologic, cardiac, and arthritic complications [1–3]. National surveillance for Lyme disease was established in the USA in 1982. Surveillance data show that Lyme disease had occurred in 43 states by 1989, although 94% of 6940 defined cases occurred in nine states, six northeastern, two north central, and one western [4]. Seroprevalence data show that as many as 10% of individuals in highly endemic areas have evidence of past exposure to *B. burgdorferi* [5].

B. burgdorferi has been isolated from blood, synovial fluid, cerebrospinal fluid, and skin tissue [6, 7]. Its isolation from blood raises the possibility of bloodborne transmission through transfusions.

Methods

To study this possibility, we collected 1 unit of blood (type B Rh+) in CPD-A1 (citrate-phosphate-dextrose with adenine added) from a donor meeting standard American Association of Blood Banks qualifications [8]. A Quad-Pack blood collection device (Fenwal Laboratories, Deerfield, IL) was used to divide the blood into four equal subsamples of 125 ml each and to maintain a closed system. Three of the subsamples were inoculated with an actively growing culture of *B. burgdorferi*, type strain B-31 (ATCC 35210), at 0.2, 20, or 2000 viable cells/ml of blood. The three subsamples were cultured immediately after inoculation and processed by conventional blood banking procedures [8] to obtain a packed cell fraction and a plasma fraction from each subsample. Each packed cell fraction received 12.5 ml of ADSOL (Fenwal).

All fractions were cultured immediately after processing to determine the effect of blood banking procedures on *B. burgdorferi* and the distribution of *B. burgdorferi* between the two fractions. The packed cell fractions were stored at 4°C and then cultured after 12, 23, 36, and 48 days. The fourth subsample of blood was inoculated with *B. burgdorferi* at 2000 cells/ml, held at 4°C throughout the study period as an unprocessed whole blood control, and cultured on the 48th day.

The numbers of *B. burgdorferi* in packed cell and plasma fractions and in whole blood were estimated by the three-tube most probable number (MPN) technique [9–11]. The MPN method involves mathematical inference of the viable count from the fraction of cultures that fail to grow in a series of tubes containing a suitable growth medium [10]. This method has particular value for estimating viable counts of bacteria that are not usually cultured on solid media or are in too small number per milliliter for limiting dilution and plating. The MPN method was used in this study because no quantitative information was available on the extent of survival of *B. burgdorferi* in human blood.

All culturing was done in duplicate. Before culturing, the subsample or fraction was thoroughly mixed to assure homogeneous distribution of *B. burgdorferi*. Each MPN determination involved the use in duplicate of nine tubes (16 × 100 mm), each containing 10 ml

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Informed consent was obtained from the donor.

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of Burgdorfer, Stoenner, and Kelly (BSK II) medium [12]. The tubes were inoculated with 1, 0.1, or 0.01 ml of the appropriate subsample or fraction at three tubes per inoculum size in each set. The cultures were incubated at 33°C for 21 days and examined for the presence of motile spirochetes by darkfield microscopy. All tubes showing the presence of motile spirochetes were scored as positive. MPN values and the 95% confidence limits were obtained from published MPN tables [9].

Results

MPN estimates of *B. burgdorferi* in whole blood inoculated at 2000 cells/ml and stored unprocessed (subsample 4) at 4°C were >240 cells/ml on the day of processing and after 48 days. When the three subsamples were processed and their fractions cultured, most *B. burgdorferi* cells were found in the packed cell fraction. Whole blood inoculated at 20 cells/ml yielded, after processing, MPN estimates of 50 and 110 cells/ml (two determinations) in the packed cell fraction (table 1) and 9 cells/ml in the plasma fraction.

Table 1 shows the extent of survival of *B. burgdorferi* in packed cell fractions. When the inoculum level before processing was 20 or 2000 cells/ml, the spirochete survived in the processed blood through 48 days. When the initial inoculum level in whole blood was 20 cells/ml, no decrease in numbers was observed in packed cells after 12 days. Most interesting, when the initial inoculum level in the whole blood was as low as 0.2 cells/ml, *B. burgdorferi* could be isolated from packed cells through 36 days. The decrease in cell numbers observed after 36 days at initial inoculum levels of 20 and 2000 cells/ml may be due to clumping and aggregation of bacteria in the blood before sampling, because the counts rose again when sampled after 48 days.

Table 1. Survival of *Borrelia burgdorferi* in blood processed for transfusion and stored at 4°C.

Days of storage	*B. burgdorferi* in packed red cell fraction after inoculation* at		
	0.2	20	2000
0	<0.3, 0.4	50, 110	>240, >240
12	0.9, <0.3	50, 110	>240, >240
23	<0.3, 0.4	29, 55	>240, >240
36	0.4, <0.3	4, 9	46, >240
48	<0.3, <0.3	15, 50	>240, >240

NOTE. Data and inocula are cells per milliliter. Results are most probable number estimates for duplicate samples, processed using three tubes each of three decimal dilutions: <0.3 = none of nine tubes positive; >240 = all nine tubes positive.

* For each inoculum, 125 ml of whole blood was used, yielding after processing 55 ml of packed red cells and 70 ml of plasma.

Discussion

Our findings demonstrate that *B. burgdorferi* can survive the blood processing procedures normally applied to transfused blood in the USA. Other investigators have made similar conclusions [13–15]. However, this is the first time that quantitative data have been presented on survival of *B. burgdorferi* in blood processed for transfusion. The possibility that *B. burgdorferi* can survive under blood processing conditions warrants a heightened awareness of this potential problem.

Although laboratory studies (including this one) indicate that transmission of *B. burgdorferi* through transfused blood can occur, epidemiologic data available to date suggest that it is unlikely to pose a major risk in transfusions. In a study of 40 consecutive patients with active Lyme disease, blood cultures were positive in only 1 patient, who was acutely ill with severe disseminated Lyme disease [16]. No confirmed cases of transfusion-associated Lyme disease have been reported to the Centers for Disease Control since surveillance was initiated in 1980.

Even though the infectious dose (ID_{50}) of *B. burgdorferi* in hamsters is $\sim 10^7$ organisms (Johnson RC, personal communication), the ID_{50} of *B. burgdorferi* in humans is not known. Therefore, it is difficult to extrapolate the laboratory observations of survival of *B. burgdorferi* in processed blood to the risk of transfusion-related Lyme disease.

Further, the results of this study should be viewed with caution. The study presents results from the investigation of only one *B. burgdorferi* strain, B-31. Viability of *B. burgdorferi* in human blood during refrigerated storage and growth upon subsequent culture may vary among strains. Also, B-31 is a long-passaged strain that may have become adapted to in vitro culture. Similar studies need to be designed and conducted using fresh clinical isolates. Also, these studies need to look at the virulence of *B. burgdorferi* strains in human blood after storage under blood banking conditions.

Serologic testing of donors for Lyme antibodies does not seem warranted at this time. Lyme disease serologic testing does not differentiate active from past infections. Because the sensitivity of such tests is low (especially early in the disease), persons most likely to transmit the infection are also most likely to be missed by serologic testing. Until the possibility of transmission of Lyme disease through transfusions is demonstrated by epidemiologic studies, and more sensitive and specific tests (such as assays based on the polymerase chain reaction) are available for rapid screening of donor blood for *B. burgdorferi*, blood centers in endemic areas should consider taking simple precautions against this potential problem.

Such precautions would include rejecting potential blood donors with symptoms consistent with acute Lyme disease and a history of skin rash characteristic of Lyme disease. Blood banking guidelines regarding patients with previous history of Lyme disease who have undergone antibiotic therapy and seropositive individuals with a past history of arthritis are beyond the scope of this paper and should be addressed by an appropriate body. Further studies on the risk of transfusion-acquired Lyme disease are clearly warranted.

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