BLOOD PRODUCTS ADVISORY COMMITTEE
98th Meeting, July 26-27, 2010
Hilton, Washington D.C./North
620 Perry Parkway, Gaithersburg, MD

Topic I: Risk of *Babesia* Infection by Blood Transfusion and Potential Strategies for Donor Testing

Issue:

FDA is seeking advice from the Blood Products Advisory Committee (BPAC) whether, based on a risk analysis, the available data support development of a regionally selective donor testing strategy to reduce the risk of transfusion transmitted babesiosis. Additionally, the Committee is asked to comment on the suitability of donor screening either by a nucleic acid-based test (NAT), an antibody test, or both, given the current technology limitations.

Background:

Babesiosis is a tick-borne zoonosis caused by infections of humans with intra-erythrocytic protozoa of the genus *Babesia.* Babesiosis is locally prevalent in diverse regions of the United States. Several species of *Babesia* are present in the U.S., but the majority of cases of babesiosis are caused by infections with *Babesia microti*. Babesiosis is characterized by a wide spectrum of clinical manifestations that range from asymptomatic to severe acute or even fatal illness. While the disease is generally mild to moderate in children and young healthy adults, it is more severe in neonates, the elderly and immuno-compromised individuals such as those undergoing treatment for cancer (1, 2). In some individuals, infections may persist for up to 27 months without overt clinical illness (3). The proportion of *Babesia* infections that persist as asymptomatic, chronic infections is not known. In one study on Block Island (Rhode Island), one third of *Babesia* infections were asymptomatic (1), although the sample size was too small to draw firm conclusions. Asymptomatic individuals are difficult to recognize and, therefore, transfusion of blood and blood components collected from them may result in transfusion-transmitted babesiosis (TTB), leading to potentially fatal clinical illness.

TTB has been recognized as a major emerging threat to blood safety in the U.S. (4). Recent serological surveys in *Babesia*-endemic areas have confirmed the existence of asymptomatic individuals who test positive for both anti-babesial antibodies by immunofluorescence (IFA) and *Babesia* DNA by the polymerase chain reaction (PCR). In a seroprevalence study conducted from July through September by the American Red Cross (ARC) in endemic and non-endemic areas in Connecticut, 30 (0.9%) of 3490 donations were positive for IgG antibodies to *B. microti* by IFA. More significant, samples from 10 (53%) of 19 antibody-positive donors were also positive by PCR indicating that a proportion of antibody- positive donors are parasitemic (5). More recently, ARC published the results of their eight-year (2000-2007) seroprevalence study in Connecticut and Massachusetts where blood samples were collected every other month during the year. Among the 23,304 donations included in the study, 267 (1.14%) were seropositive by IFA. The yearly aggregate seroprevalence rate was relatively stable over the eight-year study period. Although the highest seroprevalence was observed from July through September, seropositive donors were identified in every month of the year (6). The seropositivity rate observed was not uniform across different counties in Connecticut (average highest 1.8% and average lowest 0.3%) suggesting that, within an endemic state, *Babesia* infection is highly focal. However, data collected by the ARC Hemovigilance Program suggested that of 17 antibody-positive donors

implicated in causing TTB, 11 (65%) were residents in *Babesia*-endemic areas while four (24%) were from non-endemic areas who had a history of travel to endemic areas (7), explaining why the risk of transmitting *Babesia* infection through blood transfusion is not limited to donors living in endemic areas.

The first U.S. case of TTB was reported in 1980 (8); since then more than 100 cases of transfusion-associated infections have been documented. However, the actual numbers of U.S. TTB are thought to be much higher. Another matter of great concern is the significant increase in the reported number of TTB-associated deaths: 10 of the 11 cases of fatal TTB (primary or contributory cause of death) reported to FDA occurred between 2006 and 2008 (9, 10). Prior to that, the last fatality was reported in 1998. These cases reveal an unexpected rise in the number of fatality reports. Despite this increasing public health concern, there is currently no FDA-approved laboratory test for *Babesia*, either for blood donor screening or for diagnostic use. The only safeguard against the risk of TTB is the commonly used FDA-recognized AABB donor questionnaire that includes the question, "Have you ever had babesiosis?" However, the growing number of TTB cases reported in recent years suggests that the current donor deferral-based approach to protect the blood supply from *Babesia* is not effective. Accordingly, the AABB Transfusion Transmitted Diseases (TTD) sub-committee has identified transfusion-transmitted *Babesia* infections as having one of the highest priority among emerging infectious diseases that pose a risk to blood safety (11).

In recognition of this growing concern about blood safety from the risk of TTB, FDA has attempted to increase the awareness of TTB and to solicit public input on how best to address this threat. To this end, on September 12, 2008, FDA organized a public workshop entitled "Approaches to Reduce the Risk of Transfusion-Transmitted Babesiosis in the United States." The focus of this workshop was to discuss various aspects of TTB in the U.S. including possible strategies to identify and defer blood donors who might have been exposed to *Babesia* parasites. Other topics of discussion at this workshop included the biology, pathogenesis and epidemiology of babesiosis. The scientific sessions were followed by discussions by expert panel members on the specific topics presented during each session. Experts agreed that additional epidemiological studies were needed to identify *Babesia*-risk areas. They discussed possible donor screening strategies and reentry algorithms that included selective testing in *Babesia* high-risk states only, and a "CMV-like model," testing only those blood units intended for transfusion to especially vulnerable recipients (e.g., neonates, sickle cell patients and those undergoing treatment for malignancies, etc.). Several speakers emphasized the need to develop highly sensitive and specific laboratory tests to identify *Babesia*-infected blood donors, especially tests to distinguish between current infections from previously-resolved infections. The meeting minutes and speakers' presentations of this workshop are available at **http://www.fda.gov/BiologicsBloodVaccines/NewsEvents/WorkshopsMeetingsConferences/TranscritsMinutes/default.htm.** In addition, a detailed summary of the workshop was published in the December, 2009 issue of the journal *Transfusion* (10). Based on a recommendation by the panel members, a task force of experts from public health agencies and blood banks was established in December 2008 by the AABB to discuss and develop approaches to protect the blood supply from risk of TTB.

At this BPAC meeting, FDA plans to present the following: (1) incidence of *Babesia* infections in different parts of the U.S.; (2) information on the number of TTB cases; and (3) a description of a risk assessment model that is intended to assist in overcoming TTB reporting limitations.

The Committee will be requested to consider this information and to advise FDA on an

appropriate testing strategy to mitigate the TTB risk in blood component recipients, including the adequacy of a regional testing strategy. In addition, FDA is seeking the Committee's comments on the suitability of donor screening either by a nucleic acid-based test (NAT), an antibody test, or both, given the current technology limitations.

Clinical Incidence, Transfusion-Transmitted Babesiosis and Assessment of *Babesia* Risk in the United States:

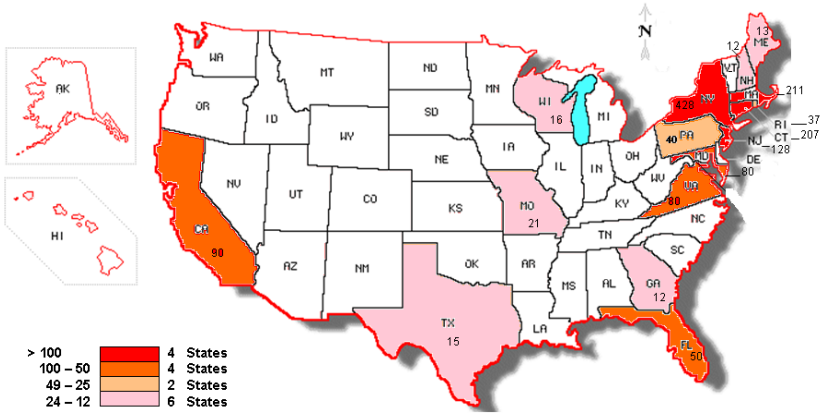
1. Incidence of Clinical Babesiosis in the United States

The first documented human case of babesiosis in the U.S. was reported in 1968 (12). The majority of U.S. babesiosis cases are caused by *B. microti*, the species that is prevalent in the Northeast and upper Midwest. A few other *Babesia* species such as *B. duncani* (formerly called WA1-type *Babesia*) and related organisms (CA1-type *Babesia*) are implicated in transmission of *Babesia* in several western U.S. states, while the other "*B. divergens*-like" agents such as MO1 have been reported in multiple U.S. states. The predominant species in Europe is *B. divergens*, reported to have caused >30 cases of babesiosis.

While reportable in several states, babesiosis is not a nationally notifiable disease in the U.S. In addition, the illness noticeably remains undiagnosed in many individuals, especially in healthy adults. Therefore, reliable data regarding the number of clinical cases of babesiosis is difficult to obtain. We searched the database of the Centers for Medicare and Medicaid Services (CMS) for the specific diagnosis of babesiosis (ICD-9 code "088.82") in order to estimate the incidence of babesiosis in different states. Incidence was defined as the number of babesiosis diagnosed patients during the period from 2006 to 2008, so long as the observed diagnosis was a patient's first babesiosis diagnosis in that period. Furthermore, a clean period of 365 days prior to the observed diagnosis was applied, during which time the diagnosed patient must have been continuously enrolled in Medicare Parts A/B Fee-for-Service, not enrolled in Medicare Part C, and not diagnosed with babesiosis by any provider.

Analysis of the CMS dataset revealed that the highest number of reports was restricted mainly to a few Northeastern and Mid-Atlantic states, namely New York, Massachusetts, Rhode Island, Connecticut, New Jersey, Maryland and Virginia. Surprisingly, California, while not considered a highly *Babesia* endemic state, reported a large number of cases. A lower number of cases were seen in several other states throughout the country (see Figure 1). These findings are consistent with the earlier reported regional bias in the *Babesia* natural transmission pattern observed in the U.S. (10). A vast majority of these CMS beneficiary claims bear treatment service dates within the months of July and August, coinciding with the peak *Babesia* transmission period.

Figure 1. Geographical distribution of babesiosis beneficiary claims in different states in 2008 (CMS dataset)*.



*States that had 11 or fewer babesiosis beneficiary claims or no reported claim are shown in white.

To normalize for the population size in different states and the District of Columbia, we also analyzed the dataset as the babesiosis incidence per 100,000 enrolled beneficiaries in each state (see Table 1). By this criterion, the highest average incidence index was seen in the state of Connecticut (44.9 per 100,000) followed by Rhode Island (34.3), New York (21.3) and Massachusetts (20.30). Incidentally, these same four states have generally been recognized as *Babesia* "hot spots" in the published literature (10), suggesting that the CMS dataset is a reliable resource for passive surveillance studies to estimate the burden of clinical babesiosis cases in the U.S. A striking feature of the CMS dataset was the wide distribution of babesiosis observed in the U.S. ─ disease was recorded in 43 of the 50 U.S. states in 2008. No cases were recorded in Nevada, Oregon, North Dakota, Kentucky, Louisiana, Montana or Wyoming. It is possible that infections reported in several low-incidence states were acquired during travels to *Babesia*-endemic areas. Nonetheless, these data clearly demonstrate that, notwithstanding the mode of acquisition, babesiosis occurs in large parts of the country, although at rates that vary greatly among different geographic regions (see Figure 1).

Table 1. Babesiosis incidence per 100,000 enrolled beneficiaries (CMS data).

State[†]	2006	2007	2008	Average
CT	40.0	45.3	49.3	44.9
RI	43.5	19.4	40.1	34.3
NY	23.4	16.9	23.6	21.3
MA	11.1	20.5	29.3	20.3
MD	9.5	8.9	13.6	10.6
NJ	9.8	8.1	12.6	10.2
VA	4.0	4.8	9.4	6.1
VT	*	*	*	4.7
DC	*	*	*	4.4
NH	*	*	6.7	4.1
ME	*	*	5.9	4.1
DE	*	*	*	3.6
PA	3.1	2.4	3.2	2.9
CA	1.7	2.0	3.7	2.4
MN	*	2.8	*	2.1

[†] Ranked based on average babesiosis cases per 100,000 enrolled beneficiaries in the state for 2006-2008.
* Fewer than 11 cases were reported in that year.

2. Transfusion-transmitted babesiosis in the United States

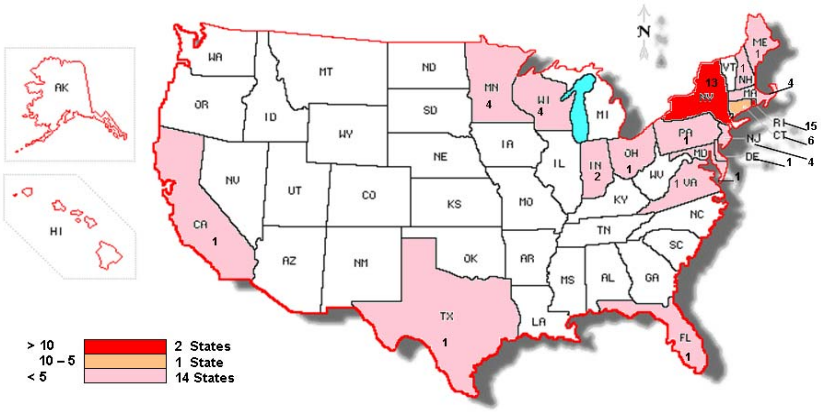
Dr. Barbara Herwaldt, Centers for Disease Control and Prevention (CDC) has compiled a dataset of TTB cases from reports to the CDC by state Departments of Health. Dr. Herwaldt generously shared her TTB dataset with FDA, and these data have been used as an input for FDA's babesiosis risk assessment.

The CDC surveillance data for 2004 through 2008 documented a total of 63 U.S. donors who were implicated in causing TTB (see Table 2 and Figure 2). For this analysis, it was assumed that each infected donor caused TTB in one recipient. Thus, an average of 12.6 TTB cases per year occurred during this five year period. Of the 63 documented cases, the state of donation and the state of blood transfusion were the same for 46 cases, but differed for 15 cases. Information regarding the state of donation was not available for 2 cases. The highest number of TTB cases ranked according to state of donation were reported from Rhode Island (15 cases) and New York (13 cases), while lower numbers of cases were recognized in Connecticut (6 cases) followed by New Jersey, Wisconsin, Minnesota and Massachusetts, each with 4 cases (see Table 2 and Figure 2). Even fewer cases (≤2) were noted in 10 other states.

Table 2. State-wise transfusion-transmitted babesiosis (TTB) cases in the United States in 2004-2008 (CDC data).

State of Donation	2004	2005	2006	2007	2008	TOTAL
RI	2	5	4	3	1	15
NY	2	1	4	1	5	13
CT		3	3			6
NJ	1	1		2		4
WI					4	4
MN			1		3	4
MA				1	3	4
IN		1		1		2
CA					1	1
MD		1				1
PA				1		1
FL					1	1
TX		1				1
OH			1			1
NH				1		1
VA				1		1
ME			1			1
Not Known			1	1		2
Total	5	13	15	12	18	63

Figure 2. Geographic distribution of transfusion-transmitted babesiosis cases in the U.S.

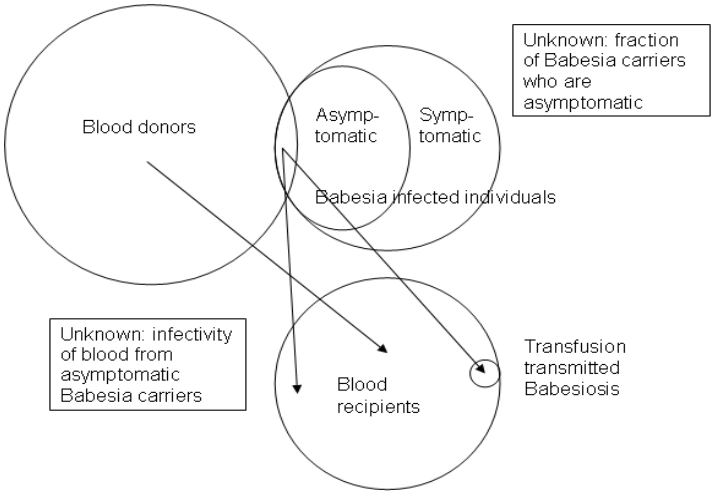


Given the absence of a robust national reporting system, and likelihood that many TTB cases remain undiagnosed, it is widely believed that there is significant underreporting of TTB. As a consequence, while the CDC dataset is the best available source of information on TTB within the U.S., it is likely to under-represent the actual numbers.

3. Assessment of *Babesia* risk in United States

We developed a probabilistic model to assess the *Babesia* risk in different states. The CMS babesiosis data coupled with the CDC TTB data were used to develop a model to predict the potential number of actual cases of babesiosis and TTB in each state. The model has uncertainties. The risk assessment estimates risks for TTB even in states where no TTB cases have been reported but babesiosis from other routes of exposure has been recognized. More accurate data for key parameters, such as the rate of asymptomatic carriers among blood donors, the probability of transmission by infected units, and the performance characteristics of potential screening tests, would improve the utility of the FDA risk assessment model.

Figure 3. A model representing a conceptualization of different elements that influence the occurrence of transfusion-transmitted babesiosis cases in the United States.



In this model, asymptomatic *Babesia*-infected individuals are represented by a single circle within a larger circle of *Babesia*-infected individuals. It is these asymptomatic carriers that represent the primary risk for TTB (Figure 3). The number of symptomatic *Babesia* infections is estimated from the annual number of diagnoses of babesiosis derived from CMS billing data for a population that comprised approximately 11% of the total state population during the three-year period studied. Consequently, the annual number of diagnoses of babesiosis derived from CMS data represents an uncertain fraction of total U.S. cases of symptomatic babesiosis. An even greater uncertainty surrounds the fraction of total *Babesia* cases that are asymptomatic carriers.

We estimated the fraction of asymptomatic carriers and used this value to predict the total number of observed TTB cases. This fraction represents the lowest potential fraction, as we believe that the true number of cases of TTB is higher than the number observed. The second important uncertainty is the likelihood of transmission of babesiosis by a unit of blood from an asymptomatic infected individual. This mean infectivity rate is assumed in the model as having a value of one, or in other words, each unit collected from an infected individual is considered infectious. Although this assumption is unlikely to occur, for the sake of simplicity, rather than incorporating two uncertainties into the model to predict the number of cases of TTB, a single value is used.

The analysis takes the *average incidence* over three years (2006-2008) of babesiosis for each state and the District of Columbia as estimated by the number of CMS beneficiaries who had a diagnosis of babesiosis (ICD-9 code: 088.82) while continuously enrolled in Medicare Part A, B, and not C, but did not have a diagnosis of babesiosis, from any provider, in the year preceding the claim. We have incorporated the uncertainty of the babesiosis incidence for these three years through a Bayesian analysis described in the Appendix. The uncertainty was carried through the calculations below using a Monte Carlo analysis with @Risk software. The *rate of babesiosis* in each state was estimated by dividing the incidence by the number of CMS beneficiaries of the state who were enrolled in Medicare Part A, B and not C continuously for 365 days ending in any Standard Analytical File year. Using this *average rate* of babesiosis per 100,000 population in each state, the ratio of asymptomatic *Babesia* carriers to symptomatic babesiosis was estimated to be 0.0175 based on subsequent calculations that yield a predicted value of TTB that equals observed TTB (CDC data). This ratio is low because there are a large number of babesiosis cases in the CMS data relative to the number of TTB cases as compiled by CDC. This ratio scales the number of babesiosis cases in the CMS data so that the FDA risk assessment model predictions match the CDC reported cases. Because of the associated uncertainties and likely underreporting of TTB cases, a sensitivity analysis was also conducted using 10 times this ratio, or 0.175 (Table 3). Although the ratio used does not affect the relative ranking of predicted number of TTB cases by state, it does affect the absolute predicted number of TTB cases.

The number of asymptomatic *Babesia* carriers was calculated by multiplying the estimated babesiosis incidence by the ratio of asymptomatic to symptomatic cases, and multiplying this by the state adult population, adjusting for the rate per 100,000. The number of asymptomatic *Babesia* carriers that donate blood was estimated by multiplying the number of asymptomatic carriers in a state by 5%, which is the approximate fraction of the U.S. population that donates blood. This number is multiplied by the average number of donations per donor per year (1.7) to yield the predicted number of average cases of TTB per state, which totals to the observed average TTB cases (see Table 3).

Table 3. Predicted numbers of transfusion transmitted babesiosis cases based on risk estimates derived from the CMS dataset*.

State**	Assumed Asymptomatic/ Symptomatic Ratio		STATE	Assumed Asymptomatic/ Symptomatic Ratio	
	1.75%	17.5%		1.75%	17.5%
NY	4.94 (4.71, 5.18)†	49.42 (47.1, 51.8)	VT	0.04 (0.02, 0.05)	0.35 (0.2, 0.5)
CT	1.87 (1.74, 2)	18.67 (17.4, 20)	DC	0.03 (0.02, 0.05)	0.32 (0.2, 0.5)
MA	1.57 (1.45, 1.7)	15.72 (14.5, 17)	UT	0.03 (0.01, 0.06)	0.31 (0.1, 0.6)
CA	1.07 (0.94, 1.21)	10.71 (9.4, 12.1)	NM	0.03 (0.01, 0.05)	0.29 (0.1, 0.5)
NJ	1.05 (0.96, 1.15)	10.53 (9.6, 11.5)	OH	0.03 (0.01, 0.05)	0.28 (0.1, 0.5)
MD	0.72 (0.63, 0.8)	7.15 (6.3, 8)	NV	0.03 (0.01, 0.05)	0.28 (0.1, 0.5)
VA	0.56 (0.49, 0.63)	5.58 (4.9, 6.3)	OK	0.03 (0.01, 0.04)	0.26 (0.1, 0.4)
FL	0.45 (0.39, 0.52)	4.50 (3.9, 5.2)	TN	0.02 (0.01, 0.04)	0.24 (0.1, 0.4)
PA	0.43 (0.37, 0.5)	4.32 (3.7, 5)	OR	0.02 (0.01, 0.04)	0.21 (0.1, 0.4)
RI	0.43 (0.36, 0.5)	4.30 (3.6, 5)	IN	0.02 (0.01, 0.03)	0.20 (0.1, 0.3)
TX	0.22 (0.17, 0.27)	2.15 (1.7, 2.7)	AL	0.02 (0.01, 0.03)	0.18 (0.1, 0.3)
MI	0.13 (0.1, 0.17)	1.35 (1, 1.7)	HI	0.02 (0.00, 0.03)	0.16 (0.0, 0.3)
MN	0.13 (0.1, 0.18)	1.34 (1, 1.8)	AR	0.01 (0.01, 0.02)	0.14 (0.1, 0.2)
WI	0.13 (0.1, 0.17)	1.29 (1, 1.7)	WV	0.01 (0.00, 0.02)	0.13 (0.0, 0.2)
CO	0.12 (0.08, 0.17)	1.24 (0.8, 1.7)	IA	0.01 (0.00, 0.02)	0.12 (0.0, 0.2)
NC	0.12 (0.09, 0.15)	1.16 (0.9, 1.5)	MS	0.01 (0.00, 0.02)	0.09 (0.0, 0.2)
AZ	0.11 (0.07, 0.15)	1.11 (0.7, 1.5)	KY	0.01 (0.00, 0.02)	0.09 (0.0, 0.2)
MO	0.09 (0.06, 0.12)	0.88 (0.6, 1.2)	SD	0.01 (0.00, 0.02)	0.09 (0.0, 0.2)
GA	0.09 (0.06, 0.12)	0.86 (0.6, 1.2)	LA	0.01 (0.00, 0.02)	0.08 (0.0, 0.2)
NH	0.07 (0.04, 0.09)	0.65 (0.4, 0.9)	NE	0.01 (0.00, 0.02)	0.07 (0.0, 0.2)
ME	0.06 (0.05, 0.09)	0.64 (0.5, 0.9)	AK	0.01 (0.00, 0.02)	0.06 (0.0, 0.2)
KS	0.06 (0.04, 0.08)	0.57 (0.4, 0.8)	ID	0.00 (0.00, 0.01)	0.05 (0.0, 0.1)
WA	0.05 (0.03, 0.07)	0.46 (0.3, 0.7)	ND	0.00 (0.00, 0.01)	0.03 (0.0, 0.1)
IL	0.04 (0.02, 0.06)	0.40 (0.2, 0.6)	MT	0.00 (0.00, 0.01)	0.01 (0.0, 0.1)
SC	0.04 (0.02, 0.06)	0.40 (0.2, 0.6)	WY	0.00 (0.00, 0.00)	0.00 (0.0, 0.0)
DE	0.04 (0.02, 0.06)	0.37 (0.2, 0.6)	Total	14.98	149.79

*The calculation for the predicted number of *Babesia* infected donors in each state is derived by using the following formula: Risk estimation was calculated as follows: Number_of_predicted TTB cases_per_state = average_symptomatic_babesiosis_per_state × ratio_asymptomatic_to_symptomatic × state_population_fraction × U.S. adult population_adjusted × fraction_that_donate × average_donations_per_year.
**States are ranked based on the degree of estimated risk.
† Numbers in parenthesis are 90% confidence intervals.

As an example of the calculation for the average number of predicted annual cases of TTB for CT: the average incidence of babesiosis per 100,000 from CMS data for the years 2006 to 2008 (44.9) was multiplied by a ratio of asymptomatic to symptomatic of 1.75%. The result is the average incidence of asymptomatic individuals per 100,000 (0.78). From this incidence, the number of asymptomatic individuals was calculated by multiplying the incidence times CT adult population and correcting the rate per 100,000 (approximately 22 individuals). Of this number we estimate that 5% will donate and this represents about 1.1 individuals donating blood that is potentially infected with *Babesia*. These 1.1 individuals donate 1.7 times per year to equal approximately 1.8 units of *Babesia* contaminated blood which we are assuming causes 1.8 cases of TTB.

A ratio of 1.75% asymptomatic carriers to symptomatic cases predicts a total number of approximately 15 TTB cases (Table 3) that closely matches the average annual number reported for 2006 to 2008 (approximately 15, Table 2). However, it is well recognized that due to the lack of rigorous reporting and a national notification system, both clinical cases and TTB cases are under-reported. To adjust for this known deficiency in the available dataset, we also calculate state-wise risk at a 10-fold higher asymptomatic carrier to symptomatic case ratio of 17.5% that predicts approximately 150 TTB cases (Table 3).

We compared the *Babesia* risk in different states based on the incidence of cases of babesiosis (CMS dataset), the observed cases of TTB (CDC dataset) and with the FDA's risk assessment data. Table 4 shows the ranking of the states by incidence rate of babesiosis per 100,000 units of population, whereas Table 5 shows a ranking of the state based upon the predicted number of TTB cases. The analysis showed a general concordance between the CMS and CDC data in *Babesia* risk with a few exceptions. Selected states in New England and the Mid Atlantic rank at the top of the incidence rates and also have a large number of observed cases of TTB; however, some states in the upper Midwest (WI, and MN) have higher numbers of TTB than are predicted by CMS data. One possible explanation is that TTB risk in these states was acquired through imported cases from endemic areas. The Midwest states of Ohio and Indiana also have a high number of observed cases of TTB despite low incidence of babesiosis in their CMS beneficiaries, but the CDC data for these cases of TTB indicate that the donor had traveled to an endemic area before donation.

Table 4. Ranking of states based on *Babesia incidence rate per 100,000 population*. The risk estimation is based on the CMS and CDC datasets.

States with *Babesia* Risk	Ranking based on the CMS data	Ranking based on the CDC data†
CT	1	2
RI	2	1
NY	3	7
MA	4	8
MD	5	11
NJ	6	9
VA	7	12
VT	8	No TTB reported
DC	9	No TTB reported
NH	10	4

†The data from Table 2 were divided by state population and the results were ranked.

Table 5. Ranking of states based on predicted number of babesiosis beneficiary claims from the CMS dataset and estimated number of transfusion transmitted babesiosis cases based on the FDA risk assessment.

States with *Babesia* Risk	Ranking for predicted TTB cases based on FDA risk assessment*
CT	2
RI	10
NY	1
MA	3
MD & DC	6
NJ	5
VA	7
PA	9
CA	4
NH	20

* Assumes an asymptomatic carrier/symptomatic case ratio of 1.75%

Results from Table 4 show a reasonable concordance in the geographical *Babesia* risk in both the CDC and CMS datasets. Overall, these results suggest that the data inputs used for the risk analysis were robust, and FDA's risk model to predict *Babesia* risk in different states (Tables 3 and 5) was valid. Finally, by taking into account a combination of risk factors that included the estimated clinical babesiosis cases (CMS data), reported TTB cases (CDC data) and estimates from the FDA's risk assessment, we prepared a list of *Babesia* risk states. The following 14 states were identified based on the FDA risk assessment data: New York, Connecticut, Massachusetts, California, New Jersey, Maryland and the District of Columbia, Virginia, Florida, Pennsylvania, Rhode Island, Texas, Michigan, Minnesota, Wisconsin. We also included New Hampshire, Maine, Ohio and Indiana in the list because these states had reported cases of TTB while Delaware, and Vermont plus the District of Columbia were included because these states had reported clinical cases of babesiosis (Table 1) and are surrounded by several *Babesia* endemic states. These 20 states and the District of Columbia account for approximately 93% of the estimated *Babesia* risk in the U.S.

Discussion:

1. Current technologies and their limitations in detecting *Babesia* infections in humans

Blood film microscopy remains the most commonly used method to detect *Babesia* parasites in humans (13). However, this method requires a trained microscopist and is not very sensitive during very early and late stages of infection when parasites are present in low numbers. Also, *Babesia* parasites can be confused easily with *Plasmodium* species. Currently *B. microti* parasites cannot be cultured *in vitro*, and only a few laboratories perform animal inoculations to amplify the parasites. This method of parasite amplification has been used to detect parasitemia in blood film-negative samples (14). However, both blood film microscopy and animal inoculation methods lack the necessary sensitivity and are not practical for donor screening purposes.

Detection of anti-babesial antibody is frequently used to confirm diagnosis. The first antibody test

developed for *Babesia* was an indirect immunofluorescence assay (IFA). Both IgM and IgG classes of anti-babesial antibodies can be detected by IFA (15, 16). The presence of IgM antibodies may be suggestive of a recent or acute infection, but the absence of IgG antibodies in subsequent testing is considered evidence of a false positive reaction. An IFA titer of 1:1024 or greater is generally considered to indicate a current infection. Titers generally drop to 1:64 or less within 8 to 12 months after exposure, except for a few cases in which antibody titers may persist for years (3, 15). The long-term presence of IgG antibodies may reflect chronic infection, re-infection or previously resolved infection. For routine diagnostic and donor screening purposes, most laboratories have used a conjugate that reacts with human antibodies of all immunoglobulin classes.

IFA is considered the most sensitive assay to identify the low grade chronic *B. microti* infections that remain asymptomatic (10). However, IFA may be inadequate to detect the early phase of *Babesia* infections (prior to seroconversion). In initial studies, the sensitivity and specificity of the IFA test for *B. microti* were determined to be 100% (17). In subsequent multi-laboratory collaborative studies, the reported sensitivities were 88% to 96% and specificities were 90% to 100% (14, 18). One limitation of IFA is the limited cross-reactivity in the test between antibodies to *B. microti* and those against other human *Babesia* species and, therefore, parasites of individual *Babesia* species must be used as antigens. It is important to note that, in the U.S, the number of babesiosis cases caused by non-*microti Babesia* species is increasing. However, the biggest limitation of the IFA test as a donor screening tool is that it has not been adapted for high throughput donor screening.

The *B. microti* immunoblot assay, using an antigen prepared from *B. microti*-infected red blood cell lysates, had a sensitivity of approximately 96% and specificity of 99% (19). Enzyme immunoassays (EIA) are sensitive but have lacked the desired specificity. A commercial laboratory developed an EIA using a whole red blood cell extract with a sensitivity of 95.5% and specificity of 94.1% compared with IFA-positive samples (20). However, the most serious limitation of current EIA tests for *Babesia* antibodies is the high level of non-specific reactivity observed in samples from non-endemic areas. (21).

Several laboratory versions of the polymerase chain reaction (PCR) have been developed for the detection of *Babesia*. A PCR test is generally considered to be more sensitive than blood film microscopy for detecting *Babesia* parasitemia. In one study, the reported sensitivity of PCR was 95 to 100 percent with specificity of 100 percent in detecting blood-film-positive, acute babesiosis cases (14). Surprisingly, the analytical sensitivity (limit of detection) of the PCR-based tests for the detection of *Babesia* parasite in human blood has not been reported. In conclusion, even with the availability of the NAT of high analytical sensitivity and specificity, major challenges for the use of any nucleic-acid-based test for donor screening are a lack of knowledge about parasite burden during the early acute phase and in asymptomatic donors, and the potential presence of a very few *Babesia* parasites that could be present in a unit of donor blood. In addition, the infectious dose of *Babesia* parasites that cause a fulminant infection in a naïve host is not known.

Based on the above discussion, FDA's considerations on the potential suitability of NAT and antibody tests for detection of *Babesia* infection in donors include the following:

- Due to the intra-erythrocytic nature of *Babesia* parasites, and the likelihood of low-grade parasitemia during the early phase of acute infection (window period), and asymptomatic infections in chronic carriers, to be effective, a NAT will require a high level of sensitivity.

- The published Limit of Detection (LOD) of NAT for *Plasmodium falciparum* (another intraerythrocytic protozoan) is 20 parasites per ml of blood [16]. Given the close genetic and biological similarity between the *Plasmodia* and *Babesia*, and recent technological advances, FDA thinks that it should be feasible in the future to develop a PCR test for *Babesia* with an LOD equal to or superior to those of PCR tests that are available for the detection of *Plasmodium* species.

- Among antibody-based tests, IFA has been demonstrated to detect 100% of blood-film-positive, acute babesiosis cases and is expected to be highly sensitive in detecting donors with asymptomatic *Babesia* infections where antibody titers are maintained by a low grade infection.

- The biggest limitations of IFA and other antibody-based tests are their potential inability to detect early phase of infections and to distinguish between active infection and previously resolved infections.

- While IFA might merit use as a "confirmatory test", and for testing in limited donor settings, due to technological limitations (low throughput, subjective readout, requirement for a highly skilled operator), this test has not been adapted for large scale donor screening purposes.

- For babesial antibody screening, highly sensitive and specific non-IFA based antibody tests are needed.

- To be useful in improving blood safety from *Babesia*-risk and in minimizing unnecessary donor loss, both NAT and antibody tests must offer very low rates of false-positive and false-negative reactions.

2. Possible Strategies for *Babesia* Screening in Blood Donors

The significant geographical disparities in risk of natural *Babesia* infection in the U.S. suggest that a region-specific approach to blood donor testing for *Babesia* infection may be more appropriate than a national testing program. The FDA risk analysis suggests that implementation of a donor screening test in 20 States and the District of Columbia would address an estimated 93% of the current risk in donors, albeit with test sensitivity that is undefined at the present time. Considering that NAT can detect some infectious units and that ELISA tests for antibody likely would result in excessive deferral of donors if applied in high risk areas (donors with resolved infections may remain seropositive), a two phased development program for *Babesia* detection in donors may have public health merit.

Phase I: As a first step, a highly sensitive and specific NAT might be used to test blood donors in *Babesia*-risk areas. *Babesia*-risk areas have been defined according to the FDA risk analysis utilizing the data inputs of clinical babesiosis cases (CMS data) and TTB cases (CDC data). Based on the FDA's risk assessment, donor screening by NAT would be established in the following states: New York, Connecticut, Massachusetts, California, New Jersey, Maryland, Virginia, Florida, Pennsylvania, Rhode Island, Texas, Michigan, Minnesota, Wisconsin, New Hampshire, Maine, Ohio, Indiana, Delaware, and Vermont, and the District of Columbia.

Those 20 *Babesia* risk states and the District of Columbia represent approximately 60% of the total U.S. population ages 18 years and older. Assuming that blood donation rates are approximately equal across states, approximately 60% of all U.S. blood supply would undergo

NAT testing for *Babesia*.

Phase II: In the next phase, testing might be extended to include donors in lower-risk areas or in areas where natural transmission of *Babesia* is not known but clinical cases and/or infected blood donors have been reported. Some residents of non-risk states are infected during travels to endemic areas. Selection of areas for Phase II testing would be based on additional information acquired during Phase I studies and new risk analysis. High-throughput antibody-based tests such as EIAs may be more suitable for donor screening in areas of medium or low endemicity.

Other Considerations: The effectiveness of screening donors by NAT alone is currently uncertain. Effectiveness of NAT could be determined from a reduction in incidence of TTB in the endemic regions after testing has been implemented. However, if TTB were to continue to occur after NAT testing were implemented, then it would be reasonable to conclude that NAT testing alone is not sufficient to detect *Babesia* infections in blood donors.

Antibody-based donor screening tests may also have a role in improving blood safety, especially to identify those donors with low-grade, asymptomatic *Babesia* infections. However, it is imperative that antibody-based tests possess sufficient sensitivity to detect low-grade early infections (to minimize the window period) and the vast majority of asymptomatic infections. To avoid unnecessary donor loss, antibody-based tests should also be highly specific for the infectious state of the donor.

Data from clinical studies will help in determining whether NAT alone or antibody tests alone will be able to offer the desired sensitivity and specificity to meet the required performance criteria as a donor screening test. It is also possible that screening with both NAT and antibody tests will be necessary to effectively detect those donors with low-grade, persistent infections and to distinguish between active infections and previously resolved infections. In the interim, in the highest risk areas, a strategy to test a subset of donors for *Babesia* infection both by NAT and IFA could be pursued to maintain an inventory of blood components at lowest risk for use in neonates and immune compromised patients.

Ultimately, use of pathogen reduced blood components may provide a more robust safeguard against transfusion transmitted babesiosis than donor testing. However, the time course for development of pathogen reduction technology is uncertain at this time.

Questions for the Committee:

1. Do the FDA risk analysis and the available CMS and CDC datasets together support the concept of regional testing of blood donors for *Babesia* infections?

2. Given the current sensitivity limitation of NAT for *Babesia*, please comment whether the public health benefits of NAT testing warrant consideration of broad-based regional testing of donors by NAT.

3. Considering the current technologies, please comment on the suitability of antibody testing for *Babesia* infections in blood donors.

References

1. Krause, P. J., K. McKay, J. Gadbaw, D. Christianson, L. Closter, T. Lepore, S. R. Telford, 3rd, V. Sikand, R. Ryan, D. Persing, J. D. Radolf, and A. Spielman. 2003. Increasing health burden of human babesiosis in endemic sites. *Am J Trop Med Hyg* 68:431-436.
2. Krause, P. J., B. E. Gewurz, D. Hill, F. M. Marty, E. Vannier, I. M. Foppa, R. R. Furman, E. Neuhaus, G. Skowron, S. Gupta, C. McCalla, E. L. Pesanti, M. Young, D. Heiman, G. Hsue, J. A. Gelfand, G. P. Wormser, J. Dickason, F. J. Bia, B. Hartman, S. R. Telford, 3rd, D. Christianson, K. Dardick, M. Coleman, J. E. Girotto, and A. Spielman. 2008. Persistent and relapsing babesiosis in immunocompromised patients. *Clin Infect Dis* 46:370-376.
3. Krause, P. J., A. Spielman, S. R. Telford, 3rd, V. K. Sikand, K. McKay, D. Christianson, R. J. Pollack, P. Brassard, J. Magera, R. Ryan, and D. H. Persing. 1998. Persistent parasitemia after acute babesiosis. *N Engl J Med* 339:160-165.
4. Leiby, D. A. 2006. Babesiosis and blood transfusion: flying under the radar. *Vox Sang* 90:157-165.
5. Leiby, D. A., A. P. Chung, J. E. Gill, R. L. Houghton, D. H. Persing, S. Badon, and R. G. Cable. 2005. Demonstrable parasitemia among Connecticut blood donors with antibodies to Babesia microti. *Transfusion* 45:1804-1810.
6. Johnson, S. T., R. G. Cable, L. Tonnetti, B. Spencer, J. Rios, and D. A. Leiby. 2009. Seroprevalence of Babesia microti in blood donors from Babesia-endemic areas of the northeastern United States: 2000 through 2007. *Transfusion* 49:2574-2582.
7. Tonnetti, L., A. F. Eder, B. Dy, J. Kennedy, P. Pisciotto, R. J. Benjamin, and D. A. Leiby. 2009. Transfusion-transmitted Babesia microti identified through hemovigilance. *Transfusion* 49:2557-2563.
8. Jacoby, G. A., J. V. Hunt, K. S. Kosinski, Z. N. Demirjian, C. Huggins, P. Etkind, L. C. Marcus, and A. Spielman. 1980. Treatment of transfusion-transmitted babesiosis by exchange transfusion. *N Engl J Med* 303:1098-1100.
9. Gubernot, D. M., C. T. Lucey, K. C. Lee, G. B. Conley, L. G. Holness, and R. P. Wise. 2009. Babesia infection through blood transfusions: reports received by the US Food and Drug Administration, 1997-2007. *Clin Infect Dis* 48:25-30.
10. Gubernot, D. M., H. L. Nakhasi, P. A. Mied, D. M. Asher, J. S. Epstein, and S. Kumar. 2009. Transfusion-transmitted babesiosis in the United States: summary of a workshop. *Transfusion* 49:2759-2771.
11. Stramer, S. L., F. B. Hollinger, L. M. Katz, S. Kleinman, P. S. Metzel, K. R. Gregory, and R. Y. Dodd. 2009. Emerging infectious disease agents and their potential threat to transfusion safety. *Transfusion* 49 Suppl 2:1S-29S.
12. Western, K. A., G. D. Benson, N. N. Gleason, G. R. Healy, and M. G. Schultz. 1970. Babesiosis in a Massachusetts resident. *N Engl J Med* 283:854-856.
13. Vannier, E., B. E. Gewurz, and P. J. Krause. 2008. Human babesiosis. *Infect Dis Clin North Am* 22:469-488, viii-ix.
14. Krause, P. J., S. Telford, 3rd, A. Spielman, R. Ryan, J. Magera, T. V. Rajan, D. Christianson, T. V. Alberghini, L. Bow, and D. Persing. 1996. Comparison of PCR with blood smear and inoculation of small animals for diagnosis of Babesia microti parasitemia. *J Clin Microbiol* 34:2791-2794.
15. Ruebush, T. K., 2nd, E. S. Chisholm, A. J. Sulzer, and G. R. Healy. 1981. Development and persistence of antibody in persons infected with Babesia microti. *Am J Trop Med Hyg* 30:291-292.

16. Krause, P. J., R. Ryan, S. Telford, 3rd, D. Persing, and A. Spielman. 1996. Efficacy of immunoglobulin M serodiagnostic test for rapid diagnosis of acute babesiosis. *J Clin Microbiol* 34:2014-2016.
17. Chisholm, E. S., T. K. Ruebush, 2nd, A. J. Sulzer, and G. R. Healy. 1978. Babesia microti infection in man: evaluation of an indirect immunofluorescent antibody test. *Am J Trop Med Hyg* 27:14-19.
18. Krause, P. J., S. R. Telford, 3rd, R. Ryan, P. A. Conrad, M. Wilson, J. W. Thomford, and A. Spielman. 1994. Diagnosis of babesiosis: evaluation of a serologic test for the detection of Babesia microti antibody. *J Infect Dis* 169:923-926.
19. Ryan, R., P. J. Krause, J. Radolf, K. Freeman, A. Spielman, R. Lenz, and A. Levin. 2001. Diagnosis of babesiosis using an immunoblot serologic test. *Clin Diagn Lab Immunol* 8:1177-1180.
20. Loa, C. C., M. E. Adelson, E. Mordechai, I. Raphaelli, and R. C. Tilton. 2004. Serological diagnosis of human babesiosis by IgG enzyme-linked immunosorbent assay. *Curr Microbiol* 49:385-389.
21. Homer, M. J., I. Aguilar-Delfin, S. R. Telford, 3rd, P. J. Krause, and D. H. Persing. 2000. Babesiosis. *Clin Microbiol Rev* 13:451-469.

APPENDIX

A Bayesian Model for the Proportion of Babesiosis Positive Cases by State

In this appendix we describe how an estimate was made and confidence intervals found for the incidence rate of Babesia. As mentioned in section B, a search of the database of the Centers for Medicare and Medicaid Services (CMS) for the specific diagnosis of Babesiosis (ICD-9 code of "088.82") enabled us to estimate the number of Babesia cases by state. The CMS dataset also included whether the patient was younger or older than 65 as well as the year in which the service was provided. This Babesia billing information along with the number of Medicare/Medicaid enrollees by state allowed for us to estimate the proportion of Babesia positive individuals which are symptomatic by state. Let the number of recorded instances of Babesia be denoted by X_{ijk} and let N_{ijk} denote the number of Medicare enrollees where the index $i = 1,...,51$ runs through the number of states including the District of Columbia, the index $j = 1,2$ denoting whether the patient was younger or older than 65 and the index $k = 1,2,3$ denotes the years 2006, 2007, 2008, respectively. The proportion of patients was then estimated by the following Bayesian logistic regression model.

In the first Bayesian model we fit the data to a logistic regression with all main effects by tacitly assuming that the number of recorded cases X_{ijk} followed a binomial distribution given by

$$X_{ijk} \sim Bin(N_{ijk}, P_{ijk}) \qquad \textbf{(1)}$$

where P_{ijk} denotes the proportion of symptomatic Babesia in the ith state, jth age group and kth year. Following the logistic regression model we assumed that the probabilities P_{ijk} were given by

$$Logit\left(P_{ijk}\right) = \alpha_i + \beta_i + \gamma\left(U_k\right)$$

where U_k denotes the year of the test. For simplicity we centered this year variable so that $U_k = \{-1, 0, 1\}$ denotes the years $\{2006, 2007, 2008\}$. To complete the model specification at the primary level of hierarchy, we assumed that the parameters $\{\alpha_i, \beta_j, \gamma\}$ had prior distributions given by

$$\alpha_i \sim N(0, \sigma_\alpha^2)$$
$$\beta_j \sim N(0, \sigma_\beta^2)$$
$$\gamma \sim N(0, \sigma_\gamma^2)$$

Moreover, to facilitate the borrowing of strength amongst parameters we placed diffuse Gelman priors (see Carlin and Louis, 2009) on the variance parameter by assuming that

$$\sigma_\alpha \sim Unif(0.1,10)$$
$$\sigma_\beta \sim Unif(0.1,10)$$
$$\sigma_\gamma \sim Unif(0.1,10)$$

Why Bayesian?

The user may wonder why we chose to adopt a Bayesian rather than classical estimator for this problem. There are 3 primary reasons for this choice.

1. First, individual level data on each patient was not available nor observed. Consequently, the data were pooled at the overall state level with $\{X_{ijk}, N_{ijk}\}$ denoting the overall number of TTB cases and population size within the state. This would have meant that we artificially create a much larger dataset with data at the individual level where the response consisted of a 1 or 0 depending on whether the artificial respondent received TTB.
2. Second and more importantly, in many instances the incidence rate was so low that there were some instances where there were no instances ($X_{ijk} = 0$) of TTB recorded for some states. In this situation, classical logistic regression has difficulty is estimating parameters as the MLE estimate may not converge when instances of complete separation occur (see Agresti, 2004). To explain in another fashion, when $X_{ijk} = 0$ the MLE estimator for P_{ijk} is $\hat{P}_{ijk} = X_{ijk} / N_{ijk} = 0$, and thus $Logit(\hat{P}_{ijk}) = -\infty$. This can lead to results where convergence via the Newton-Raphson iteration process fails to converge when the MLE is computed.
3. Thirdly, the ultimate objective of the study was to develop estimates for the proportion so that a sample could be made in a Monte-Carlo simulation. As the result of Bayesian posterior inference is a posterior sample, the use of Bayesian posterior sample nicely fits into the overall objective of the study.

To conduct posterior inference we generated a production run of 50,000 MCMC posterior samples subsequent to a burn-in sample of 1,000 MCMC simulations. Convergence of the posterior sampling was observed almost immediately. Several tests were conducted which showed convergence. The posterior sampling was performed in WinBUGS which performed Gibbs sampling of the model parameters $\{\alpha_i, \beta_j, \gamma\}$.

Subsequent to this analysis it was decided for pragmatic purposes that the effects due to year and age $\{\beta_j, \gamma\}$ were not important for this analysis and so were set to zero. It

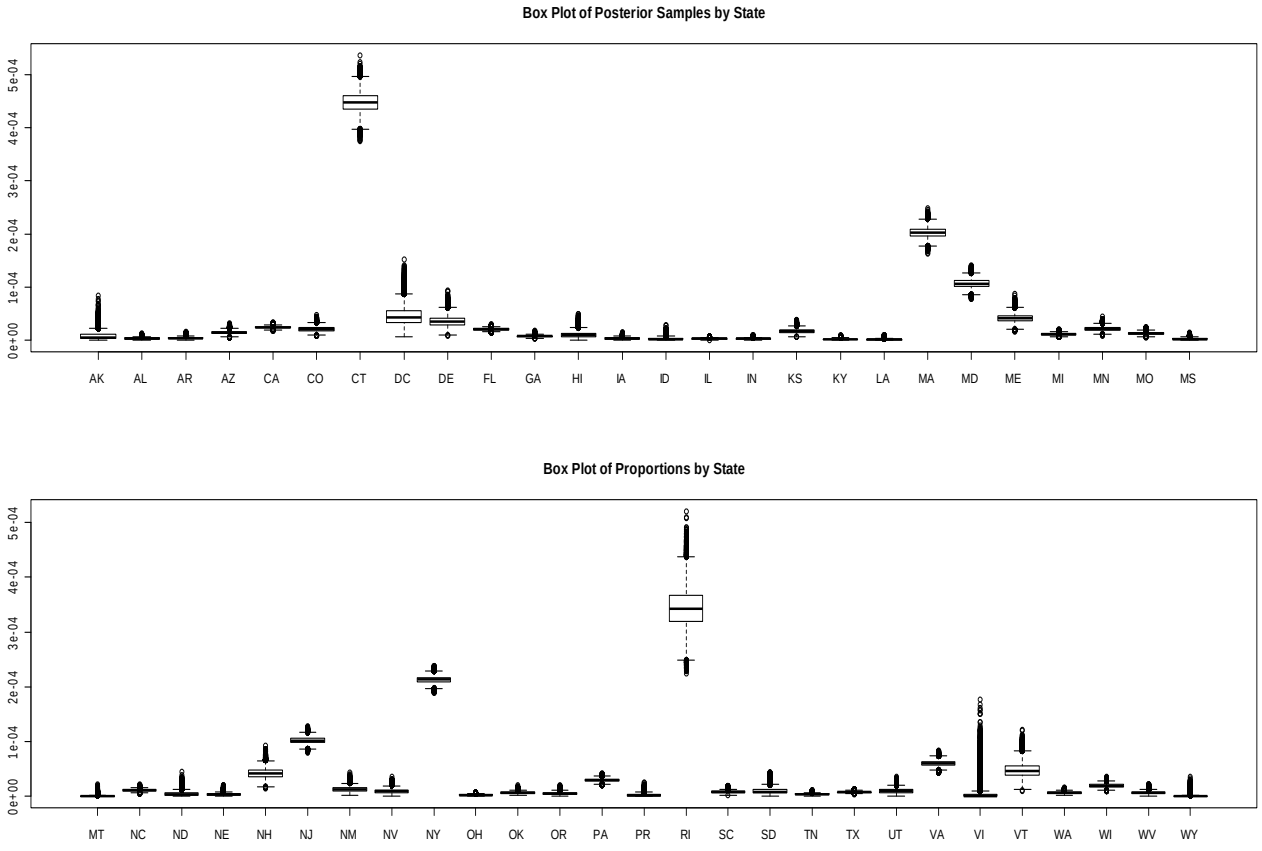
should be mentioned, however, that the variables related to age and year were both found to be statistically significant, with the younger population having a higher rate than the older population and the rate of Babesia slightly increasing with time. When the variables $\{\beta_j, \gamma\}$ associated with age and year were set to zero, this lead to the second reduced logistic regression model whereby

$$Logit\left(P_{ijk}\right) = Logit\left(P_i\right) = \alpha_i$$

with

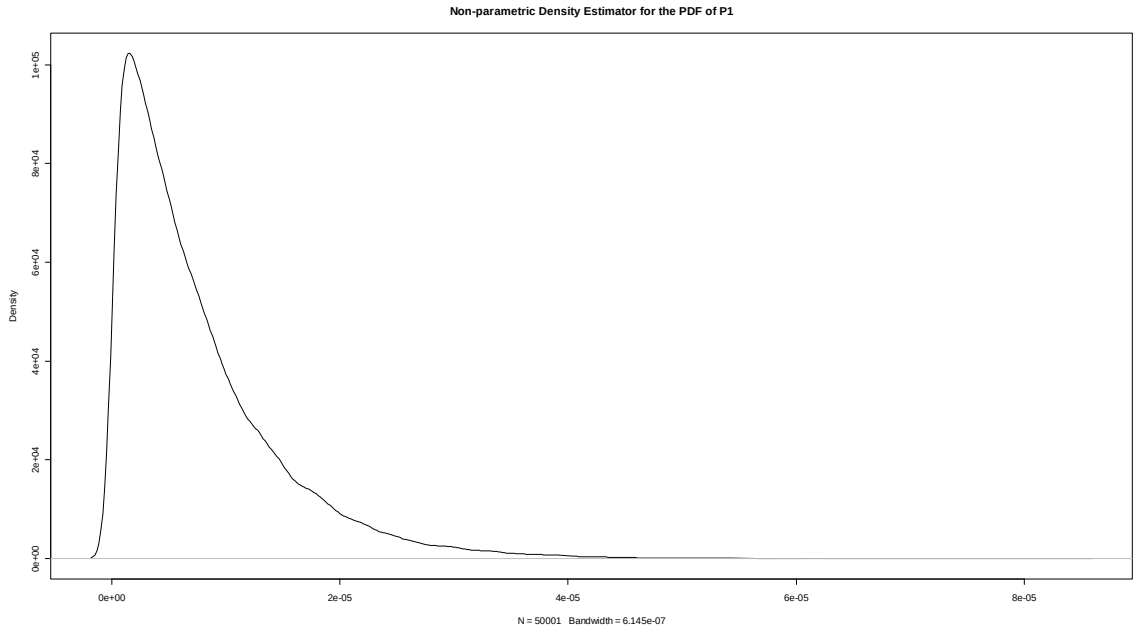
$$\alpha_i \sim N(0, \sigma_\alpha^2)$$
$$\sigma_\alpha \sim Unif\,(0.1, 10)\,.$$

The box and whisker plots below are of the posterior predictive proportions by state. One can see that certain states have a much higher incidence rate of Babesia than others.



Next we wanted to find the best parametric distribution for the proportion of TTB positive individuals P_i by state, so that this summary could be utilized in the @Risk

software. To come up with these model parameters, it was more reasonable to use all 50,000 posterior samples to estimate the distribution for P_i rather than merely utilize the classical MLE point estimate for the mean and corresponding confidence limits. This is particularly true since the distribution for P_i is skewed heavily to the right as the non-parametric density plot for P_1 shows below



One can see from this plot that any two sided confidence interval for the proportion P_1 would necessarily exclude more area in the right tail than the left. Often, if one obtained classical estimates for the 95% confidence interval $[L,U]$ enclosing the mean value of P_1, one would construct such intervals symmetrically and with large coverage. However, the skewness involved with these low probability events is so large that often that the coverage can either be conservative or anti-conservative depending on how one formulates such an interval. It is also arguable that the parameters estimated from a Bayesian posterior sample of 50,000 will always be more accurate than a distribution constructed on the basis of a classical point estimate for the mean and two confidence intervals $\left[\hat{L},\hat{U}\right]$. To see a concrete example of this we note that when the number of TTB cases for a state is zero ($X_{ijk}=0$), both the MLE estimator $\hat{P}_{ijk}$ and the lower confidence limit $\hat{L}_{ijk}$ equal 0 which means that the parameters of the distribution must be determined on the basis of a single point estimator for the upper confidence limit $\hat{U}_{ijk}$ alone. Naturally, this would lead to an ill posed problem if one had to estimate, for example, two parameters to a distribution on the basis of one single point estimator. Naturally, Bayesians would argue that if the ultimate aim of a study involves MC simulation, then a natural choice for all statistical models would be Bayesian ones, as the output of all Bayesian techniques is after all a posterior sample. Moreover, the posterior

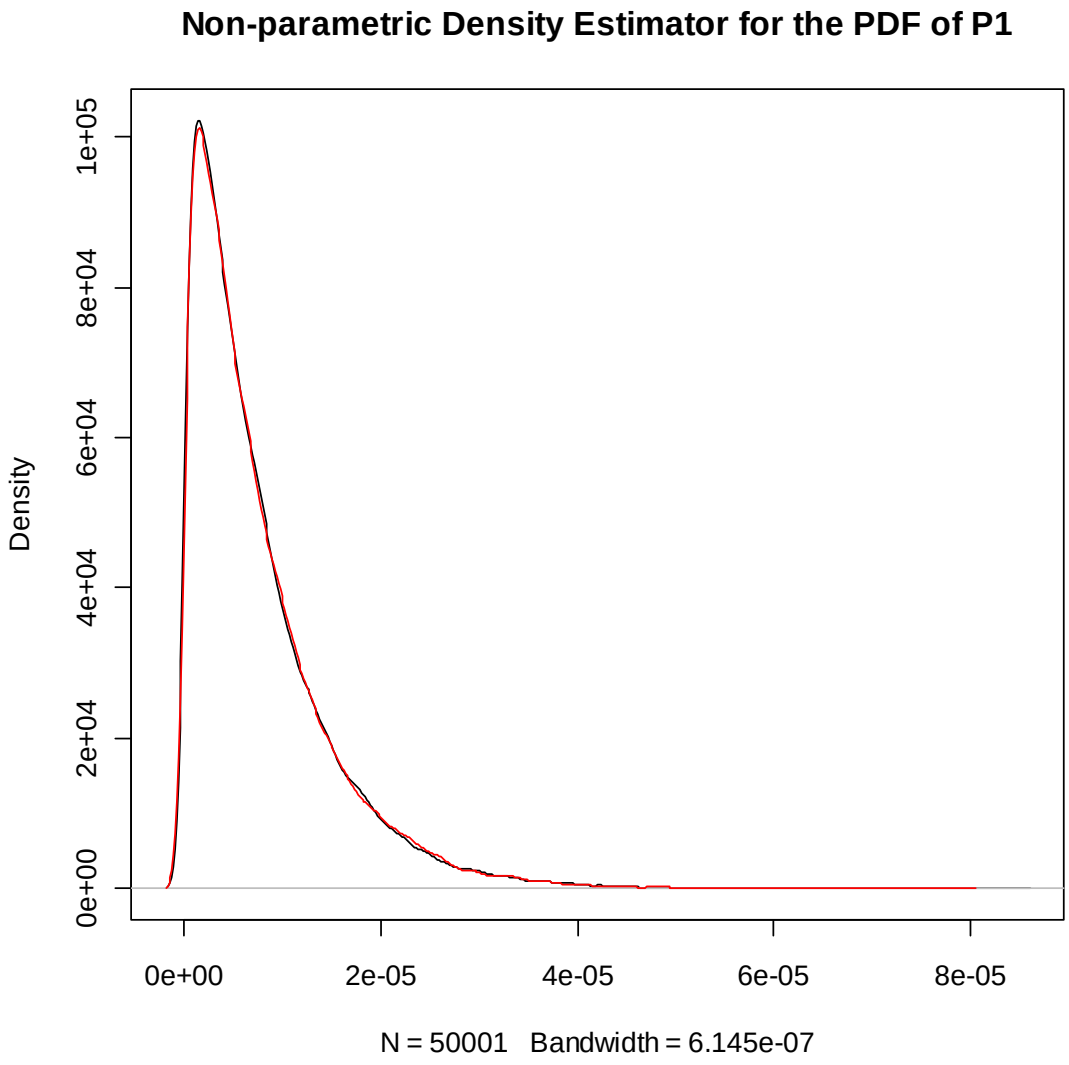
sample provides the analyst with a natural means of fitting distributions and nonparametric estimators to the posterior sample converge to the posterior density as the MCMC sample size grows larger.

Next, in order to facilitate subsequent analysis in the @Risk program, we wanted to find the best approximate beta distribution for each state. That is we wanted to find the best parameters $\{\theta_i, \xi_i\}$ such that the P_i were approximately distributed by

$$P_i \sim Beta(\theta_i, \xi_i).$$

To facilitate this, we utilized the "fitdistplus" package in R. This package is freely available off the web and can estimate the distributional parameters using any number of methods. We utilized the function $fitdist(\bullet, dist = "beta", method = "mle")$ which finds the best fit parameters $\left\{\hat{\theta}_i, \hat{\xi}_i\right\}$ for a sample of data by maximizing the likelihood under the null hypothesis that the data truly came from the beta distribution. We reasoned that the beta distribution was a reasonable choice for the proportion as this distribution is confined to the unit interval $[0,1]$. Provided that the posterior density for P_i was truly $Beta(\theta_i, \xi_i)$ the MLE procedure is tantamount to finding the posterior mode.

The closeness of fit to the posterior density is illustrated below. When the 50,000 posterior samples for P_1 were estimated using the "fitdist" program it produced the estimators $\hat{\theta}_1 = 1.143$ and $\hat{\xi}_1 = 151109.25$. We then generated 50,000 beta distributed random variables with the like parameters and overlaid the non-parametric density estimator for these against the non-parametric density estimator for the posterior sample. In the plot below the red line represents the non-parametric density estimator for the assumed beta distribution and the black line represents that for the posterior sample. Notice that the two coincide nearly exactly.



Subsequent analysis from the MC simulation was based upon utilizing these $\left\{\hat{\theta}_i, \hat{\xi}_i\right\}$ parameters. This further analysis entailed generating Monte Carlo simulations for the other uncertainties involved in the overall model such as the ratio of asymptomatic to symptomatic donors as well as the proportion of asymptomatic donors who will donate blood.