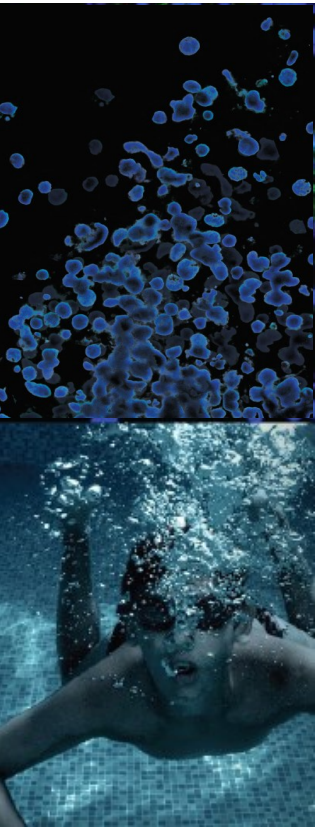
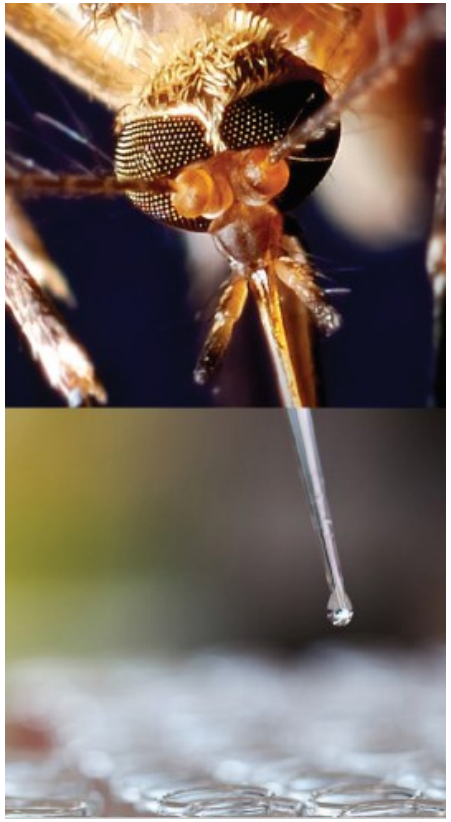
Impact of Tick-borne Diseases on Human Health in the United States with a Special Emphasis on Babesiosis



Robert Molestina, PhD
Senior Scientist
Protistology Laboratory

Credible Leads to Incredible™



ATCC – Credible leads to incredible

- ATCC has provided credible biomaterials for over 90 years
- We continue to cultivate collaboration
  - Among scientists across disciplines
  - Essential for accelerating innovative research
  - Leading to incredible, high-impact results
- Our Cultivating Collaboration pledge: We bring scientists together to discuss
  - Breakthroughs in the state of science
  - Multidisciplinary approaches to key areas of research
  - Breaking the silos that impede research
- Our partnership with you, the scientific community, allows us all to reach the incredible

Outline

- Overview of tick-borne diseases
- Needs and gaps in tick-borne disease research
- ATCC resources for tick-borne disease research and diagnostics
- Tick-borne disease research projects at ATCC: A focus on babesiosis

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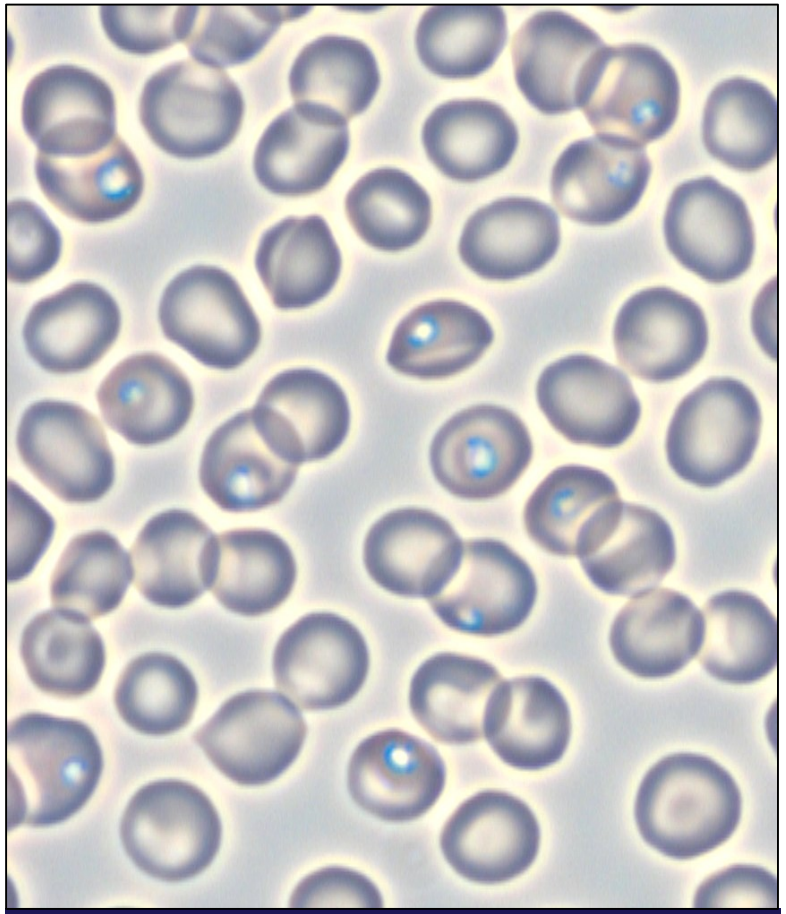
Tick-borne diseases (TBDs) in the United States

- TBDs are an emerging public health epidemic in the United States.
- TBDs can be bacterial, parasitic, or viral.
- Infections may occur with more than one tick-borne pathogen at a time.
- The CDC currently recognizes 13 unique human tick-borne illnesses caused by 18 different pathogens in the United States.



Giemsa stained red blood cells infected with *Babesia microti*.

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TBDs in the United States

Bacterial

- Anaplasmosis*
- *Borrelia miyamotoi* Disease
- Ehrlichiosis*
- Lyme Disease*
- Rocky Mountain Spotted Fever*
- *Rickettsia parkeri* Rickettsiosis
- Tick-borne Relapsing Fever
- Tularemia*

Parasitic

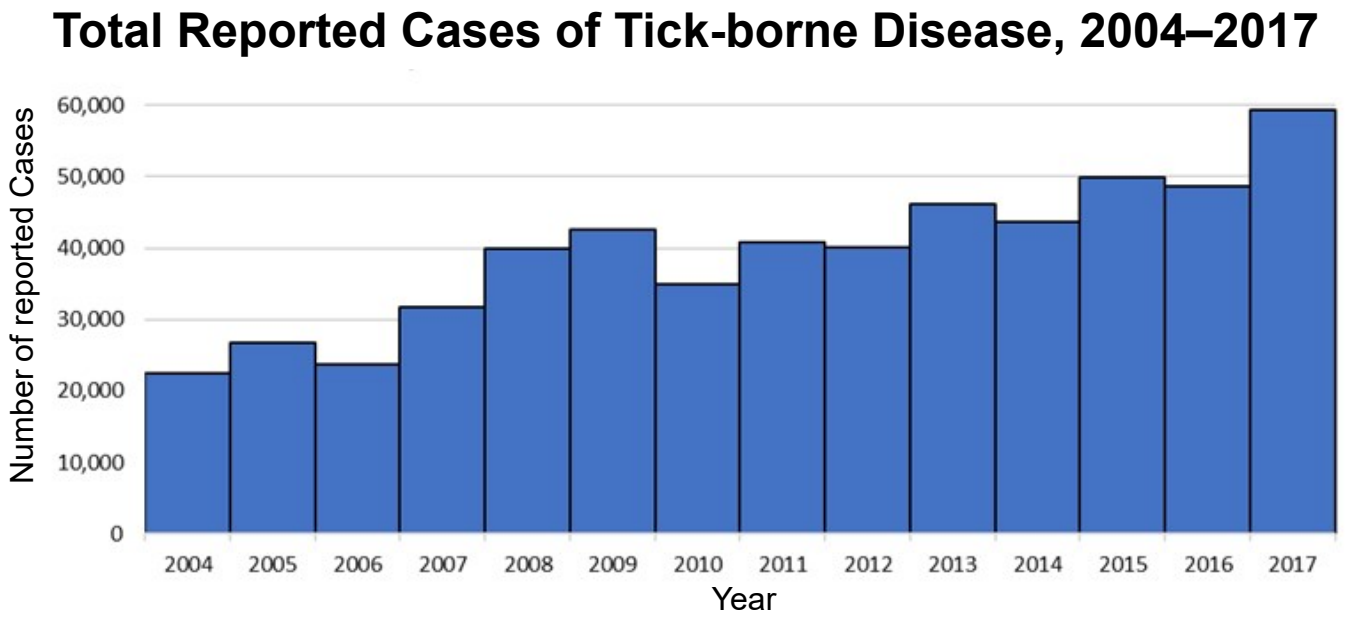
- Babesiosis*

Viral

- Bourbon Virus Disease
- Colorado Tick Fever
- Heartland Virus Disease
- Powassan Virus Disease*

* Nationally notifiable to the National Notifiable Diseases Surveillance System

TBD surveillance in the United States

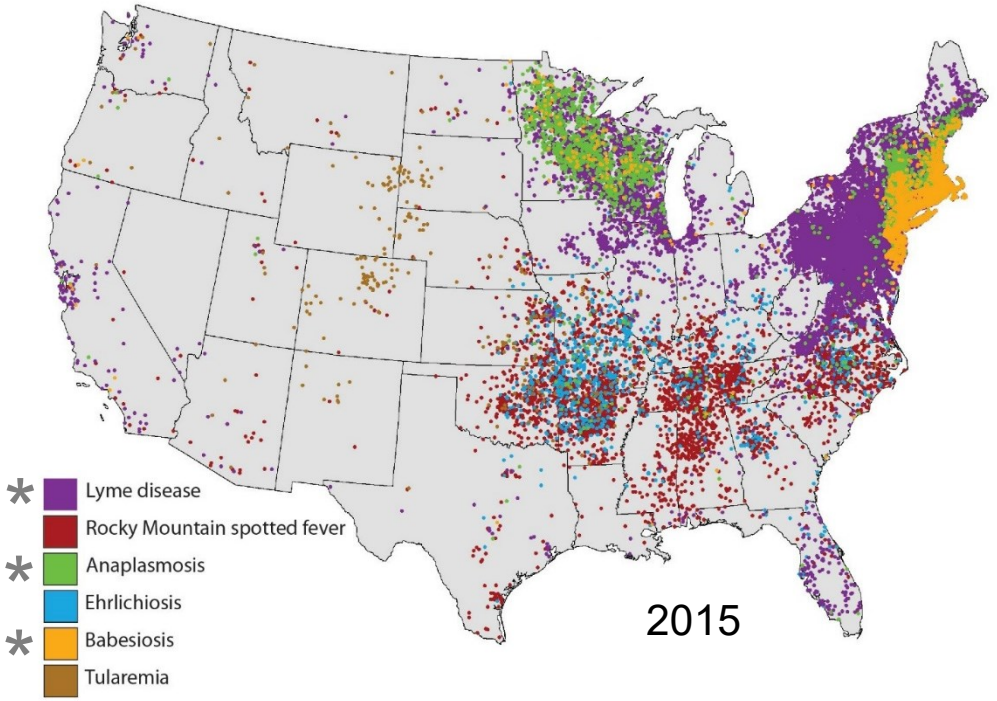


Reported Tick-borne Diseases (2016-2017)		
Tick-borne Disease	**2016**	**2017**
Lyme Disease[1] (confirmed and probable)	36,429	42,743
Anaplasmosis/ Ehrlichiosis	5,750	7,718
Spotted Fever Rickettsiosis	4,269	6,248
Babesiosis	1,910	2,368
Tularemia	230	239
Powassan virus	22	33
Total	**48,610**	**59,349**

[1]Estimated annual cases ~300,000

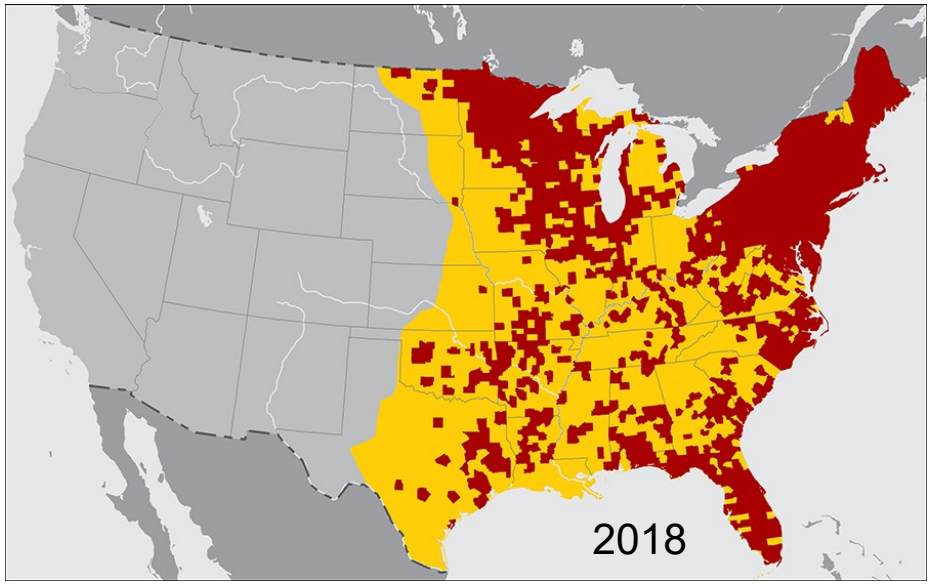
Geographic distribution of TBDs

Distribution of Selected TBDs



* Transmitted by the Blacklegged tick *Ixodes scapularis*

Distribution of Blacklegged Tick *Ixodes scapularis*



Pathogens transmitted by *I. scapularis:*

- *Borrelia burgdorferi* and *B. mayonii* (Lyme disease)
- *Anaplasma phagocytophilum* (Anaplasmosis)
- *B. miyamotoi* (Relapsing Fever)
- *Ehrlichia muris* (Ehrlichiosis)
- *Babesia microti* (Babesiosis)
- Powassan virus

www.cdc.gov

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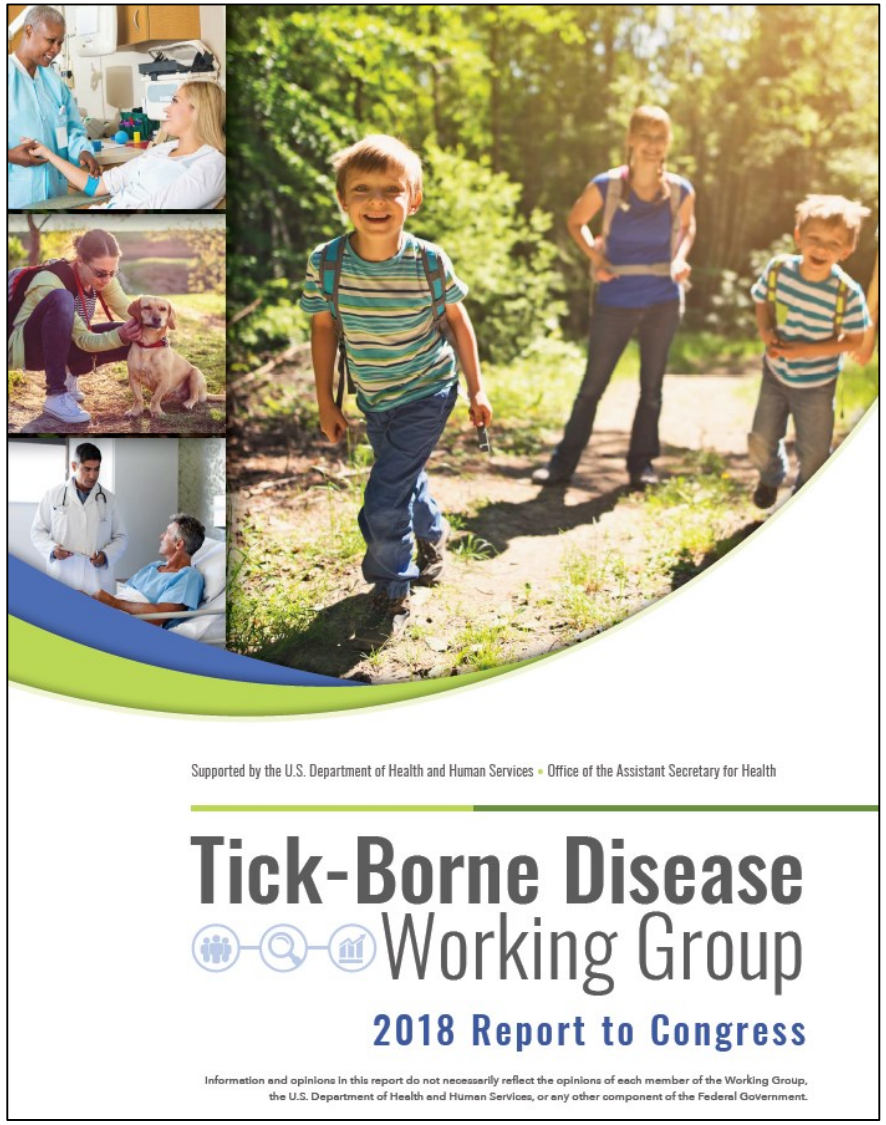
Current needs and gaps in TBD research



- The *21st Century Cures Act* was passed by Congress in 2016 to promote new health care innovations.
- Part of the legislation pertained to advancing research on TBDs.
- The Act required the U.S. Department of Health and Human Services (HHS) to establish a Federal Advisory Committee.
- The HHS formed the Tick-Borne Disease Working Group.

TBD Working Group Report to Congress (www.hhs.gov)

Current needs and gaps in TBD research

- Improve early and accurate diagnosis and treatment.
- Strengthen national surveillance.
- Understand the immunological mechanisms (*i.e.*, pathogen-host interactions) of immune protection for Lyme disease and other TBDs.
- Develop new rapid and accurate lab tests.
- Develop antibiotic combinations and/or therapeutic options for treating acute and persistent illness.
- Encourage the development of strategic plans for TBD federal investments.
- Characterize how TBD affects U.S. national security, military readiness, and the health and wellness of active duty Service members, Veterans, and their families.

TBD Working Group Report to Congress (www.hhs.gov)

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ATCC resources that support TBD research

Needs and Gaps in TBD Research[1]
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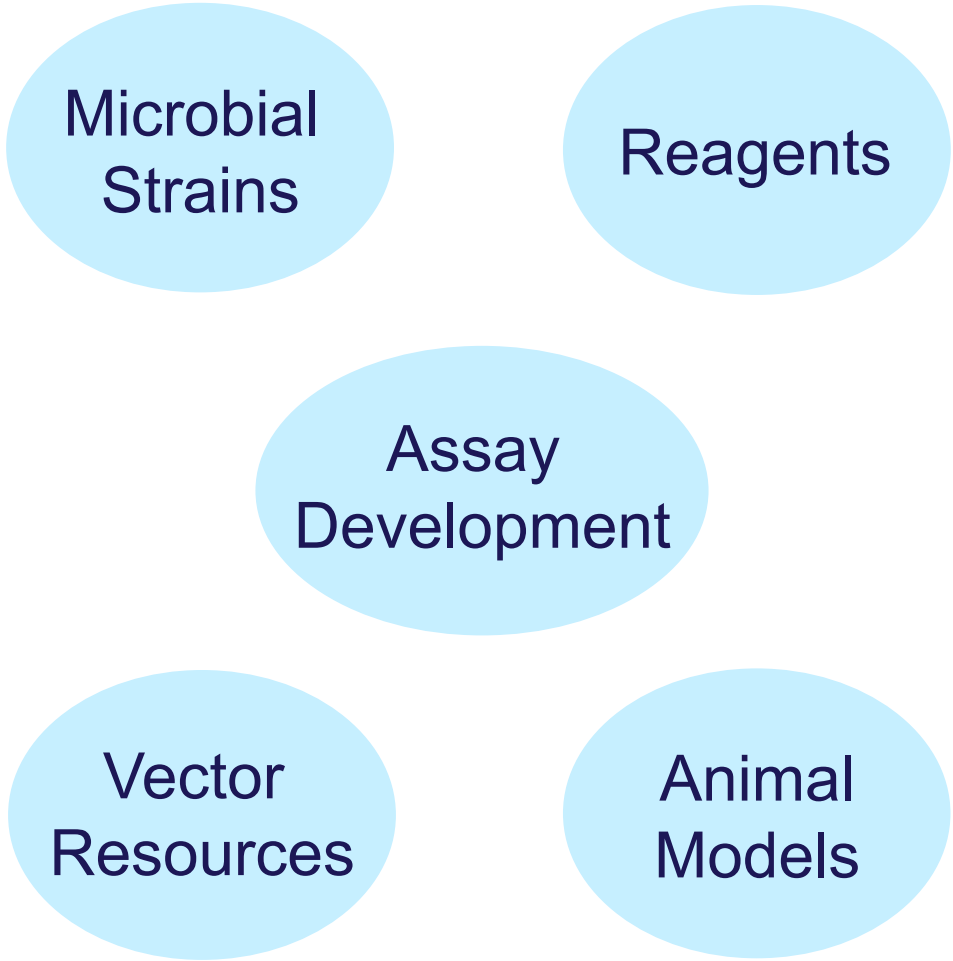
Develop new rapid and accurate lab tests.
Develop antibiotic combinations and/or therapeutic options for treating acute and persistent illness.



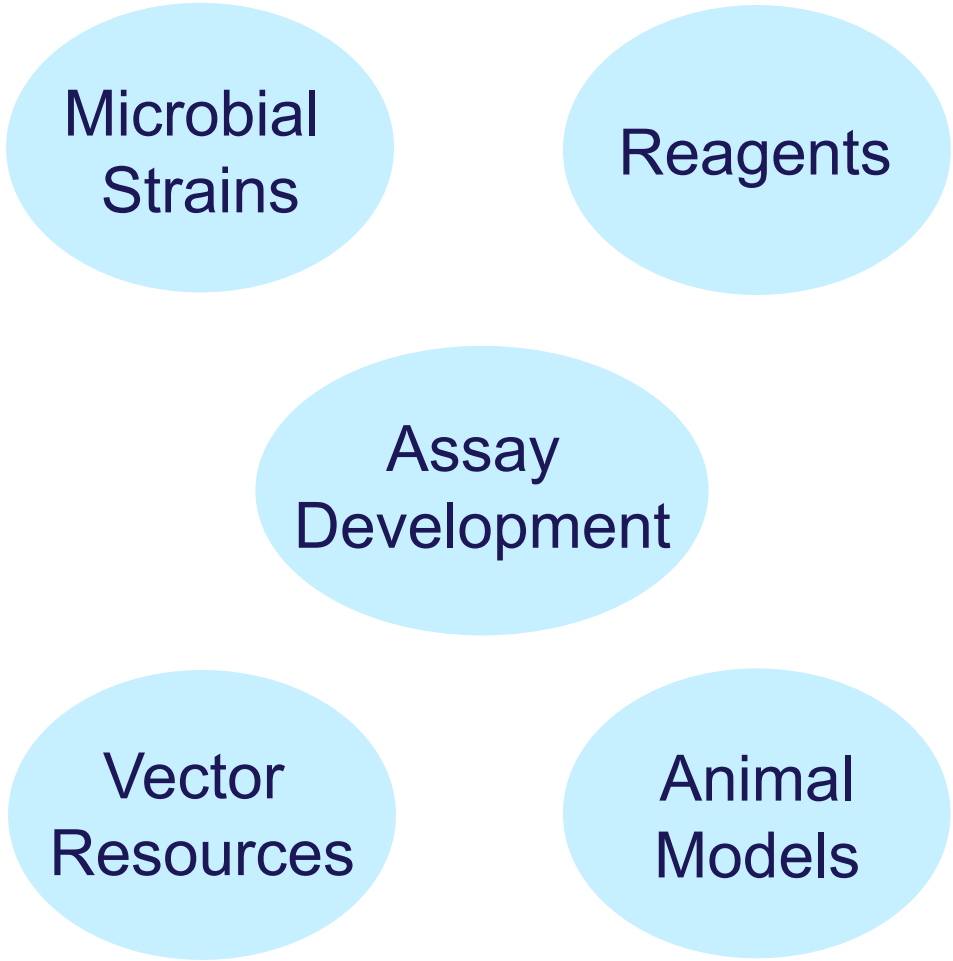
[1]*TBD Working Group Report to Congress (www.hhs.gov)*

ATCC resources that support TBD research

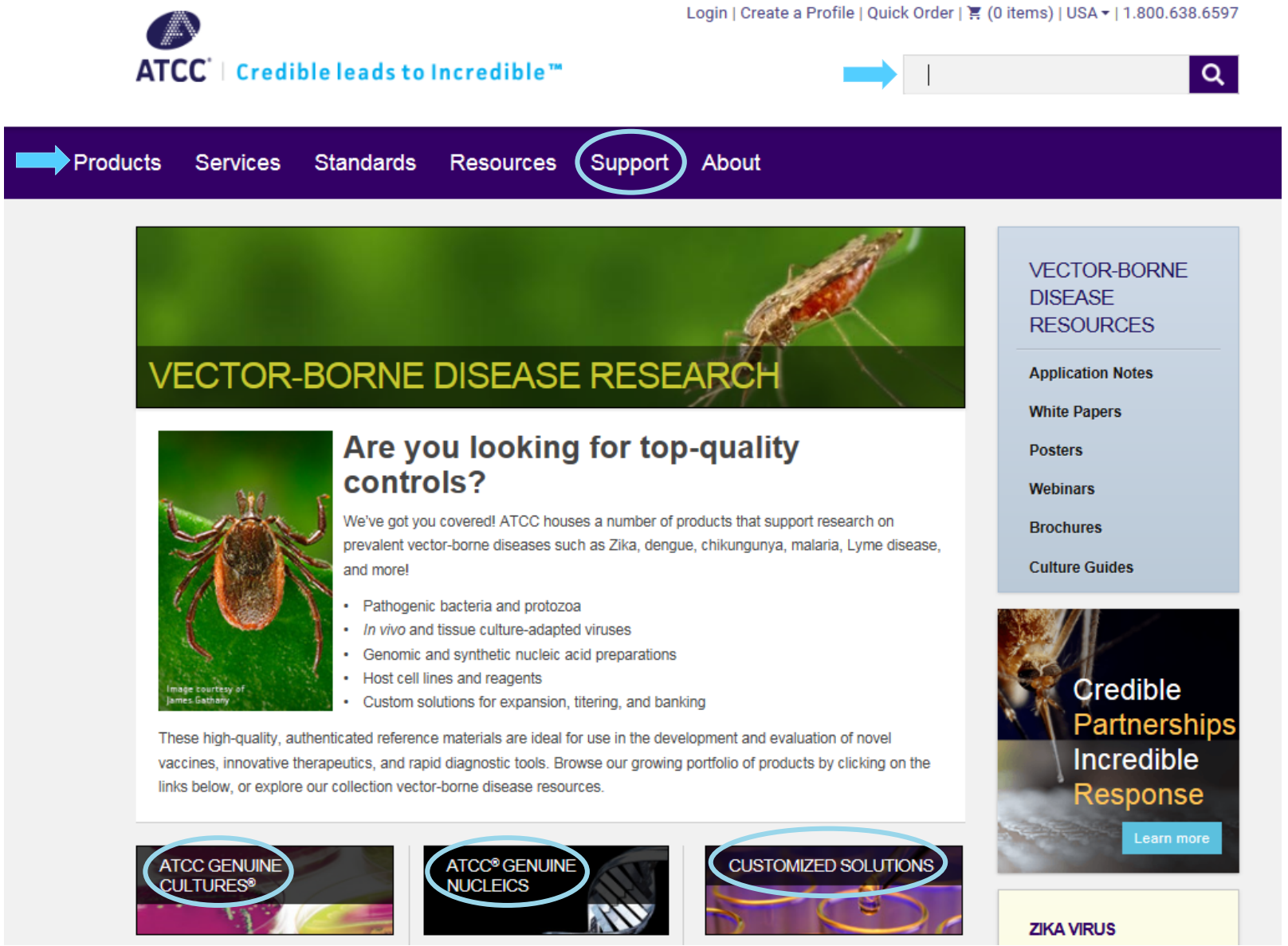
Bacterial
Anaplasmosis
Ehrlichiosis
Lyme Disease
Rickettsiosis
Tularemia

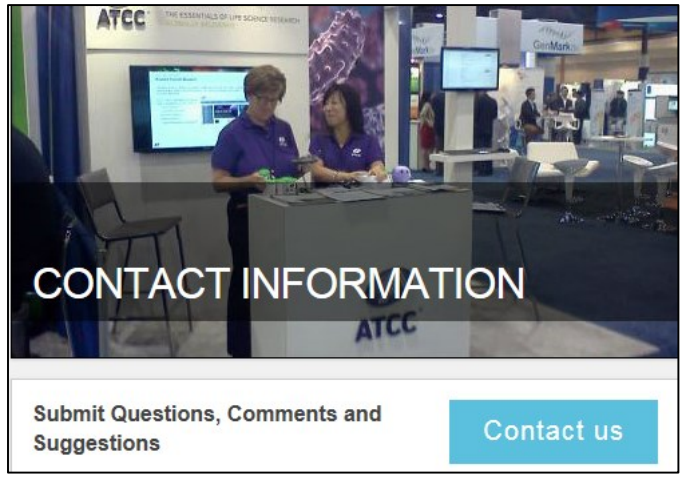
Parasitic
Babesiosis

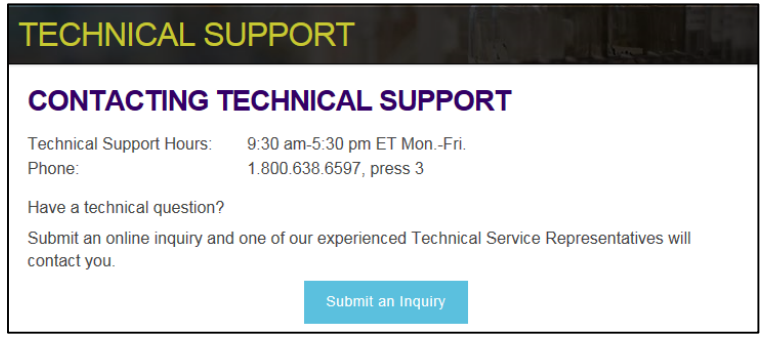
Viral
Bourbon virus
Colorado Tick Fever
Powassan virus



Where to find ATCC TBD research resources







www.atcc.org/Vectorborne

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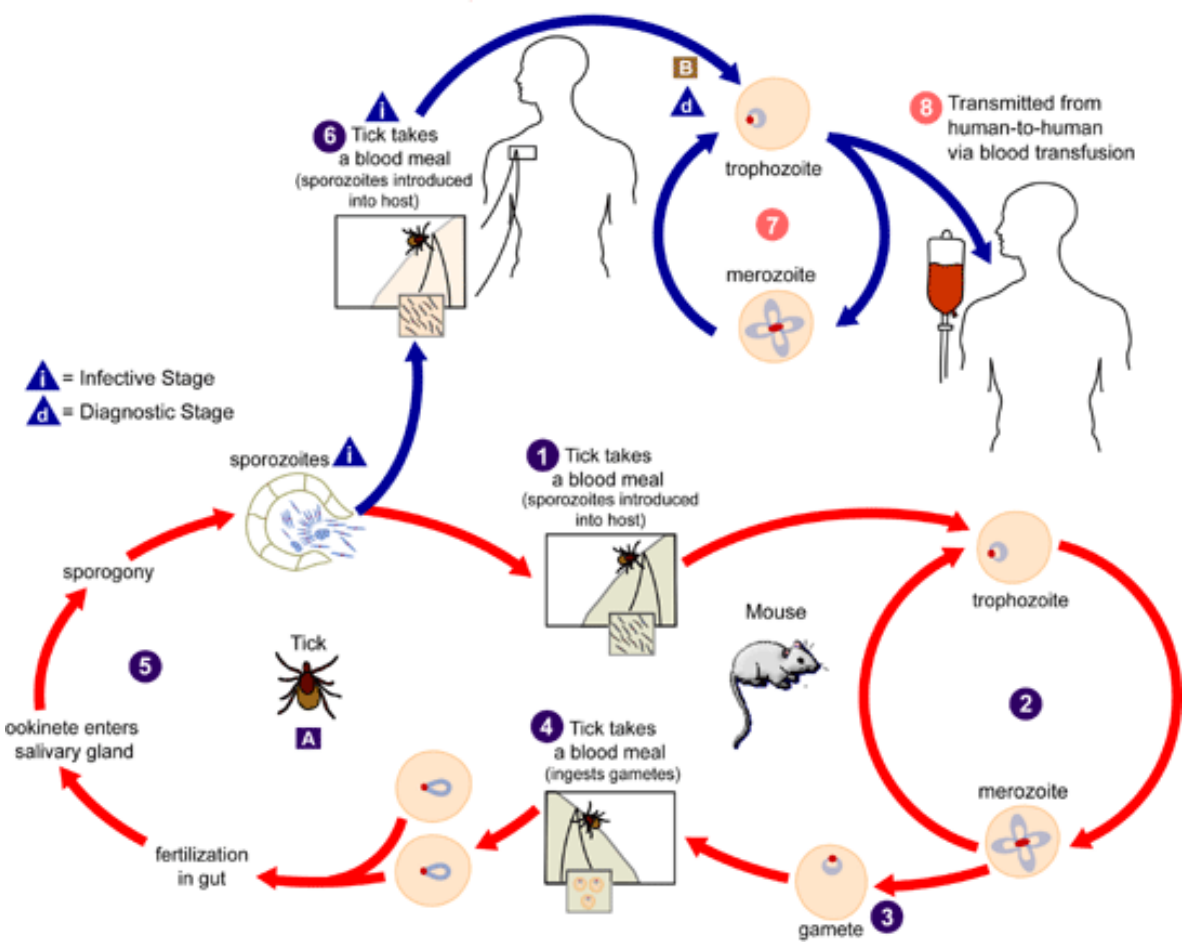
TBD research projects in ATCC: babesiosis

- Emerging TBD in the U.S.
- Caused by RBC-infecting *Babesia microti*
- Transmitted via *Ixodes* ticks, pregnancy, transfusion (less common)
- High prevalence in Northeast and Upper Midwest
- Infections usually asymptomatic or mild among healthy
- Severe infections in immunocompromised
- Worsening of Lyme disease symptoms in co-infections
- <u>New specific and sensitive lab tests for diagnosis and blood screening are needed</u>



TBD research projects in ATCC: babesiosis

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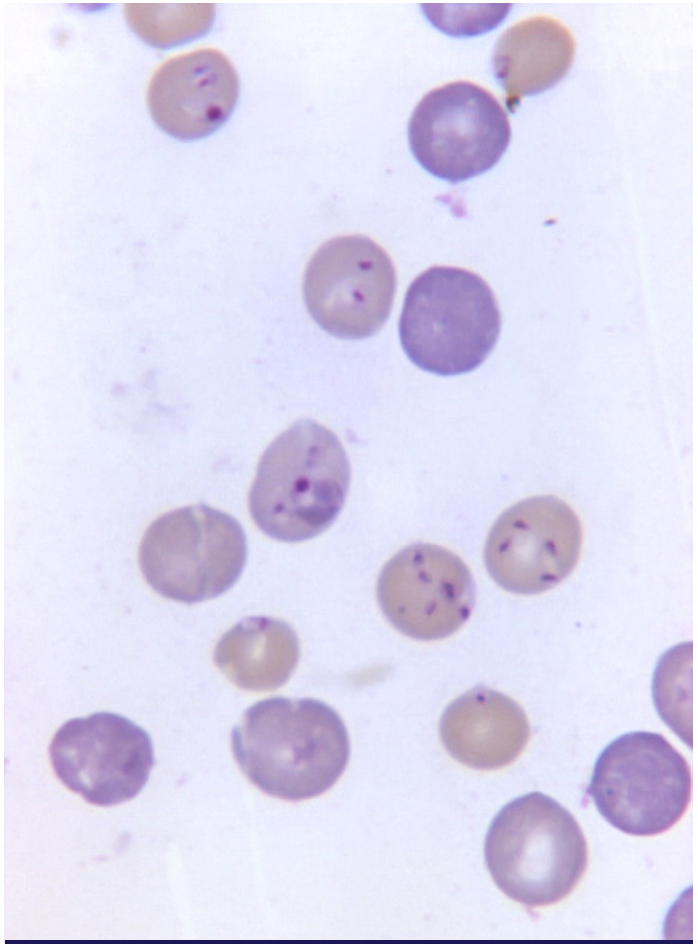
Development of quantitative PCR assays for *Babesia*

- The current gold standard for detection of *Babesia microti* infections is microscopic examination of blood smears.
- Antibody detection by serology can provide supportive evidence for diagnosis but does not reliably distinguish between active and prior infection.
- Recent PCR-based assays, including real-time PCR, have been developed for *Babesia microti*.
- Molecular assays that detect and distinguish between *B. microti* and other agents of human babesiosis (*i.e.*, *B. duncani*) are lacking.
- These closely related species can be differentiated due to sequence variation within the internal transcribed spacer (ITS) regions of nuclear ribosomal RNAs.

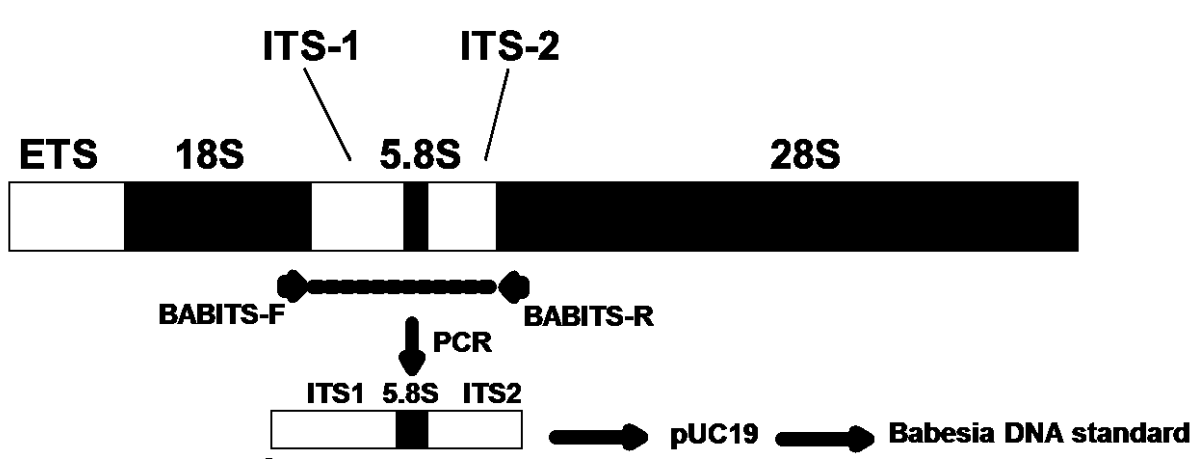


Giemsa stained red blood cells containing both *B microti* and *P berghei*.

Image courtesy of Dr. George Healy, CDC

Targeting *Babesia* ITS regions for quantitative PCR assays

Babesia nuclear ribosomal RNA



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Development of real time PCR assays for *Babesia*

Real-time PCR detection and quantitation of *Babesia* DNA standards

B. microti standard[a]	Cq		*B. duncani* standard[a]	Cq	
	Mean	SD		Mean	SD
1×10^8	12.85	0.155	1×10^8	10.90	0.194
1×10^7	16.36	0.059	1×10^7	14.15	0.257
1×10^6	19.82	0.075	1×10^6	17.39	0.058
1×10^5	23.44	0.042	1×10^5	21.06	0.202
1×10^4	27.03	0.049	1×10^4	24.78	0.112
1×10^3	30.54	0.184	1×10^3	28.53	0.414
100	34.24	0.529	100	31.71	0.127
10	37.60	0.408	10	34.13	1.057
1	0.0	0.0	1	0.0	0.0
H_2O	0.0	0.0	H_2O	0.0	0.0

[a] Values reflect copy numbers per reaction based on serial dilutions of plasmid DNA standard by DNA concentration and known molecular mass. Data represent the means ± standard deviations of each dilution tested in triplicate from a representative experiment of three performed.

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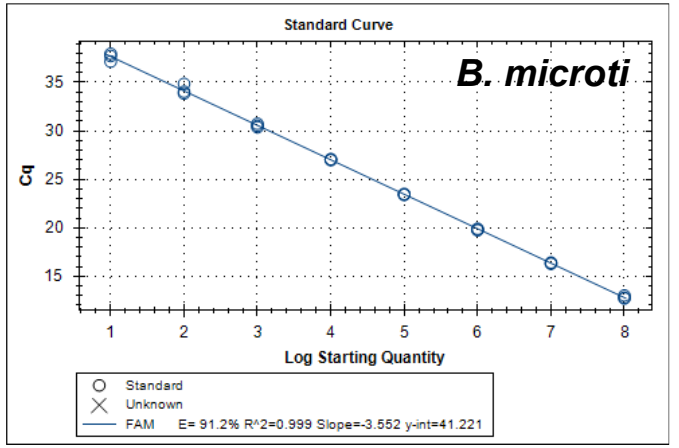

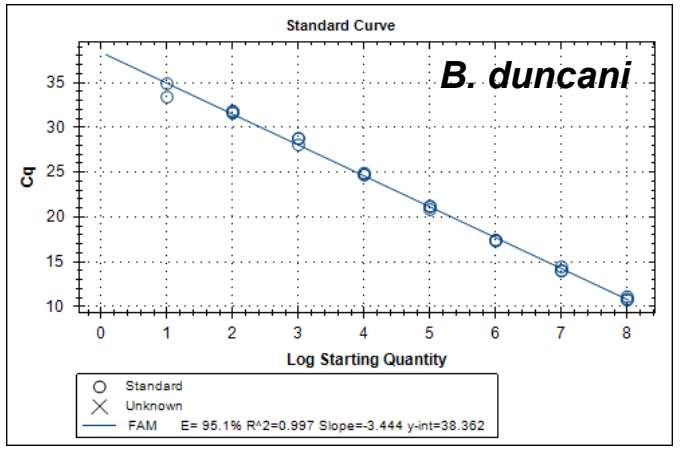

Wilson M, et al. Exp. Parasitol. 149: 24-31, 2015.

Development of droplet digital™ PCR assays for *Babesia*

ddPCR™ detection and quantitation of *Babesia* DNA standards

B. microti standard[a]	ddPCR value[b]		*B. duncani* standard[a]	ddPCR value[b]	
	Mean	SD		Mean	SD
1×10^5	38,200	721	1×10^5	51,733	1,858
1×10^4	3,393	170	1×10^4	4,827	50
1×10^3	344	26.5	1×10^3	414	31.4
100	38.5	3.8	100	44.3	3.4
10	1.2	0	10	7.5	1.1
1	0	0	1	0	0
H_2O	0	0	H_2O	0	0

[a]Values reflect copy numbers added per reaction based on serial dilutions of plasmid DNA standard by DNA concentration and known molecular mass. [b]Copies per reaction detected. Data represent the means ± standard deviations of each dilution tested as five replicates from a representative experiment of three performed.

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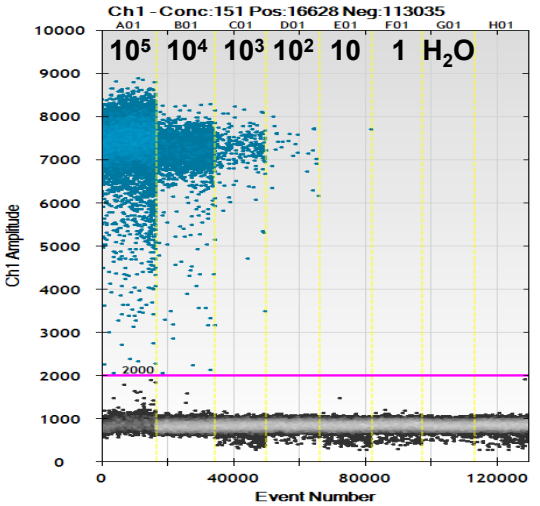
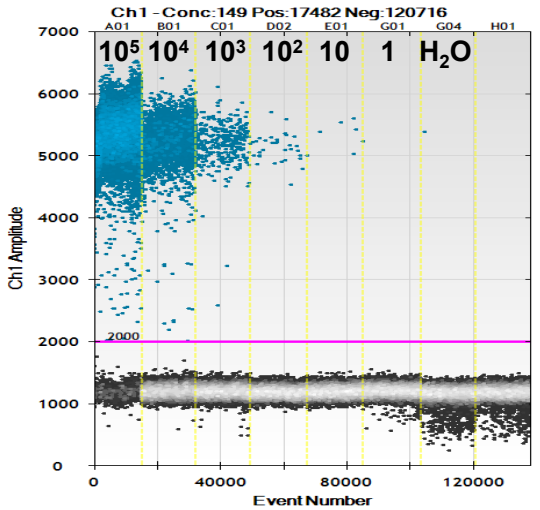
B. microti



B. duncani



Wilson M, et al. Exp. Parasitol. 149: 24-31, 2015.

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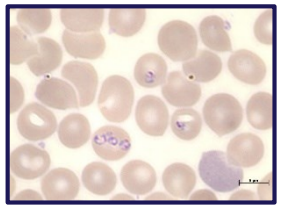
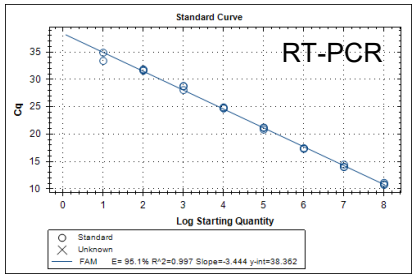
Detection of *Babesia* DNA in hamster blood by qPCR

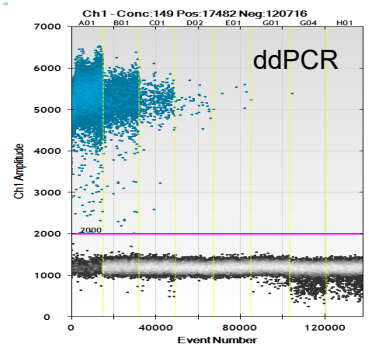
Infection of hamsters with *Babesia microti* or *B. duncani*

Assessment of parasitemia by microscopy

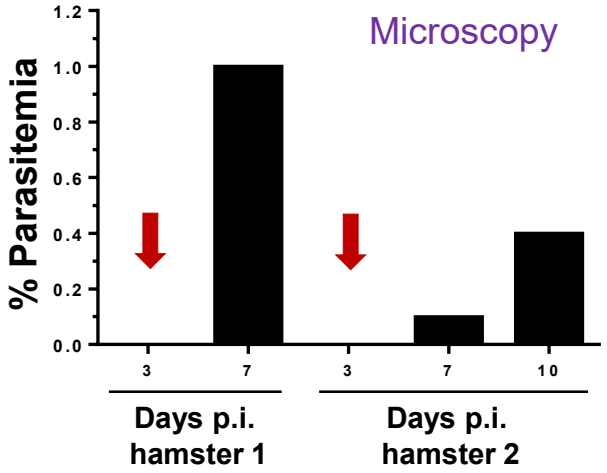


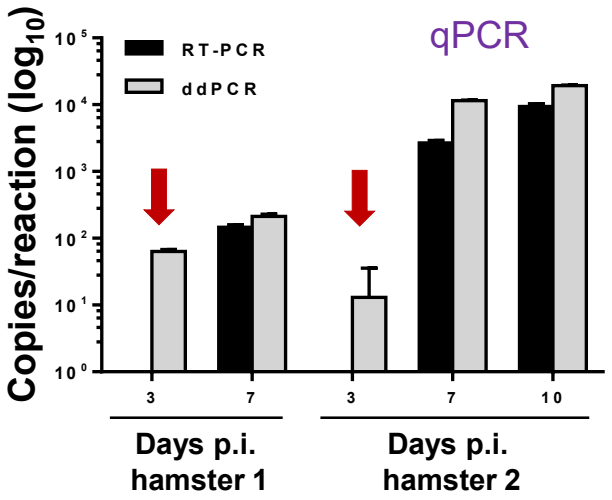
Comparison with qPCR



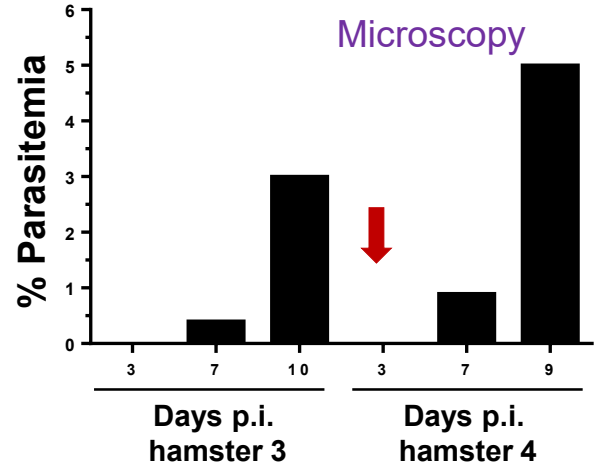


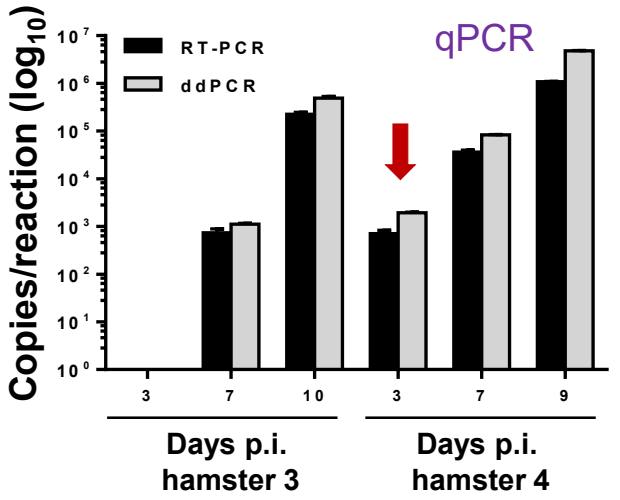
B. microti





B. duncani

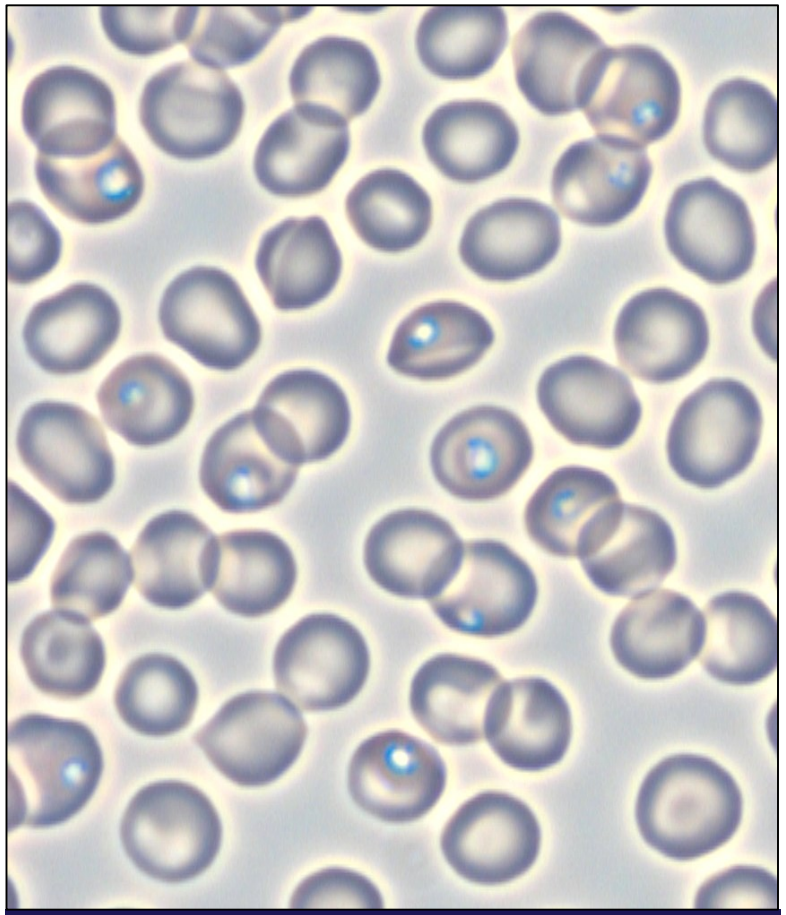




Wilson M, et al. Exp. Parasitol. 149: 24-31, 2015.

Summary of qPCR assays for *Babesia*

- We report the development of RT-PCR and ddPCR™ assays for the detection and discrimination of *B. microti* and *B. duncani* in blood samples.
- Pending further validation, the assays could be used as highly specific and sensitive molecular tests for screening human blood samples.
- Wilson M, *et al.* Development of droplet digital PCR for the detection of *Babesia microti* and *Babesia duncani*. *Exp Parasitol*, 149: 24-31, 2015.
- Quantitated DNA products for use as standards in *Babesia* qPCR assays are available in the ATCC catalog (ATCC® PRA-398DQ™ and ATCC® PRA-302DQ™)



Giemsa stained red blood cells infected with *Babesia microti*.

TBD research projects in ATCC: babesiosis

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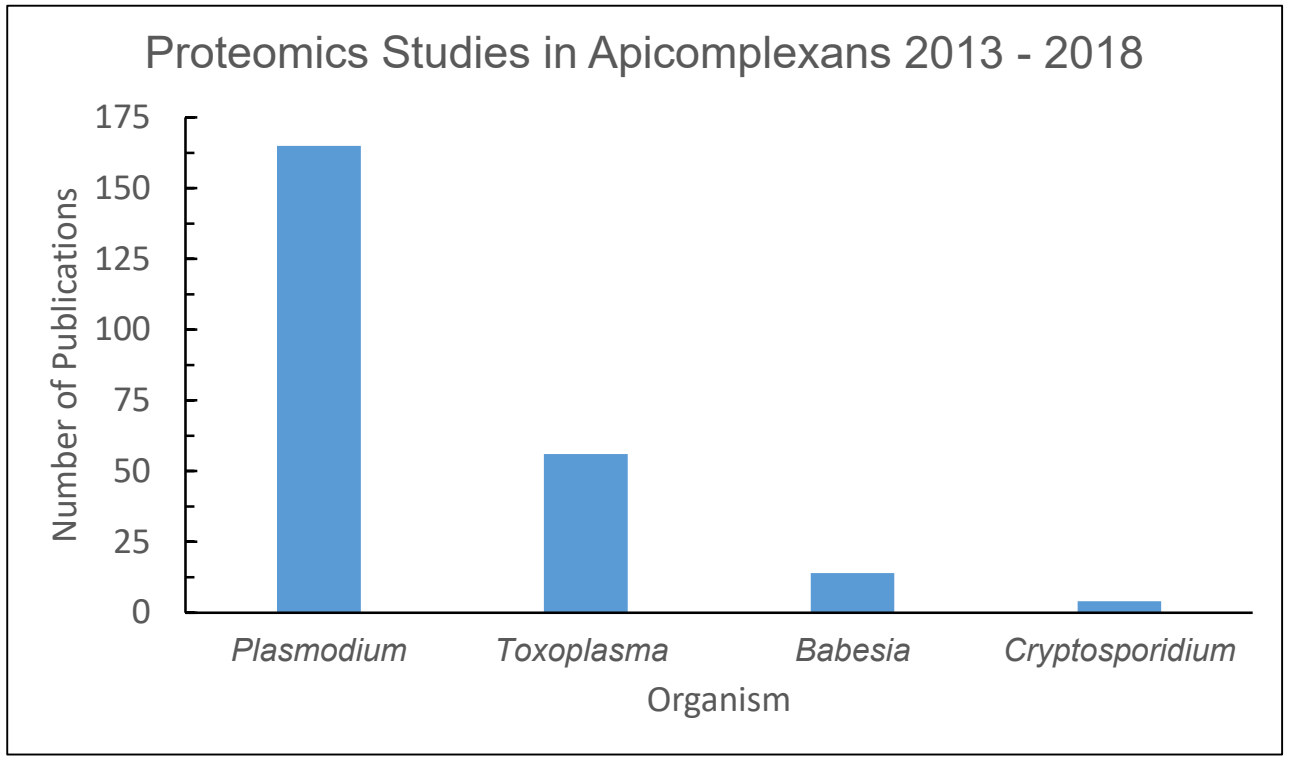
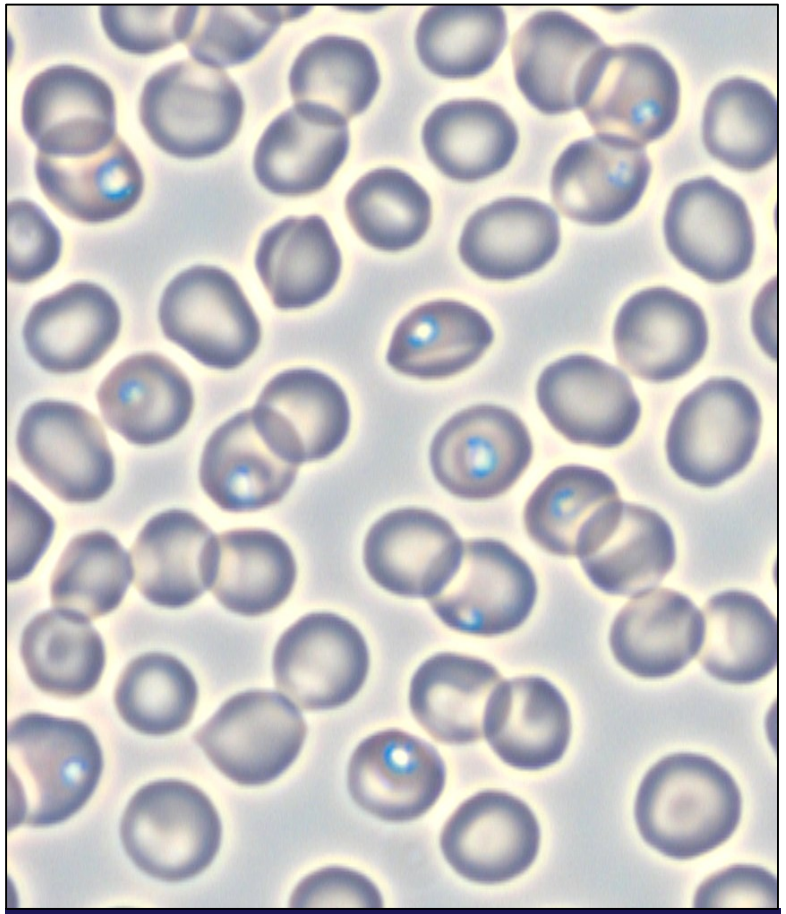
Proteomics of *Babesia microti* infection



- Proteomics research on *Babesia* lags behind other Apicomplexans.
- *In vitro* culture techniques used for *Plasmodium* and *Toxoplasma* are not readily available for *B. microti.*
- Proteomic analysis of *B. microti* is currently restricted to animal models.
- Benefits of analyzing blood and other body fluids include:
  - Discovery of new biomarkers of infection
  - Correlation of the progression of infection with the detection of specific biomarkers

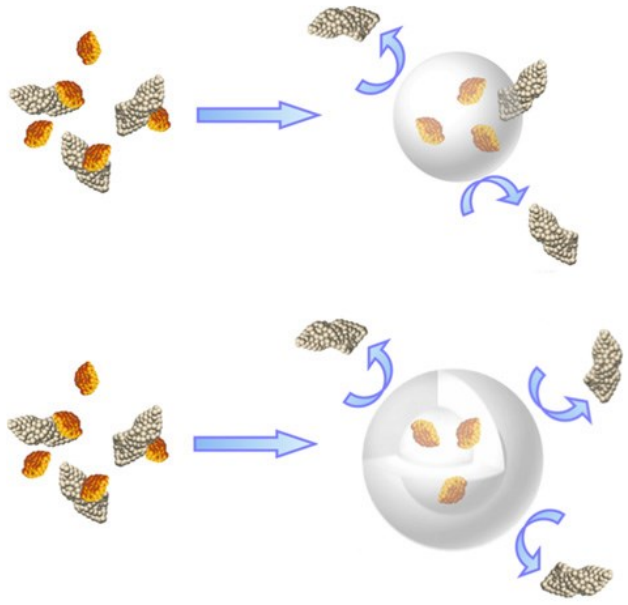
Major challenges in biomarker discovery by proteomics

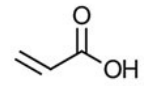
- Detection of biomarkers during early stages of disease is difficult due to their low abundance in blood and other body fluids.
- There is an overwhelming abundance of host resident proteins such as albumin and immunoglobulins in plasma (~90% of circulating proteins), which masks the isolation of rare biomarkers.
- There is a possibility for biomarkers to become rapidly degraded by proteases immediately after collection of the biological sample.



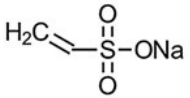
Giemsa stained red blood cells infected with *Babesia microti*.

Application of nanoparticle capture in proteomics

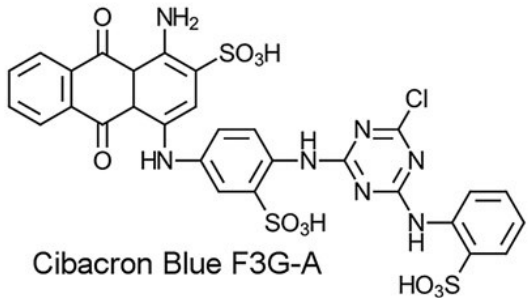




Acrylic acid



Vinyl sulfonic acid



Cibacron Blue F3G-A

- Hydrogel nanospheres concentrate and preserve small molecular-weight peptides and proteins.
- Nanoparticles are based on a cross-linked N-isopropyl-acrylamide (NIPAm) core containing a charge-based bait.
- A shell surrounding the NIPAm core acts as a sieve to exclude proteins too large to penetrate the porosity of the shell.
- The nanoparticles achieve three essential functions:
  - Capturing and concentrating dilute amounts of target analytes
  - Sieving of interfering proteins
  - Protection from proteolysis
- Proteins sequestered by the particles can be analyzed by mass spectrometry, Western blotting, and other immunoassays.

Shafagati N, et al. Pathog Dis 71:164-176, 2014.

Applications of nanoparticle capture in infectious diseases

Lyme Disease

- Douglas TA, *et al*. The use of hydrogel microparticles to sequester and concentrate bacterial antigens in a urine test for Lyme disease. *Biomaterials* 32(4): 1157-66, 2011.
- Magni R, *et al*., Application of Nanotrap technology for high sensitivity measurement of urinary outer surface protein A carboxyl-terminus domain in early stage Lyme borreliosis. *J Transl Med* 13: 346, 2015.

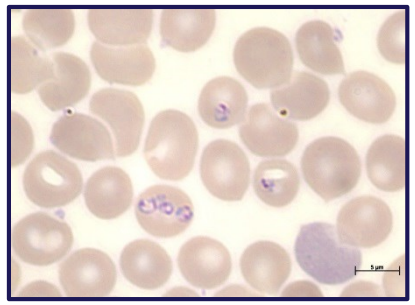
Chagas Disease

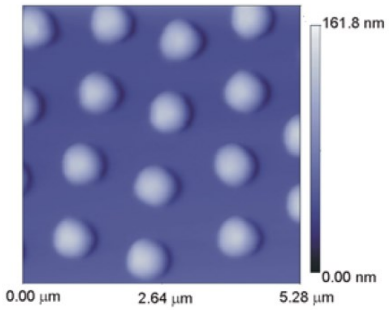
- Castro-Sesquen YE, *et al*. Use of a novel chagas urine nanoparticle test (chunap) for diagnosis of congenital chagas disease. *PLoS Negl Trop Dis* 8(10): e3211, 2014.
- Castro-Sesquen YE, *et al*. Use of a chagas urine nanoparticle test (Chunap) to correlate with parasitemia levels in *T. cruzi*/HIV co-infected patients. *PLoS Negl Trop Dis* 10(2): e0004407, 2016.

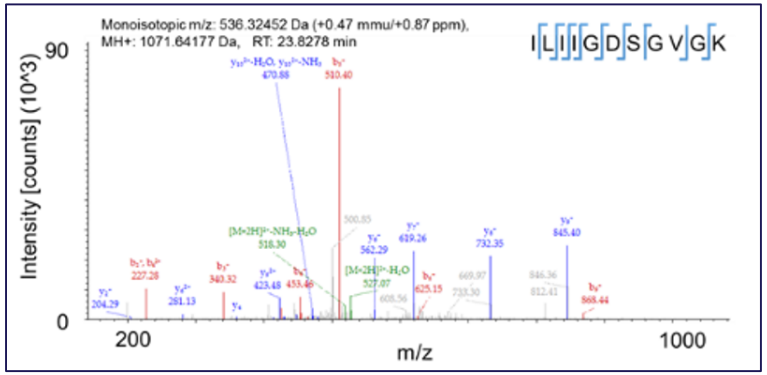
Toxoplasmosis

- Steinberg HE, *et al.* Toward detection of toxoplasmosis from urine in mice using hydro-gel nanoparticles concentration and parallel reaction monitoring mass spectrometry. *Nanomedicine* 14(2): 461-469, 2018.

Application of nanoparticle capture in babesiosis

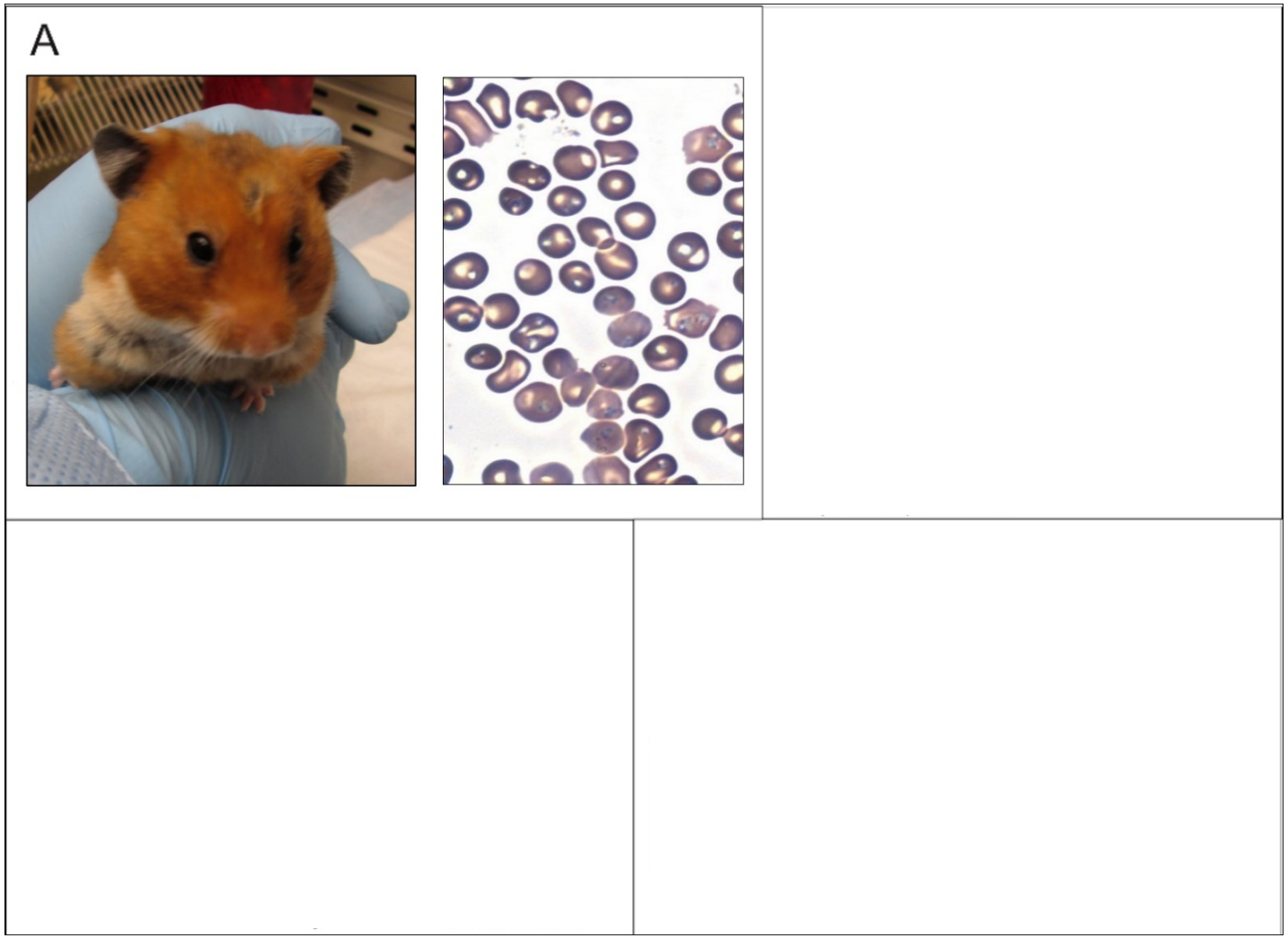






- Determine the capability of nanoparticles to sequester proteins of *Babesia microti* from whole blood by using the hamster model of babesiosis.
- Identify captured proteins by mass spectrometry.
- Present a preliminary description of the *B. microti* proteome to complement previous genomic and transcriptomic studies.
- Contribute to the development of newer diagnostic tools.

Experimental strategy



A) Whole blood is collected from experimentally infected hamsters (≥30% parasitemia; *B. microti* G1; ATCC® PRA-398™).

B) Hydrogel nanoparticles sequester parasite proteins and exclude high abundance proteins from the host.

C) Proteins are enriched and eluted from nanoparticles.

D) MS is performed using a LTQ™ Fusion™ Mass Spectrometer (Thermo Fisher Scientific™). Tandem mass spectra are searched against a *Babesia* protein database (Piroplasma.db).

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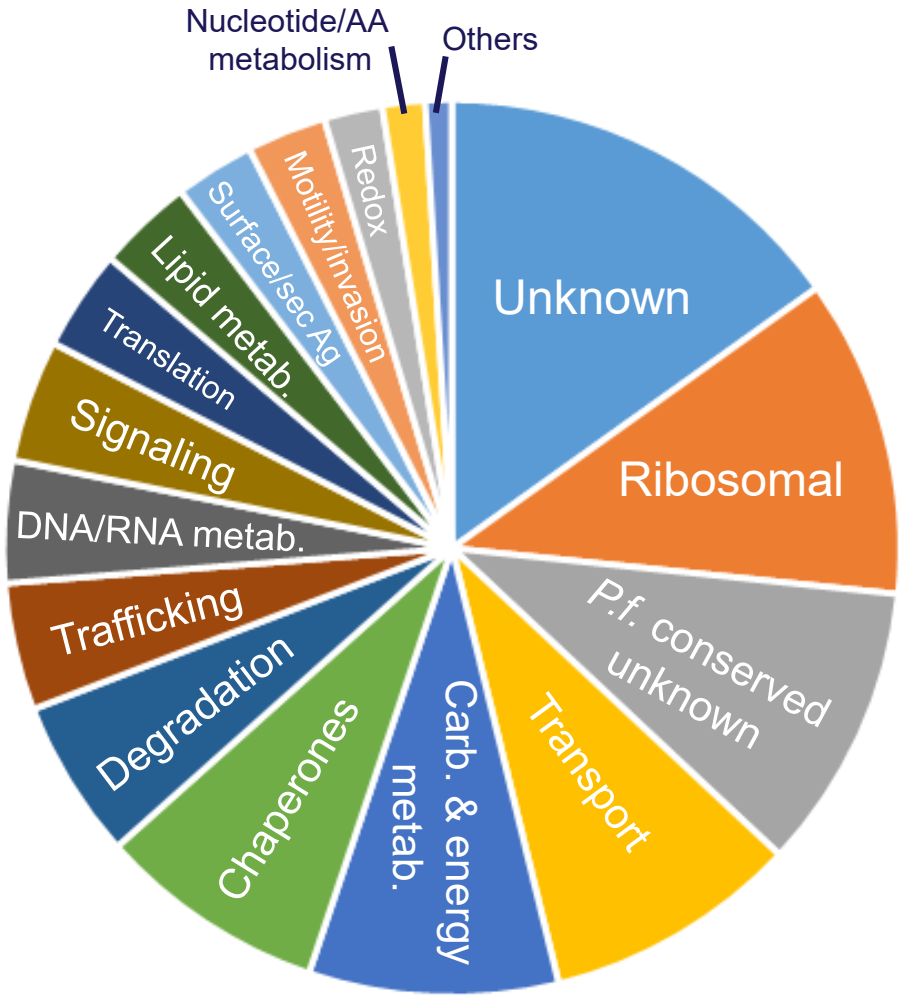
Biological function

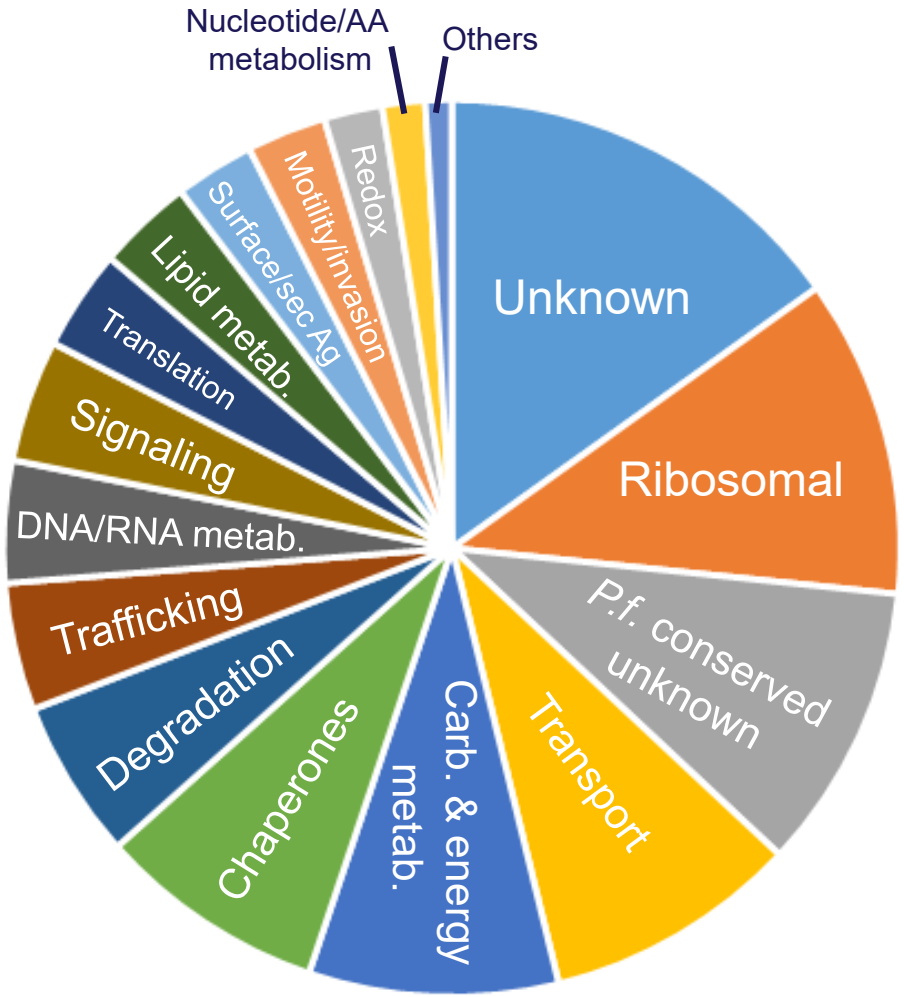


> 500 *Babesia* proteins

Biological Function	No. of proteins	Examples
Unknown	79	Uncharacterized proteins matching to hypothetical genes in the genome
Ribosomal	60	Components of small subunit and large subunit ribosomal proteins
Plasmodium conserved, unknown	54	Uncharacterized *Plasmodium* conserved proteins
Transport	48	Ion transporters, carbohydrate transporters, protein transporters, ABC transporters
Carbohydrate and energy metabolism	47	Glycolysis proteins, TCA cycle proteins, electron transport chain proteins, mitochondrial importers, ATP synthases
Protein folding/chaperones	43	Heat shock proteins, SEC63, DnaK family proteins
Protein degradation	30	Cysteine proteases, metalloproteases, proteasome subunits, peptidases
Trafficking	24	Vesicular transport proteins, Rab proteins, SNARE-like proteins, ER lumen proteins, translocation proteins
DNA/RNA metabolism	23	DNA synthesis, replication, degradation, and repair proteins. RNA synthesis, transcription, modification, processing, and degradation proteins. Includes histones.

Magni RL, et al. Int J Parasitol 49(2): 139-144, 2019.

Biological function



> 500 *Babesia* proteins

Biological Function	No. of proteins	Examples
Signal transduction	23	Kinases, phosphatases, GTP-binding proteins
Protein translation and modification	19	Aminoacyl tRNA synthetases, elongation factors, ubiquitin protein ligases, glycosyltransferases
Lipid metabolism	18	Proteins involved in lipid transport, biosynthesis, and degradation
Surface/secreted antigens	15	BMN family proteins, BmSA1 surface antigen
Motility and invasion	15	Actin, profilin, myosin, microneme and rhoptry proteins
Redox homeostasis	11	Thioredoxins, oxidoreductases, and other proteins that maintain the redox environment of the cell
Nucleotide and AA metabolism	8	Proteins involved in biosynthesis and degradation of nucleic acids and amino acids
Others	5	Proteins involved in porphyrin biosynthesis, chloroquine resistance, ubiquinone biosynthesis, and peripheral plastid proteins
Total	**522**	

Magni RL, et al. Int J Parasitol 49(2): 139-144, 2019.

Top 20 ranking of identified proteins

BMN: Surface/ secreted antigens

HSP: Heat shock proteins

Gene ID	Description	No. PSMs[1]	No. Peptides[2]	No. Unique Peptides[3]	Coverage[4]	AA length[5]	MW (kDa)[5]
BMR1_03g00785	BmGPI12, BMN1 family, BMN1-9, BmSA1 orthologue BMN	104	26	26	53.0	328	35.4
BMR1_03g03720	Myosin ATPase	79	44	44	35.3	1629	183.6
BmR1_04g06050	Heat shock 70kDa protein 5 HSP	64	24	24	45.7	647	71.5
BMR1_03g03315	Chaperonin GroEL HSP	50	23	23	56.5	568	60.7
BMR1_03g04636	F-type H+-transporting ATPase oligomycin sensitivity conferral	44	24	24	40.7	759	86.2
BmR1_04g05605	F-type H+-transporting ATPase subunit alpha	40	19	17	48.1	532	58.4
BMR1_01G02545	HSPA1_8, heat shock 70kDa protein 1/8 HSP	38	23	23	43.9	644	70.2
BmR1_04g08040	Molecular chaperone DnaK protein HSP	36	21	21	42.7	663	72.5
BMR1_02g01245	F-type H+-transporting ATP synthase subunit beta	36	15	10	46.7	514	55.3
BMR1_03g00005	BMN1 family (Fragment) BMN	29	9	2	44.3	291	32.8
BMR1_03g00960	Uncharacterized protein	27	15	15	42.8	439	49.9
BMR1_03g04270	Chaperonin GroEL HSP	27	14	14	28.8	650	70.9
BmR1_04g09925	Papain family cysteine protease	26	9	9	35.5	445	49.8
BMR1_03g01005	HAD ATPase P-type family IC	25	18	18	22.9	1234	134.2
BmR1_04g05480	Uncharacterized protein	25	15	15	66.8	298	33.5
BMR1_02g04275	BmGPI10, BMN1 family, N1-21a orthologue BMN	25	9	2	40.1	304	34.4
BMR1_01G02590	Solute carrier family 25 (Mitochondrial carrier, adenine nucleotide)	23	15	15	55.2	306	33.6
BMR1_01G03280	BMN1-20 BMN	22	11	11	29.5	535	59.6
BMR1_01G01670	Protein disulfide-isomerase A1	21	15	15	39.7	471	53.8
BMR1_02g04100	Hsp70 protein HSP	19	13	13	27.0	649	72.8

[1] The total number of identified peptide spectra matched for the protein. [2] The total number of distinct peptide sequences identified in the protein.

[3] The number of peptide sequences that are unique to a protein and which do not occur in the proteins of any other group.

[4] The percentage of the protein sequence covered by identified peptides. [5] Amino acid length and calculated molecular weight of the protein.

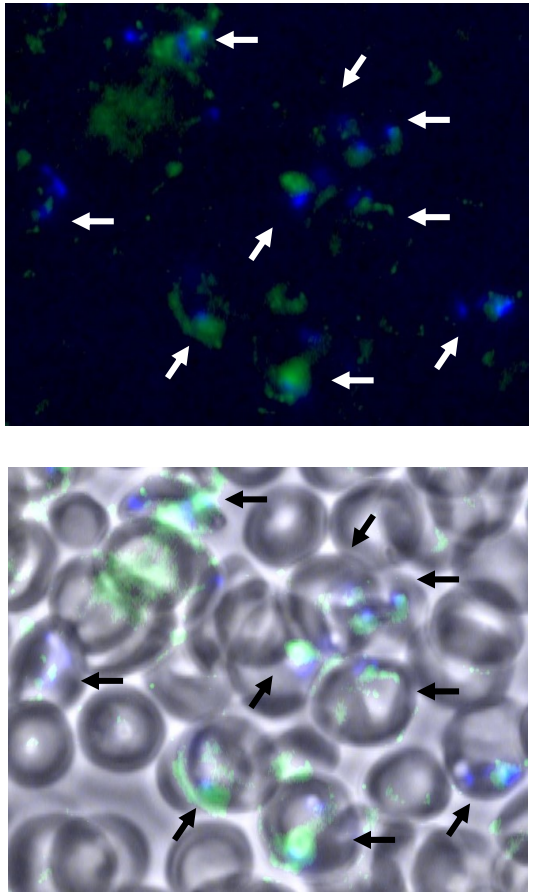
ATCC

Seroreactive antigens

Identification of *B. microti* proteins with reported immune responses *in vivo*[1]						
Gene ID	**Description**	**Class**[2]	**Ig Response**[1]	**AA length**	**MW (kDa)**	***P. falciparum* ortholog**
BMR1_03g00785	BmGPI12, BMN1 family, BMN1-9, BmSA1 orthologue	GPI	IgM/IgG	328	35.4	
BMR1_02g04275	BmGPI10, BMN1 family, N1-21a orthologue	GPI	IgG	304	34.4	
BMR1_02g03140	S1/P1 Nuclease	SEC	IgM/IgG	373	42.8	PF3D7_1411900
BMR1_03g00947	conserved Plasmodium protein, unknown function	SEC	IgM/IgG	438	48.7	PF3D7_1324300
BMR1_01G00985	conserved Plasmodium protein, unknown function	SEC	IgM/IgG	1025	114.4	PF3D7_0822900
BMR1_02g04280	BMN2 family, possible orthologue of N1-10-2	TM	IgM	519	60.9	
BmR1_04g07535	N1-15 protein, maltese-cross seroactive antigen	SEC	IgG	2396	265.7	
BMR1_03g04695	Rhoptry neck protein 2		IgM/IgG	1483	165.7	PF3D7_1452000
BMR1_03g00020	BMN2 family	SEC	IgM	474	55.4	
BmR1_04g07556	Uncharacterized protein	TM	IgM	218	24.4	
BmR1_04g05531	MDN1, REA1, midasin		IgG	4337	493.1	PF3D7_1434500

[1] As reported in Silva, J.C., et al. 2016. Sci. Rep. 6: 35284. [2] GPI, Glycosylphosphatidylinositol; SEC, secreted; TM, transmembrane

BMR1_03g00785 IFA



Magni RL, et al. Int J Parasitol 49(2): 139-144, 2019.

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BMR1_02g03140	S1/P1 Nuclease	SEC	IgM/IgG	373	42.8	PF3D7_1411900
BMR1_03g00947	conserved Plasmodium protein, unknown function	SEC	IgM/IgG	438	48.7	PF3D7_1324300
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BmR1_04g07535	N1-15 protein, maltese-cross seroactive antigen	SEC	IgG	2396	265.7	
BMR1_03g04695	Rhoptry neck protein 2		IgM/IgG	1483	165.7	PF3D7_1452000
BMR1_03g00020	BMN2 family	SEC	IgM	474	55.4	
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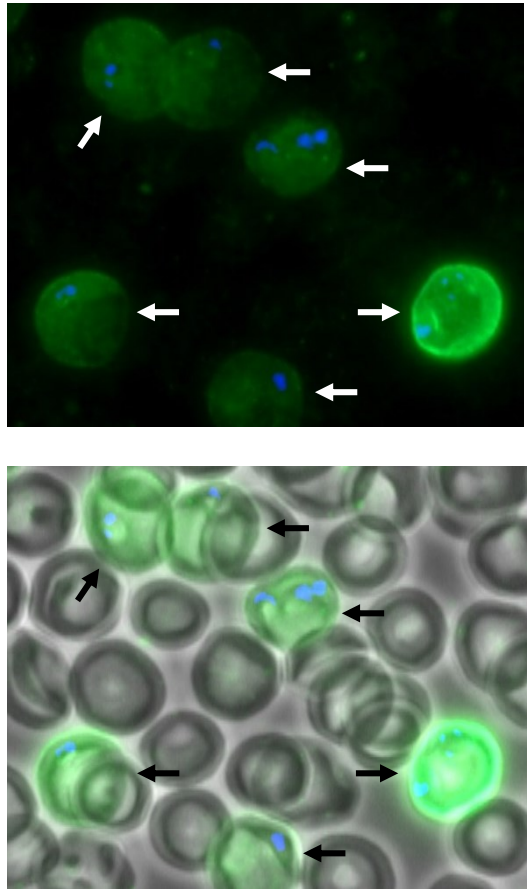
BMR1_03g00947 IFA



Magni RL, et al. Int J Parasitol 49(2): 139-144, 2019.

Summary of proteomics of *B. microti* study

- We present a partial description of the *B. microti* proteome by successfully capturing and identifying parasite proteins from whole blood by using a combination of nanotechnology and mass spectrometry.
- The catalog of proteins mirrors the metabolically active trophozoite stage.
- Surface/secreted antigens are highly abundant in the *B. microti* proteome and serve as useful biomarkers of infection.
- The nanotechnology approach avoids the cumbersome separation of infected erythrocytes and/or free parasites from the sample.
- The molecular sieve and affinity functions minimizes the capture of highly abundant interfering proteins from the host.
- Magni RL, *et al*. Analysis of the *Babesia microti* proteome in infected red blood cells by a combination of nanotechnology and mass spectrometry. *Int J Parasitol* 49(2): 139-144, 2019.

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Conclusions

- Diseases transmitted by ticks are a serious and growing public health concern in the United States.
- There remain several gaps in TBD research, education, prevention, and treatment.
- ATCC offers a variety of resources critical to advancing TBD research priorities.
- Ongoing research projects in ATCC are focused on improving diagnostic methods for babesiosis.

Cultivating collaboration to support global health

Upcoming webinars:

- Making Sense Out of Microbiome Data – The Importance of Standards | August 22, 12:00 ET
- STR Profiling for Mouse Cell Lines: Another Tool to Combat Cell Line Misidentification | September 12, 12:00 ET

www.atcc.org/webinars