# Transfusion-Transmitted Babesiosis in Washington State: First Reported Case Caused by a WA1-Type Parasite

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**Most cases of babesiosis reported in the United States have been tickborne and caused by *Babesia microti,* the etiologic agent of all previously described transfusion-transmitted cases. A 76-year-old man with the first recognized case of transfusion-transmitted infection with the recently identified WA1-type *Babesia* parasite is described. The subject received multiple blood transfusions in 1994. Indirect immunofluorescent antibody testing of serum from 57 blood donors implicated a 34-year-old man (WA1 titer, 1:65,536) whose donation had been used for packed red cells. Isolates of the organisms that infected the recipient and the donor, both of whom were spleen-intact residents of Washington State, were obtained by hamster inoculation. The DNA sequence of a 536-bp region of the nuclear small subunit–rRNA gene of both isolates was identical to that of WA1 (isolated in 1991 from the index WA1 case-patient). Effective measures for preventing transmission of babesiosis by blood transfusion are needed.**

Most of the hundreds of reported human cases of babesiosis in the United States have been tickborne, acquired in the Northeast, and caused by infection with *Babesia microti,* an intraerythrocytic parasite of rodents. Recently, other zoonotic pathogens have been identified (WA1-type parasites in Washington State and California [1–3] and MO1 in Missouri [4]), and the importance of bloodborne transmission [5] has been recognized. The previously reported transfusion-transmitted cases (≥15) were all attributed to *B. microti* [6, 7] (Cable R, personal communication), which can be associated with protracted, asymptomatic, and thus undiagnosed parasitemia. The parasite remains viable under blood bank conditions [8] and is transmissible by transfusion of erythrocytes (frozen-deglycerolized and liquid-stored) and platelet concentrates (which have residual erythrocytes) [5]. Currently, prevention of transfusion-transmitted babesiosis depends on questioning of donors (e.g., history of babesiosis) and on assessment of temperature and hematocrit.

We report a transfusion-transmitted case of infection with a WA1-type parasite. This case occurred in 1994 and was the second recognized symptomatic case of babesiosis acquired in Washington State. Whereas we termed the organism isolated in 1991 from the first symptomatic case-patient "WA1" [1], the WA1-type organisms that we isolated from the transfusion recipient and the implicated donor will be referred to here as WA2 and WA3, respectively.

## Case Reports of Blood Recipient and Implicated Donor, Investigations of Blood Donors, and Molecular Studies

*Case report of blood recipient.* The recipient, a 76-year-old spleen-intact man, had lived his entire life in a rural area of coastal, northwestern Washington State (Whatcom County). He did not travel outside western Washington in 1994 but in previous years had traveled to eastern Washington, elsewhere in the western United States, and to Mexican border towns. He did not farm, hunt, fish, have pets, or recall any tick bites.

His medical problems included megaloblastic anemia, myelodysplasia, and angina pectoris. On 28 April 1994, his hematocrit was 0.25; because of increasing angina and fatigue, he was transfused with 2 U of packed red cells, and his condition improved. On 24 May, he received another unit of packed red cells because of worsening fatigue. He was multiply transfused (see below) during hospitalizations from 14–27 July and 9–20 August for aortic valve replacement and coronary artery bypass graft surgery and for a sternal wound infection.

He noted fatigue in mid-September, became febrile on about 22 September, and was rehospitalized on 28 September because of

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Human experimentation guidelines of the CDC were followed in the conduct of clinical research. Survey participants signed informed consent. Animal experimentation guidelines were followed in accordance with the CDC Animal Care and Use Committee or the University of California, Davis.

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increasing weakness, anorexia, and weight loss. His medications on admission included aspirin (325 mg daily), quinine sulfate as needed for nocturnal leg cramps, and hydrocodone as needed for pain. His initial evaluation was notable for a temperature of 37.4°C, blood pressure of 80/40 mm Hg, pulse of 100/min, and a palpable spleen. Laboratory findings were hematocrit, 0.28; leukocyte count, 5600/$\mu$L; and platelet count, 235,000/$\mu$L. He had normal renal function, urinalysis, and lactate dehydrogenase level; negative blood cultures; and no antibody to human immunodeficiency virus. No reticulocyte count was obtained.

A blood smear done on admission showed stable abnormalities of erythrocyte morphology and ring forms that were attributed to *Plasmodium* species in <1% of the erythrocytes. He was treated with empiric antibacterial therapy (ampicillin-sulbactam and vancomycin) for several days and with oral chloroquine phosphate (1 g initially, 500 mg 6 h later and at 24 and 48 h), followed by a 14-day course of oral primaquine phosphate (26.3 mg daily). On 3 October, ring forms were noted on a follow-up blood smear (parasitemia not determined), his hematocrit was 0.22, and he was transfused with 3 U of packed red cells. His temperature, which had risen to 40°C with chills on 29 September, essentially was normal by 4 October. He was discharged on 6 October.

The Centers for Disease Control and Prevention (CDC) confirmed the presence of rare intraerythrocytic ring forms on the blood smear from 28 September (received at CDC 21 November; in October, the Armed Forces Institute of Pathology examined the slide and noted one tetrad as well as ring forms). Subsequent laboratory testing by the CDC of whole blood and serum obtained from the patient on 12 December confirmed that he had babesiosis rather than malaria (table 1). Although his blood smear was negative, an isolate of his parasite (WA2) was obtained by hamster *(Mesocricetus auratus)* inoculation and was used to generate WA2 antigen for indirect immunofluorescent antibody (IFA) testing [1, 9] (table 1). The patient had strikingly high IFA titers to WA1 and WA2 antigen, no detectable antibody to *B. microti,* an IFA titer of 1:64 to *P. falciparum* [10], a negative result with a malaria-specific polymerase chain reaction (PCR) assay [11], and negative results with IFA testing for antibody to *Ehrlichia chaffeensis* [12] and the agent of human granulocytic ehrlichiosis (Nicholson WL, unpublished methodology). A serum specimen obtained from his wife on 4 January 1995 did not react to WA1 or *B. microti* antigen.

In early January 1995, because of persistent subpatent parasitemia and subjective fever, he was treated for babesiosis with an 8-day course of thrice-daily clindamycin (3 days of oral and 5 days of intravenous therapy; 600 mg/dose) and thrice daily quinine (650 mg/dose). Thereafter, he transiently felt somewhat better and had increased energy. His IFA titers to WA1 and WA2 antigen progressively waned, and hamsters inoculated with posttreatment blood specimens did not develop demonstrable parasitemia (table 1). However, fatigue and intermittent chills, without documented fever, of unknown etiology persisted.

*Investigation of blood donors.* During hospitalizations in July and August 1994, the patient received blood products (15 U of packed red blood cells, 36 U of platelets, 4 U of fresh frozen plasma, and 2 U of modified whole blood) from 57 donors. To investigate the possibility of transfusion-transmitted babesiosis, we tested residual aliquots of serum from all donations. Of the 57 donors, 1 had a specimen with a titer of 1:256 to WA2 antigen, and another donor had a titer of ≥1:4096. The latter had donated blood on 16 August 1994. This donation was used for packed red cells that were transfused into the patient on 19 August, platelets (recipient unavailable for testing), and for manufacture into plasma derivatives. The implicated donor had also donated blood on 21 February 1994, when, in retrospect, he was seropositive to WA1 and WA2 antigen (table 1); the donation was used for packed red cells (recipient died of cancer), platelets (recipient died of cancer), and for manufacture into plasma derivatives.

*Case report of implicated donor.* The donor, a 34-year-old lifelong resident of Washington State, had lived in a rural area of King County (western foothills of the Cascade Mountains) since he was 5 years old. At the time of his implicated donation, he lived on 0.4 hectare of wooded land that was frequented by deer. Although he did not hunt and seldom hiked, he frequently walked on wooded land in the vicinity of his home in the fall of 1993 and January 1994. The last time before his first donation (February 1994) that he left the area in which he lived was March 1993 for a trip to Hawaii. He did not recall any tick bites and had never had a blood transfusion.

The donor had been healthy except for intermittent fatigue, two syncopal episodes of unknown etiology during the winter of 1994–1995, and a brief, flu-like illness several years before he donated blood. He had spun hematocrits of at least 0.38 when he donated blood in February and August 1994 but was deferred from donating on 15 December 1994 (he was not yet known to have babesiosis), because his spun hematocrit was 0.37. On 26 April 1995, he had a hematocrit of 0.39, a leukocyte count of 6400/$\mu$L, and a platelet count of 231,000/$\mu$L.

After he was implicated as the donor of interest, testing of serial blood specimens (table 1) showed strikingly high IFA titers to WA1 and WA2 antigen that waned over time, even though he was not treated. Hamsters that were inoculated with his blood 7 months after his implicated donation became parasitemic (source of the WA3 isolate and antigen). IFA testing for antibody to *E. chaffeensis* and the agent of human granulocytic ehrlichiosis was negative.

The donor's wife and 3 children (ages 8–10 years) were seronegative to WA2 and *B. microti* antigen and had negative blood smears (specimens obtained in February or March 1995).

*Investigation of area blood donors.* From 20 May through 1 June 1995, serum specimens were obtained from 111 blood donors who lived in communities in the general vicinity of the implicated donor's residence. The donors had a median age of 42 years (range, 18–76); 54 (48.6%) were female. In IFA testing of the 111 specimens to WA1 and WA2 antigen, 5 specimens (4.5%) had at least one titer of ≥1:256 and one had a WA1 titer of 1:1024. The donors were from Pierce County (4) and King County (1). When additional specimens obtained in July 1996 from 3 of the donors (including a specimen from the person with the highest titer) were tested in parallel with the original specimens, the specimens from the two time points had comparable titers. Hamsters inoculated with 1 mL of whole blood obtained in July 1996 from each of these donors did not become demonstrably parasitemic during the 2-month monitoring period (i.e., Giemsa-stained smears of blood obtained weekly by tail snip were negative).

*Molecular studies.* DNA sequence analysis was done to determine if WA1, WA2, and WA3 were distinguishable. Merozoites of each were obtained from infected hamster blood as described previously [2]. A nucleic acid extraction kit (Isoquick; Microprobe,

**Table 1.** Results of serologic testing, blood smear analysis, and hamster inoculation for serial specimens from the recipient with transfusion-transmitted babesiosis and the implicated blood donor.

| Date of specimen | Indirect immunofluorescent antibody titer to* | | Blood smear† | Hamster inoculation†‡ |
|---|---|---|---|---|
| | WA1 antigen | WA2 antigen | | |
| Blood recipient (infected with WA2; transfused 19 August 1994) | | | | |
| 12 December 1994§ | 65,536 | 65,536 | Negative | Positive‖ |
| 4 January 1995§ | 65,536 | 65,536 | Negative | Positive¶ |
| 3 April 1995** | 4096 | 16,384 | Negative | Negative |
| 12 March 1996** | 1024 | 1024 | Negative | Negative |
| Blood donor (infected with WA3) | | | | |
| 21 February 1994 | 16,384 | 65,536 | Not done | Not done |
| 16 August 1994†† | 65,536 | 65,536 | Not done | Not done |
| 2 February 1995‡ | 65,536 | 16,384 | Negative | Negative |
| 17 March 1995 | 16,384 | 16,384 | Negative | Positive‡‡ |
| 25 March 1996‡ | 1024 | 1024 | Negative | Negative |

* All specimens were tested in parallel at CDC in serial 4-fold dilutions [1, 9] and had titers of ≤1:8 to *Babesia microti* antigen. Reciprocal titers are shown.

† Negative indicates no intraerythrocytic parasites were noted on blood smear. For hamster inoculations, Giemsa-stained smears were made from hamster blood obtained weekly by tail snip. Hamsters that did not become demonstrably parasitemic were monitored 7–8 weeks.

‡ Human blood from recipient or donor was inoculated intraperitoneally (1 mL inocula) into hamsters 1 day after it was obtained, except for donor's 2 February 1995 and 25 March 1996 specimens, which were inoculated 2 days after they were obtained (refrigerated in interim).

§ Before treatment for babesiosis. No pretransfusion specimens were available for testing.

‖ Inoculated hamsters ($n$ = 3) became parasitemic and died by postinoculation day 14 or 15. Parasitemic blood from hamsters subinoculated with ground-up heart tissue from dead hamsters was used to obtain isolate of parasite (WA2).

¶ Inoculated hamsters ($n$ = 2) were parasitemic by postinoculation day 12; 1 died 3 days later; other was exsanguinated to provide ample supply of parasitized blood.

** After treatment for babesiosis.

†† Date implicated blood was donated, which was transfused (packed red cells) 19 August 1994; donor had also donated blood 21 February 1994.

‡‡ Inoculated hamsters ($n$ = 2) became parasitemic and died within several weeks of inoculation.

Bothell, WA) was used as recommended by the manufacturer to extract DNA from the merozoite preparations. A 1.8-kb amplification product of the nuclear small subunit (nss)–rRNA gene was isolated using broad-range PCR primers [13]. DNA cycle sequencing was done on the product using a DNA sequencer protocol (ABI Prism 377; Perkin-Elmer, Foster City, CA).

Briefly, a 3.2 p$M$ concentration of each universal primer A (5′-3′) and C (3′-5′) directed against a 589-bp region of the highly conserved portion of the eukaryotic nss-rRNA gene [14] was used in a 20-$\mu$L PCR reaction mix, which contained 8.0 $\mu$L of terminator premix (Perkin-Elmer) and 20 ng of the 1.8-kb rRNA gene amplification product. Amplification, terminator mix removal, and gel electrophoresis were done per the sequencer protocol. The resulting chromatogram was read and analyzed using Sequencher 3.0 (Gene Codes, Ann Arbor, MI) on a Macintosh II si computer. To verify the sequence, each isolate was analyzed at least three times, using either the A or C primer. WA1 was sequenced as a positive control, and its sequence was compared with that in GenBank (accession no. L13730) [2].

A 536-bp region was successfully sequenced from each isolate, corresponding to nt 3–558 of the *Saccharomyces cerevisiae* nss-rRNA gene (GenBank JO1353). The sequence obtained for WA1 was identical to that in GenBank (L13730) and to those obtained for WA2 and WA3.

## Discussion

We report here the first transfusion-transmitted case of infection with a WA1-type parasite and the second recognized symptomatic case of babesiosis acquired in Washington State. The first case was moderately severe, despite occurring in a 41-year-old immunocompetent man with an intact spleen [1]. However, the donor's case in the present report demonstrates that, like *B. microti,* WA1-type parasites can cause chronic asymptomatic or mildly symptomatic parasitemia; the donor was parasitemic (by hamster inoculation) 7 months after he donated the implicated blood, and he was markedly seropositive ≥6 months before that donation.

We demonstrated not only that the recipient's and donor's isolates (WA2 and WA3) had identical DNA sequences, which was expected, but also that this sequence was identical to that of WA1 in a phylogenetically informative region of the nss-

rRNA gene of piroplasms [2]. This suggests that WA2 and WA3 are closely related to WA1. However, strain differences may be more apparent with sequence analysis of the internal transcribed spacer, in which divergence may have occurred more recently [15].

The public health importance of WA1-type parasites requires further elucidation, and the tick vector and animal reservoir have yet to be identified. Unrecognized transfusion-transmitted cases caused by WA1-type parasites may have occurred, perhaps even in association with this donor. The recipient's case could have been missed had he not been elderly, had various medical problems, had a blood smear examined, had low-grade parasitemia noted, and had his misdiagnosis (malaria) corrected. The severity of his illness may have been moderated by the quinine sulfate that he periodically took because of leg cramps. We do not know whether any other donors we tested who had IFA titers to WA1 or WA2 antigen of $\leq$1:1024 had ever been infected with WA1-type parasites. Further experience with this IFA test is needed to determine the appropriate threshold for positivity.

In conclusion, babesiosis in the United States is not limited to tickborne cases of *B. microti* infection in the Northeast; the possibilities of other modes of transmission, other *Babesia* species, and other babesiosis-endemic geographic areas should be considered when febrile patients, particularly those who have hemolytic anemia, are evaluated. Effective measures for preventing transfusion-transmitted cases of babesiosis are needed.

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