Seroprevalence of *Babesia microti* in blood donors from *Babesia*-endemic areas of the northeastern United States: 2000 through 2007

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BACKGROUND: Current estimates of 70 cases of transfusion-transmitted *Babesia microti*, with 12 associated deaths, suggest that *Babesia* is a growing blood safety concern. The extent of *Babesia* infections among blood donors has not been well defined. To determine how common exposure to *B. microti* is among blood donors, a seroprevalence study was undertaken in the American Red Cross Northeast Division.

STUDY DESIGN AND METHODS: Blood donations at selected blood drives in Connecticut and Massachusetts (2000 through 2007) were tested for the presence of immunoglobulin (Ig)G antibodies to *B. microti* using immunofluorescence assay. Geographic and temporal trends of *B. microti* seroprevalence were estimated for donor's zip code of residence.

RESULTS: Overall, a 1.1% seroprevalence was identified in Connecticut, with the highest levels found in two Southeastern counties (Middlesex and New London). Observed seroprevalence for offshore islands of Massachusetts was 1.4%. Seropositive donations were identified from donors residing in all eight counties in Connecticut and three counties in Massachusetts. Although a seasonal peak was found between July and September, seropositive donations were identified in every month of the year.

CONCLUSIONS: Foci of statistically higher *B. microti* seroprevalence among blood donors were observed; however, *B. microti* transfusion transmission risk exists for blood collected throughout Connecticut and portions of Massachusetts. Similarly, a seasonal peak was identified; nevertheless, seropositive donations were found year-round. Thus, geographic and/or seasonal exclusion methods are insufficient to fully safeguard the blood supply from *Babesia* transmission. Steps should be taken to reduce risk of transfusion-transmitted *B. microti*, perhaps through implementation of year-round, regional testing.

Babesia microti, an intraerythrocytic protozoan parasite belonging to the phylum Apicomplexa, is the primary cause of human babesiosis in the United States. The first reported US case occurred in 1969 and involved a resident of Nantucket Island, Massachusetts.[1] The parasite is primarily transmitted to humans via a tick vector, *Ixodes scapularis*, commonly known as the black-legged or deer tick. Human babesiosis is often asymptomatic in healthy individuals; however, symptoms including fever, chills, sweating, myalgias, fatigue, hepatosplenomegaly, and hemolytic anemia may develop 1 to 6 weeks after an infective tick bite.[2] Among immunocompromised individuals, such as those who are elderly or asplenic, the symptoms can be severe, with mortality rates of up to 5% reported.[3]

Humans are generally considered to be incidental and dead-end hosts for *B. microti* infections, unless they donate blood and the parasite is transmitted to the recipient of a blood transfusion. Indeed, *B. microti* is of

ABBREVIATIONS: GIS = geographic information system; IFA = immunofluorescence assay.

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increasing concern for transfusion medicine because asymptomatic blood donors can unwittingly transmit the parasite to susceptible blood recipients.[4] *B. microti* is known to survive and remain viable under blood storage conditions (4°C) for up to 35 days in red blood cells (RBCs)[5] and indefinitely in cryopreserved RBCs.[6,7] Current estimates suggest that there have been at least 70 cases of transfusion-transmitted *B. microti* during the period from 1979 through 2008, with 12 associated fatalities.[4,8,9] Despite the increase in reported cases of transfusion transmission,[8] including seven recent cases reported in New York City,[10] there are limited measures available to mitigate transmission risk (e.g., blood screening), with blood centers currently relying on a question regarding a history of babesiosis, which appears to be ineffective.[9]

The challenges inherent to implementing measures that reduce transmission risk were emphasized at a recent (September 2008) Food and Drug Administration (FDA)-sponsored meeting, "Workshop to Consider Approaches to Reduce the Risk of Transfusion-Transmitted Babesiosis in the United States" (FDA Workshop). Discussion at this workshop focused on the need to define the geographic distribution of *B. microti* and the prevalence of exposure among blood donors. Herein we report on our *Babesia* seroprevalence study conducted over the past 8 years in Northeastern US blood donors. These data were collected with the expectation that they may be used to support the development of a screening algorithm to improve blood safety.

MATERIALS AND METHODS

Donor enrollment and sample collection

Between May 2000 and December 2007, blood donors at drives in targeted areas of Connecticut and Massachusetts were selected for enrollment in the study (Fig. 1A). All donors at designated blood drives were provided an information sheet describing *B. microti*, the research study, and participant benefits and risks. The donors' signature on the blood donation record served as consent for the study.

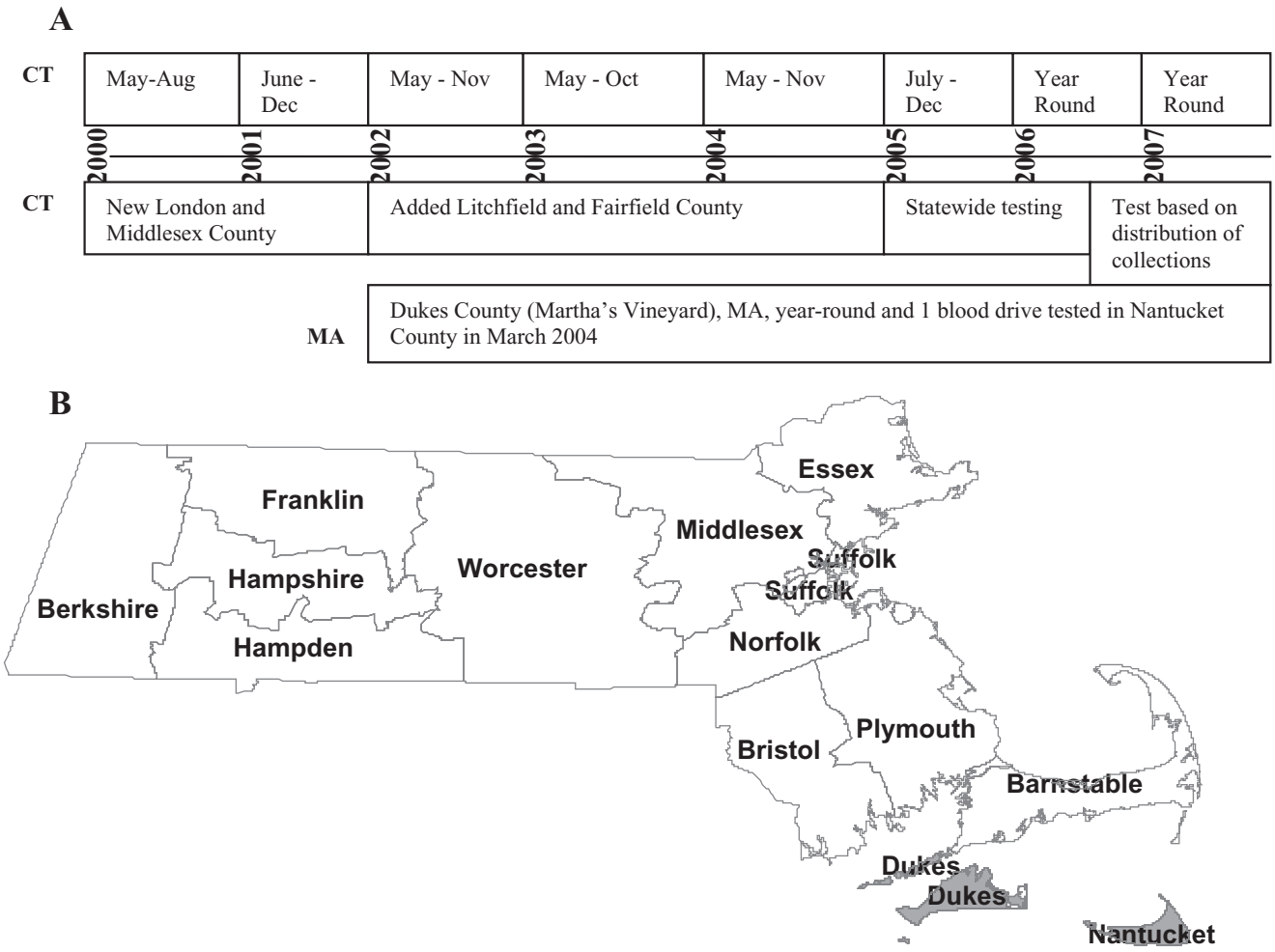

Fig. 1. (A) Timeline depicting annual changes in months of testing as well as geographic location of blood drive selection by county. (B) Massachusetts map depicting county boundaries.

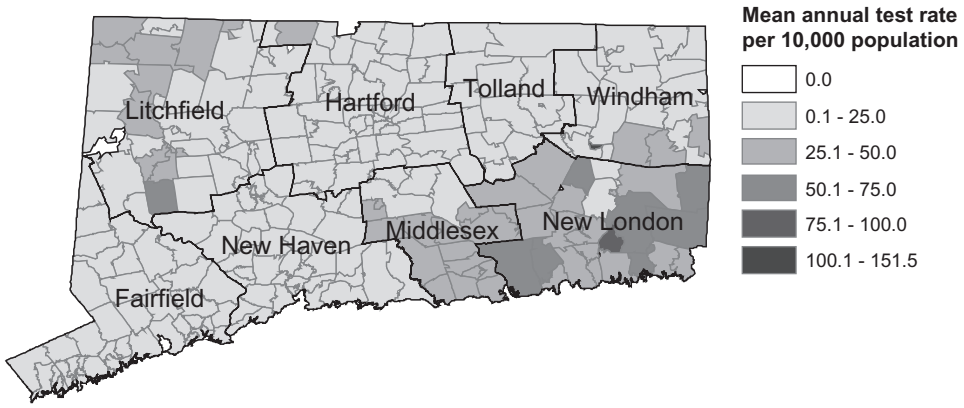

Fig. 2. Connecticut map depicting the *B. microti* mean annual IFA test rate, per 10,000 population, by zip code of residence, during the years 2000 through 2007.

Donors wishing not to be tested remained eligible for blood donation. After the review of data from a related study, demonstrating that 50% of seropositive donors tested were parasitemic,[11] all units at blood drives selected for testing (beginning on November 27, 2000) were quarantined until testing was complete. After this time, all seropositive donors were then notified of test results and deferred from future blood donation, and any associated products were discarded. Before November 27, 2000, quarantine and deferral were not in place; identified seropositive donors were retroactively deferred and any associated products withdrawn and discarded. All aspects of this study were reviewed and approved by the American Red Cross Institutional Review Board, but the study was not conducted under an investigational new drug application.

The geographic areas tested and the temporality of testing both expanded over time. In 2000, testing focused on blood drives in southeastern Connecticut, specifically Middlesex and New London Counties. These counties were chosen because clinical case reports of babesiosis[12] and the localized presence of *Babesia*-infected mice[13] suggested that they would be the most productive areas for identifying seropositive donors. In time, additional areas in Connecticut and two offshore islands in Massachusetts (Martha's Vineyard in 2002; Nantucket in 2004) were added as study sites (Figs. 1A and 1B). By 2005 testing was distributed throughout Connecticut (Fig. 2). For several years, testing was limited to blood drives held during peak tick-borne disease transmission season in Connecticut, defined as May through August. Beginning in 2006, testing was performed during all months of the year to determine year round seroprevalence.

Serologic testing

Red-top serum tubes, routinely collected from consenting donors, were used for testing by indirect immunofluorescence assay (IFA) for immunoglobulin (Ig)G antibodies to *B. microti*. IgG was utilized because it provides an overall measure of exposure to *Babesia* and therefore risk, whereas IgM was thought to be of low yield since it is short-lived. In addition, our companion donor follow-up study shows that many persistently IgG positive donors may be chronically infected with the parasite.[14] Testing was conducted as per the manufacturer's instructions (Focus Technologies, Inc., Cypress, CA). The IFA procedure utilizes microscope slide wells coated with *B. microti*-infected hamster RBCs as an antigen source. Briefly, serum samples were diluted 1 in 64 in phosphate-buffered saline (PBS) and 20 μL was added to each slide well containing fixed *B. microti* antigen and incubated at 37°C for 30 minutes in a humid chamber. After incubation, slides were washed for 10 minutes in PBS by agitation, rinsed in distilled water, and air-dried. Diluted fluorescein-labeled goat anti-human IgG conjugate (Focus Technologies) was added to each well and again incubated at 37°C for 30 minutes in a humid chamber. Slides were then washed for 10 minutes in PBS by agitation, rinsed in distilled water, and air-dried. Samples were examined by fluorescence microscopy at 400× magnification, considered positive at 1 in 64 or greater and titered to endpoint. Appropriate negative and positive controls were included in all IFA testing.

Statistical analysis

Statistical comparisons were performed using chi-square and/or Fisher's exact tests, unless otherwise stated. A p value of 0.05 or less was considered significant. The seroconversion rate was calculated by dividing the number of seroconverters, namely, donors that initially tested IFA negative and later tested IFA positive, by the total number of donors that were tested more than once. Total number of person-years of follow-up contributed by the donors donating more than once was calculated by determining

the interval from first (negative) test to the first positive test (and after deferral was implemented in November 2000, the sole positive test) for each donor, summing these numbers and dividing by 365.25. Incidence was calculated by dividing the total number of observed person-years by the observed number of seroconverters.

Additional analyses were conducted using a geographic information system (GIS) and SaTScan. GIS is a computer-based system that is capable of managing, analyzing and displaying spatial data. GIS and SaTScan were utilized for this analysis due to their ability to perform complex spatial analyses and project them in an easily viewable format. Connecticut data were analyzed using computer software (ArcView 9.3, Environmental Systems Research Institute, Inc., Redlands, CA). Layers were projected to World Geodetic System (WGS) 1984 and datum WGS 1984. Mapping was conducted based on each individual donation's associated donor zip code of residence, rather than zip code of the donation site, since published evidence suggests peridomestic transmission.[13] Donations from out-of-state resident donors were removed from the data set to work within state boundaries. Rates of testing were calculated by dividing the number of tested donations within each zip code by the zip code population (reported in census data for 2000) and then multiplied by 10,000. This number was then divided by the number of years of testing to get a mean annual test rate per 10,000 population (Fig. 2). Seroprevalence was calculated by dividing the number of IFA-positive donations by the number of tested blood donations per zip code and multiplying by 10,000. A smooth map of seroprevalence was created using ArcView 9.3 spatial analysis extension inverse distance weighted method. Inverse distance weighted is a method of spatial interpolation that assigns values (here, calculated seroprevalence levels), at locations where the value is not known, using the seroprevalence at known locations. Due to localized testing within the state of Massachusetts (confined to offshore islands of Martha's Vineyard and one drive in Nantucket) spatial analysis was not performed for Massachusetts zip codes.

Data were also analyzed using SaTScan, which is capable of conducting statistical analyses on spatial data. Cluster analyses were performed for Connecticut data using the Poisson Model of SaTScan 7.0.3 (Martin Kuldorff, with Information Management Services, Inc., Silver Spring, MD) to identify spatial clusters of statistical significance within the state. Cluster sizes were limited to 10% of the population at risk. Population at risk is defined as all tested donations for the purpose of this analysis. A total of 999 computer-generated replications (i.e., Monte Carlo method) were conducted. The identified statistically significant clusters were then joined to the existing seroprevalence table and overlaid on the seroprevalence map.

RESULTS

Seroprevalence, endpoint titers, seroconversion, and incidence data

From May 2000 to December 2007, a total of 27,592 donations collected in Connecticut and Massachusetts were tested by IFA. Ninety-two additional donations (0.3% of all the donations chosen for testing) were not tested because the donors declined to participate in the research study. Donations from out-of-state donors (n = 4288) were removed from further analysis, leaving 21,523 from Connecticut residents and 1781 from Massachusetts residents (total of 23,304 donations). These donations were made by 17,465 donors donating one or more times. Overall, 267 seropositive donations were identified (114.6 per 10,000 donations): 242 from Connecticut (112.4 per 10,000 donations) and 25 from Massachusetts (140.4 per 10,000 donations; Table 1). Annual seroprevalence values fluctuated moderately, ranging from a low of 75.3 per 10,000 donations in 2002 to a high of 182.3 in 2003. However, using linear regression analysis, no significant change was noted over the 8-year period. All 267 seropositive samples were titered to endpoint with reported titers ranging from 64 to 1024 or greater. A total of 127 (47.6%) had a reported endpoint titer of 64, and the remaining 140 (52.4%) of 128 or greater (Table 2).

During the 8 years of testing, 3471 of 17,465 donors were tested more than once, contributing 9310 of the 23,304 donations. Of these donors tested and found positive, 71 had previously tested negative, which represents a 204.5 per 10,000 seroconversion rate. The 3471 donors accounted for 7986 person-years of observation. The range of time from first to last tested donation was 56 to 2770 days. The 71 seroconversions represent 1 per 112 person-years.

Geographic analyses

Seroprevalence levels for the eight counties of Connecticut were compared to each other, with a significant difference found across them ($p < 0.01$). When Middlesex and New London counties were each compared to the remaining six counties, they both had significantly higher seroprevalence than all other counties ($p < 0.01$). A comparison of New London and Middlesex counties demonstrated a significant difference ($p < 0.04$), with New London having a greater number of observed than expected seropositive donations. Although the levels for the remaining six counties were not significantly different from each other, each county had a seroprevalence of 29.2 or greater per 10,000 donations (Table 1). Figure 3 illustrates the smoothed seroprevalence across individual Connecticut zip codes, determined using inverse distance weighted. Two geographic clusters of significance were identified in Connecticut ($p < 0.01$), one incorporating

TABLE 1. Annual serology (IFA) results by county of residence

	Number positive/number tested (positive per 10,000 donations)										
	Connecticut							Massachusetts			
Year	Fairfield	Hartford	Litchfield	Middlesex	New Haven	New London	Tolland	Windham	Dukes	Nantucket	Other
2000	0/23	0/53	0/5	4/453	0/68	24/1842	0/30	0/111	0/0	0/0	0/14
	(0)	(0)	(0)	(88.3)	(0)	(130.3)	(0)	(0)	(0)	(0)	(0)
2001	0/6	0/31	0/6	5/446	0/47	24/1442	0/12	0/43	0/0	0/0	0/27
	(0)	(0)	(0)	(112.1)	(0)	(166.4)	(0)	(0)	(0)	(0)	(0)
2002	0/116	0/33	1/418	1/180	0/59	13/1185	0/11	1/160	1/88	0/0	0/8
	(0)	(0)	(23.9)	(55.6)	(0)	(109.7)	(0)	(62.5)	(113.6)	(0)	(0)
2003	0/83	0/31	0/164	8/506	0/85	25/900	0/15	1/150	7/300	0/0	0/15
	(0)	(0)	(0)	(158.1)	(0)	(277.8)	(0)	(66.7)	(233.3)	(0)	(0)
2004	2/686	1/23	1/150	5/383	0/67	30/1355	0/9	2/111	3/275	3/41	1/41
	(29.2)	(434.8)	(66.7)	(130.5)	(0)	(221.4)	(0)	(180.2)	(109.1)	(731.7)	(243.9)
2005	0/132	4/415	0/146	0/132	4/263	12/401	1/159	3/148	6/235	0/0	0/18
	(0)	(1.0)	(0)	(0)	(152.1)	(299.3)	(62.9)	(202.7)	(255.3)	(0)	(0)
2006	3/519	6/668	2/147	4/383	2/538	9/592	4/200	0/93	2/271	0/0	0/73
	(57.8)	(89.8)	(136.1)	(104.4)	(37.2)	(152.0)	(200.0)	(0)	(73.8)	(0)	(0)
2007	5/917	5/1489	0/335	8/333	8/1055	13/437	1/335	0/188	2/312	0/0	0/63
	(54.5)	(33.6)	(0)	(240.2)	(75.8)	(297.5)	(29.9)	(0)	(64.1)	(0)	(0)
Total	10/2482	16/2743	4/1371	35/2816	14/2182	150/8154	6/771	7/1004	21/1481	3/41	1/259
	(40.3)	(58.3)	(29.2)	(124.3)	(64.2)	(184.0)	(77.8)	(69.7)	(141.8)	(731.7)	(38.6)
State total				242/21523 (112.4)						25/1781 (140.4)	
Combined total						267/23304 (114.6)					

portions of New London and Middlesex Counties and one restricted to New London County (Fig. 4).

Testing in Massachusetts focused almost solely on blood donations collected in Dukes County (Fig. 1B), which incorporates the island of Martha's Vineyard, with the exception of a single drive held in Nantucket in 2004. For this reason, it was not possible to statistically compare numbers for counties within the state of Massachusetts. A total of 1481 donations from residents of Dukes County were tested and 21 (141.8 per 10,000 donations) were seropositive. For Nantucket County, 3 of 41 (731.7 per 10,000 donations) donations tested were seropositive. An additional 259 donations were tested from residents dispersed throughout mainland Massachusetts counties, and 1 (38.6 per 10,000 donations) was seropositive. The sole seropositive donation was from a donor residing in Berkshire County (Table 1).

Seasonal trends

In addition to the previously discussed geographic analyses, temporal data analyses were also conducted. Connecticut and Massachusetts aggregate data (2000-2007) were divided into monthly quarters (January-March, April-June, etc.) and assessed for seasonality of *Babesia* seropositivity using chi-square contingency tables. A significant difference ($p < 0.05$) between the quarters was observed; the seroprevalence for the July through September period was significantly higher than for the remaining three quarters ($p < 0.03$; Fig. 5). Although not significantly different from one another, each remaining quarter had a 77.9 per 10,000 seropositive rate or greater. Furthermore, one or more seropositive donors were identified in every month of the year.

Demographic factors

Males contributed 55% of the 23,304 donations tested, a proportion consistent with blood collections across the American Red Cross system. Seroprevalence in male donors was almost 50% higher than in females, 130.3 versus 95.6 per 10,000 donations ($p < 0.02$). The mean age for the tested population was 46 years and the range was 16 to 88 years. The age distribution of seropositive donors was not different from that of seronegative donors when comparing age categories (<21, 21-30, 31-40, 41-50, 51-60, 61-70, >71 years). Roughly 10% of all donations tested were from first-time donors, and the proportion that was seropositive (108.8 per 10,000 donations) was not significantly different from that for donations from repeat donors (115.2 per 10,000 donations).

DISCUSSION

Currently there are no approved and viable measures in place for eliminating the risk of transfusion-transmitted *B.*

microti; specifically, an FDA-licensed screening test for *B. microti* is not available. There have been an estimated 70 transfusion transmission cases reported in the United States, with eight associated deaths reported to the FDA in the past 3 years alone;[4,8,9] however, the number of cases of transfusion transmission is believed to be grossly underreported.[9,14] There are large gaps in knowledge regarding the true rate of transfusion-transmitted *B. microti*, the geographic range of infections, and the frequency of chronic carriers who pose a risk for transmitting the agent. The recent FDA-sponsored workshop highlighted these and other critical issues as they relate to *B. microti* transmission and blood safety. As established herein, significant numbers of blood donors in the northeastern United States demonstrate exposure to *B. microti*, supporting recent calls to mitigate the risk associated with this transfusion-transmitted parasite.

TABLE 2. Annual *B. microti* IFA-positive donors by titer

		Total positive	Titer				
Year	Number tested	≥64	64	128	256	512	>1024
2000	2,599	28	9	9	3	7	0
2001	2,060	29	17	6	5	1	0
2002	2,258	17	10	4	0	1	2
2003	2,249	41	24	10	1	4	3
2004	3,141	48	37	4	1	2	3
2005	2,049	30	14	8	5	3	0
2006	3,484	32	5	15	6	5	1
2007	5,464	42	11	21	7	2	1
Total	23,304	267	127	77	28	25	10
Percent			47.6	28.8	10.5	9.4	3.7

After 8 years of study, the observed *B. microti* seroprevalence in blood donations from Connecticut residents was 112.4 per 10,000 donations and 140.4 per 10,000 donations from Massachusetts residents with no significant change in seroprevalence over the 8-year study period. The highest seroprevalence levels were observed in Middlesex and New London Counties

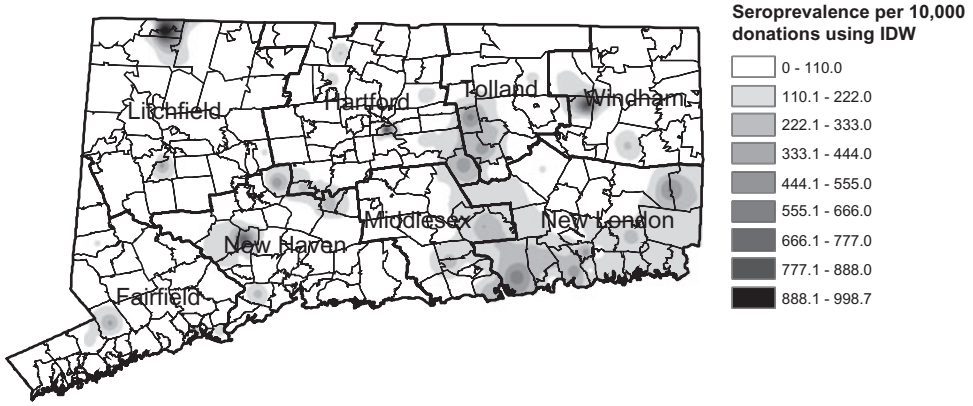

Fig. 3. Connecticut map depicting *B. microti* seroprevalence, per 10,000 donations, based on zip code of residence during the years 2000 through 2007. This map was smoothed using the inverse distance weighted method.

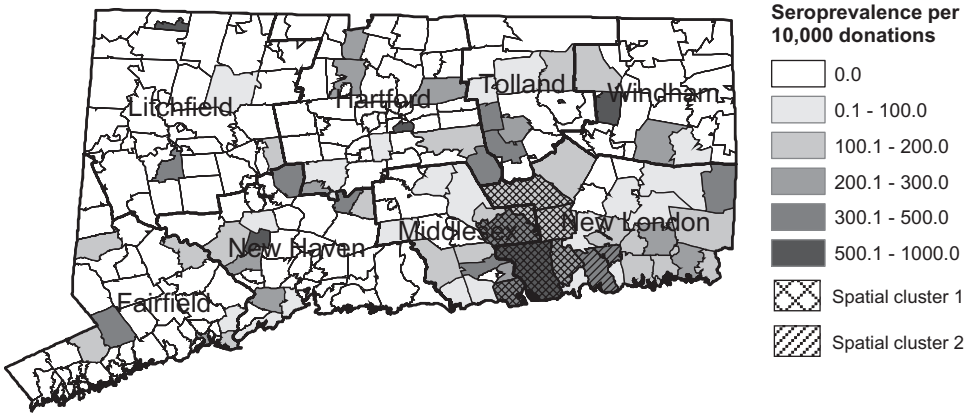

Fig. 4. Connecticut map depicting *B. microti* seroprevalence, per 10,000 donations, based on zip code of residence during the years 2000 through 2007. Two significant spatial clusters (using 10% of population at risk) are identified.

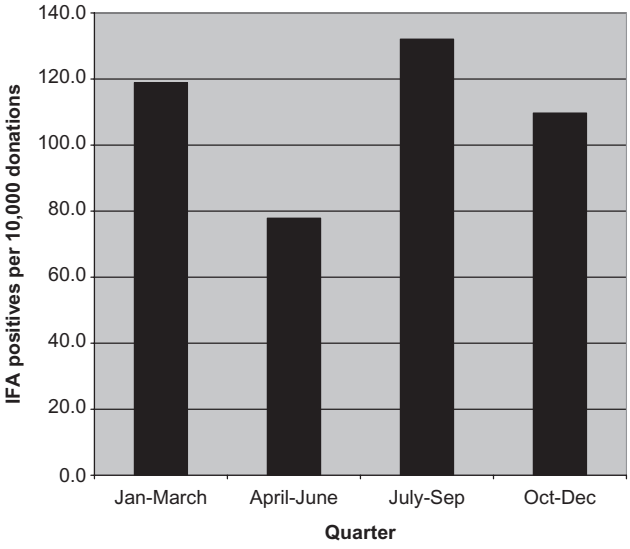

Fig. 5. Connecticut and Massachusetts serology (IFA) positives per 10,000 donations by seasonal quarter for the period 2000 through 2007.

from Connecticut and Dukes County from Massachusetts. Using cluster analysis, two significant spatial clusters were identified within the focal area of New London and Middlesex County, Connecticut, incorporating nine separate zip codes. However, seropositive individuals were not restricted to these areas; seropositive donations were identified from residents of all eight counties in Connecticut and from three counties in Massachusetts, suggesting broader geographic risk for *B. microti* infection. In addition to the geographic areas detailed in this study, *B. microti* has been identified from residents of several other areas in the United States, including, but not limited to, Minnesota,[15] New Jersey,[16] New York,[17] Rhode Island,[18] and Wisconsin.[19]

Geographic exclusion has been suggested as a viable method for reducing the risk of transfusion-transmitted *B. microti*.[20] As shown by this present study, defining areas of geographic risk is problematic. While focal areas of high endemicity are identifiable, defining the broader boundaries of geographic risk becomes increasingly difficult as one moves distally from endemic hot spots. Further, blood donors living within a defined endemic area are not restricted by state boundaries and may donate outside of their area of residence. Conversely, those living in nonendemic areas may travel to an endemic area and become infected, only to return home and donate.[21] Indeed, Tonnetti and colleagues[9] demonstrated that 24% of donors implicated in recent transfusion cases resided and donated in nonendemic areas. Considering that large swaths of the Northeast are endemic for *B. microti*, a geographic exclusion method of deferring donors that reside in or travel to a predefined endemic area would be impractical, leading to an unacceptably large loss of donors.

Like geographic deferrals, seasonal exclusion does not appear to be a practical method for preventing transfusion-transmitted *B. microti*. In this study, the quarter containing July through September had the highest seroprevalence rate (132.1 per 10,000 donations). Seasonality of *B. microti* transmission is directly related to epidemiology of the vector and human hosts. Infected ticks are more active during the spring and summer months, therefore increasing the likelihood of transmission during this period.[22] For this reason, some blood centers have chosen not to collect blood in highly endemic areas during the summer months;[20] however, our analyses demonstrate that there is the potential for transmission throughout the year because seropositive donors were identified during all months of the year. Anecdotal reports suggest that ticks can emerge on warm days outside of the normal transmission season and may be capable of transmitting *B. microti*. Additionally, through a separate ongoing study, we have identified individuals who appear to be chronic carriers of the parasite, maintaining high antibody titers and intermittent parasitemia for up to 3 years.[23]

Limitations of the study include the lack of data on the IgG immune response curve and a lack of simultaneous measures of infectivity. It is possible that the 1-in-64 donations could be in the early stages of the infection or in the convalescent phase. While IgG positivity does not correlate directly with infectivity, our ongoing follow-up study suggests that a significant percentage of persistently IgG-positive donors may be chronically infected with the parasite.[14,23] An additional limitation is the lack of rigorous data on IFA reproducibility. Finally, there is no confirmatory test available for *Babesia*. PCR is limited by sample volume and difficulties in testing large numbers of samples, but PCR also may be intermittently positive in chronically infected donors.[14] Because of these limitations the seroprevalence rates reported in this publication likely overestimate the true prevalence of *Babesia* infection in Connecticut and Massachusetts blood donors.

Additional methods suggested to safeguard the blood supply from *B. microti* include leukoreduction, pathogen reduction, and gamma irradiation. Unfortunately, each of these approaches has pitfalls that prevent them from being effective and/or viable options. Leukoreduction can at best only marginally reduce the parasite burden in blood products because *Babesia* is primarily an intraerythrocytic organism. Pathogen reduction has shown feasibility for plasma and platelet products, but has not yet proven to be effective for RBC products, which are associated with the vast majority of *Babesia* transmission cases.[24,25] Finally, gamma irradiation has not been demonstrated to be an effective measure; transfusion transmission associated with a gamma-irradiated product has been reported.[26]

In the absence of other sound alternatives, implementation of donor screening by an antibody and/or nucleic acid test (NAT) may be prudent. Any discussion of testing must acknowledge that a licensed assay for *B. microti* is presently not available; however, several blood organizations have suggested implementing a testing algorithm under investigational new drug application.[9,14] Given the restricted geographic distribution of *B. microti*, universal screening does not appear to be a reasonable or cost-effective approach. A selective approach, based on the CMV model, whereby a defined population of at-risk patients receives blood products determined to be *B. microti* negative, has also been suggested.[4] However, this option is complex and problematic, particularly when defining a population of at-risk patients.[27] Perhaps the most effective approach would be regional testing for *B. microti*, based on known endemic regions of the United States. While we earlier discussed the problems with precisely defining geographic areas of endemicity, a "superregional" testing approach may make sense. In essence, testing could initially focus on two primary regions, the Upper Midwest and the Northeast. While testing in these regions would certainly extend beyond the highly endemic areas and would likely include low-level and nonendemic areas, it provides the best option at this time for mitigating risk. If testing is limited to serology, there is the potential that window period positives will be missed and enter the blood supply; thus, at some point NAT should be considered in addition to antibody testing. Although the superregional approach will not capture all travelers and will result in the deferral of false-positive donors, the current approach of "benign neglect" is no longer acceptable in light of a well-defined and growing blood safety issue. No approach provides zero risk, nor will it address all possible *Babesia* pitfalls; however, the time has come to take the next step in protecting the blood supply from transfusion-transmitted *Babesia*.

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CONFLICT OF INTEREST

There are no conflicts of interest to report.

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