Current Research in the Tickborne Disease Babesiosis at the ATCC

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Lead Scientist

American Type Culture Collection, Manassas, VA

Outline

- Overview of ATCC
- Tickborne Diseases in the U.S.
- Human Babesiosis Overview
- Role of the CD47-SIRPα axis in the clearance of *Babesia microti* in vivo
- Role of Babesia extracellular vesicles in the immune response

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ATCC Overview

- Non-profit founded in 1925, providing the world's scientific community with a range of biomaterials (e.g., cells, parasites, bacteria, viruses), laboratory services, and repository operations
- 600+ employees providing resources for Government programs performing disease research and surveillance
- Support to federal Government programs for >60 years focused on global health, clinical study support, and biodefense
  - Clients include NIH (NCI, NIAID), CDC, FDA, DHS, USDA, DoD

ATCC Federal Solutions focuses on clinical study support, global health, and biodefense.

- Material storage and resource operations
- Laboratory services
- Research & development
- Laboratory staffing and subject matter expertise

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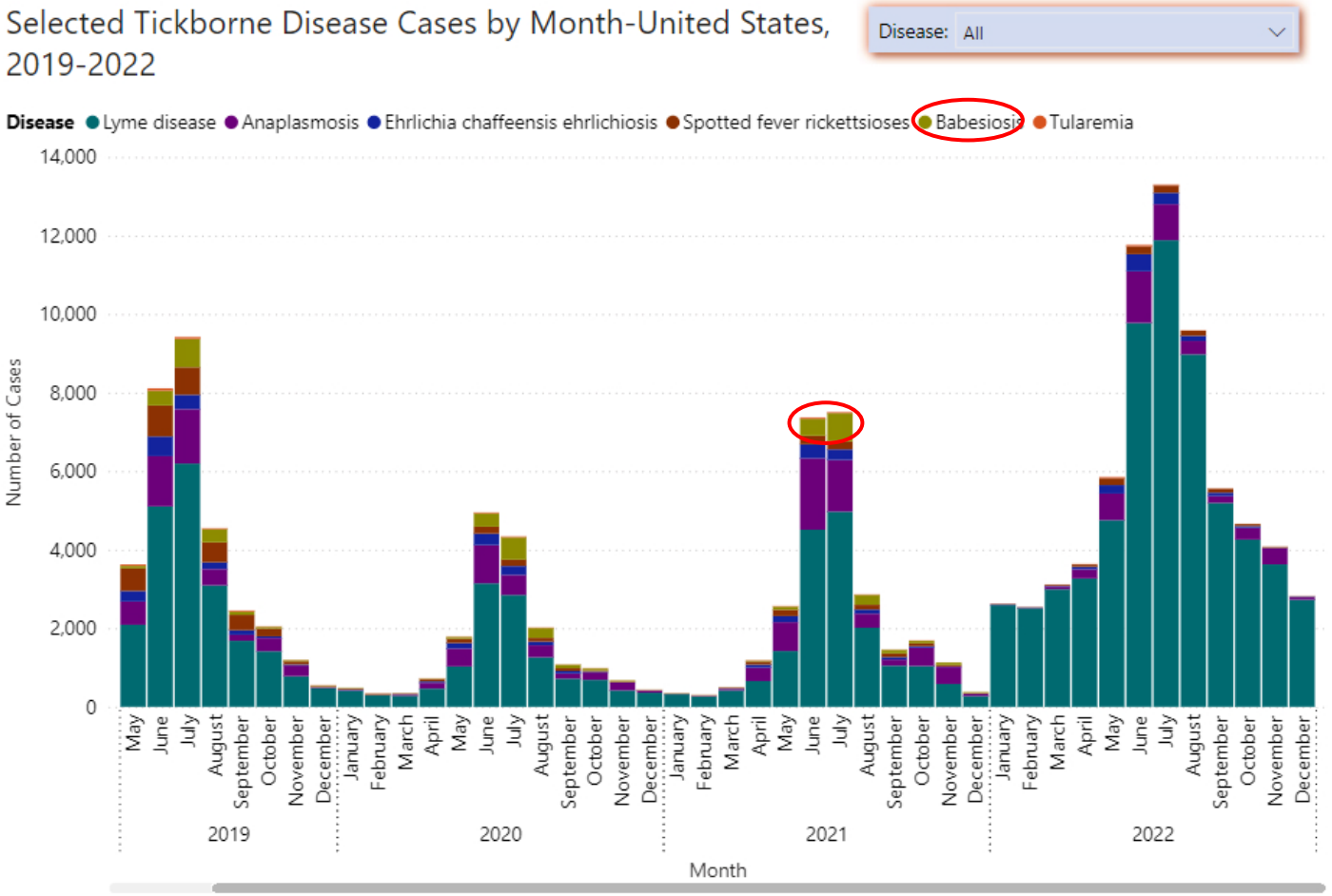
Tickborne Diseases in the U.S.

- Tickborne diseases (TBD) are an emerging public health epidemic in the United States.
- Tickborne diseases can be caused by bacteria, parasites, or viruses.
- Infections may occur with more than one tickborne pathogen at a time.
- The CDC currently recognizes 13 unique human tickborne illnesses caused by 18 different pathogens in the United States.

Surveillance of Babesiosis and other TBD in the U.S.



www.cdc.gov

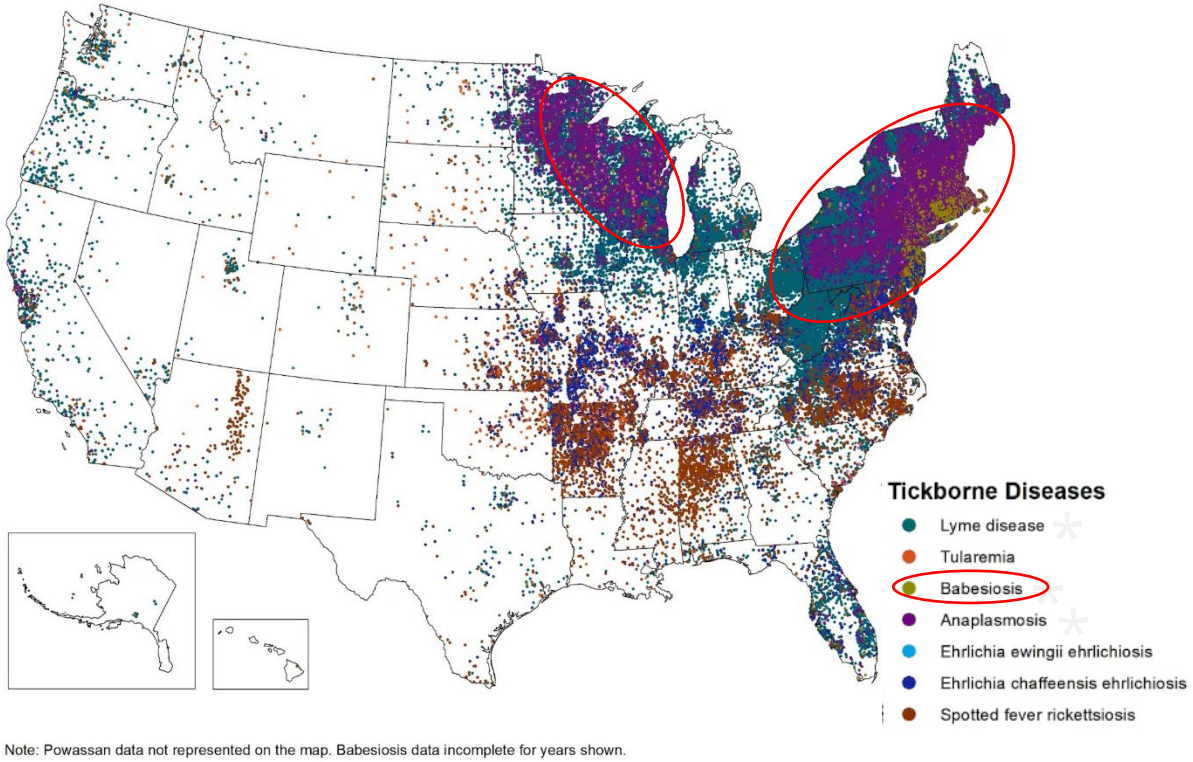
Total Reported Cases by Tickborne Disease, 2019-2022

Disease	2019	2020	2021	2022
Lyme disease[1]	34,945	18,010	24,611	62,551
Anaplasmosis	5,655	3,639	6,744	5,633
Spotted fever rickettsioses	5,207	1,175	1,278	1,271
Ehrlichia chaffeensis ehrlichiosis	2,093	1,180	1,347	1,557
Babesiosis	2,418	1,827	1,915	
Tularemia	274	150	162	167
Undetermined ehrlichiosis/anaplasmosis	185	50	77	95
Powassan virus disease	43	21	24	47
Ehrlichia ewingii ehrlichiosis	43	21	19	25
Total	**50,863**	**26,073**	**36,177**	**71,346**

[1] Estimated annual cases ~300,000

Geographic Distribution of Babesiosis and other TBD

Distribution of Selected Tickborne Diseases

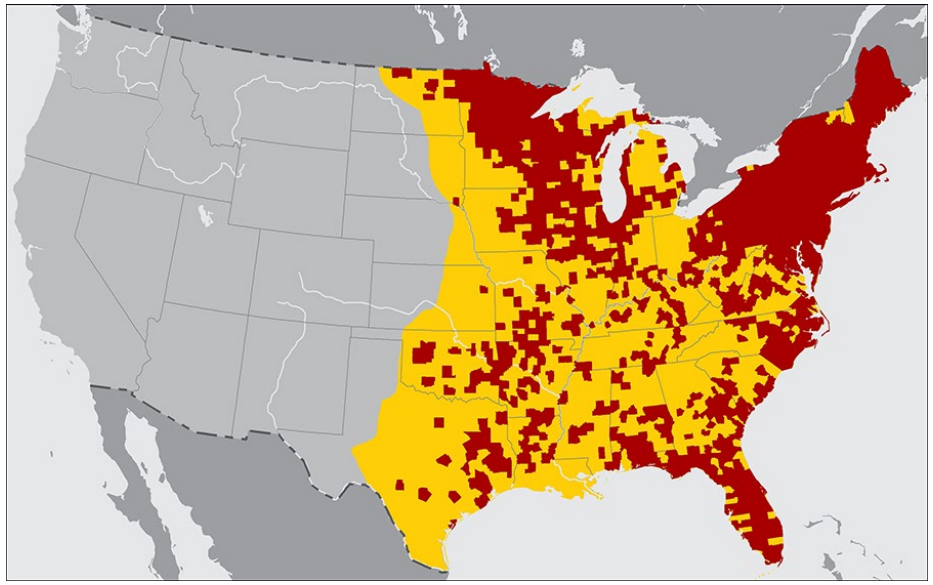


Note: Powassan data not represented on the map. Babesiosis data incomplete for years shown.

Babesiosis overlaps with Lyme and anaplasmosis, all transmitted by the Blacklegged tick *Ixodes scapularis*

www.cdc.gov

Distribution of Blacklegged Tick *Ixodes scapularis*



Established
Estimated

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

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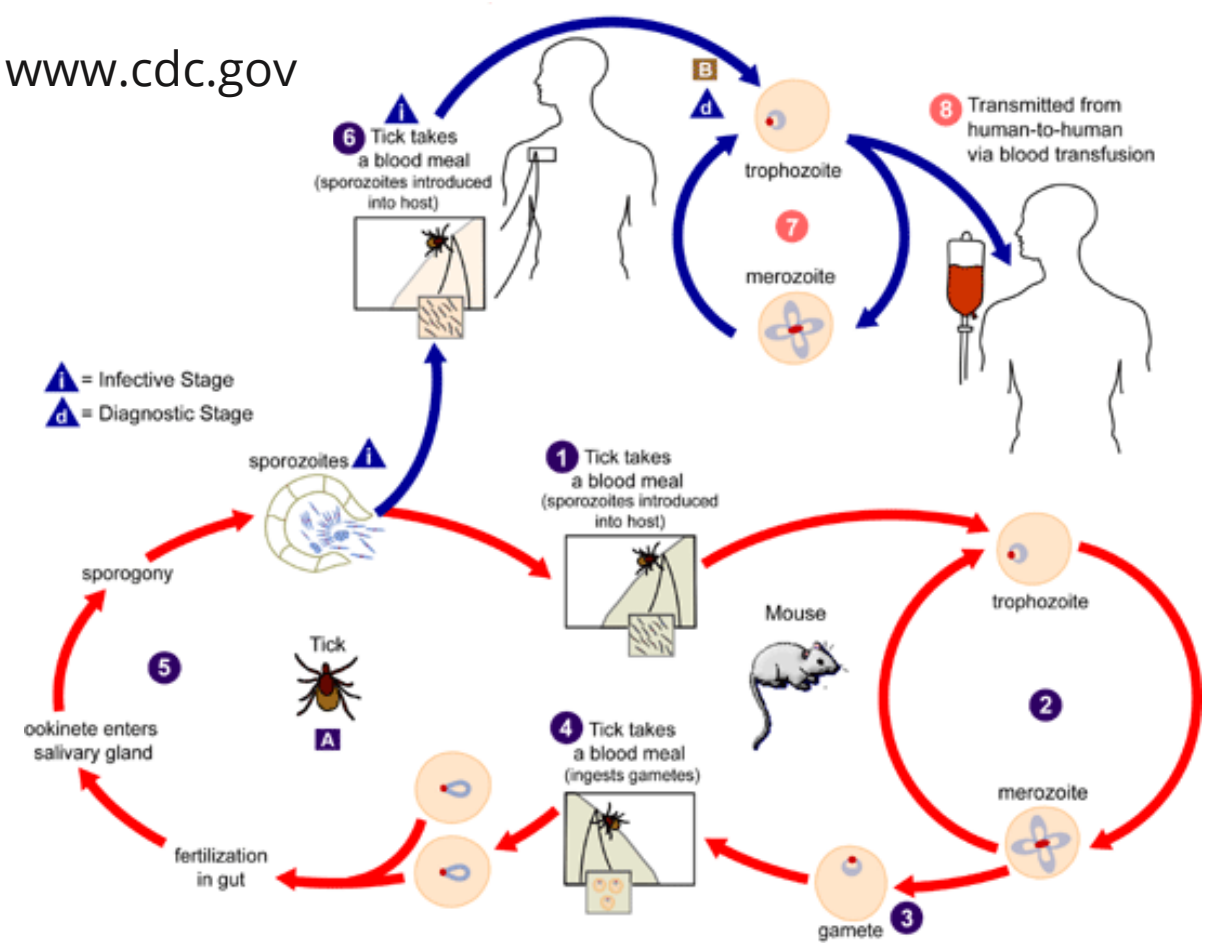
Human Babesiosis Overview

- Emerging tickborne disease in the U.S.
- Caused by RBC infecting *Babesia microti*
- Transmitted by *Ixodes* ticks, pregnancy, transfusion (less common)
- Common in the Northeast and Upper Midwest regions of the U.S.
- Infections usually asymptomatic or mild among healthy individuals
- Severe infections in immuno-compromised
- Worsening of Lyme disease symptoms in co-infections
- Mechanisms underlying pathogenesis and immune response remain unresolved

www.cdc.gov



Human Babesiosis Overview

- Atovaquone in combination with azithromycin or clindamycin combined with quinine are commonly effective in the treatment of human babesiosis.
- Persistent illness may occur in immunocompromised hosts with a mortality rate as high as 20%.
- Treatment with tafenoquine can be an alternative for immunocompromised patients who experience severe, relapsing babesiosis.
- Given the increased incidence of human babesiosis and the risk for antimicrobial resistance among the immunocompromised, novel therapeutic approaches are needed.

Kumar, A., et al. 2021. Pathogens. 10: 1447.
Rogers, R. 2023. Clin. Infect. Dis. 76: 741-744.

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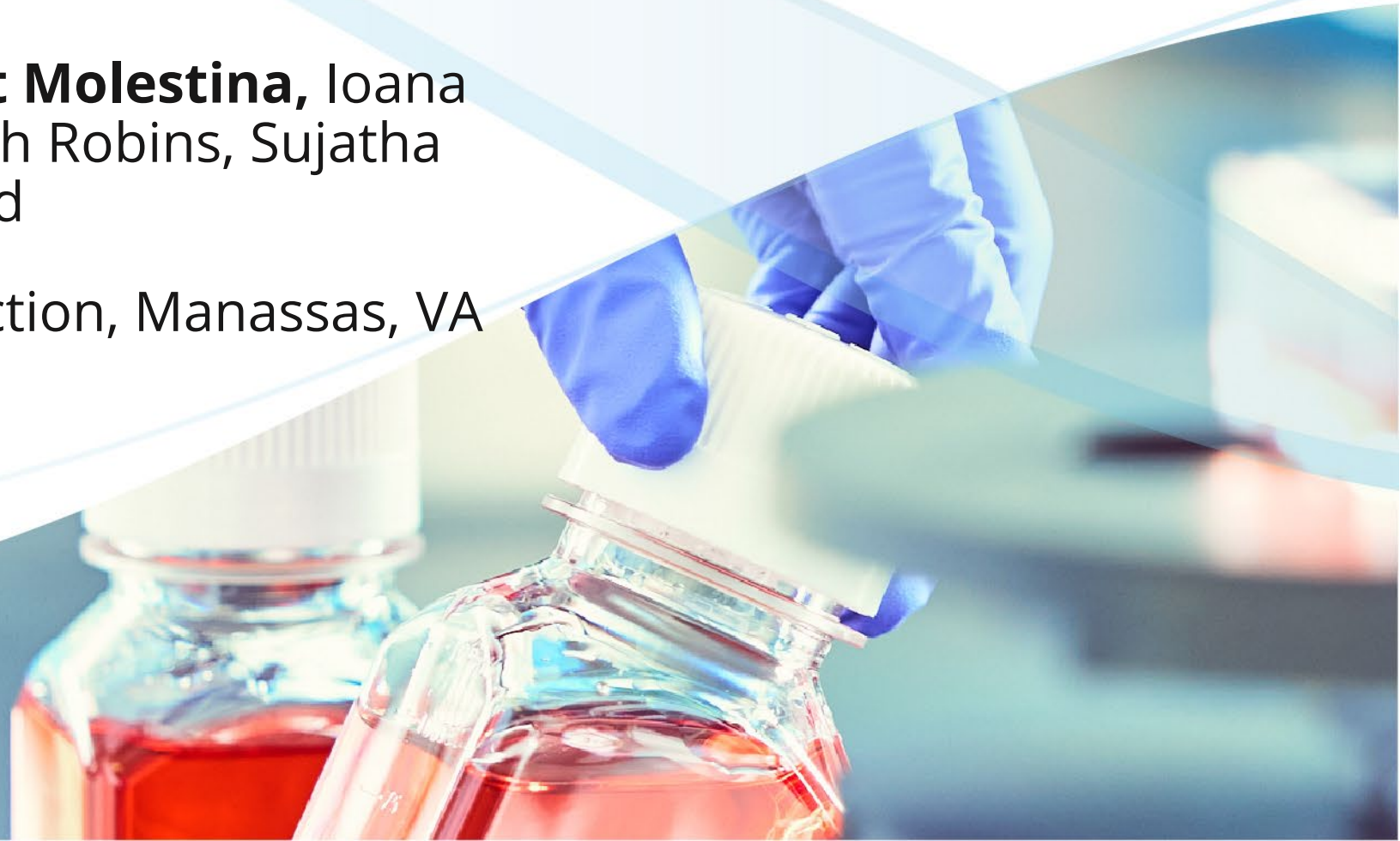
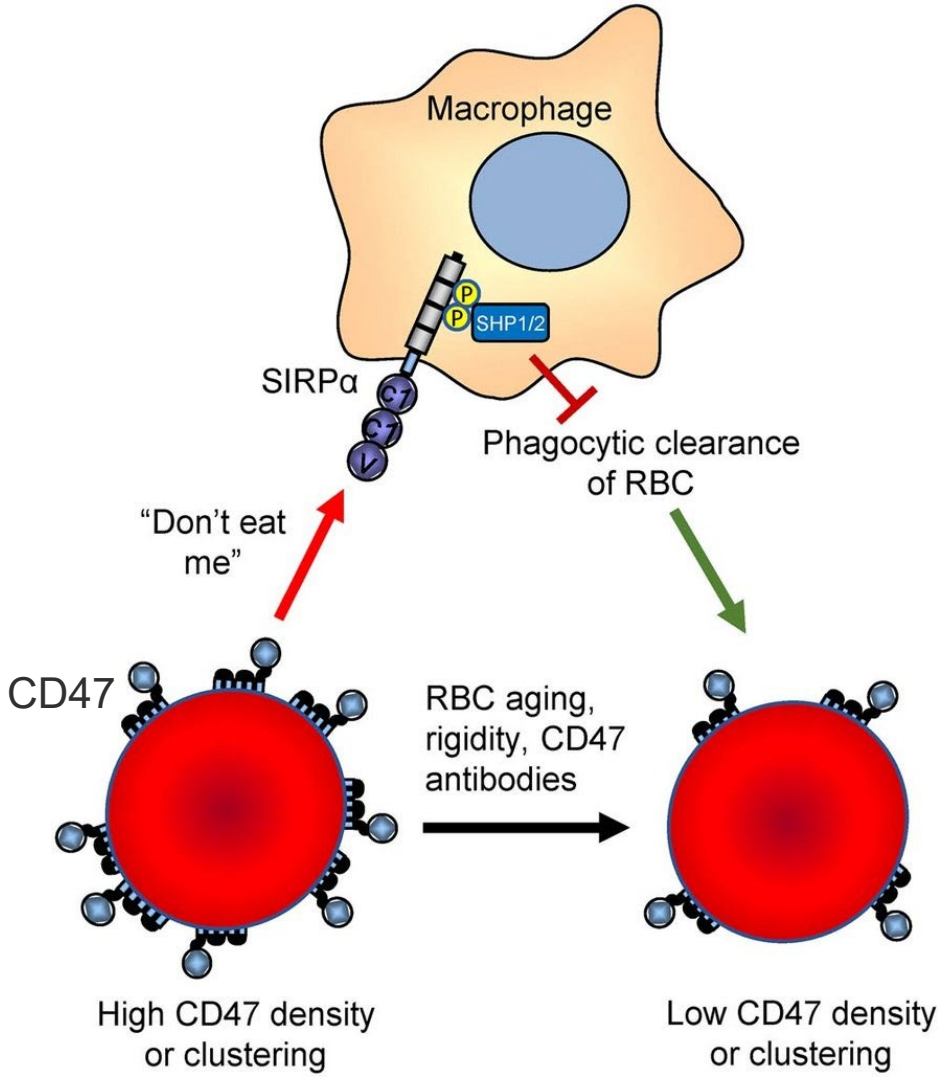
Role of the CD47-SIRPα axis in the clearance of *Babesia microti* in vivo

Shamim Mohammad, **Robert Molestina,** Ioana Brasov, Susan Gottshall, Sarah Robins, Sujatha Rashid, and Rebecca Bradford

American Type Culture Collection, Manassas, VA



Role of CD47 in self-recognition



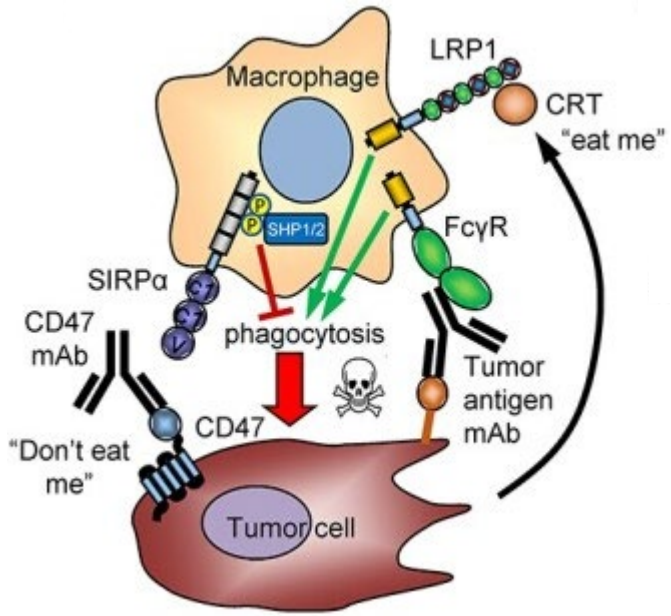
- CD47 is expressed on both hematopoietic and non-hematopoietic cells.
- Plays crucial role in immune regulation and maintenance of homeostasis.
- CD47 interacts with signal regulatory protein alpha (SIRPα) and thrombospodin-1 (TSP-1).
- The CD47-SIRPα interaction induces an antiphagocytic "don't eat me" signal in macrophages.
- Loss of CD47-SIRPα signaling in results in more rapid turnover of circulating platelets and red blood cells.

Kaur, S. 2020. Antib Ther. 3: 179–192.
Cham, L.B. 2020. Antibodies. 9: 44.

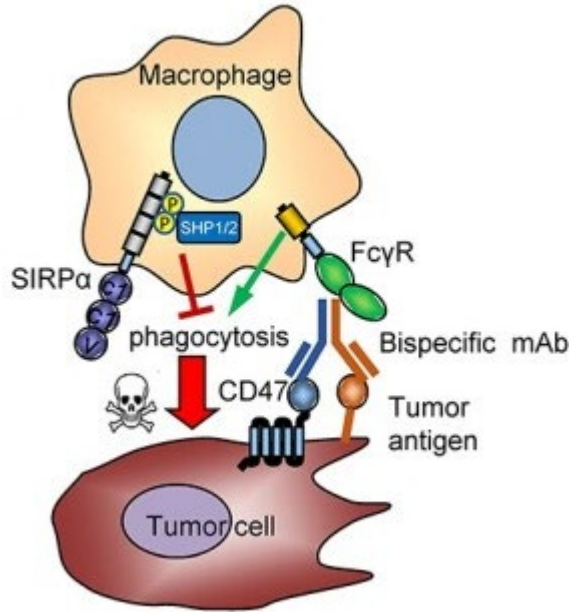
CD47 as a potential therapeutic target

- Increased CD47 expression on some cancer cells limits their phagocytic clearance by macrophages
- The CD47/SIRPα interaction is postulated as a major innate immune checkpoint in cancer.
- Several antibodies and other antagonists of CD47 binding to SIRPα have entered Phase 1 and 2 clinical trials.

Use of CD47 mAb and/or tumor-specific antibodies

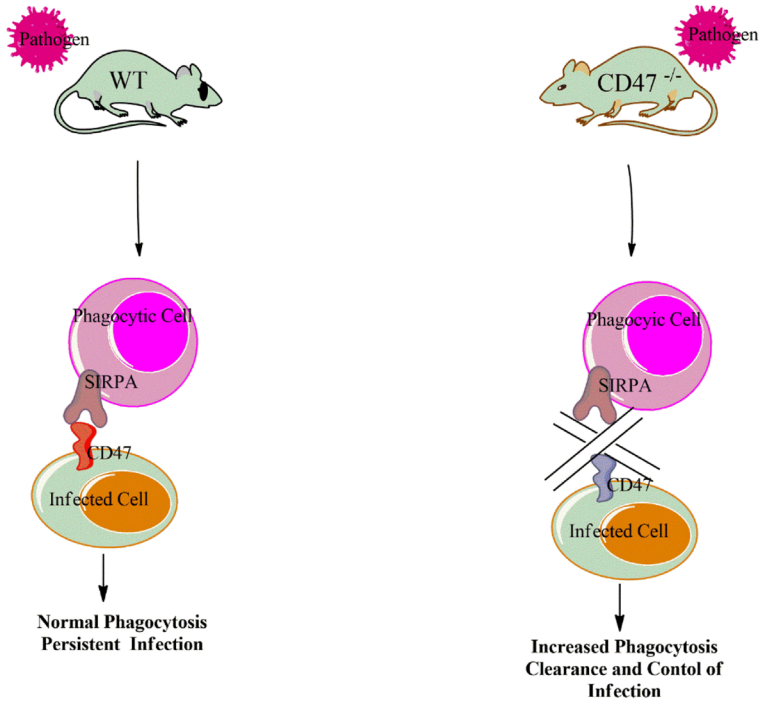


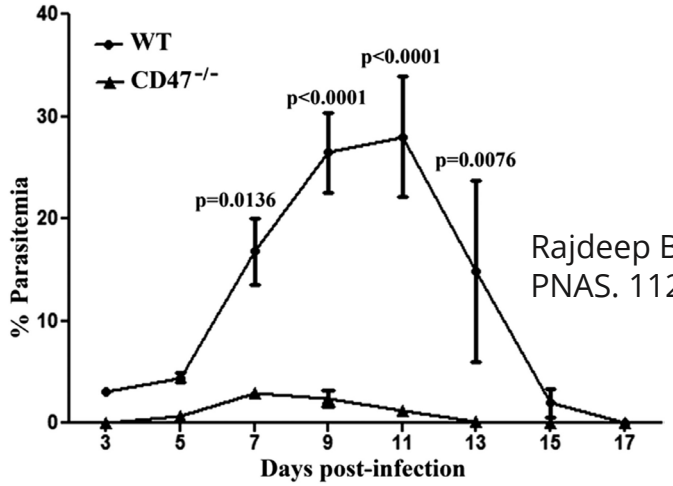
Use of Bi-specific mAb to CD47 and tumor-specific antigen



Kaur, S. 2020. Antib Ther. 3: 179–192.

Role of CD47 during parasitic infections





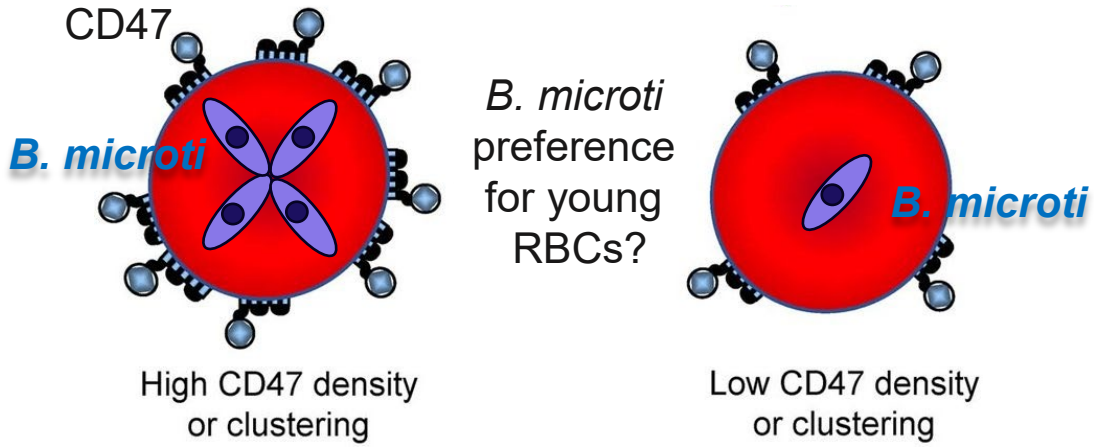
Rajdeep Banerjee, R. 2015. PNAS. 112: 3062-3067

- A role for CD47 has been shown in the clearance of the Babesia-related parasite Plasmodium in mice.
- *Plasmodium yoelii* preferentially infects young RBCs with high expression of CD47 and is protected from phagocytosis.
- Disruption of SIRPα has been shown to increase phagocytosis of *P. falciparum*-infected RBCs.
- CD47 blockade by mAb decreases parasite burden and promotes survival in mice infected with *P. berghei*
- Blockade of the CD47 “don’t eat me” signal can serve as therapeutic approach against the Babesia-related parasite Plasmodium.

Cham, L.B. 2020. Antibodies. 9: 44.
Ayi K., et al. 2016. Infect. Immun., IAI.01426-15.
Laughing Bear Torrez Dulgeroff, L.B. 2021. PNAS. 118: e1907653118

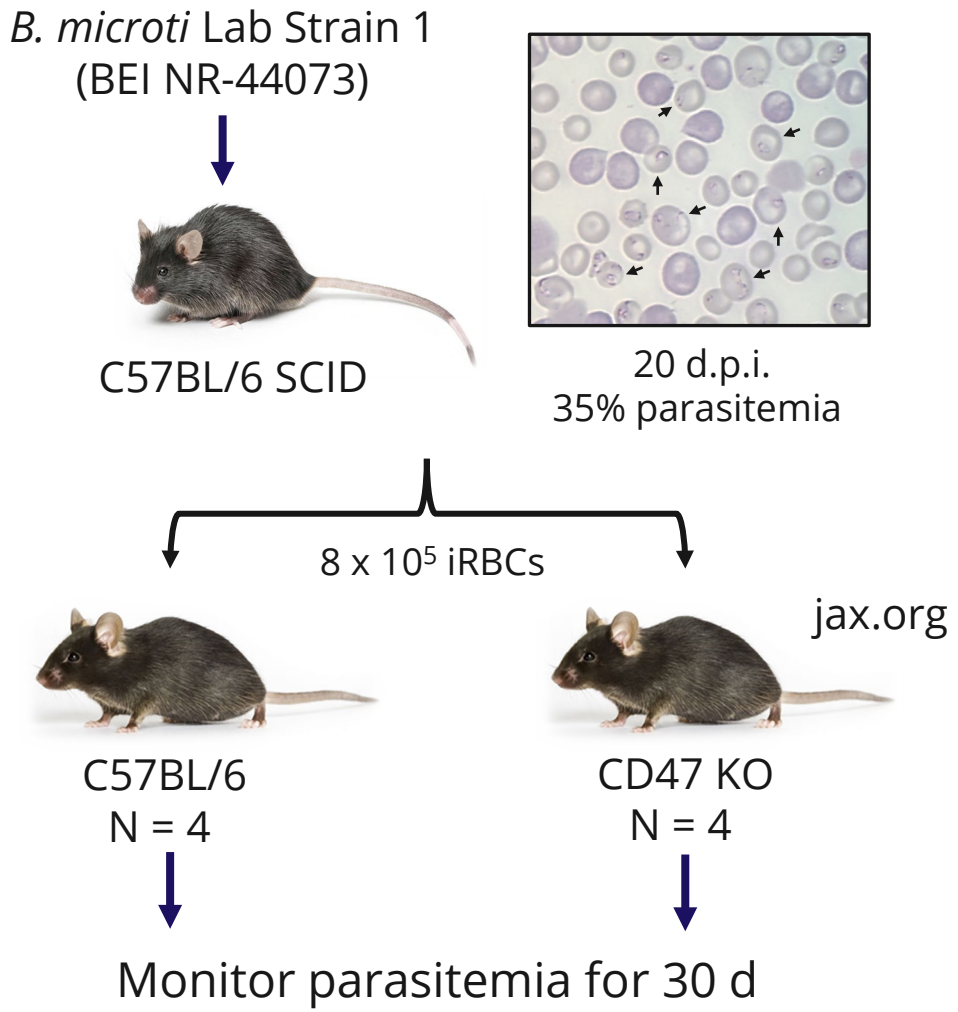
A potential role of CD47 in Babesiosis?

- Macrophages are important for immunity to babesiosis (removal of parasitized RBCs, participation as antigen-presenting cells for T cells, production of cytokines)
- We posit that, similar to *Plasmodium, B. microti* preferentially infects young RBCs with high expression of CD47.
- *B. microti* is protected from phagocytosis during early stages of infection.
- A disruption of CD47 signaling is likely to increase the clearance of *B. microti*-infected RBCs.
- A better understanding of the CD47-SIRPα axis in babesiosis can provide additional insights of the immune response and develop new therapeutic strategies to infection.



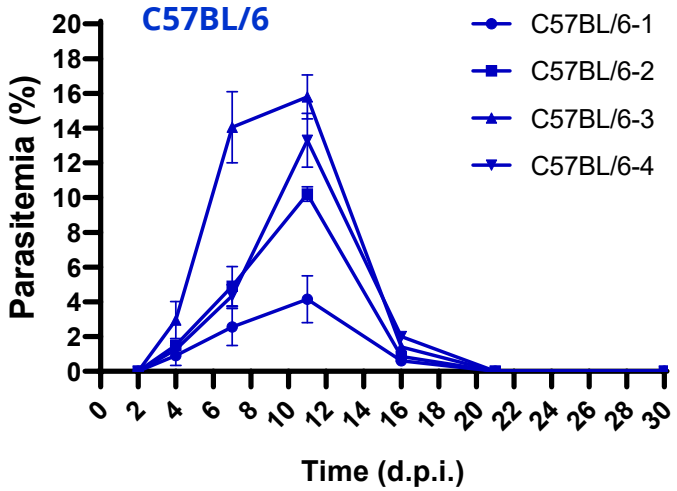
Objective and Experimental Approach

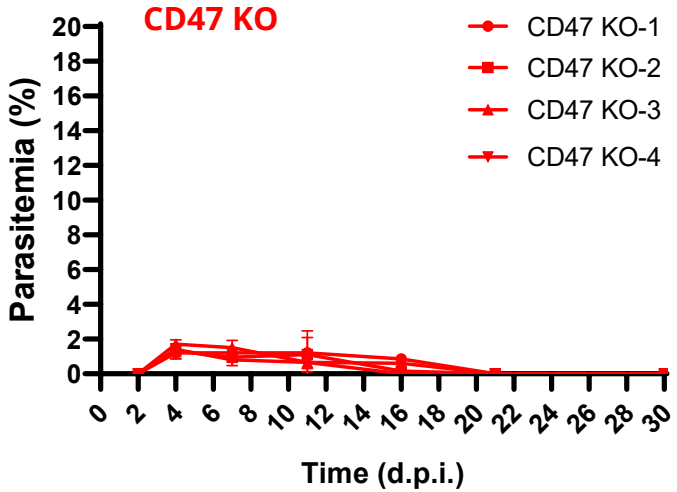
- **Objective.** To investigate the role of CD47 on the growth of *Babesia microti* in mice.
- **Experimental Approach**
- *B. microti* Lab Strain 1 (BEI NR-44073) was propagated in C57BL/6 SCID mice.
- Eight-week-old female C57BL/6 and CD47 KO (B6.129S7-Cd47^{tm1Fpl}) mice were injected i.p. with 8 x 10^5 infected red blood cells (iRBCs).
- Blood samples were collected from the tail veins and parasitemia was monitored over a 30-d period of infection by microscopic examination.



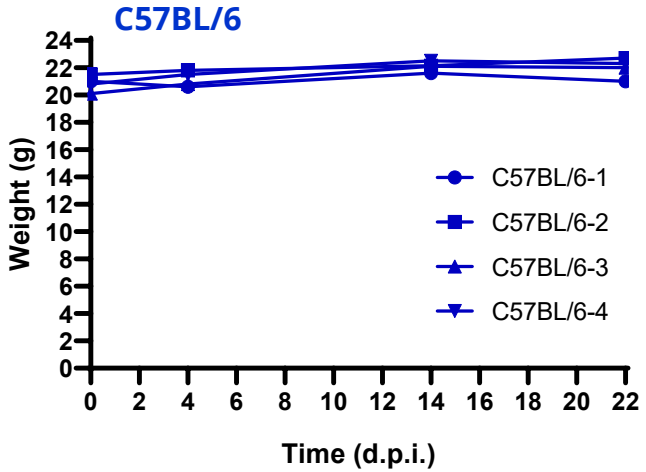
Susceptibility of CD47 KO mice to *B. microti*

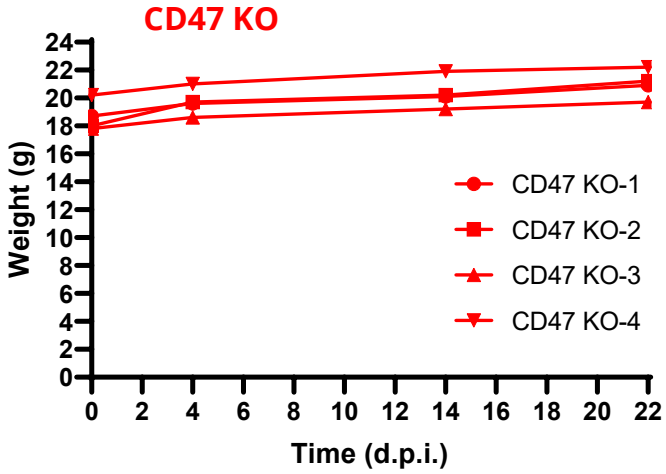
Parasitemia

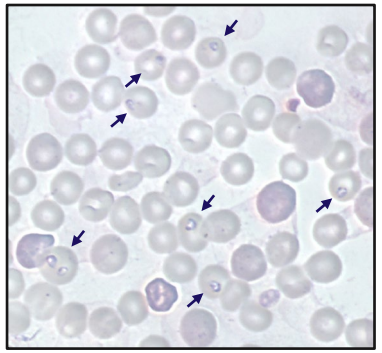




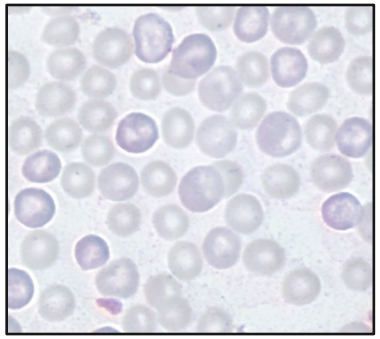
Animal weight







C57BL/6-1 mouse infected with *B. microti* Lab Strain 1 (11 d.p.i.)



CD47 KO-1 mouse infected with *B. microti* Lab Strain 1 (11 d.p.i.)

Summary - Role of the CD47-SIRPα in Babesiosis

- We determined the effect of CD47 loss on *B. microti* growth by comparing parasitemia in wild-type C57BL/6 and CD47 KO mice by microscopic examination of blood smears.
- C57BL/6 mice developed an average parasitemia of 6% on day 7 and reached a peak parasitemia of 11% on day 11, with infection self-resolving by day 21.
- In contrast, CD47 KO mice developed markedly low parasitemia that on average did not reach >1.5% throughout infection and resolved by day 16.
- Future studies will examine the correlation between CD47 level on RBCs and parasite burden, the capacity of *B. microti* to invade CD47 KO RBCs, and the immunological basis underlying the observed CD47-mediated resistance from babesiosis.

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Role of Babesia Secreted Extracellular Vesicles in the Modulation of the Immune Response

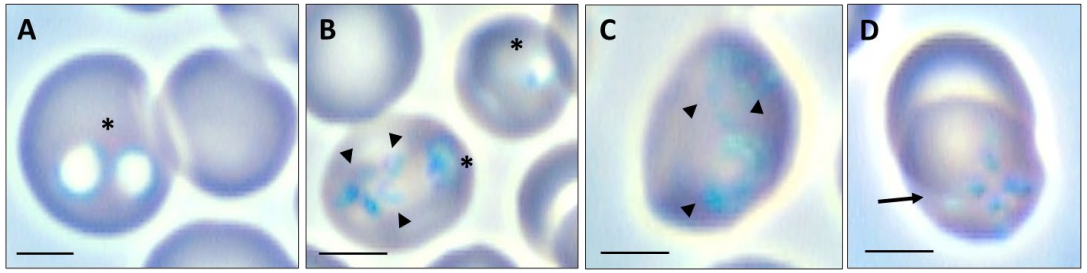
Choukri Ben Mamoun[1], Fatah Kashanchi[2], Heather Branscome[3], and **Robert E. Molestina[3]**

[1] Section of Infectious Disease, Yale University School of Medicine, New Haven, CT

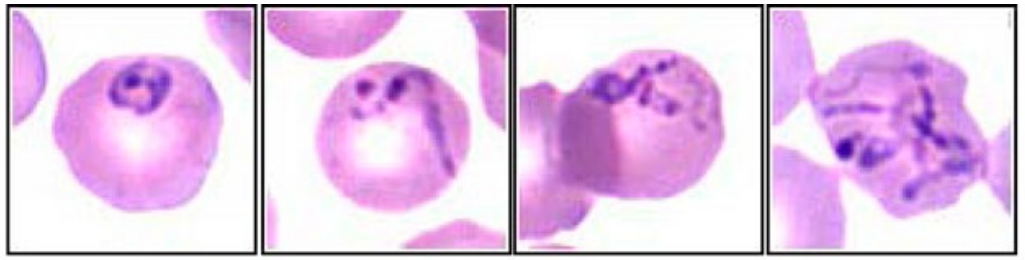
[2] School of Systems Biology, George Mason University, Manassas, VA

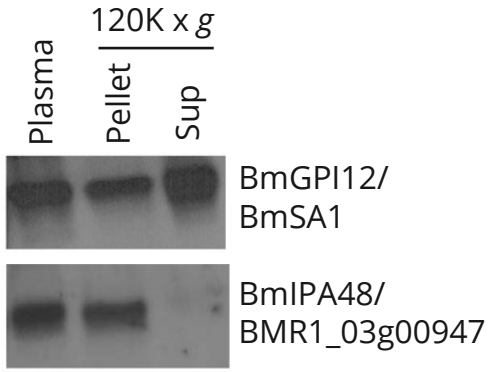
[3] ATCC Federal Solutions, American Type Culture Collection, Manassas, VA

A Novel Protein Export Mechanism in *B. microti*

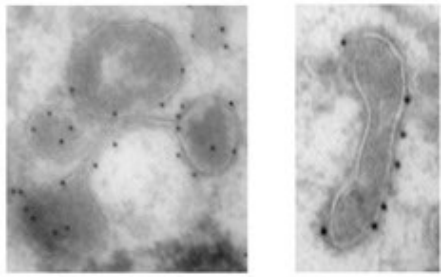


Molestina, R. et al. Unpublished.





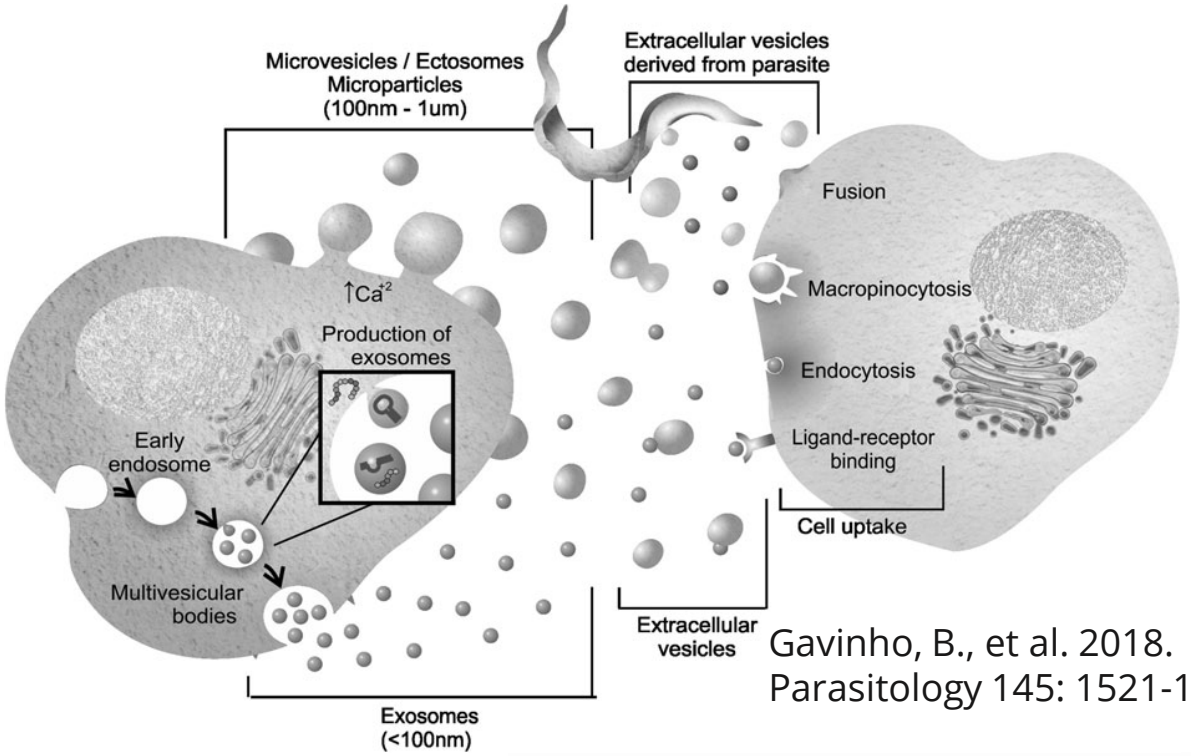
BmSA1 in vesicle pellets



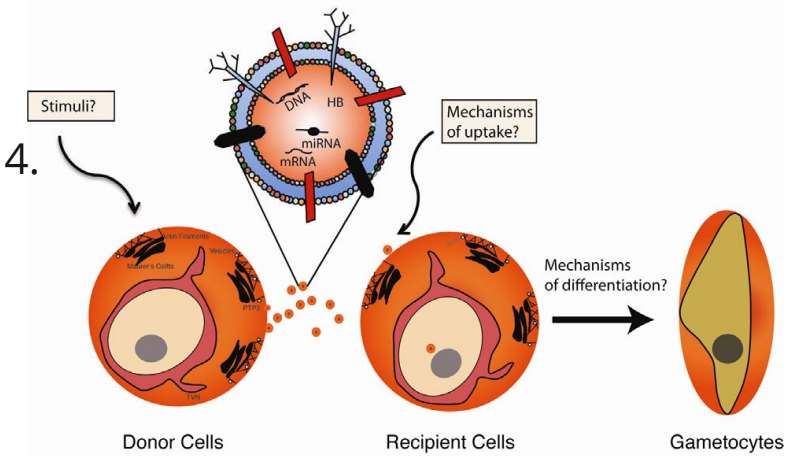
Thekkiniath, J. et al. 2019.
Life Sci. Alliance 2: e201900382.

- *B. microti* forms a transient parasitophorous vacuole upon invasion of the RBC.
- Infected RBCs show membranous extensions protruding from the parasite into the cytoplasm.
- These extensions are known as tubes of vesicles (TOVs).
- *B. microti* antigens are detected in extracellular vesicles (EVs) isolated from the plasma of infected lab mice (Thekkiniath et al. 2019).
- EVs isolated from both *in vivo* and *in vitro* infections of *B. microti* and *B. divergens* harbor several parasite proteins and microRNAs (Beri et al., 2022).

Roles of extracellular vesicles in host-parasite interactions



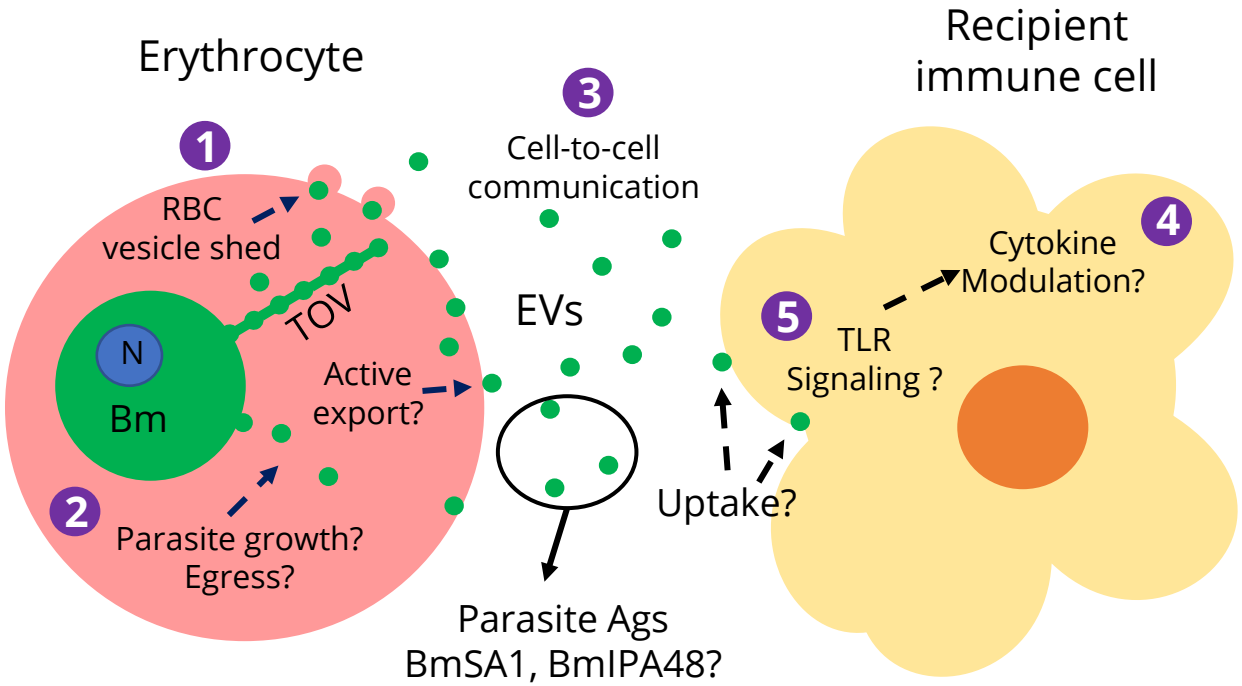
Gavinho, B., et al. 2018. Parasitology 145: 1521-1530.



Ankarklev, J., et al. 2014. J. Circ. Biomark. 3: 3

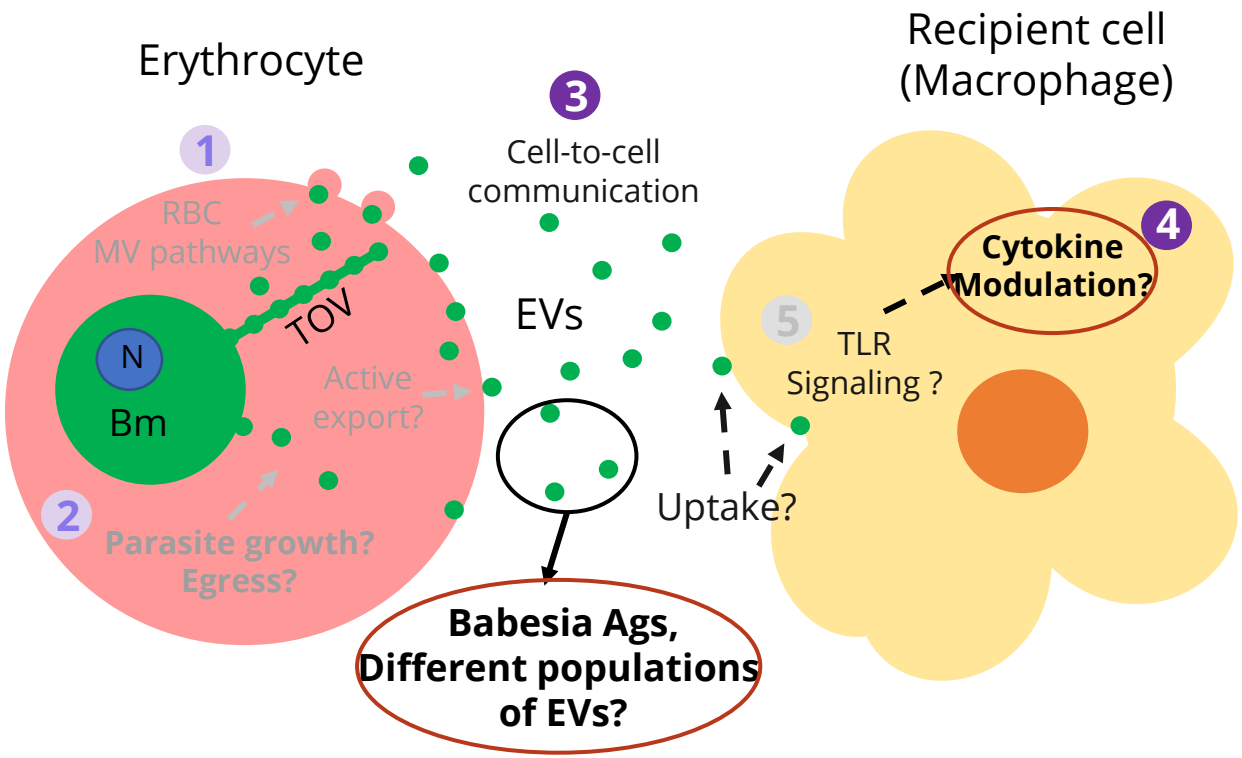
- EVs are released from the membranes of parasites and parasite-infected cells.
- Sizes vary in the range of microvesicles (0.1-1 µm) and exosomes (30-150 nm).
- Parasite EVs play roles in host-pathogen interactions.
- In malaria, EVs induce macrophage activation in mice, and mediate intercellular communication between RBCs.
- In Babesia, EVs from iRBCs have different sizes, harbor parasite antigens, and miRNAs - potential role as disease biomarkers? (Beri et al., 2022).

What are the Biological Roles of EVs in Babesiosis?



1. Does *B. microti* benefit from vesicle shedding from the RBC to release parasite EVs?
2. Is the extent of EV release dependent on parasite growth and egress?
3. Do EVs participate in cell-to-cell communication causing changes in the phenotypes of recipient cells?
4. When immune cells function as recipients, are there changes in the production of cytokines with key roles in the host immune response?
5. What parasite antigens enclosed within EVs and host signaling pathways participate in this response?

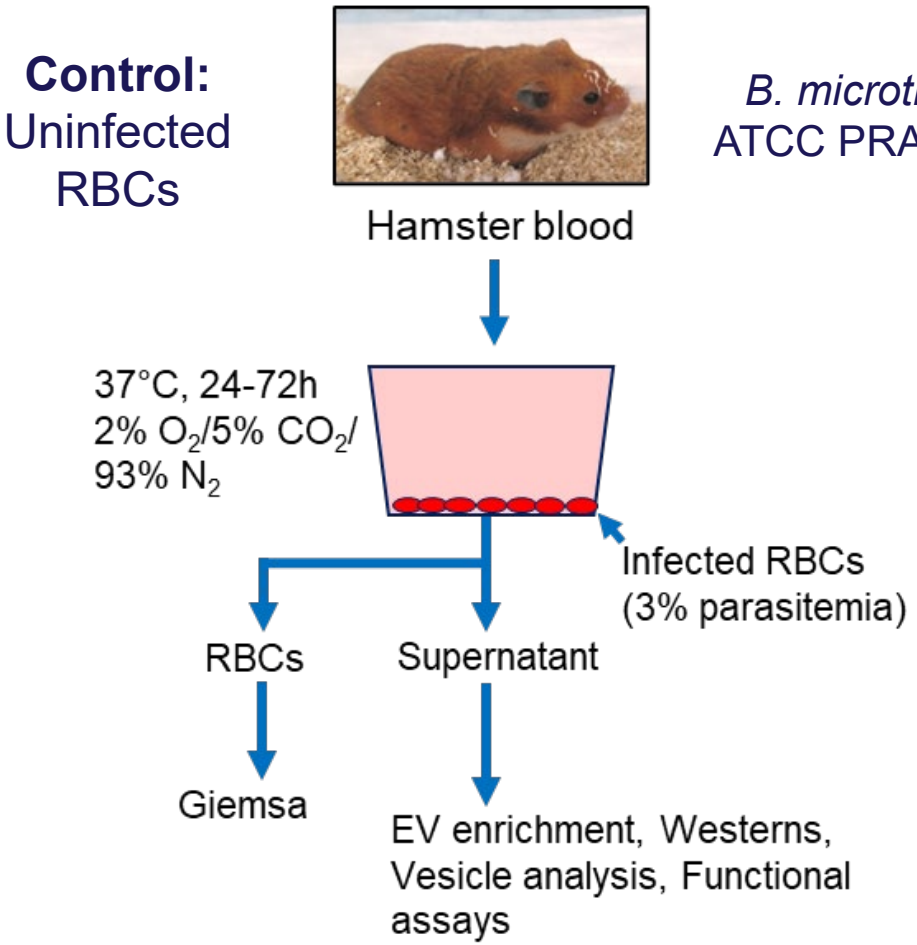
Aims of this study



- **Hypothesis:** Babesia-derived vesicles (BDVs) released from infected erythrocytes cause phenotypic changes in recipient immune cells.
- Examine the expression of parasite antigens in enriched EV fractions collected from *in vitro* cultured iRBC supernatants.
- Evaluate the presence of different populations of EVs by size distribution analysis (NTA).
- Examine the uptake of EVs isolated from RBC culture supernatants by mouse macrophages.
- Determine whether treatment of macrophages with BDVs results in cytokine regulation.
- Macrophages are critical in the innate immune response to Babesia infection.

Experimental Approach

Short-term *in vitro* culture of *B. microti*-infected RBCs

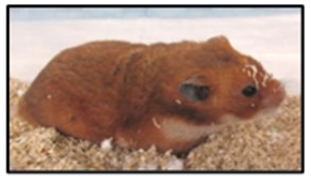


- Blood is collected from *B. microti*-infected hamsters.
- RBC cultures are established.
- Parasitemia is checked daily by microscopic examination.
- EVs are enriched from RBC culture supernatants (SBI ExoMax or sequential centrifugation).
- Vesicles are analyzed by Western Blots, size distribution, and functional assays in macrophages.

Hagos, B. 2025. Infect. Immun. 93: e0033324.

Isolation of EVs from *in vitro* cultures of *B. microti*-infected RBCs.

Short-term *in vitro* culture of *B. microti*



Hamster blood

37°C, 24-72h
2% O_2/5% CO_2/
93% N_2

Infected RBCs
(3% parasitemia)

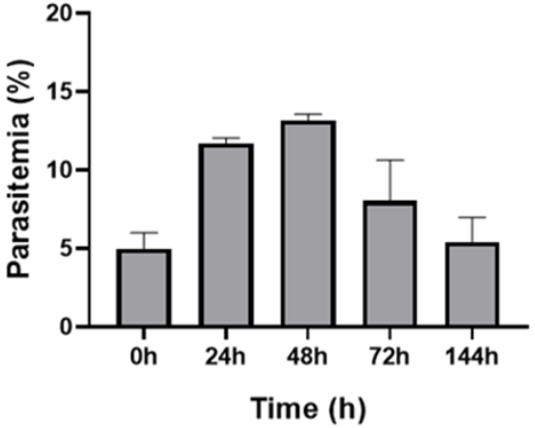
RBCs

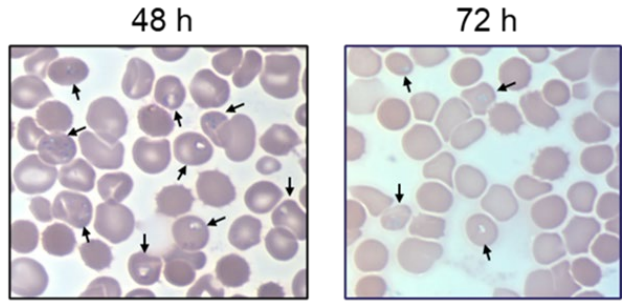
Supernatant

Giemsa

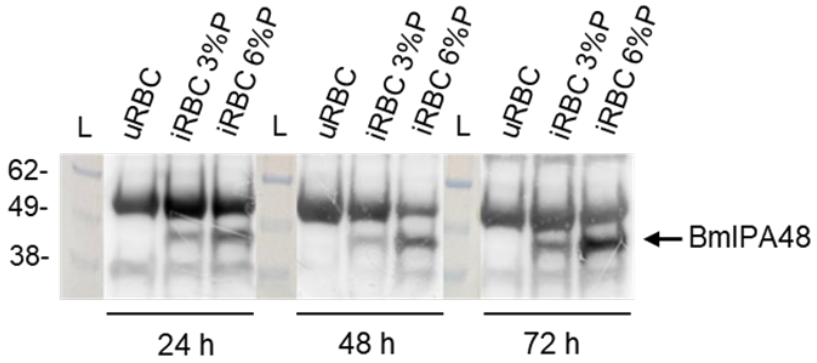
EV enrichment, Westerns, Vesicle analysis, Functional assays

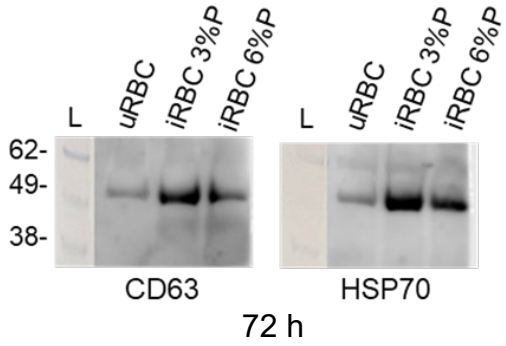
Assessment of parasitemia *in vitro*





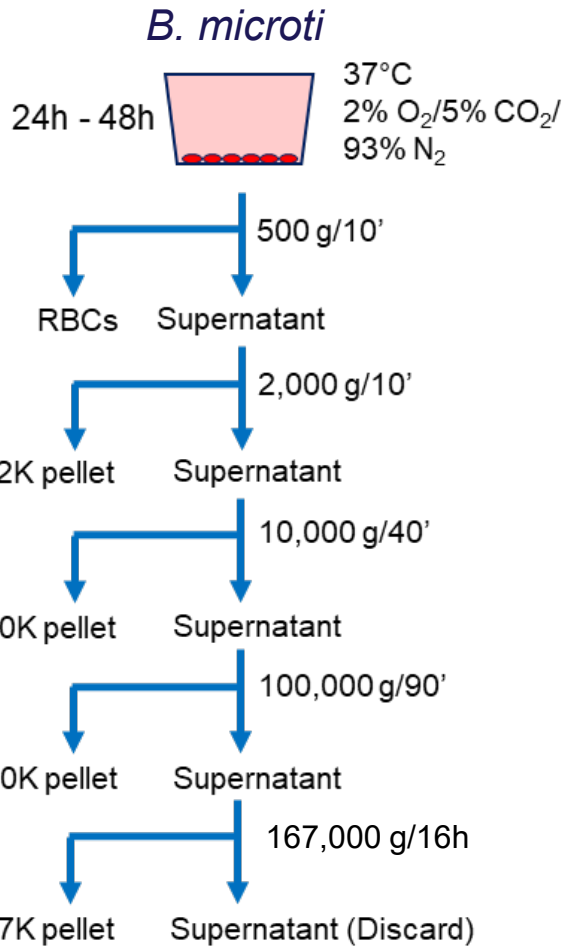
Detection of parasite Ag and host vesicular markers in EV preps



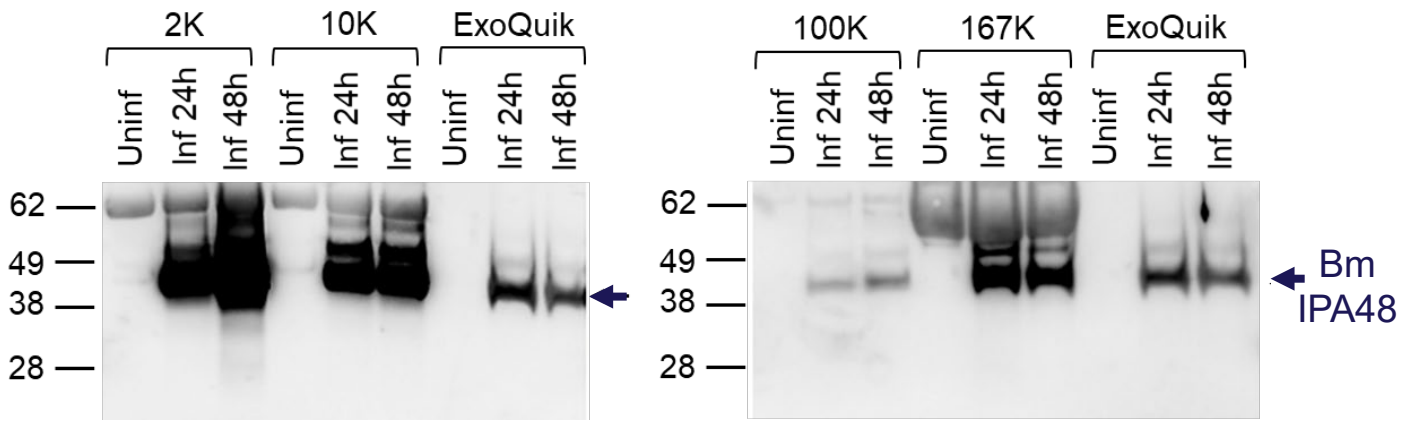


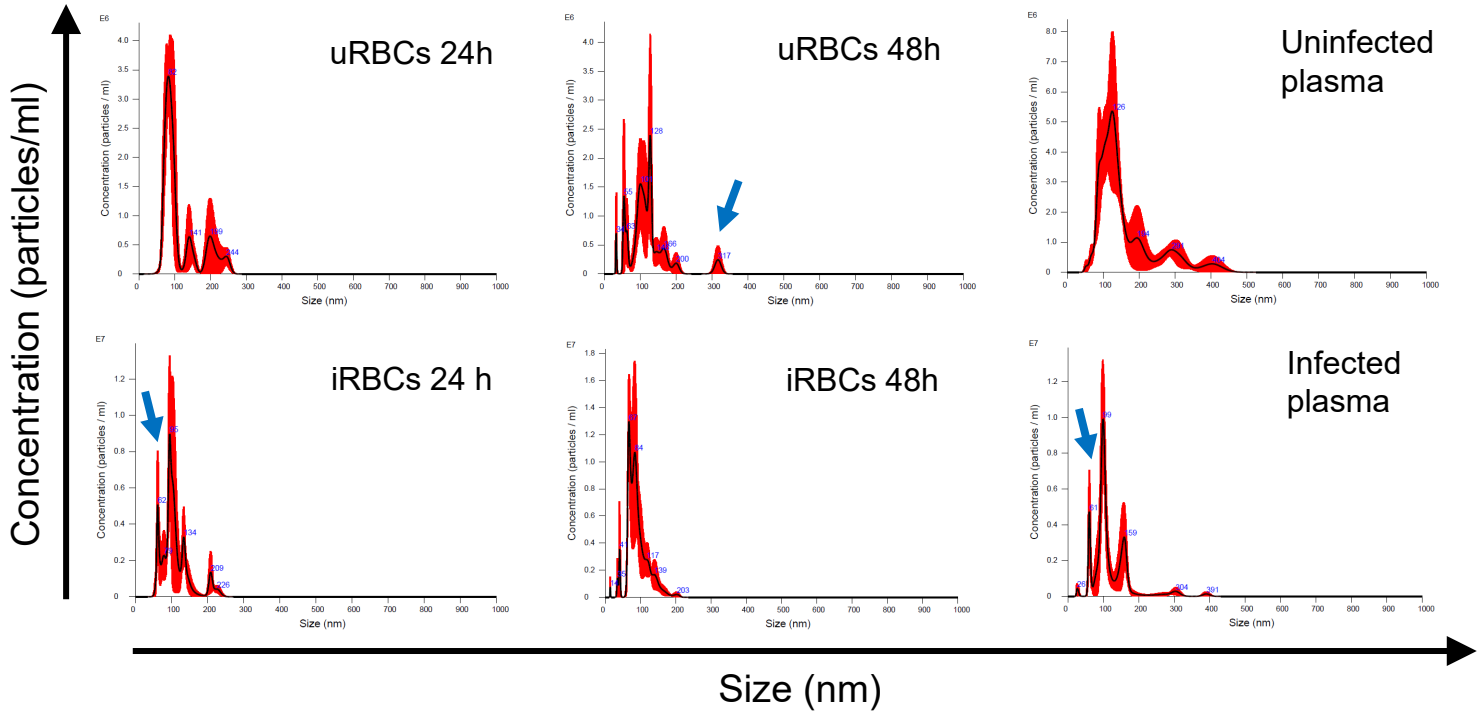
EVs were isolated from RBC supernatants using SBI ExoMax

Size distribution of EVs isolated from iRBC supernatants and plasma



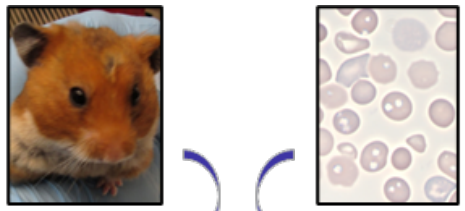
Hagos, B. 2025. Infect. Immun. 93: e0033324.





NTA of EVs in 167K pellets

Analysis of EV uptake by macrophages



Control:
Uninfected RBCs

B. microti in vitro culture

24h - 48h

37°C
2% O_2/5% CO_2/
93% N_2

EV isolation by centrifugation

500 g/10' → 2K g/10' → 10K g/40' → 100K g/90' → 167K g/16h

167K pellet

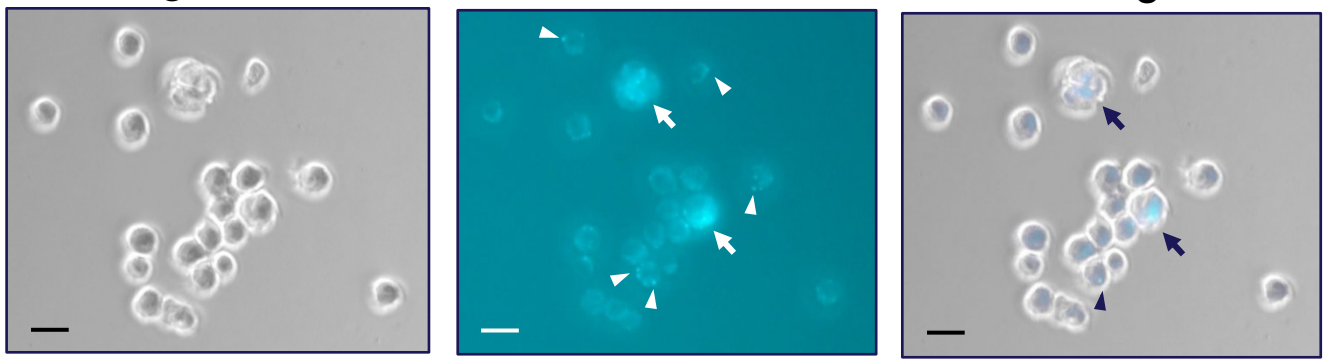
Label EVs with BODIPY

Uptake assays

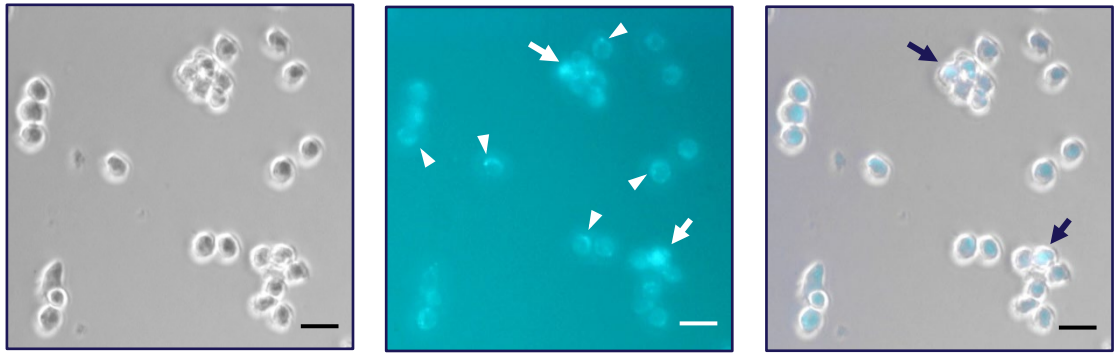
- NF-κB Regulation
- Cytokine production

Macrophages

EVs from uRBC supernatants

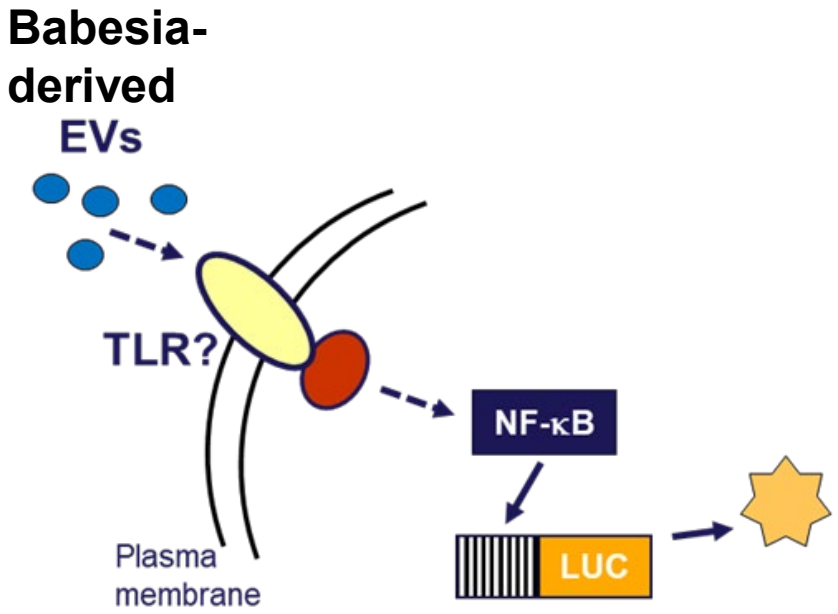


EVs from iRBC supernatants



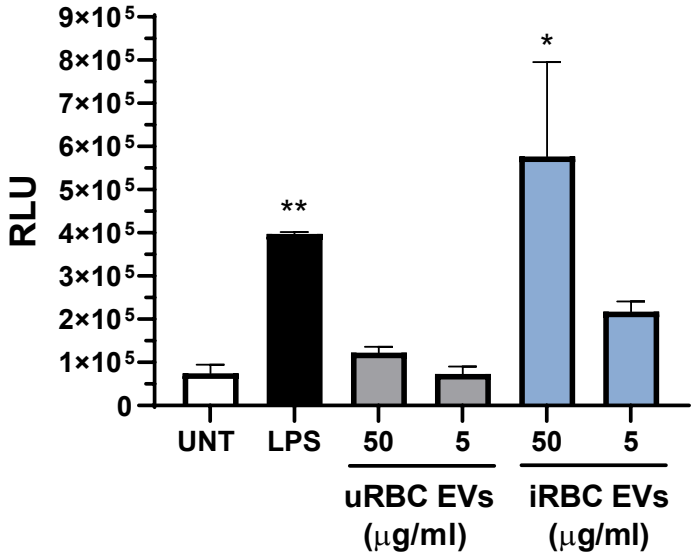
NF-κB and cytokine regulation in macrophages treated with EVs

Hypothesis



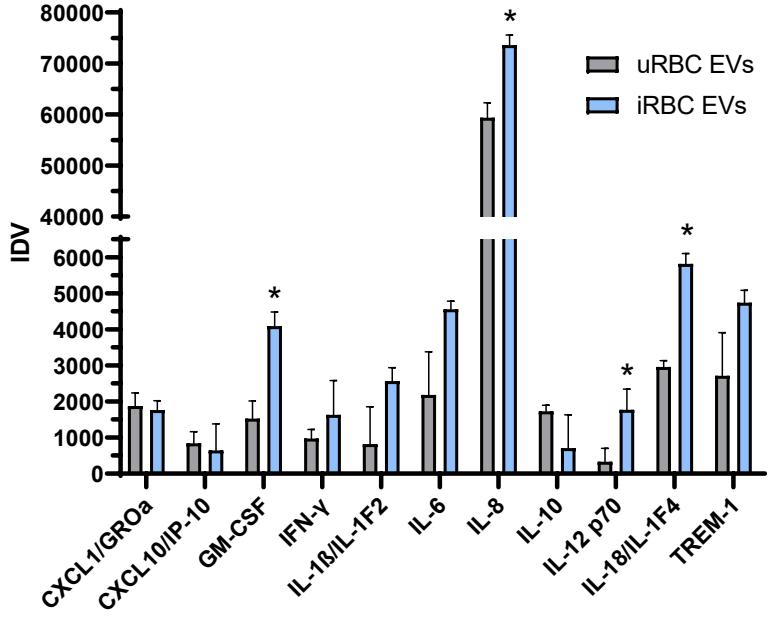
ATCC
THP-1 NF-κB-Luc2

NF-κB Activation by EVs from *B. microti* iRBCs



EVs isolated from 48 h cultures in 167K pellets

Cytokine regulation by EVs from *B. microti* iRBCs



Proteome Profiler Mouse Cytokine Array

Hagos, B. 2025. Infect. Immun. 93: e0033324.

Summary – Role of Babesia EVs

- The present study provides further evidence that supports the secretion of Babesia-derived vesicles (BDVs) from infected RBCs.
- An analysis of vesicle size in 167K fractions showed diverse populations in the <100 nm size range between EVs from uRBCs and Babesia iRBCs. Further separation of parasite-specific BDVs from these fractions requires further studies.
- Macrophage uptake of EVs was indistinguishable between vesicles isolated from uRBC and iRBC cultures. However, EVs isolated from Babesia iRBCs induced a significant increase in macrophage NF-κB activity.
- Concomitant with the activation of NF-κB, cytokines were upregulated in macrophages in response to EVs isolated from Babesia iRBC supernatants.
- Our model allows the examination of signaling pathways participating in the response of immune cells to EVs derived from *Babesia microti.*

Future Directions

- Optimize our EV isolation methodology to obtain highly enriched parasite-derived vesicles.
- Identify parasite proteins in enriched EV fractions by proteomics.
- Investigate the mechanisms of EV-mediated intercellular communication between iRBCs and macrophages.
- Identify parasite, host factors, and signaling pathways involved in macrophage cytokine activation.

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Thank You