

Interventions for *Babesia* (and *Plasmodium*)



Susan L. Stramer, Ph.D.

May 16 2017



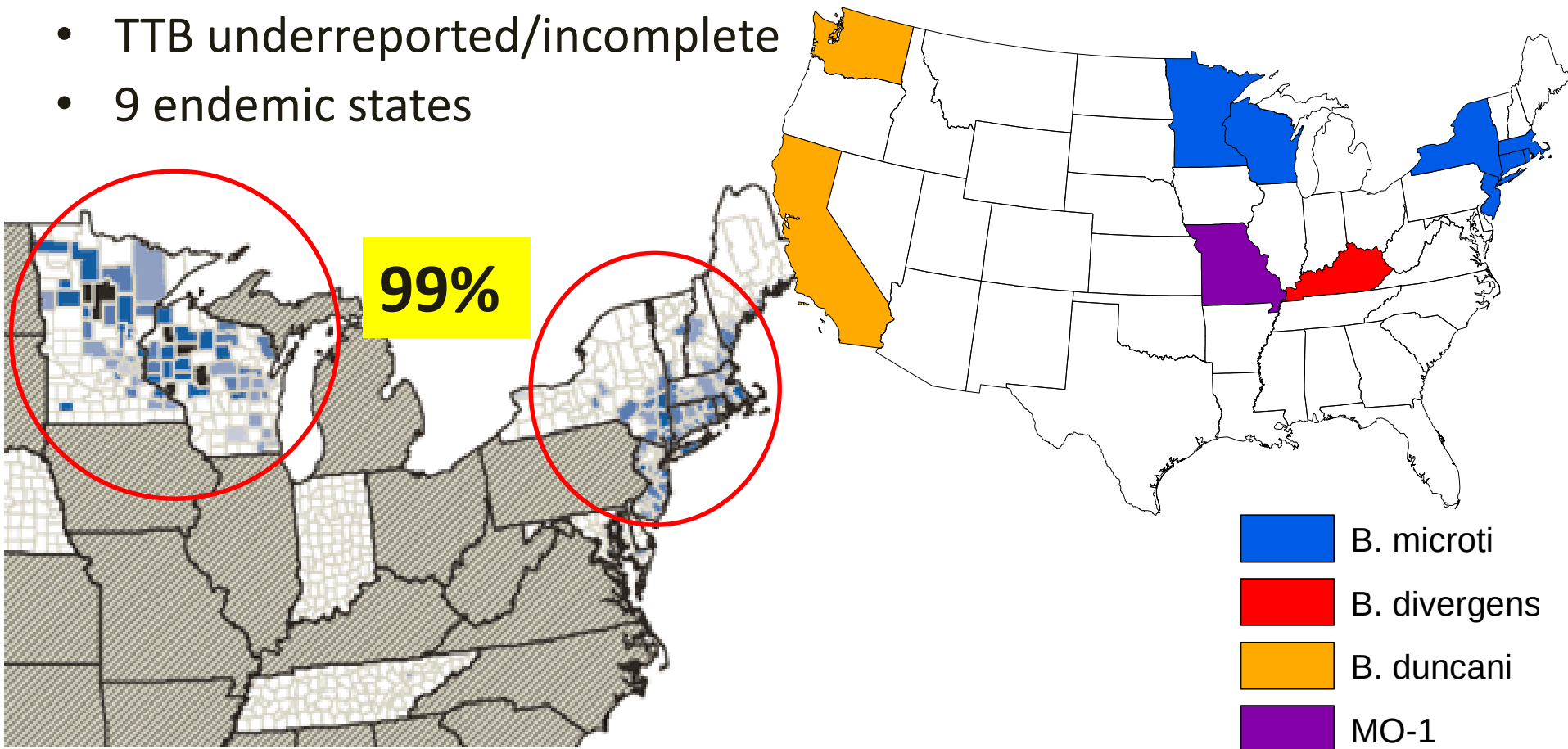
**American
Red Cross**

Babesiosis

- **Malaria-like illness caused by *Babesia* spp.**
 - Asymptomatic  fatal
 - Non-specific symptoms (malaise, fever, etc.)
 - Hemolytic anemia
 - Onset 1-9 weeks after exposure
- **General mortality 5-9%**
 - 21% immunocompromised
- **At risk:** infants, elderly, immunocompromised, asplenic, red cell disorders
- **However, risk groups not limited to above**

Babesia microti and *Babesia* spp.

- Intraerythrocytic tick-borne parasite
- Most frequent cause of tick-borne TT-fatalities reported to FDA
- 162 CDC (1979-2009) + 72 ARC (2010-2017) TTB cases
 - All *B. microti* except 4 *B. duncani*
- TTB underreported/incomplete
- 9 endemic states



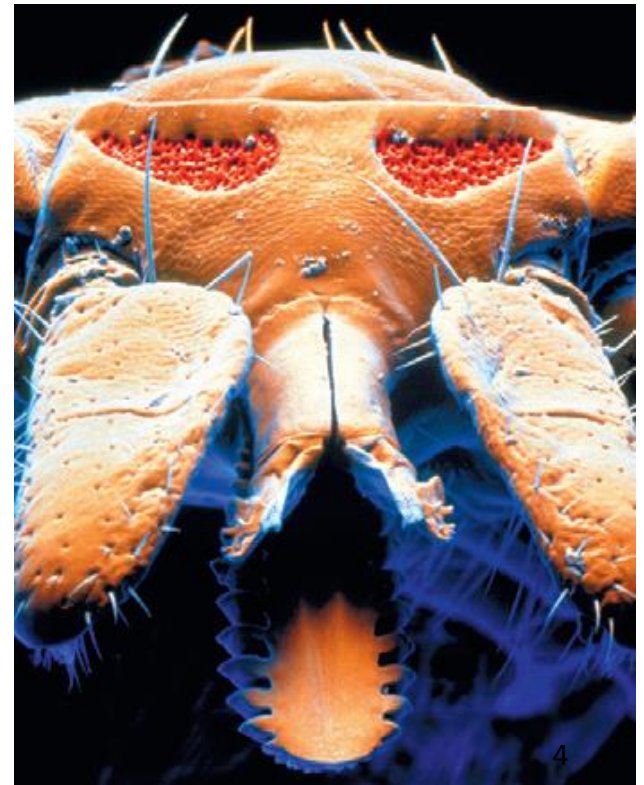
Incidence of reported cases of babesiosis by county of residence (2011)



The tick that gives you Lyme Disease is smaller than a poppyseed.

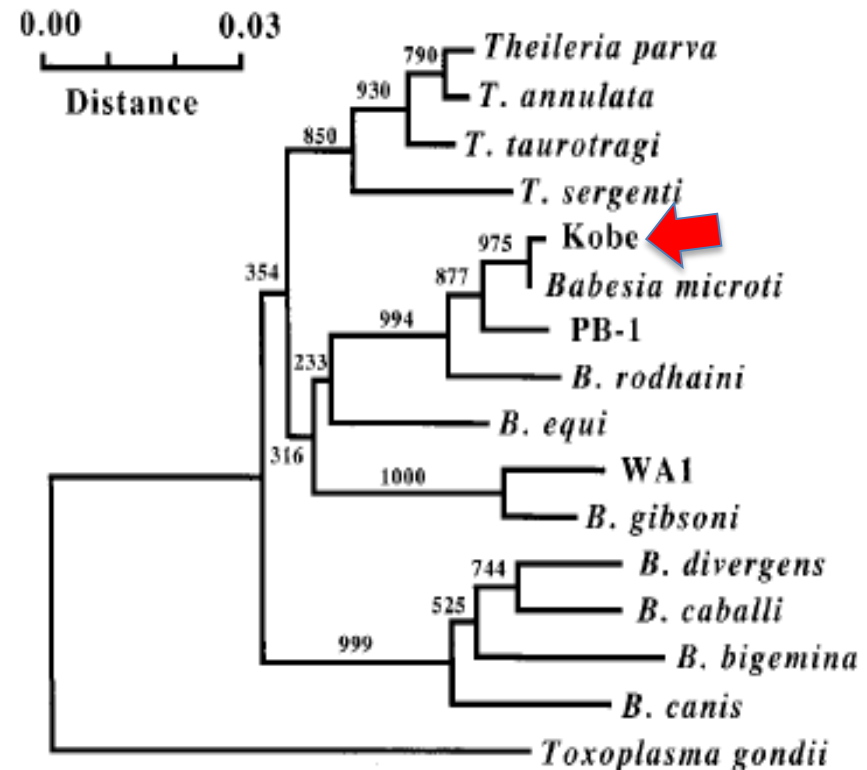


Deer tick nymphs



Outside of the US

- Reports of babesiosis worldwide (>100 babesia sp):
 - Japan, Taiwan, Europe, S Africa, S America, Australia
- 39 human cases published in Europe; all clinically severe and involved immunocompromised patients:
 - B. divergens* (mainly a bovine parasite)
 - B. venatorum* (EU1), and *B. microti*
- *Babesia* variants are found in Korea (KO1) and Taiwan (TW1)
- *B. microti*-like transfusion transmission in Japan - donor presumably infected in Japan
- Canada – first reported case babesiosis in 1999; however, in 2001, TTB case reported (actually occurred in 1998); 1 *B. microti* pos donor ID'd
 - RBCs transfused @ 6 mos following donor's travel to Cape Cod, MA (endemic area - US)
 - On f/u, donor remained PCR pos/Ab pos (IFA 1:1024)



REVIEW ARTICLE

CURRENT CONCEPTS

Human Babesiosis

Edouard Vannier, Ph.D., and Peter J. Krause, M.D.

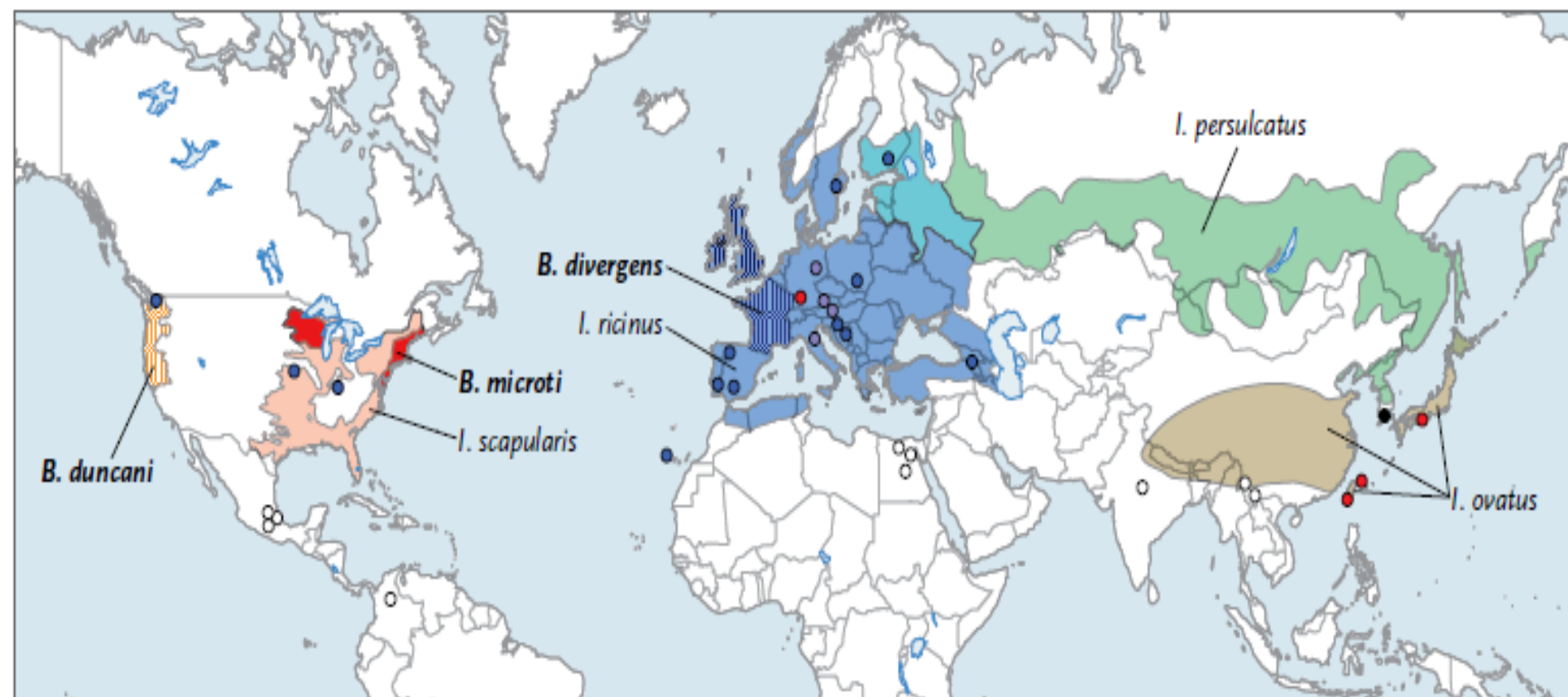


Figure 1. Geographic Areas Where Human Babesiosis and Ixodes Tick Vectors Are Endemic.

Screening for *Babesia microti* in the U.S. Blood Supply

N ENGL J MED 375:23 NEJM.ORG DECEMBER 8, 2016

Erin D. Moritz, Ph.D., Colleen S. Winton, S.B.B. (A.S.C.P.), Laura Tonnetti, Ph.D.,
Rebecca L. Townsend, B.A., Victor P. Berardi, Mary-Ellen Hewins, B.S.,
Karen E. Weeks, B.S., Roger Y. Dodd, Ph.D., and Susan L. Stramer, Ph.D.

- June 2012-Sept 2014 tested donations from 4 states (CT, MA, MN, WI) by investigational antibody (AFIA) and DNA (PCR)
- Determined parasite loads (qPCR) and infectivity (parasitemia in hamsters)
- Followed donors for Ab/DNA clearance
- Using our HV system, compared rates of TTB: screened vs unscreened blood

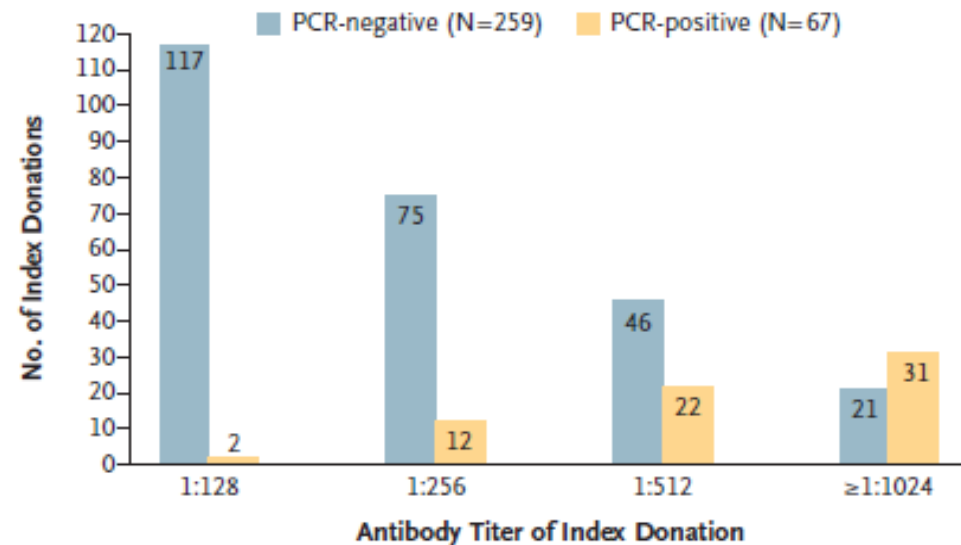
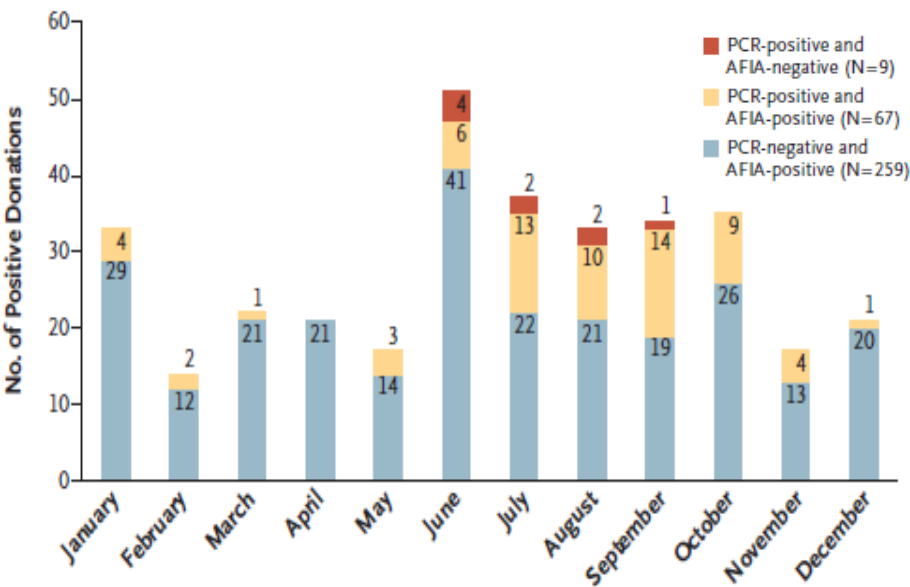
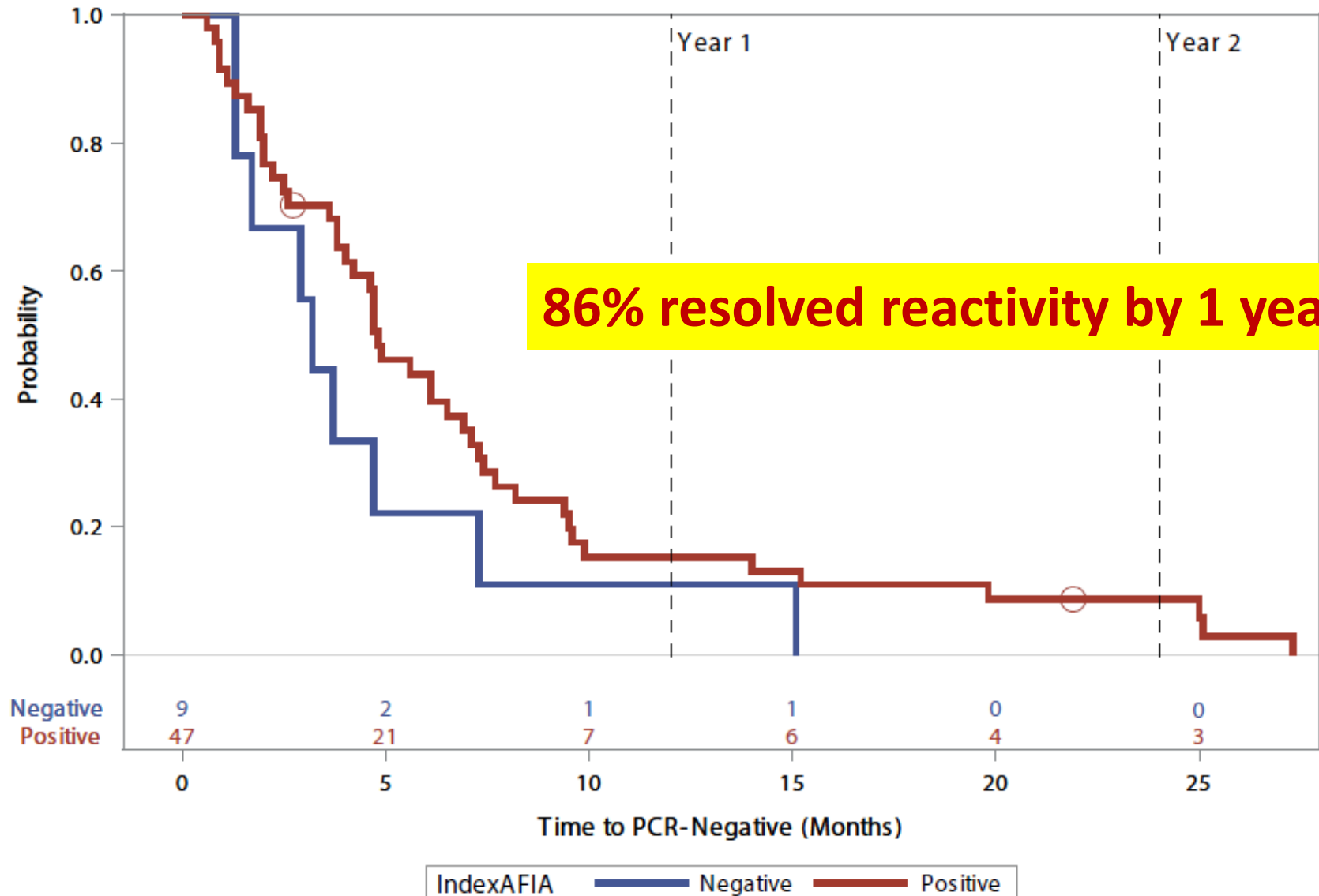


Figure 3. Positive Donation Samples from June 4, 2012, to September 30, 2014, According to

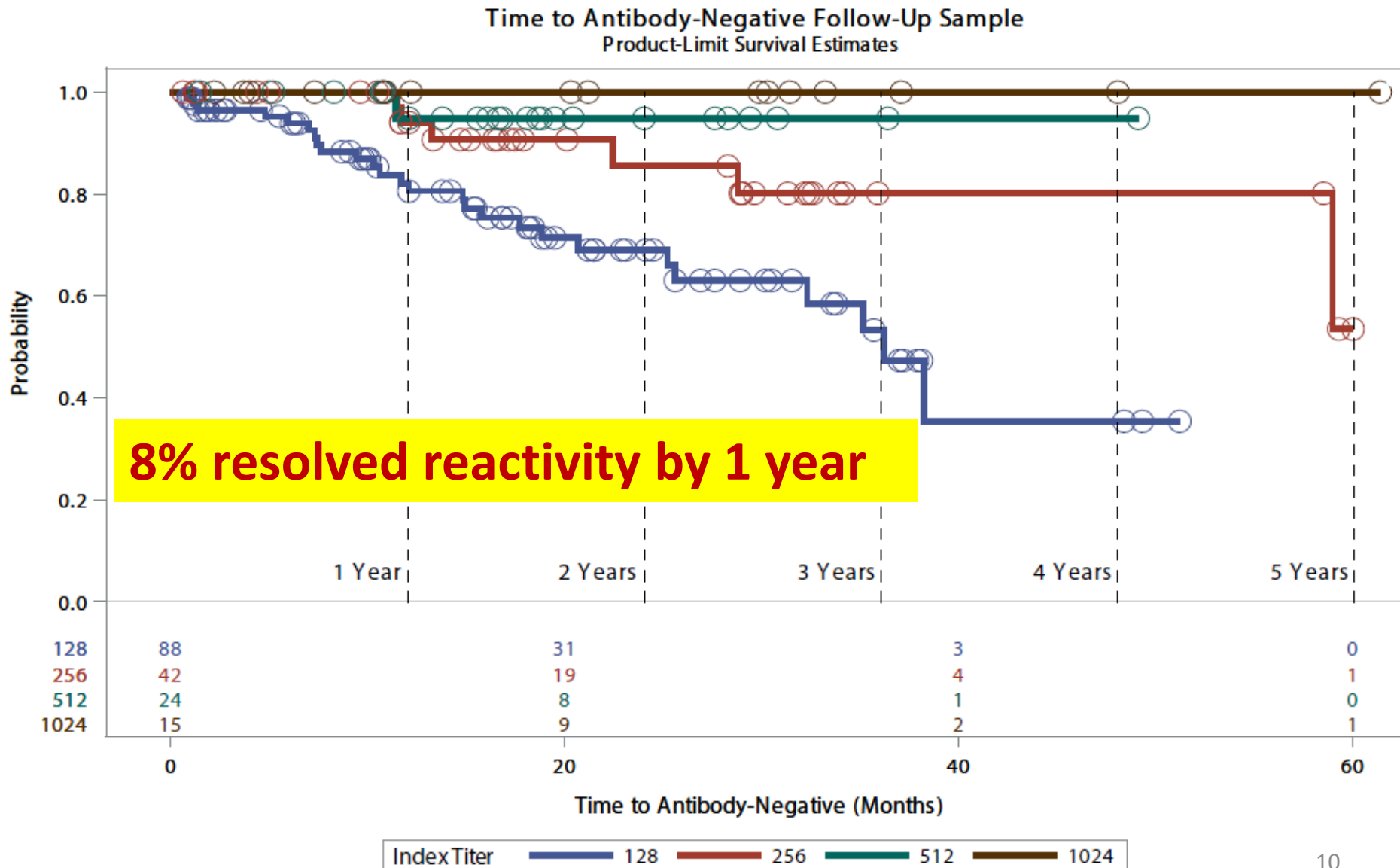
Figure 4. Relationship between AFIA Titer and Result on PCR Assay in Index Donation Samples That Tested Positive.

PCR-Positive Donors – DNA Decline

Time to PCR-Negative Follow-Up Sample
Product-Limit Survival Estimates



PCR-Negative Donors – Ab Decline



	NEJM Time period (June 2012-Sept 2014)	Extended to March 31 2017
# Donations Screened	89,153	312,473
# Total Pos # DNA Pos # WP Pos	335 (0.38%) 67 (20%) 9 (1:9900; 13% PCR pos)	964 (0.30%) 144 (15%) 16 (1:19,500; 11% PCR pos)
# PCR Infectious/total # Infectious/total	25/46 (54.3%) 27/93 (29.0%)	29/54 (53.7%) 31/107 (29.0%) Difference p=0.025
DNA Resolution @ 1y Ab Seroreversion @ 1y	86% 8.0%	86% 8.3%
In high-risk counties, TTB screened blood, TTB unscreened blood	0/75,331 14/253,031 OR=8.6; p=0.05	0/312,473 23/1,254,819 OR=11.7; p=0.01
TTB cases overall during study period	29; 10 PCR pos (34.5%) 2-7 mos donor f/u	51; 20 PCR pos (39.2%) 2-7 mos donor f/u

Transfusion 2017 early on-line

≥ 1024

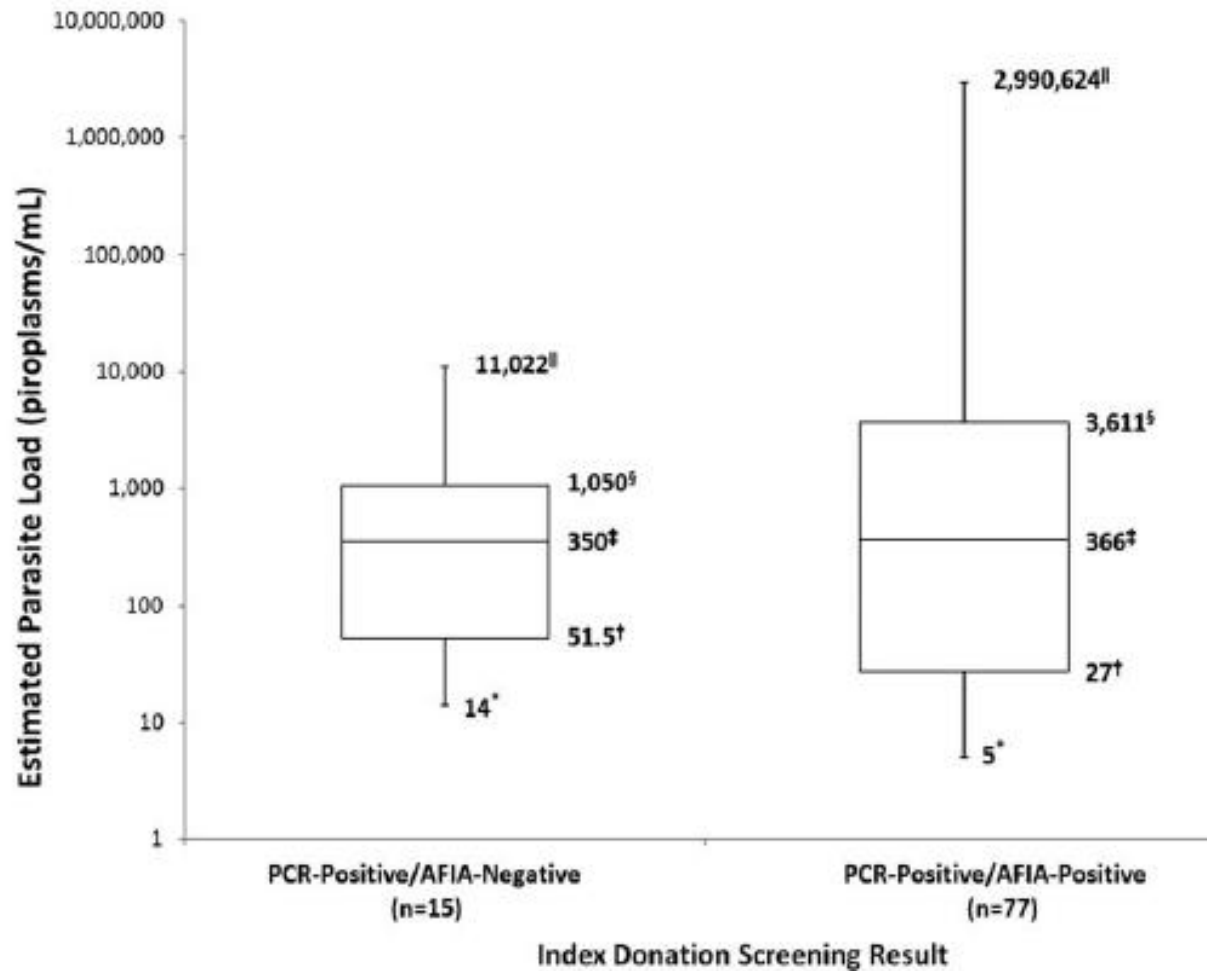


Fig. 1. Box-and-whisker plots depicting the distribution of parasite loads in index donations screening PCR positive and AFIA negative (i.e., WP donations) and PCR positive and AFIA positive for DNA from and antibodies to *B. microti* in blood donors. Note the parasite loads are plotted on a logarithmic scale. *Minimum value; †first quartile; ‡median value; §third quartile; ||maximum value.

Babesia Investigational Testing Results

Specificity = 99.997%

PPV = 98.26%

	<u>2012</u>	<u>2013</u>	<u>2014</u>	<u>2015</u>	<u>2016</u>	<u>2017</u>	<u>Totals</u>
Testing Totals	22,174	39,375	31,178	40,521	137,981	27,189	298,778

Index Reactive Testing Category								
IFA and PCR	Confirmed	13	22	23	11	48	6	123
IFA only	Confirmed	91	114	86	86	325	72	780
	Non-confirmed	1		1	2	3		
PCR only	Confirmed	5	3	1	3	3	1	16
	Non-confirmed or NT							

Total Reactives	110	139	111	101	379	79	919
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For 2015 & 2016 numbers, reactives for 13 IFA only are not shown.
(11 without additional sample to test and 2 pending testing.)

2/28/2017

Case 27-2016: A 71-Year-Old Woman with Müllerian Carcinoma, Fever, Fatigue, and Myalgias

N Engl J Med 2016;375:981-91.
DOI: 10.1056/NEJMcpc1607091

Farrin A. Manian, M.D., M.P.H., Miriam B. Barshak, M.D., Kathryn P. Lowry, M.D.,
Kristen M. Basnet, M.D., and Christopher P. Stowell, M.D., Ph.D.

CASE RECORDS of the MASSACHUSETTS GENERAL HOSPITAL

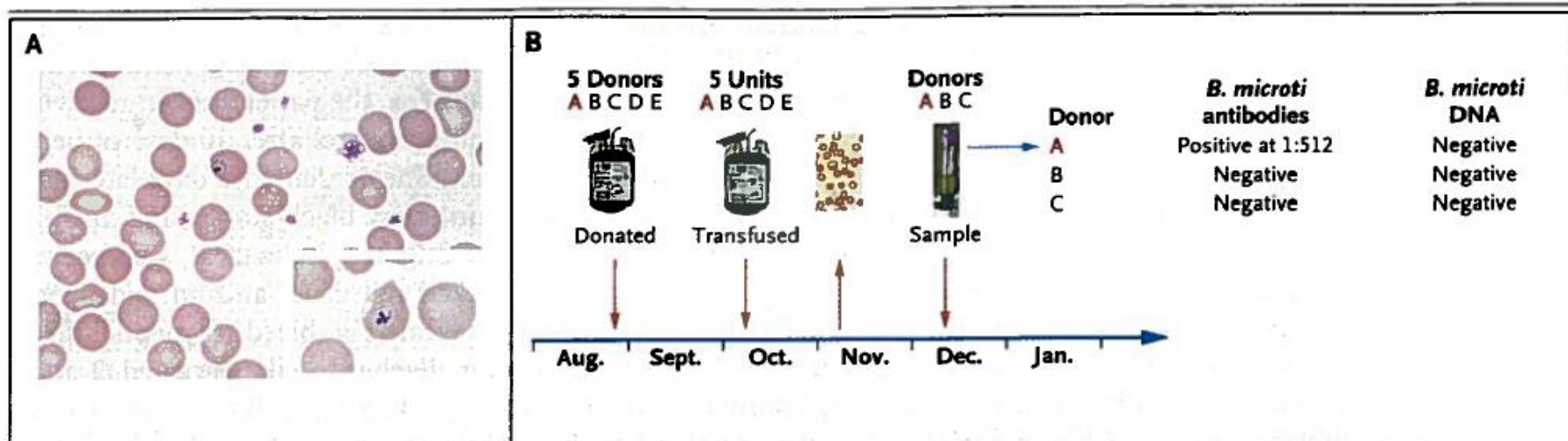


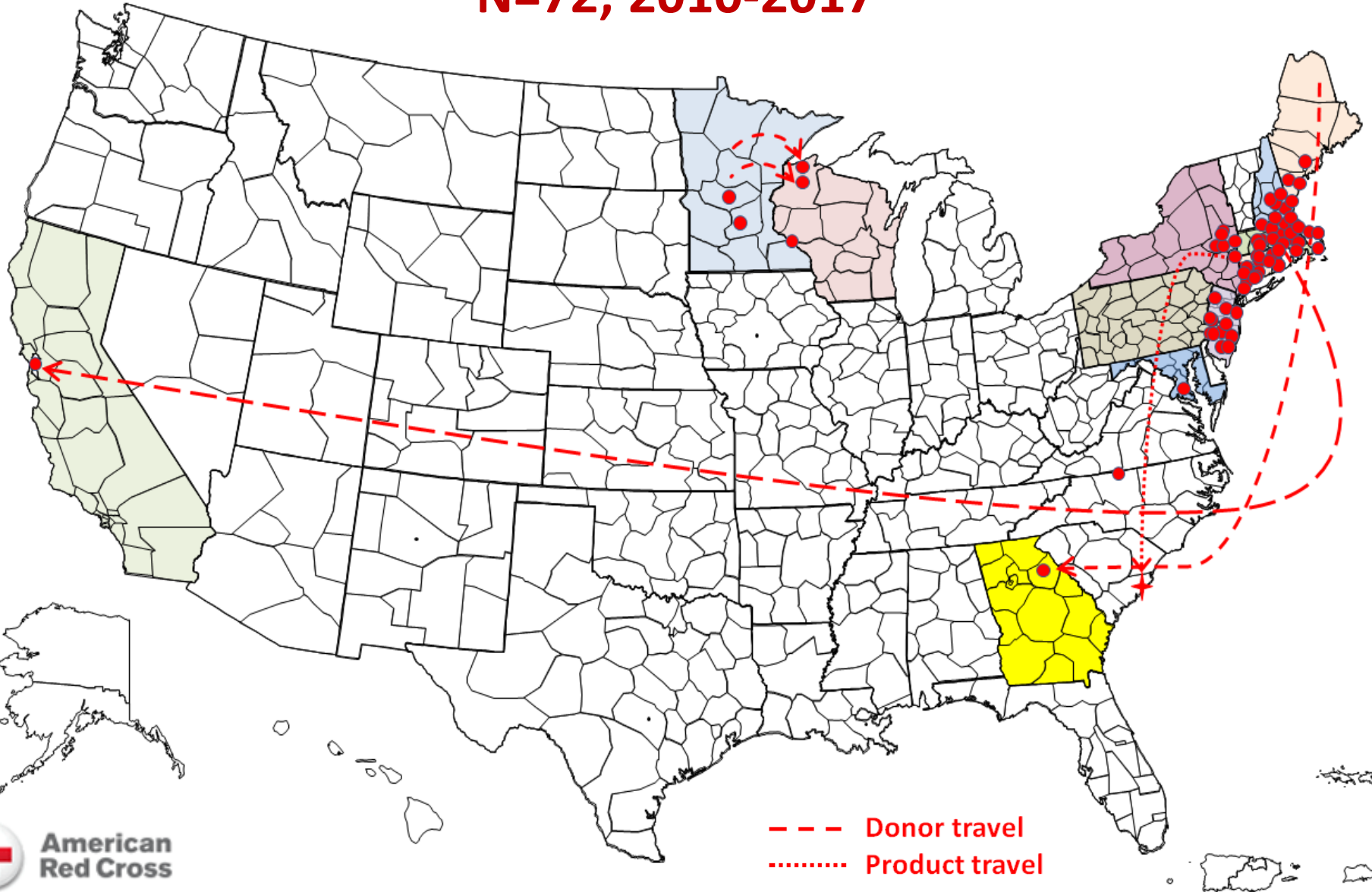
Figure 4. Peripheral-Blood Smear and Timeline of Transfusion-Related Events.

A smear of thin peripheral blood (Panel A, Giemsa stain) shows intraerythrocytic ring forms characterized by a ring (stained pale purple) with central clearing and a peripheral purple chromatin dot. Some erythrocytes contain tetrads, a formation that is characteristic of babesia (Panel A, inset). Taken together with the clinical and epidemiologic features present in this patient, the intraerythrocytic ring forms were thought to represent babesia. A timeline of transfusion-related events (Panel B) shows that the 5 units of packed red cells that were transfused perioperatively (approximately 3.5 weeks before babesiosis was diagnosed in this patient) had all been donated on the same date in late August. Three of the five blood donors returned for additional testing in December; all three donors had tested negative for *Babesia microti* DNA by means of a nucleic-acid-amplification technique. Donors B and C had negative tests for *B. microti* antibodies by means of immunofluorescence assay, but Donor A was positive for *B. microti* antibodies at a titer of 1:512.

Donors Implicated in ARC Recipient Complications

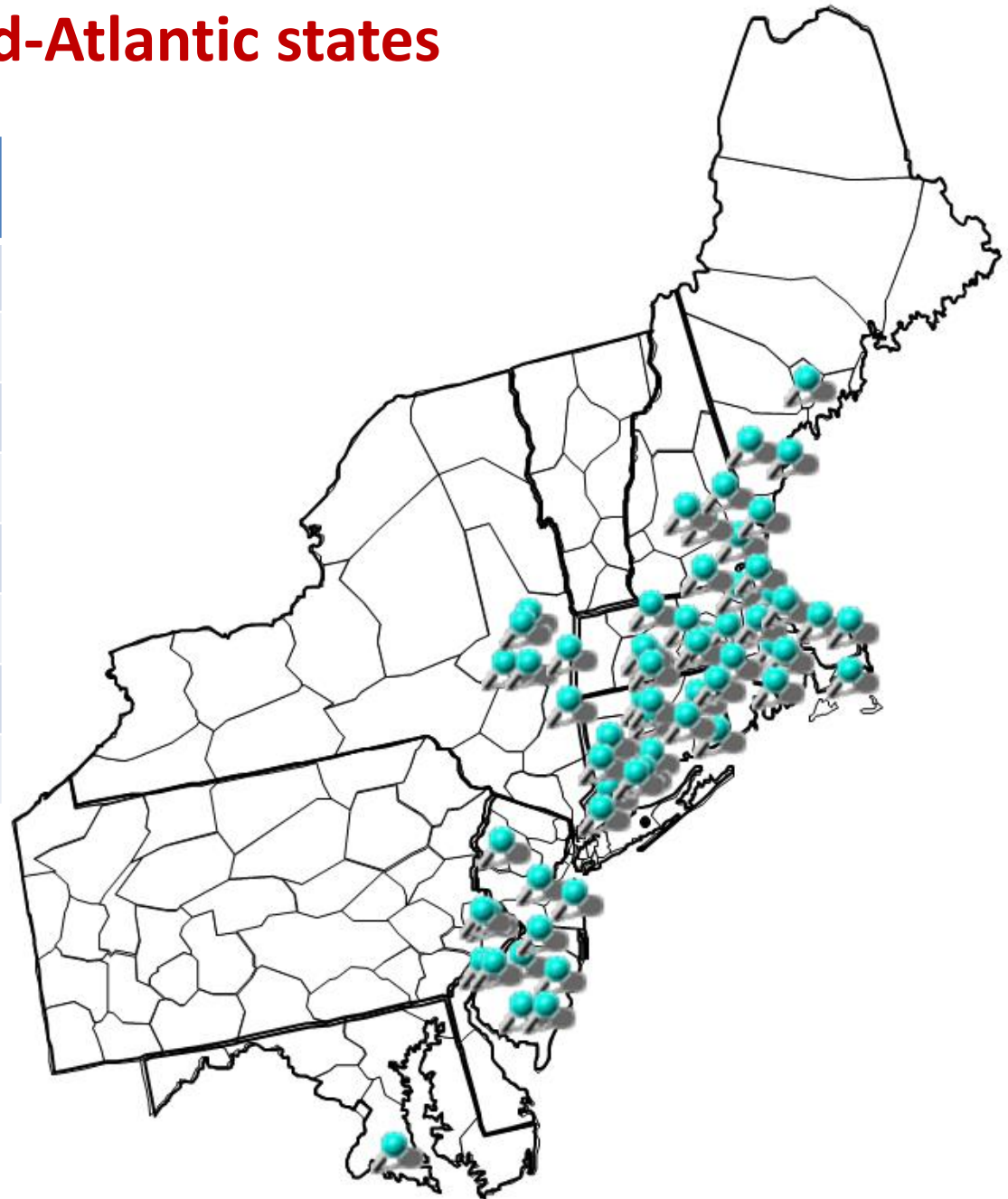
Residence of Babesia-Positive Donors (Pins)

N=72; 2010-2017

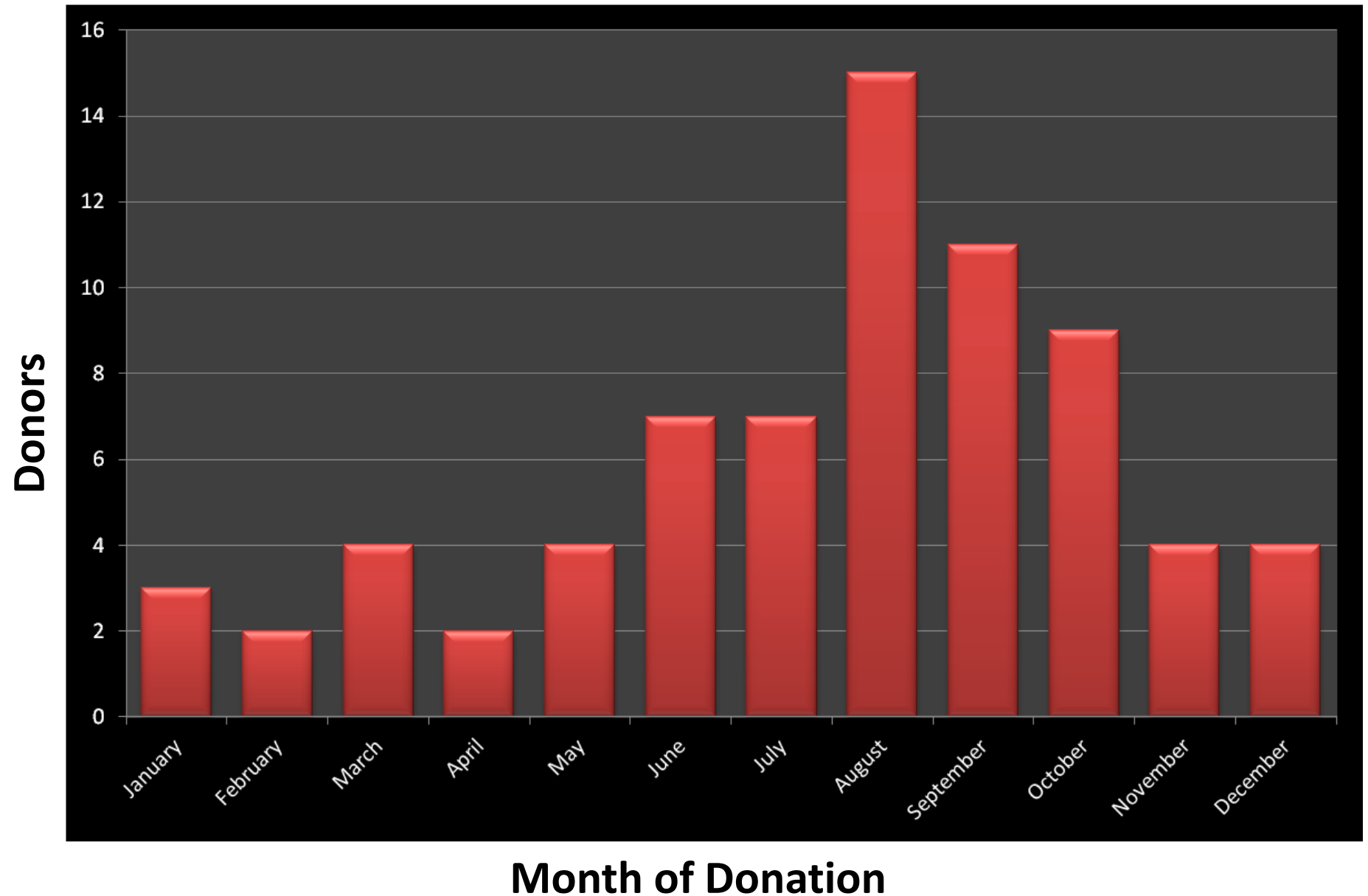


64 of 72 (89%) positive donors reside in New England or Mid-Atlantic states

State	# Positive donors
Connecticut	19
Massachusetts	18
Maine	4
Maryland	1
New Hampshire	4
New Jersey	8
New York	6
Pennsylvania	4



Distribution by month of the blood donations associated with *B. microti* transfusion cases (N=72); 2010-2017



Procleix Babesia Assay on the Procleix Panther System

Assay in development / Panther not available for commercial use

Preliminary Performance Characteristics

- **Sample preparation:** method for **whole blood** specimens
 - Compatible with target capture/TMA, and end-point chemiluminescent or real-time fluorescent detection
 - Compatible with current fully automated system: **Procleix Panther System**
- **Species detection:** detect all species known to cause human disease world-wide
 - ***B. microti*, *B. divergens*, *B. duncani*, and *B. venatorum***
- **Analytical sensitivity:** comparable to other TMA blood screening assays
 - **100% detection at 30 copies/mL**
 - **95% detection at 7.1-13.5 copies/mL**
- **Assay specificity:** comparable to current blood screening assays
 - > 99.95% specificity with no cross reactivity to other blood-borne pathogens
- **Testing formats:** individual donor samples/qualifying in pools of 4, 8, and 16 donations



Assay Workflow: Procleix Babesia Assay

Procleix Panther system

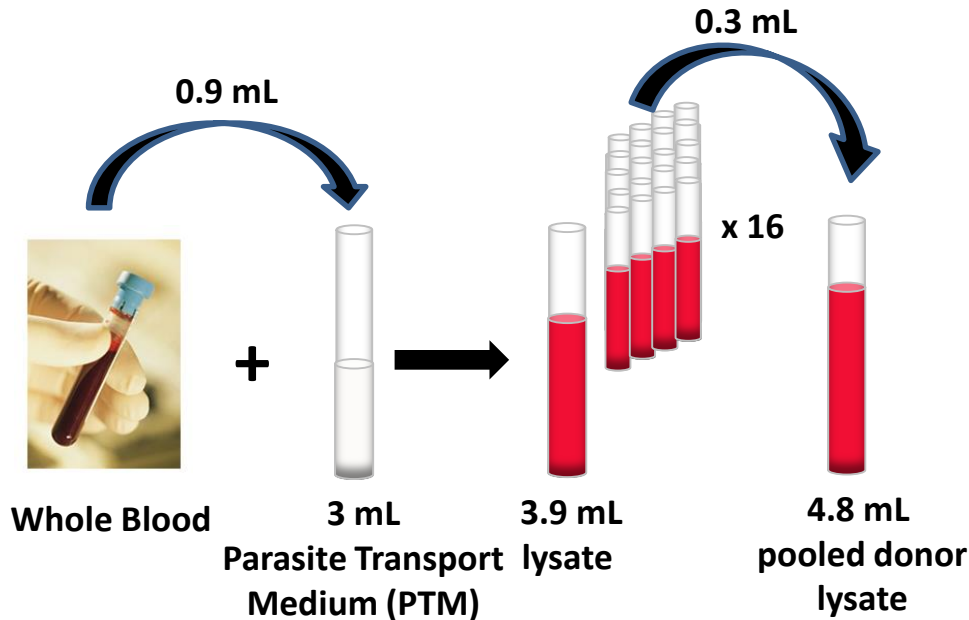


Modified Procleix Xpress System

Individual donor lysates
(IDL) or pooled donor
lysates (PDL)



Procleix Panther System



- Sample traceability by Procleix NAT Manager
- Deconvolution of reactive PDLs by testing IDLs

Overall Babesia RNA Reactivity: ARC Testing

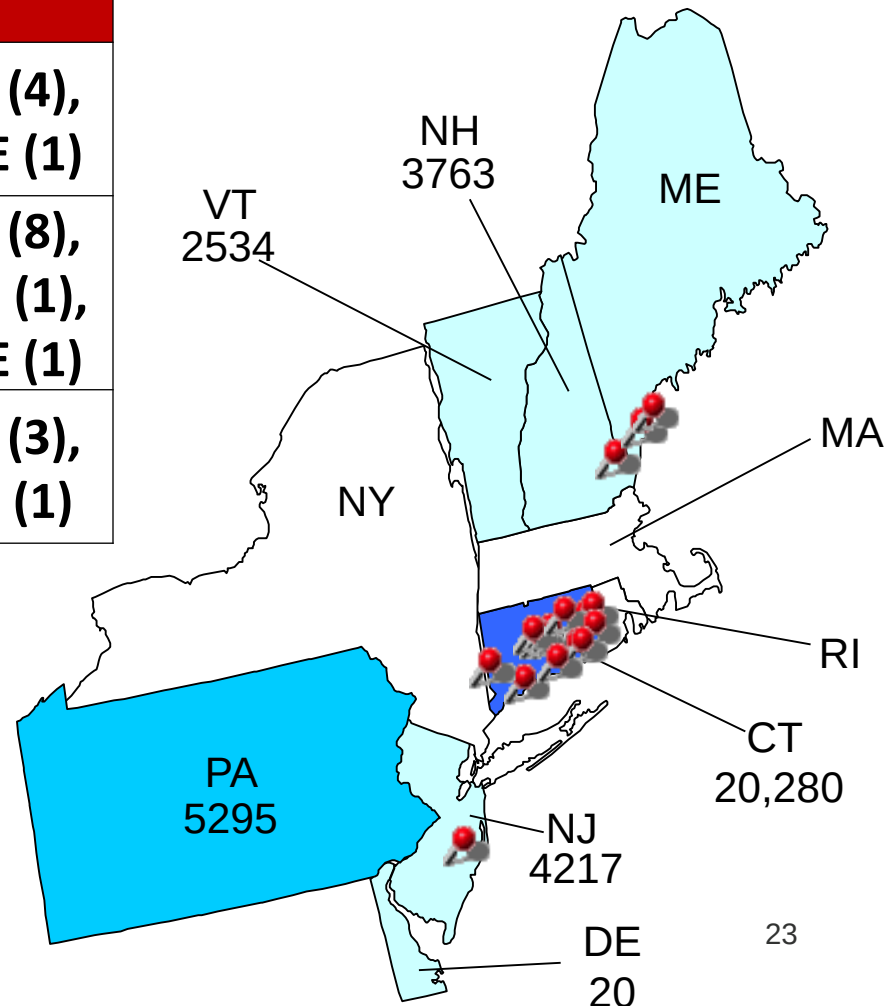
	Jul 22-Sep 20 2016		Jan 14-Apr 19 2017	Total
Lot(s)	1	2 - 4	5 - 6	1 - 6
Formulation	A	B	B (+ new cutoff)	A, B
Number Tested	5,791	15,619	18,577	39,987
Number Confirmed Positives / Number Reactivities (%)	5/21 (24)	10/18 (56)	4/5 (80)	19/45 (42)
% Specificity	99.72	99.95	99.995	
Positive Frequency	1:1158	1:1562	1:4644	1:2104
States Tested	CT, ME, NH, VT	CT, ME, NH, VT	CT, PA, NJ, DE	CT, ME, NH, VT, PA, NJ, DE

Details of Reactivity of Confirmed Positives

# Conf'd Positive by Lot	# Rx/6 reps tested neat	# Rx/6 reps tested 1:16	Ab Status (IFA $\geq$ 1:64)	State of Positive Donors
Lot 1	5/5	4/5*	3/5	CT (4), ME (1)
Lots 2 - 4	10/10	10/10	9/10	CT (8), NH (1), ME (1)
Lots 5 - 6	4/4	4/4	4/4	CT (3), NJ (1)

*1 discrepant sample – Reactivity:
neat (4/6), pool of 4 (1/3), pool of 8 (0/3),
pool of 16 (0/3), Ab Neg, CT resident

1 to 5100	5	71.4%
5101 to 10200	1	14.3%
10201 to 15300	0	0.0%
15301 to 20400	1	14.3%

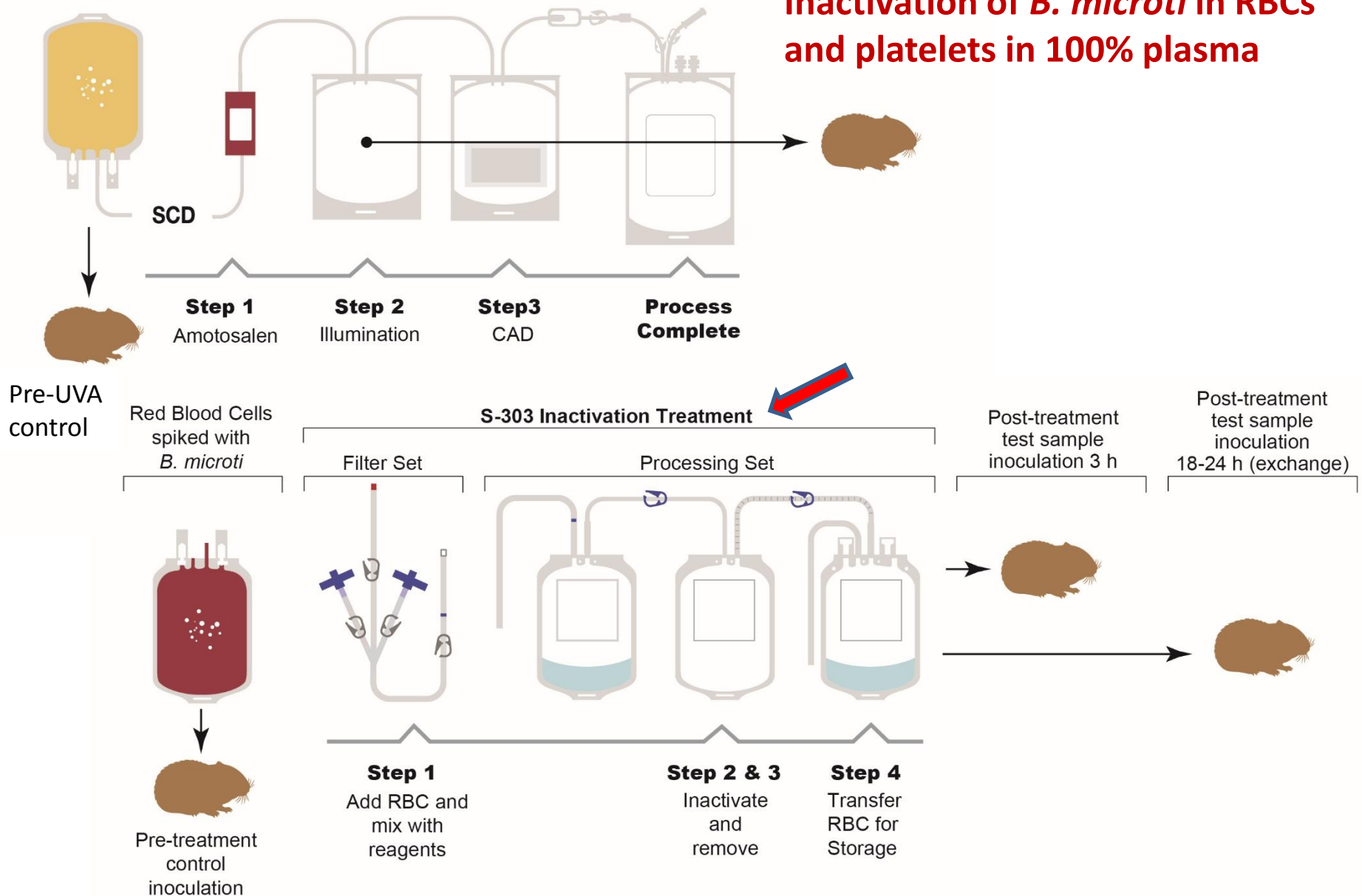


Platelets in 100%
plasma spiked with
B. microti

INTERCEPT Blood System for Platelets

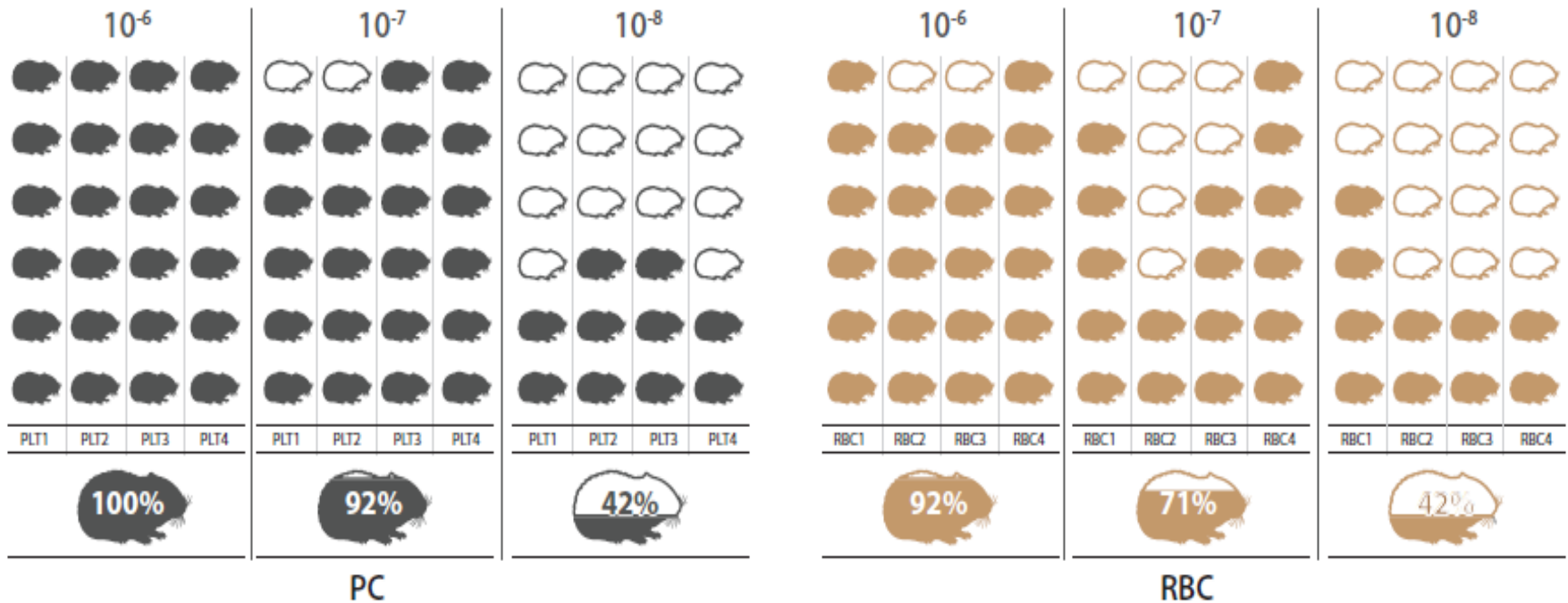
Post-UVA illumination
sample inoculation

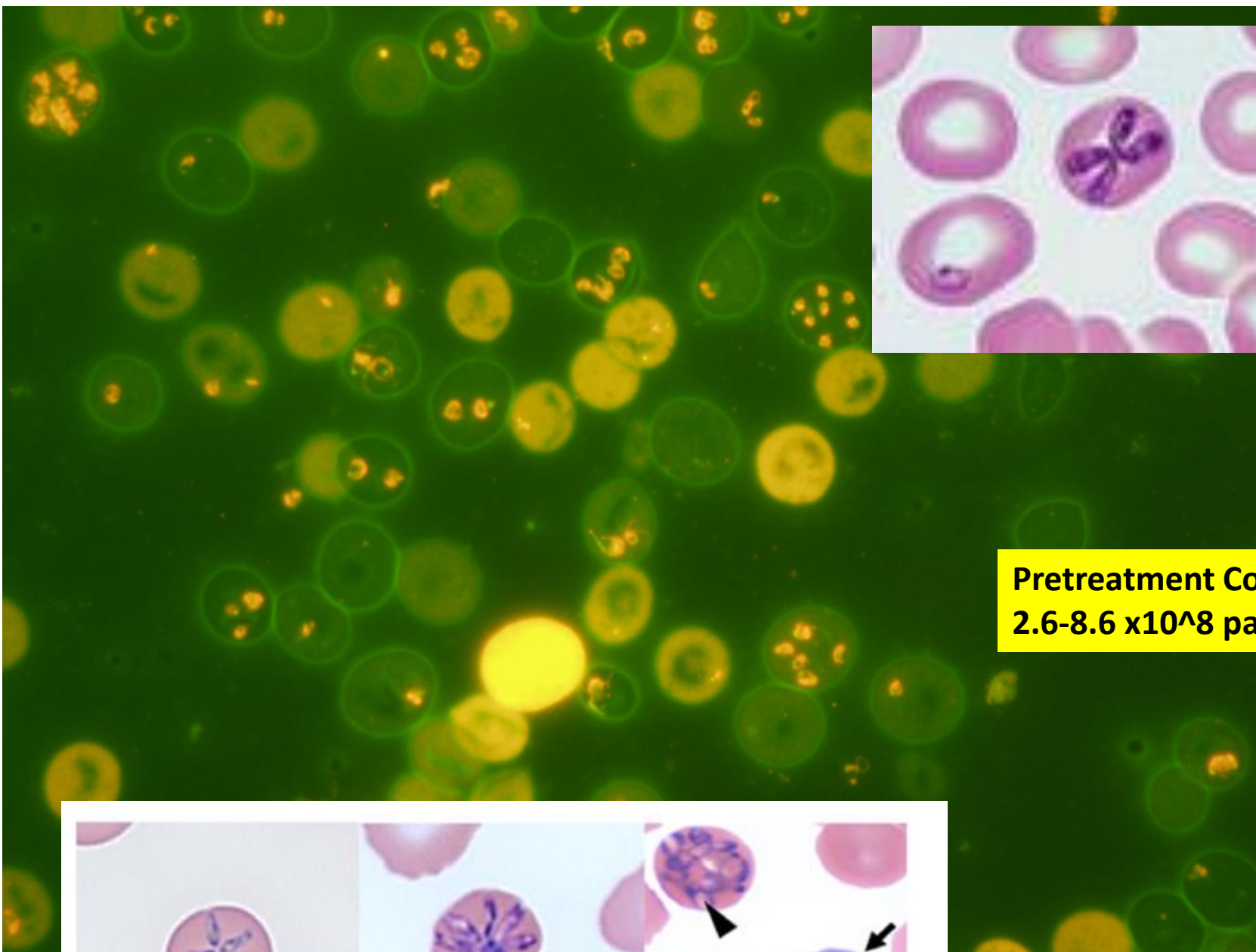
**Inactivation of *B. microti* in RBCs
and platelets in 100% plasma**



Infectivity of stock aliquots. Blood was collected from highly parasitemic hamsters ($\geq 35\%$ of red cells parasitized) and serially diluted in PBS. The three highest dilutions were injected into naïve hamsters and the development of parasitemia monitored up to five weeks after injection.

Percent infected of 24 total hamsters: platelets and red cells





Pretreatment Control
2.6-8.6 x10⁸ parasites/mL



Inactivation of *Babesia microti* with Amustaline/GSH in Red Blood Cells

¹Laura Tonnetti, ²Andrew Laughunn, ¹Aaron M. Thorp, ¹Irina Vasilyeva,, ²Kent Dupuis,
²Adonis Stassinopoulos, ¹Susan Stramer

¹American Red Cross Holland Laboratory, Scientific Affairs, Rockville, MD; ²Cerus Corporation, Concord, CA

Parasitemia was detected in hamsters injected with up to 10⁻⁵ dilution of the control samples, while no parasites were detectable in the blood smears of any hamsters receiving neat test samples.

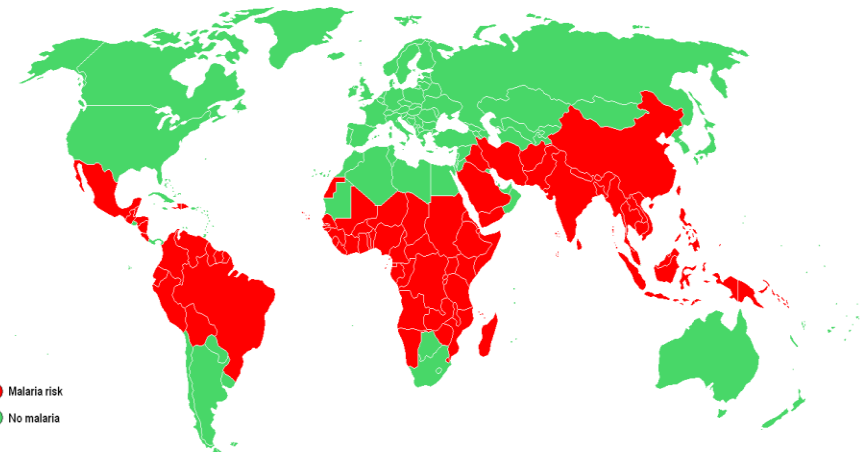
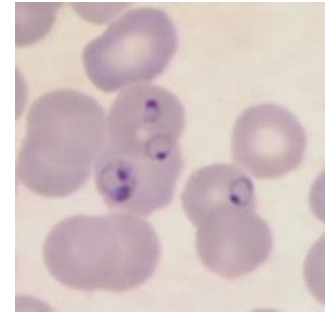
Replicate	Log Titers (ID ₅₀ /mL) ^a			Log Reduction ^b	
	Control T=0	Test T=3h	Test Post- exchange T=24h	Test T=3h	Test Post- exchange T=24h
1	4.8	<-1.0	<-1.0	>5.8	>5.8
2	5.0	<-1.0	<-1.0	>5.9	>5.9
3	4.8	<-1.0	<-1.0	>5.8	>5.8
4	5.2	<-1.0	<-1.0	>6.2	>6.2
Mean ± SD	5.0 ± 0.2	<-1.0	<-1.0	>5.9 ± 0.2	>5.9 ± 0.2

a. 1.5 mL injected/hamster; 1.5 mL×6 hamsters or 9 mL total inoculum/replicate).

b. Log reduction is calculated as Log (Control T=0 titer ÷ Test titer).

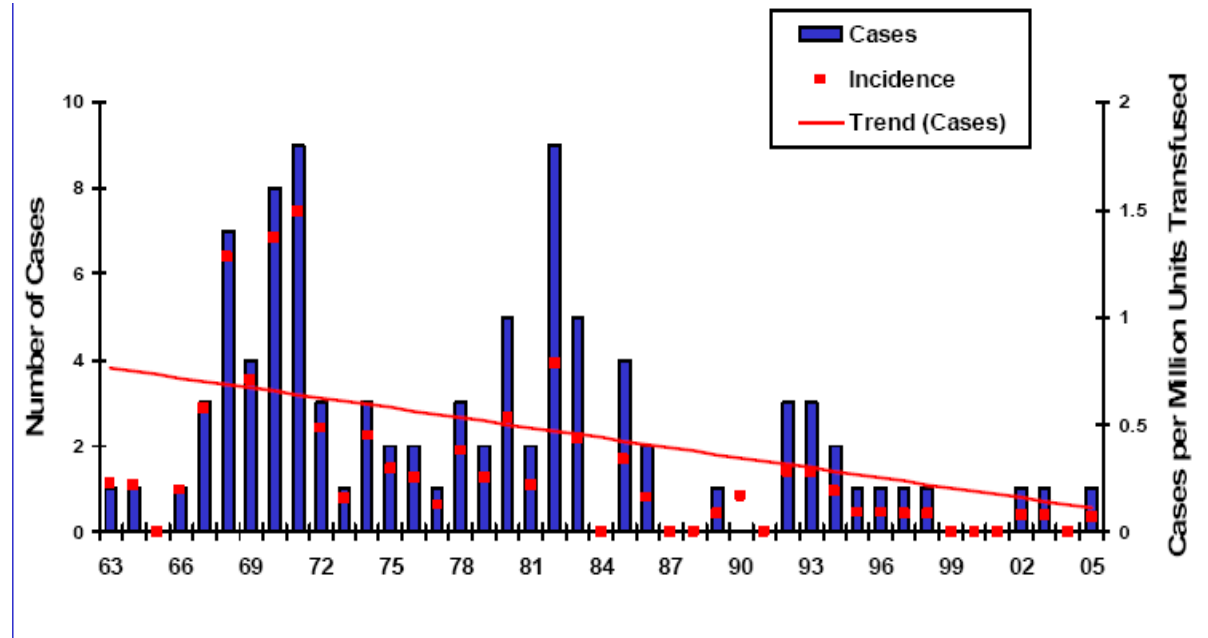
Plasmodium spp.

- Agents of human malaria
 - *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* & *P. knowlesi*
- Found inside red and liver cells
- Transmitted by female anopholene mosquitoes
- Usually found in tropical or subtropical areas
 - >200 million cases/year
 - 665,000 to 1.24 million deaths/year
 - > 80% occur in sub-Saharan Africa
 - 90% is *P. falciparum*
 - Periods of fever & chills
- Blood banks
 - challenges differ by country
 - blood safety vs. availability



Transfusion-Transmitted Malaria (TTM) in the US

- 93 cases 1963-99*
 - WB:63%
 - RBCs:31%
 - Platelets:6%
- Since: TTM is rare
- Only 7 cases since 2002

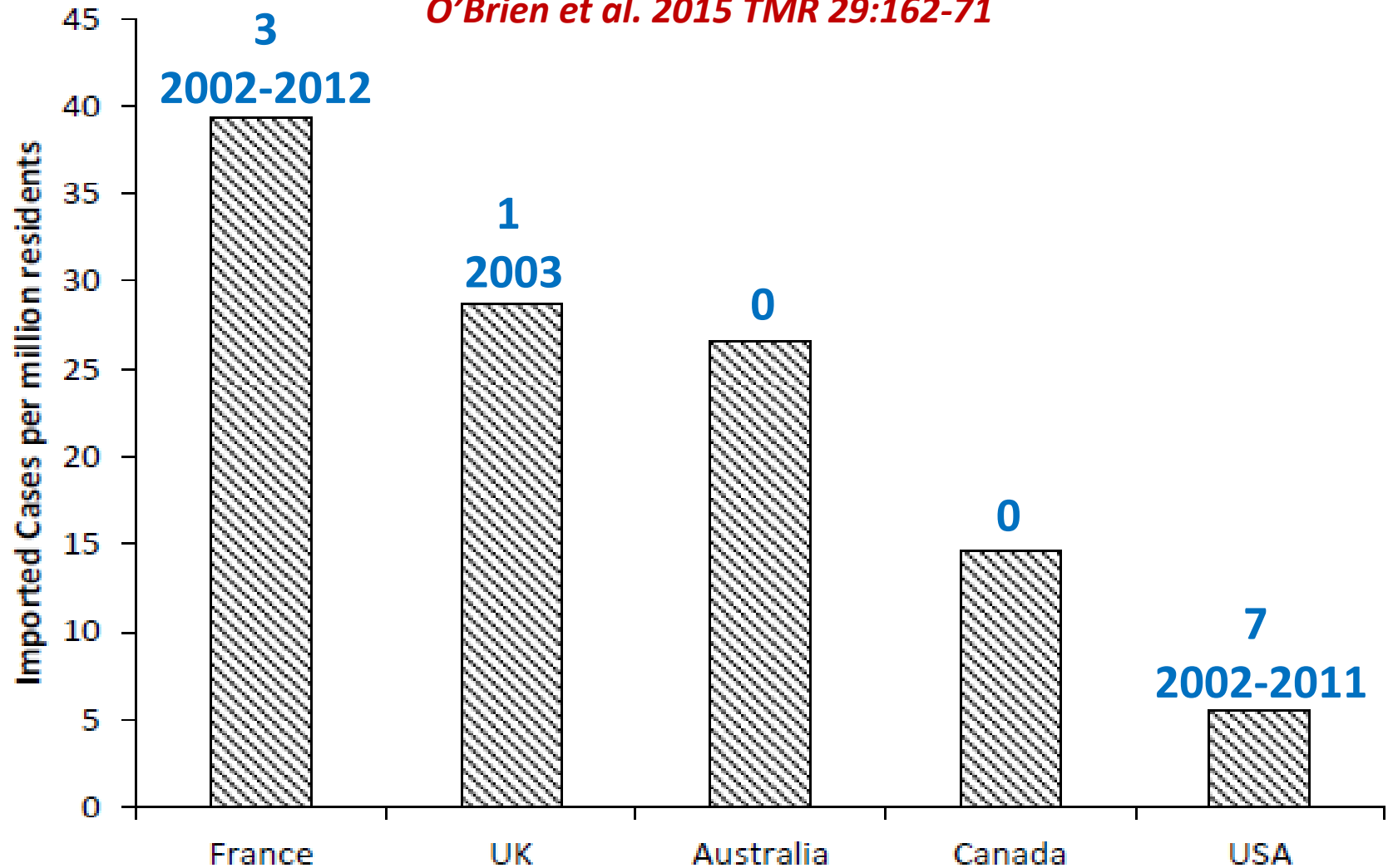


- $\geq 2/3$ rd's of cases attributed to donors with a previous history of malaria (i.e., semi-immune)
 - associated with residence in an endemic country
 - military service in Vietnam

Imported malaria cases by country and TTM

TTM: 11 all from West Africa, 10 *P. falciparum* (1 *P. malariae*)
10 emigrated, 5 had or had been treated for malaria

O'Brien et al. 2015 TMR 29:162-71



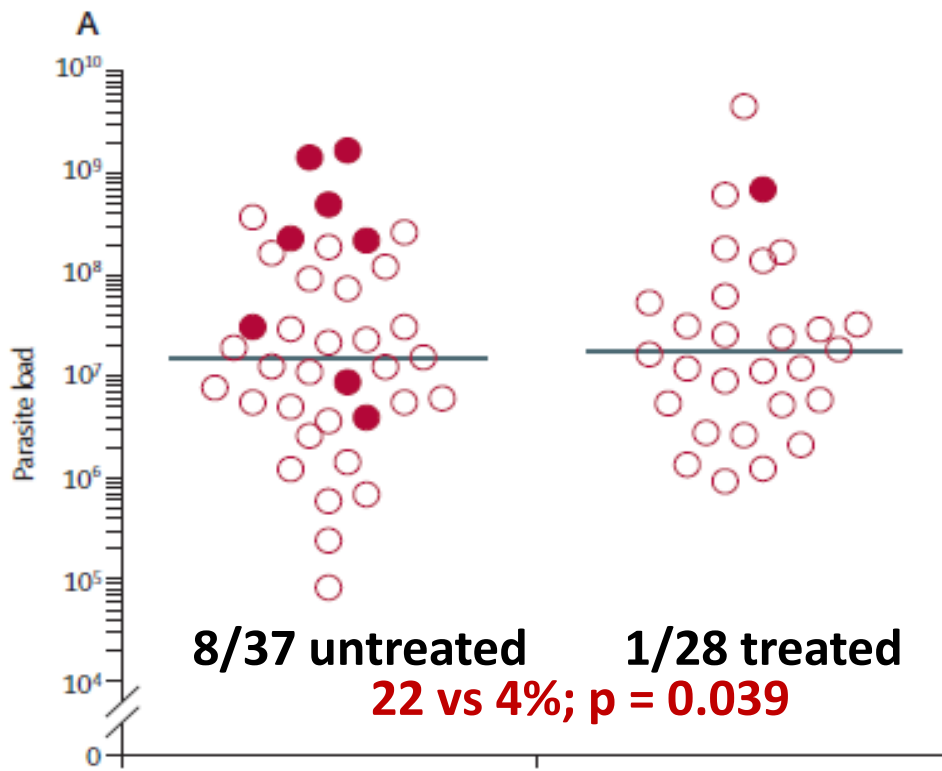
Effect of *Plasmodium* inactivation in whole blood on the incidence of blood transfusion-transmitted malaria in endemic regions: the African Investigation of the Mirasol System (AIMS) randomised controlled trial

Jean-Pierre Allain, Alex K Owusu-Ofori, Sonny Michael Assennato, Susanne Marschner, Raymond P Goodrich, Shirley Owusu-Ofori

Summary

Lancet 2016; 387: 1753–61

Background Transfusion-transmitted malaria is a frequent but neglected adverse event in Ghana. We did a randomised controlled clinical trial to assess the efficacy and safety of a whole blood pathogen reduction technology at preventing transfusion transmission of *Plasmodium* spp parasites.



**111 patients rec'd treated WB;
112 rec'd untreated WB...**

**65 patients exposed to
parasitemic blood...**

**Closed circles show TTM cases
relative to donor parasite loads
when allelic discrimination was
used in the definition of TTM**

Summary

- Prospective blood donation screening for *B. microti* is feasible and has removed ~1000 potentially infectious units of blood including
 - 15-20% PCR positive => infectious
 - 1:10,000-1:20,000 window-period units => infectious
 - Ab positivity persists for years => donations from such donors unlikely to be infectious in absence of RNA/DNA
- Other technologies in development
 - **Highly sensitive RNA NAT** methods (vs DNA PCR)
 - Would a highly sensitive NAT method be adequate in the absence of Ab testing?
 - Eliminates likely all infectious donations (and avoids deferral of high numbers of antibody pos donors with resolved infections)
 - **RBC Pathogen Inactivation** is highly effective in preventing both TTB and TTM