CHRONIC BARTONELLOSIS AND BABESIOSIS:

IT'S NOT JUST ABOUT LYME ANYMORE

Henry Lindner, MD

Tunkhannock, Wyoming County, Pennsylvania

PA Senate HHS Hearing on SB 1188, Sept 20, 2022

DAUGHTER: TICK BITES @AGE 10

No rash or fever, no antibiotic prophylaxis

Gradually became depressed, fatigued, suicidal, disabled

Finally considered tick-borne encephalitis @ age 25

Initial testing @LabCorp and IGeneX Negative for "Lyme and coinfections"

Empiric antimicrobial treatment caused Herxing*

Then:

Galaxy Diagnostics found *Bartonella* antibodies

Target	Sample	Collection	Type	Result	Titer	Reference
B. henselae	GH18-3406-1-S	10/29/2018	Serum	Reactive	1:128	Non-reactive
B. quintana	GH18-3406-1-S	10/29/2018	Serum	Reactive	1:64	Non-reactive

***Named after Jarisch-Herxheimer reaction—Illness that occurs when immune evading parasites are killed, and thereby exposed to the immune system.**

Galaxy Diagnostics, 6 Davis Drive, Suite 201, Research Triangle Park, NC 27709, https://www.galaxydx.com/

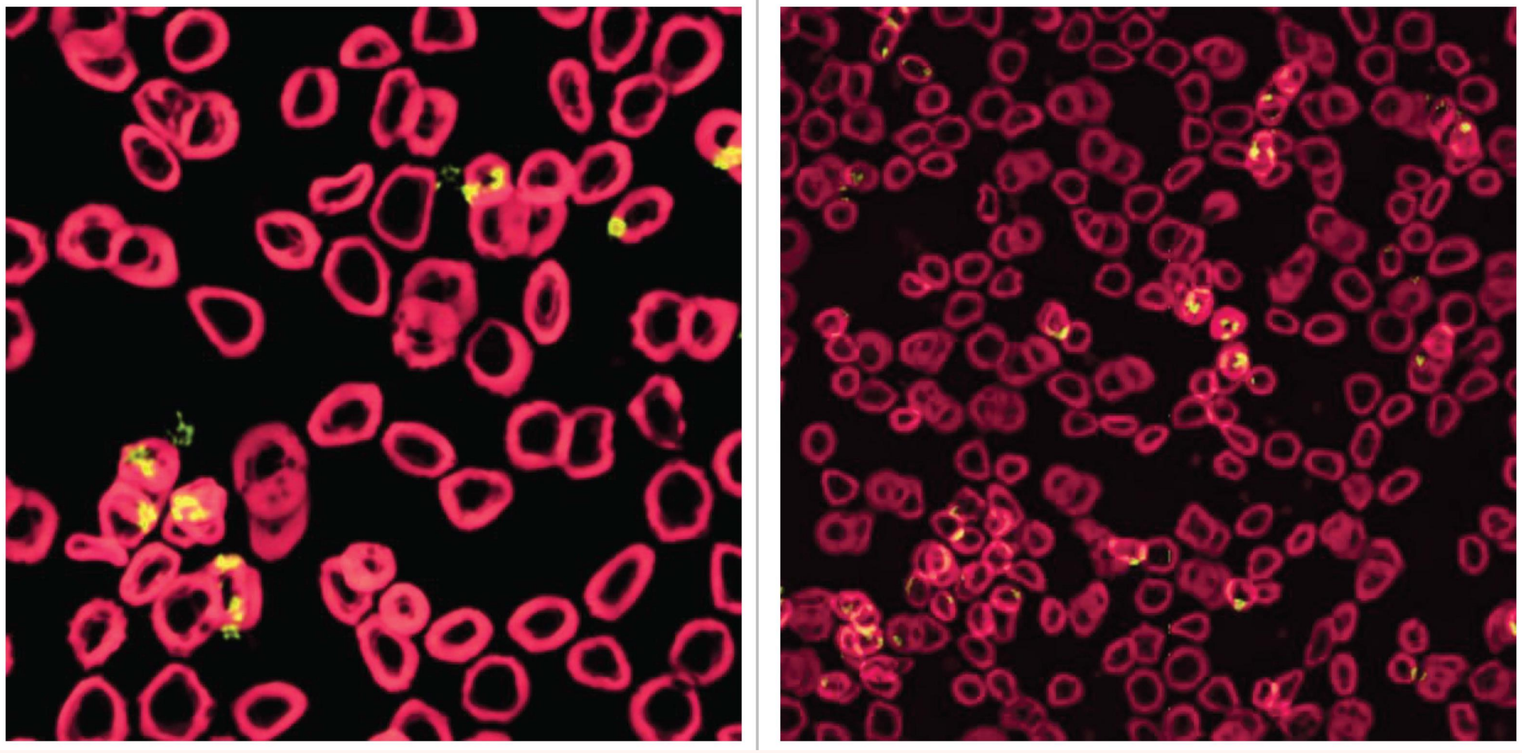
TLAB: *BARTONELLA HENSELAE* IN HER BLOOD

FISH: Florescence in situ Hybridization

TEST RESULT		
Target	**Method**	**Result**
B. henselae 23s rRNA* **	***in situ* hybridization and Confocal Laser Microscopy**	Your result is: **Positive** (Research Use Only) (Reference value is “negative”)

Indicates ongoing Infection

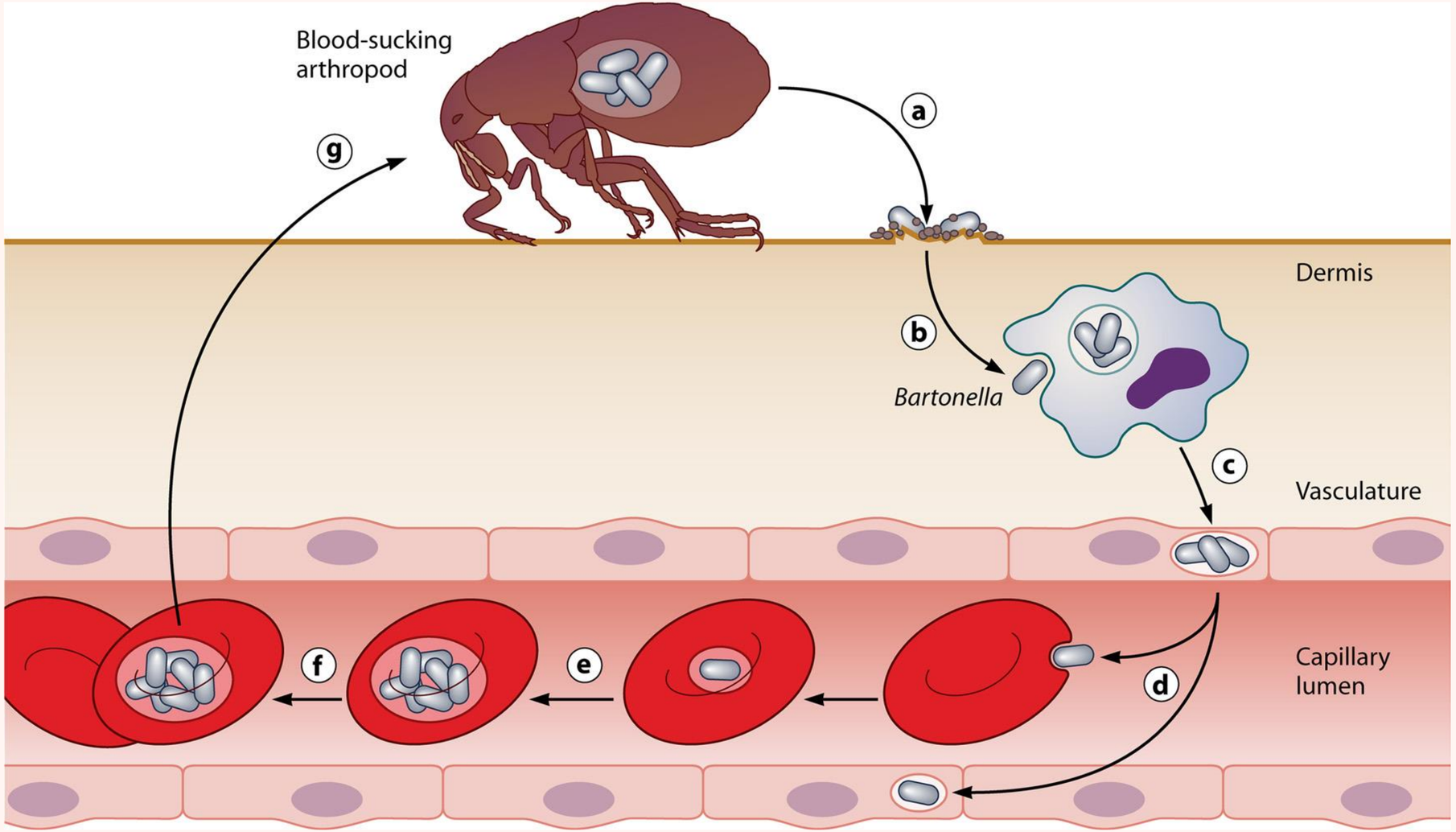
REPRESENTATIVE IMAGES



TLab Inc., 910 Clopper Road, Suite 220S, Gaithersburg, MD 20878, BR Mozayeni,, director. https://www.tlabdx.com/

BARTONELLA: INTRAVASCULAR BACTERIAL PARASITE

***Bartonella henselae*:**
Cat-scratch fever



<u>Common in humans</u>: many arthropod, animal <u>vectors</u>

Harms A, Dehio C. Intruders below the radar: molecular pathogenesis of *Bartonella* spp. *Clin Microbiol Rev*. 2012 Jan;25(1):42-78. PMID: 22232371
Chiaraviglio L et al. An immunocompromised murine model of chronic *Bartonella* infection. *Am J Pathol*. 2010 Jun;176(6):2753-63. PMID: 20395436

2016:
BARTONELLA BACTEREMIA ASSOC. WITH TICK BITES

Identification of Novel Zoonotic Activity of *Bartonella* spp., France

Muriel Vayssier-Taussat, Sara Moutailler, Françoise Féménia, Philippe Raymond, Olivier Croce, Bernard La Scola, Pierre-Edouard Fournier, Didier Raoult

Certain *Bartonella* species are known to cause afebrile bacteremia in humans and other mammals, including *B. quintana*, the agent of trench fever, and *B. henselae*, the agent of cat scratch disease. Reports have indicated that animal-associated *Bartonella* species may cause paucisymptomatic bacteremia and endocarditis in humans. We identified potentially zoonotic strains from 6 *Bartonella* species in samples from patients who had chronic, subjective symptoms and who reported tick bites. Three strains were *B. henselae* and 3 were from other animal-associated *Bartonella* spp. (*B. doshiae*, *B. schoenbuchensis*, and *B. tribocorum*). Genomic analysis of the isolated strains revealed differences from previously sequenced *Bartonella* strains. Our investigation identifed 3 novel *Bartonella* spp. strains with human pathogenic potential and showed that *Bartonella* spp. may be the cause of undifferentiated chronic illness in humans who have been bitten by ticks.

Nonreservoir hosts were considered incidentally infected without bacteria being detected in blood. Recently, these assumptions has been contradicted by studies describing animal-associated *Bartonella* spp. indirectly associated with bacteremia and a spectrum of diverse symptoms in immune-competent persons who had contact with animals, arthropods, or both, which are natural routes of *Bartonella* transmission (*5–7*). In some cases, the source of infection remains unknown; ticks have been suggested as a possible source of animal-associated *Bartonella* infection in humans (*6,8–10*).

Related to a patient's history of tick bites, it is common for physicians to suspect Lyme disease, some rickettsial diseases, or tickborne encephalitis. However, in many cases, the diagnosis is not confirmed by serologic or DNA-based tests. In recent years, alternate interpreta-

Vayssier-Taussat M et al. Identification of Novel Zoonotic Activity of *Bartonella* spp., France. *Emerg Infect Dis*. 2016 Mar;22(3):457-62. PMID: 26885624
Billeter SA et al. Vector transmission of *Bartonella* species with emphasis on the potential for tick transmission. *Med Vet Entomol*. 2008 Mar;22(1):1-15. PMID: 18380649
Maggi RG et al. *Bartonella henselae* bacteremia in a mother and son potentially associated with tick exposure. *Parasit Vectors*. 2013 Apr 15;6:101. PMID: 23587194
Cotté V et al. Transmission of *Bartonella henselae* by *Ixodes ricinus*. *Emerg Infect Dis.* 2008 Jul;14(7):1074-80. PMID: 18598628
Angelakis E et al. Potential for tick-borne bartonelloses. *Emerg Infect Dis*. 2010 Mar;16(3):385-91. PMID: 20202411

SINCE 2008: *BARTONELLA* SPECIES CULTURED FROM PATIENTS' BLOOD

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2008, p. 2856–2861 Vol. 46, No. 9
0095-1137/08/$08.00+0 doi:10.1128/JCM.00832-08
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Bartonella sp. Bacteremia in Patients with Neurological and Neurocognitive Dysfunction▽

E. B. Breitschwerdt,[1]* R. G. Maggi,[1] W. L. Nicholson,[2] N. A. Cherry,[1] and C. W. Woods[3]

Intracellular Pathogens Research Laboratory, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina[1]; Rickettsial Zoonoses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia[2]; and Duke University Medical Center, Durham, North Carolina[3]

Received 1 May 2008/Returned for modification 16 June 2008/Accepted 10 July 2008

We detected infection with a *Bartonella* species (*B. henselae* or *B. vinsonii* subsp. *berkhoffii*) in blood samples from six immunocompetent patients who presented with a chronic neurological or neurocognitive syndrome including seizures, ataxia, memory loss, and/or tremors. Each of these patients had substantial animal contact or recent arthropod exposure as a potential risk factor for *Bartonella* infection. Additional studies should be performed to clarify the potential role of *Bartonella* spp. as a cause of chronic neurological and neurocognitive dysfunction.

Breitschwerdt EB et al. *Bartonella* sp. bacteremia in patients with neurological and neurocognitive dysfunction. *J Clin Microbiol*. 2008 Sep;46(9):2856-61. PMID: 18632903
Maggi RG et al. *Bartonella* spp. bacteremia and rheumatic symptoms in patients from Lyme disease-endemic region. *Emerg Infect Dis*. 2012 May;18(5):783-91. PMID: 22516098
Breitschwerdt EB et al. *Bartonella* Associated Cutaneous Lesions (BACL) in People with Neuropsychiatric Symptoms. *Pathogens*. 2020 Dec 4;9(12):1023. PMID: 33291688
Maggi RG et al. Bartonella spp. bacteremia in high-risk immunocompetent patients. *Diagn Microbiol Infect Dis.* 2011 Dec;71(4):430-7. PMID: 21996096

DAUGHTER: REPEAT IGENEX TEST FINDS *BABESIA*

Remained Lyme-Negative

BABESIOSIS

B. microti IFA - IgM	Serum	<20	< 20 : Negative = 20 : May or may not indicate active infection >=40 : Indicates active infection	Titer
B. microti IFA - IgG	Serum	<40	< 40 : Negative < 160 : May or may not suggest active infection >=160 : Indicates active infection	Titer
Babesia FISH	W blood	**Pos**		

Ongoing infection with a *Babesia* species

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
Babesia PCR				
B. microti	W blood	Neg		
B. duncani	W blood	Neg		
B. duncani IFA - IgM	Serum	**80**	< 20 : Negative = 20 : May or may not indicate active infection >=40 : Indicates active infection	Titer
B. duncani IFA - IgG	Serum	<40	< 40 : Negative < 160 : May or may not suggest active infection >=160 : Indicates active infection	Titer

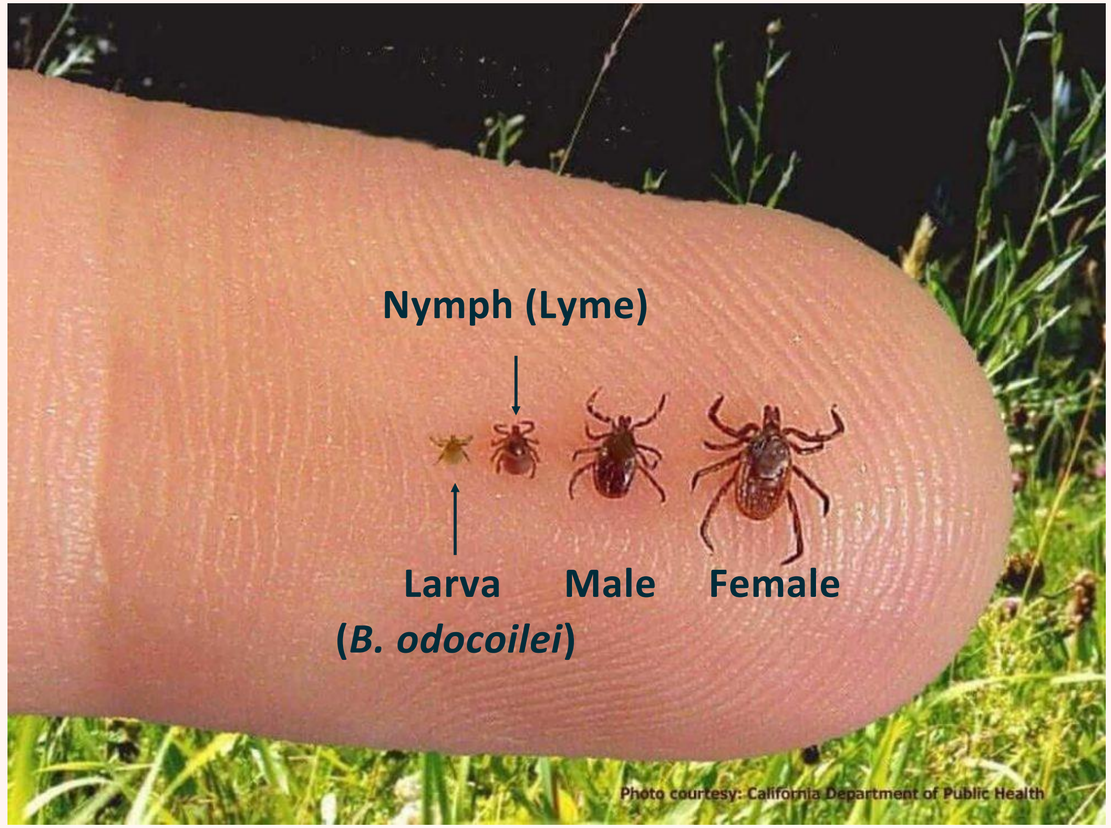
***Babesia duncani* antibodies**

WHAT'S IN THE DEER TICKS IN PA?

30%: *Borrelia burgdorferi* (Lyme Disease)

28%: *Bartonella* species

<3%: *Babesia microti*



No *Babesia duncani* (US West coast only)

***B. duncani* antibodies due to cross-reaction:**

***Babesia odocoilei*—20% of deer ticks**

Transovarial transmission: Pregnant female tick produces 1000 infected larvae

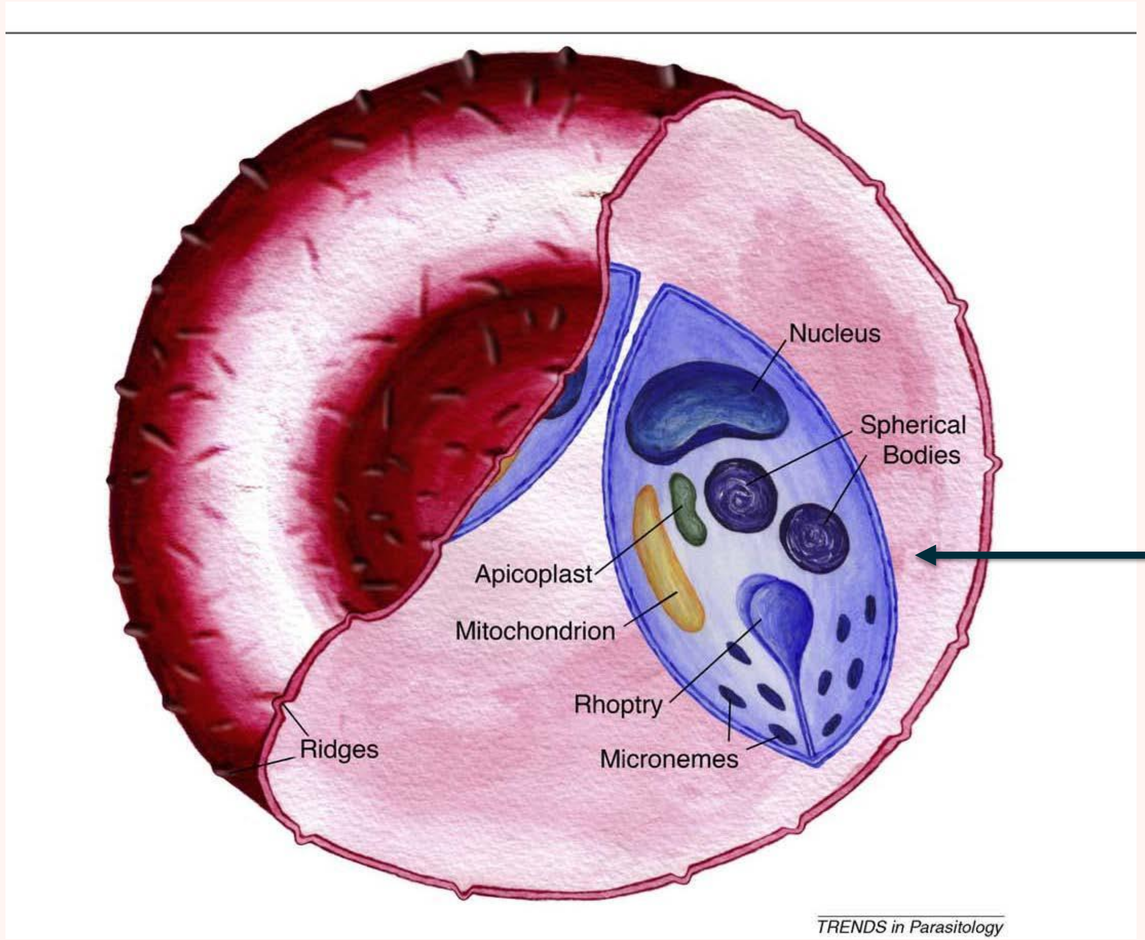
Data from the Tick Research Lab of Pennsylvania, Nicole Chinnici, Director

Also: Livengood J et al. Detection of *Babesia, Borrelia, Anaplasma*, and *Rickettsia* spp. in Adult Black-Legged Ticks from Pennsylvania…*Vector Borne Zoonotic Dis*. 2020;20(6):406-411. PMID: 31976829

Scott JD, Scott CM. Human Babesiosis Caused by *Babesia duncani* Has Widespread Distribution across Canada. Healthcare (Basel). 2018 May 17;6(2):49. PMID: 29772759

Milnes EL et al. *Babesia odocoilei* and zoonotic pathogens identified from *Ixodes scapularis* ticks in southern Ontario, Canada. *Ticks Tick Borne Dis*. 2019 Apr;10(3):670-676. PMID: 30833200

BABESIA: PROTOZOA RELATED TO MALARIA



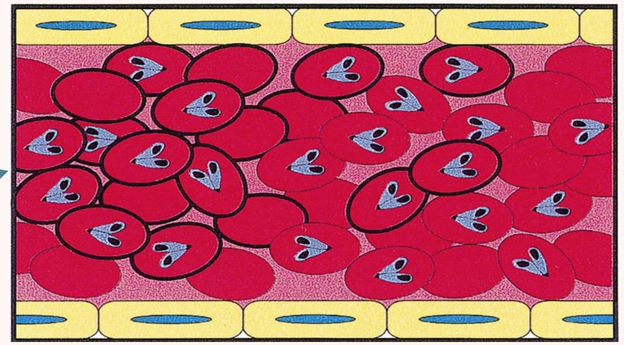
Merozoite

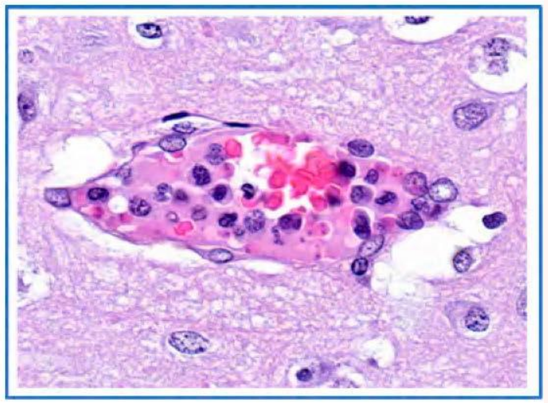
Gohil S et al. Recent insights into alteration of red blood cells by *Babesia bovis*: moovin' forward. *Trends Parasitol*. 2010;26(12):591-9. PMID: 20598944

B. ODOCOILEI IN THE VETERINARY LITERATURE

HOST: White-tailed deer (*Odocoileus virginianus*)

Sequestration: Infested red blood cells lodge in capillaries→NESTS





Uses fibrin for adherence (like *Babesia canis*)

Organisms rarely found in venous blood!

Chauvin A et al. Babesia and its hosts: adaptation to long-lasting interactions as a way to achieve efficient transmission. *Vet Res*. 2009;40(2):37. PMID: 19379662

Thomford JW et al. Isolation and in vitro cultivation of Babesia parasites from free-ranging desert bighorn sheep and mule deer in California. *J Parasitol.* 1993;79(1):77-84. PMID: 8437062

Schetters T. Mechanisms Involved in the Persistence of *Babesia canis* Infection in Dogs. *Pathogens*. 2019;8(3):94. PMID: 31261942

Sanders, DA. Observations on (chronic) Canine Babesiosis (piroplasmosis). *J Am Vet Med Assoc*. 1937;90:27-40

Malherbe, W. The manifestations and diagnosis of *Babesia* infections. *Ann. N. Y. Acad. Sci.* 1956;64(2):128-146. 10.1111/j.1749-6632.1956.tb36609.x

2021: *BABESIA ODOCOILEI* FOUND IN HUMANS

diagnostics

MDPI

Article

Detection of *Babesia odocoilei* in Humans with Babesiosis Symptoms

John D. Scott [1,*], Muhammad S. Sajid [1,2], Emily L. Pascoe [1] and Janet E. Foley [1]

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2 Faculty of Veterinary Medicine, University of Agriculture, Faisalabad 38040, Pakistan
* Correspondence: jkscott@bserv.com

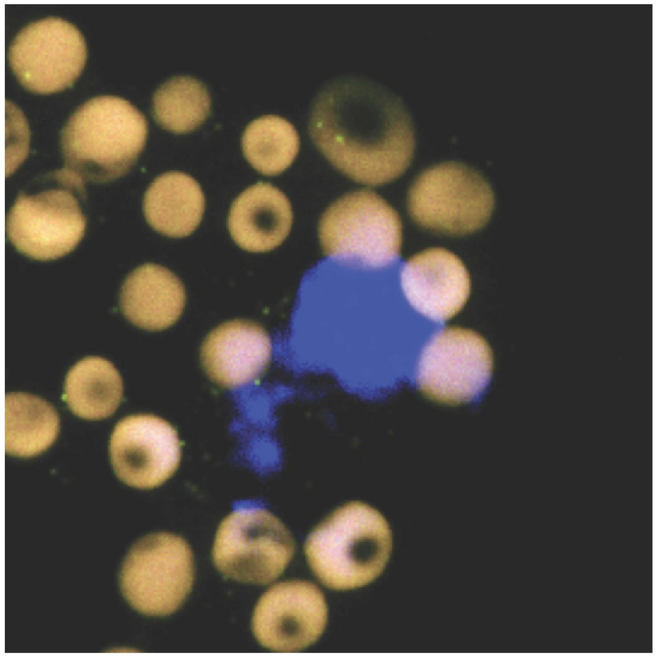
Scott JD et al, Detection of *Babesia odocoilei* in Humans with Babesiosis Symptoms. *Diagnostics (Basel)*. 2021;11(6):947. PMID: 34070625

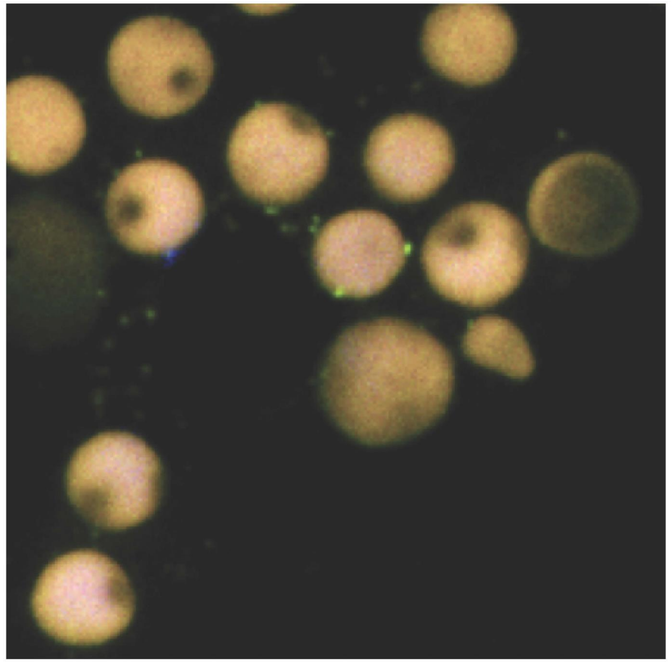
DAUGHTER: TLAB CONFIRMS *B. ODOCOILEI*

TEST RESULT		
Target	**Method**	**Result**
Babesia odocoilei* 18s rRNA*	***in situ* hybridization and Confocal Laser Microscopy**	Your result is: **Positive** (Research Use Only) (Reference value is "negative")

Ongoing infection

REPRESENTATIVE IMAGES





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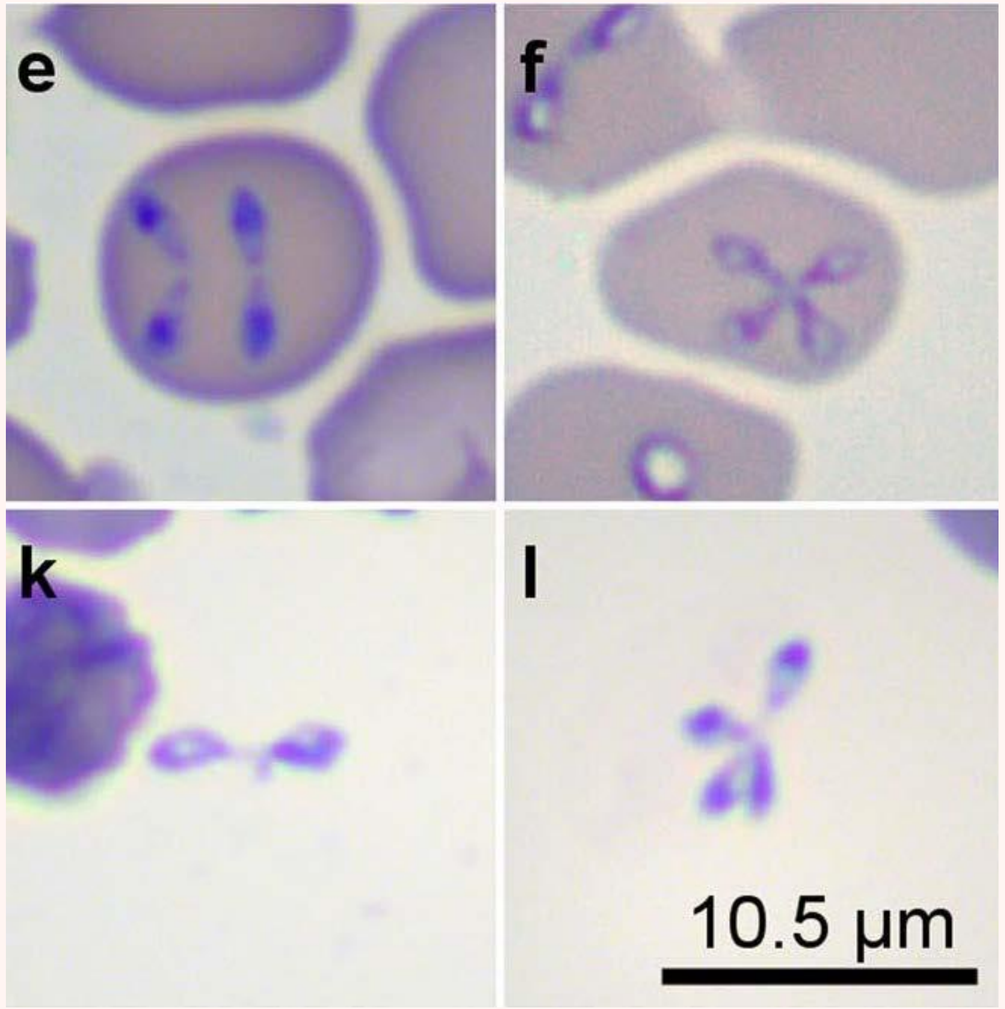
LIGHT MICROSCOPY: *BABESIA DIVERGENS*

Veterinary Literature:

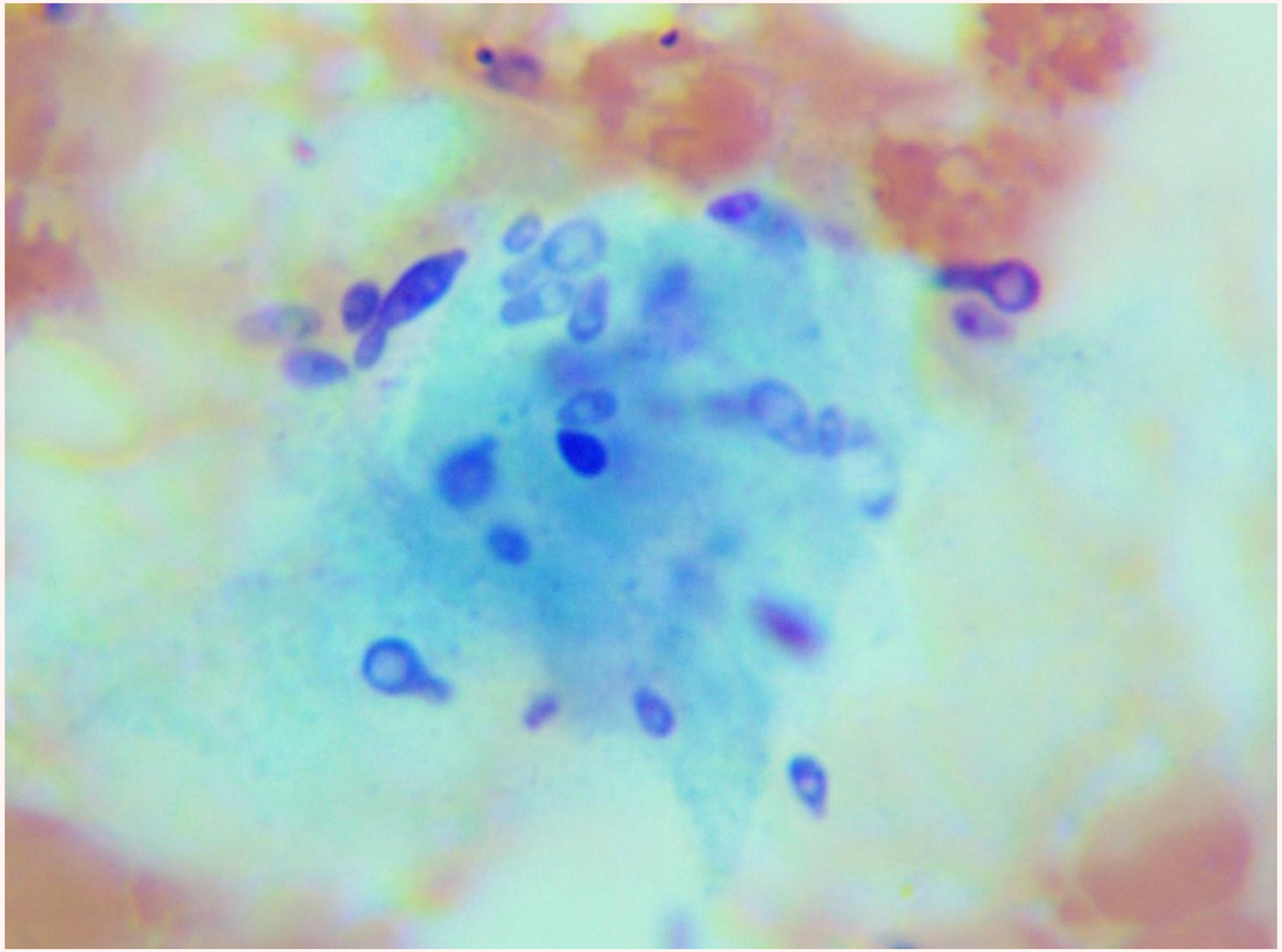
For sequestering *Babesia* species, one must look at capillary blood



Healthy
"Piriforms"
(Pear-shaped merozoites)

Sevilla E et al. Kinetics of the invasion and egress processes of *Babesia divergens*, observed by time-lapse video microscopy. *Sci Rep*. 2018;8(1):14116. PMID: 30237573

DAUGHTER: AFTER 6 MONTHS OF AGGRESSIVE *BABESIA* TREATMENT



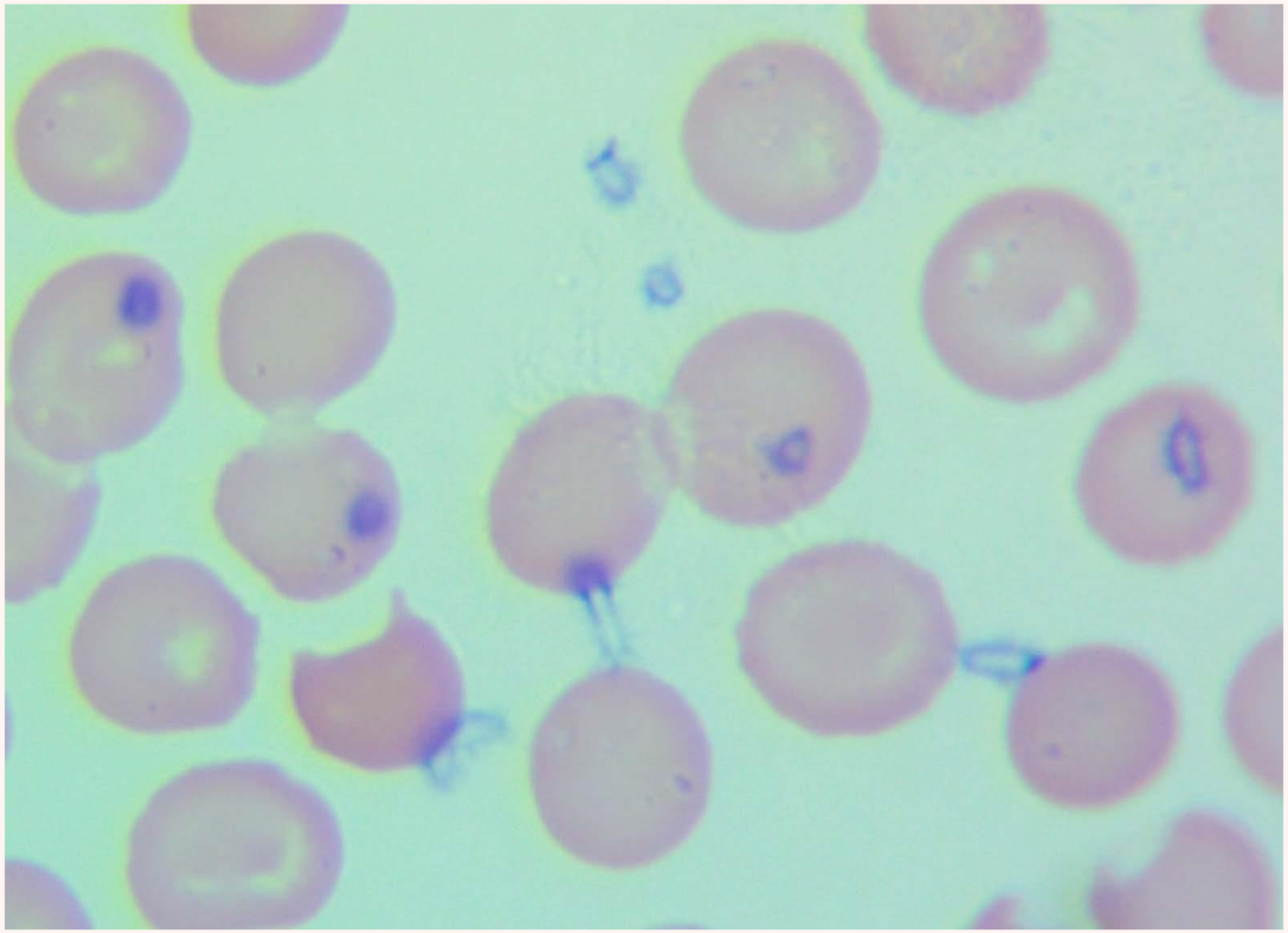
Blood from earlobe

Capillary Nest with many piriforms

Bock R et al. Babesiosis of cattle. *Parasitology*. 2004;129 Suppl:S247-69. PMID: 15938514

Wright IG. An electron microscopic study of intravascular agglutination in the cerebral cortex due to *Babesia argentina* infection. *Int J Parasitol*. 1972;2(2):209-15. PMID: 4652608

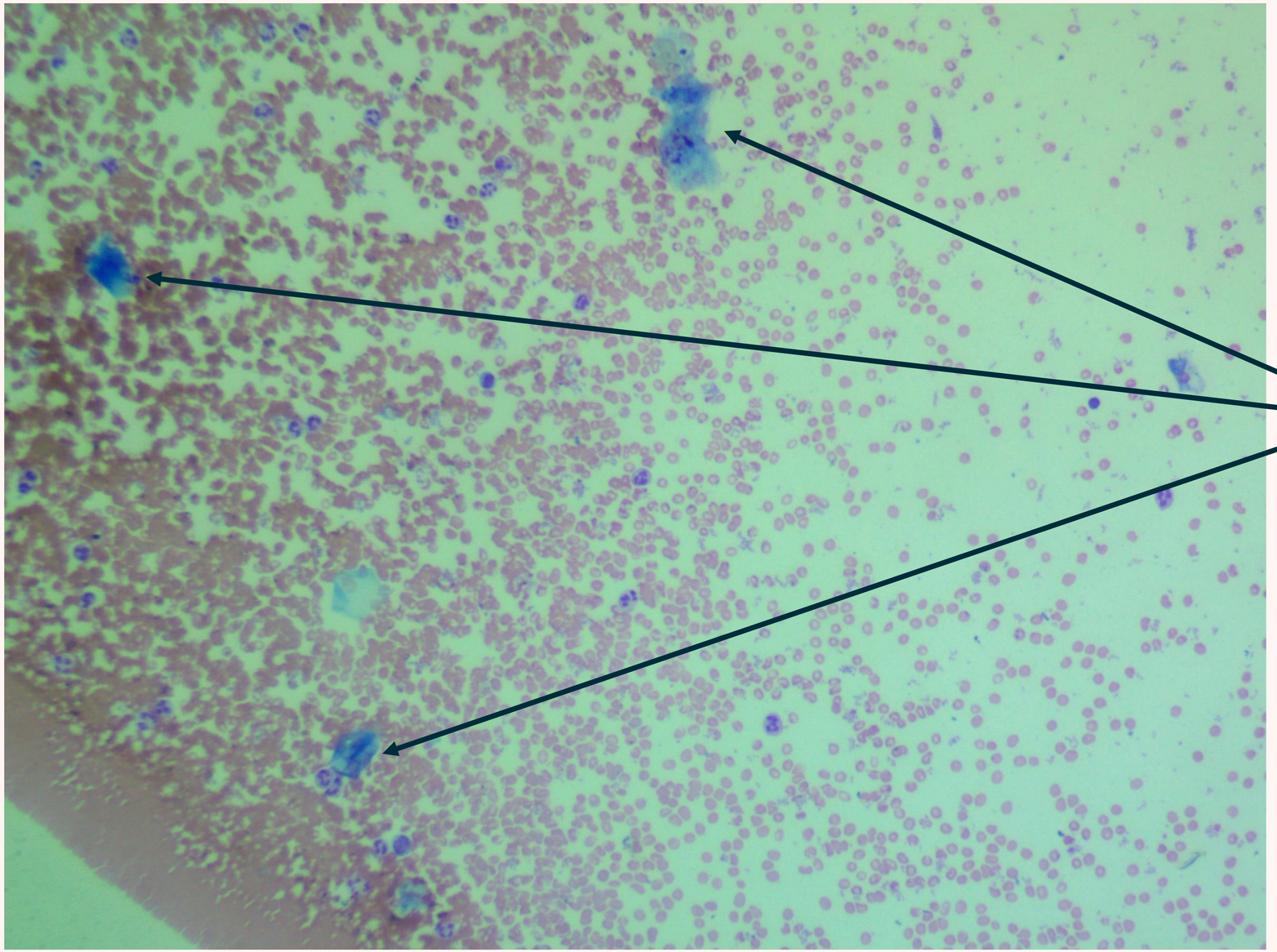
DAUGHTER: ACTIVE MEROZOITES ALSO SEEN



Seen only in Corticosteroid-treated patients

Wright IG. An electron microscopic study of intravascular agglutination in the cerebral cortex due to *Babesia argentina* infection. *Int J Parasitol*. 1972;2(2):209-15. PMID: 4652608

NESTS AT LOW MAGNIFICATION

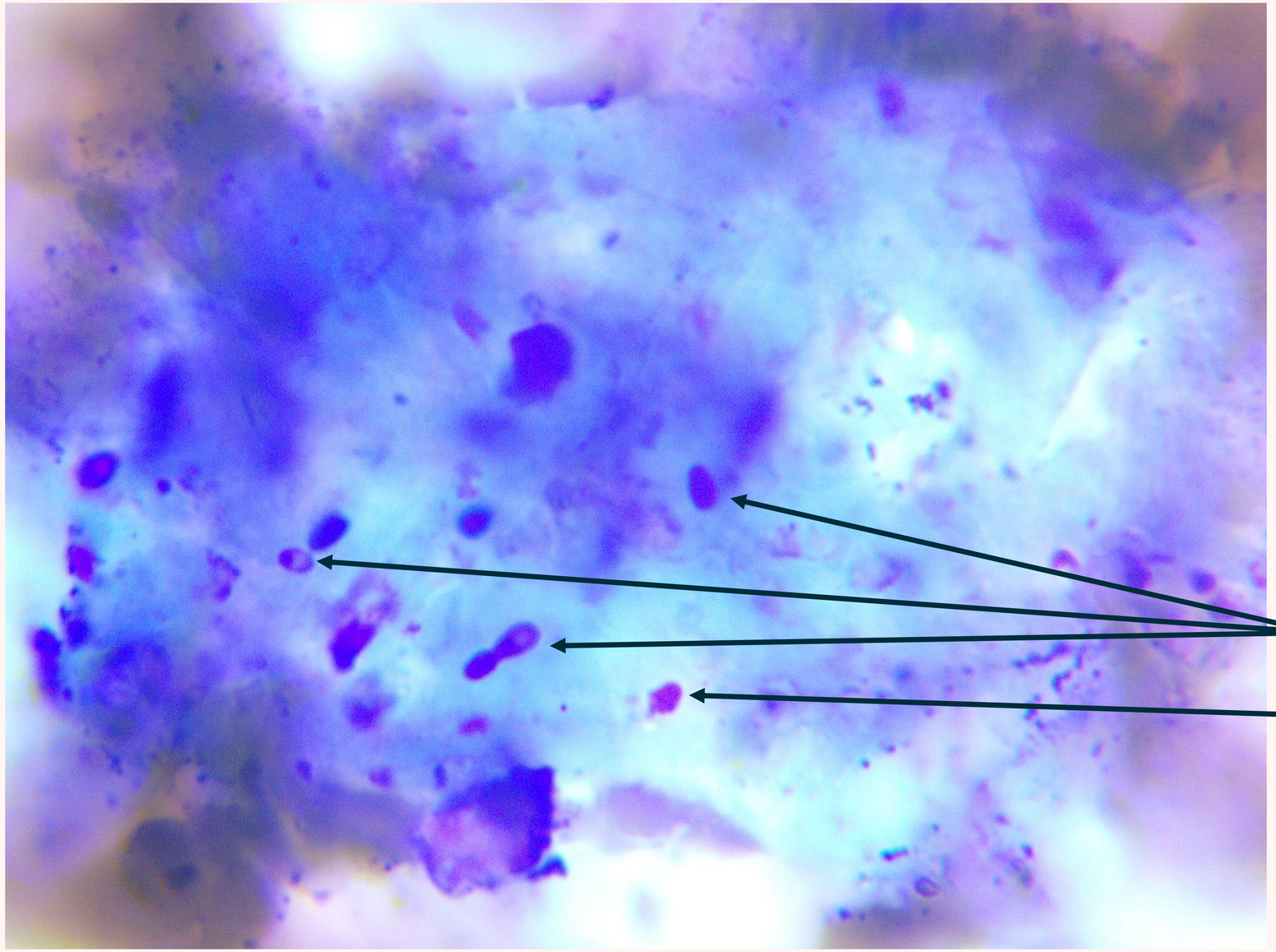


100x magnification

Babesia **capillary/venule NEST fragments**

Thomford JW et al. Isolation and in vitro cultivation of Babesia parasites from free-ranging desert bighorn sheep and mule deer in California. *J Parasitol.* 1993;79(1):77-84. PMID: 8437062
Bock R et al. Babesiosis of cattle. *Parasitology*. 2004;129 Suppl:S247-69. PMID: 15938514

ZOOM IN: *BABESIA* PERSISTERS IN NEST



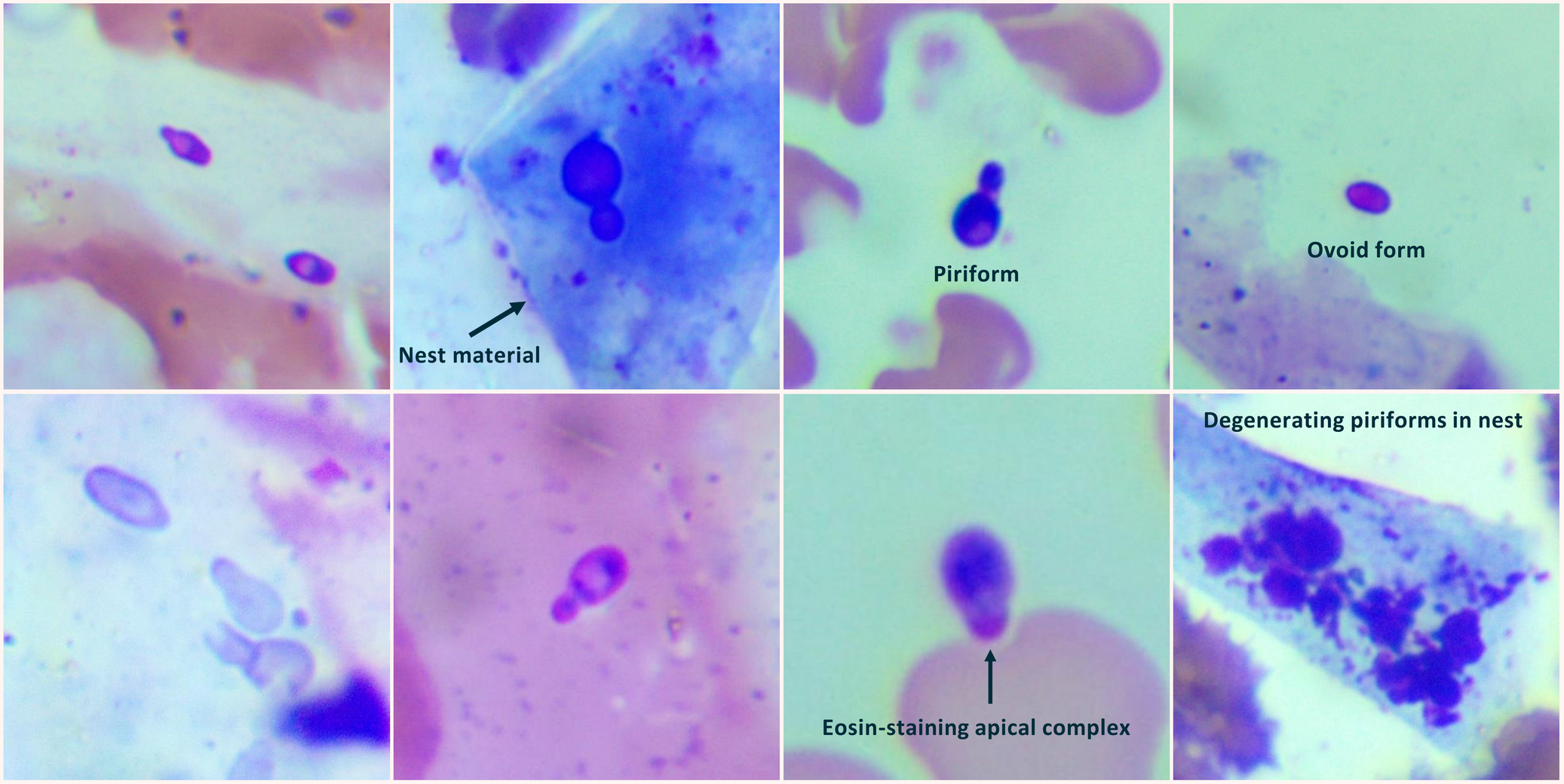
1000x
oil immersion

***Babesia* Persisters**

Degenerating,
fragmented

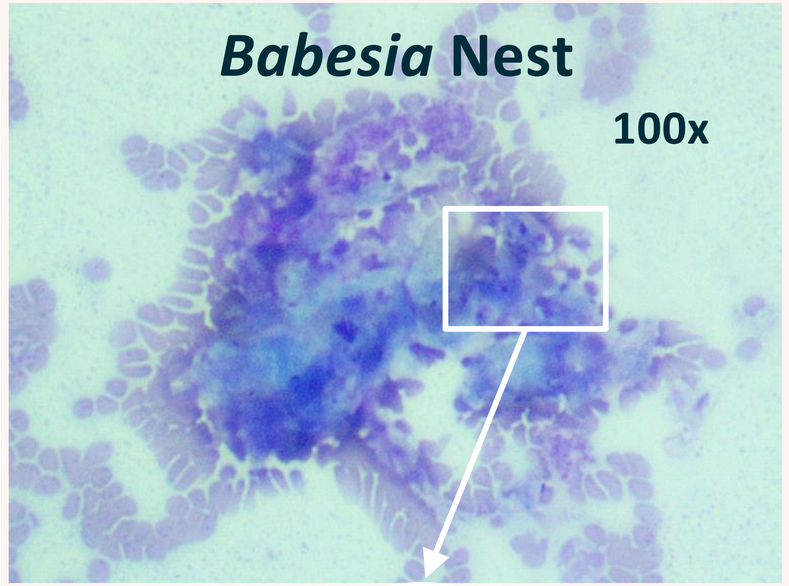
Wright IG. An electron microscopic study of intravascular agglutination in the cerebral cortex due to *Babesia argentina* infection. *Int J Parasitol*. 1972;2(2):209-15. PMID: 4652608

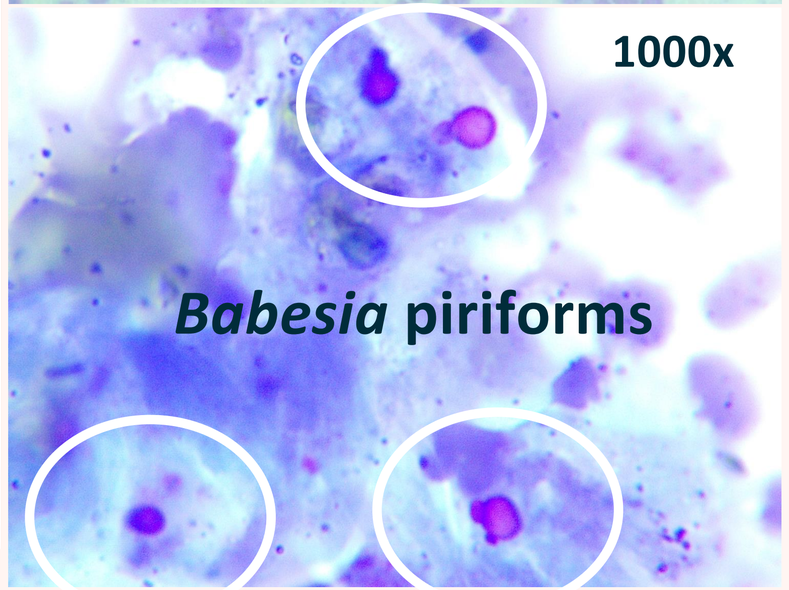
TO DATE: *BABESIA* FOUND IN >80 FATIGUED PATIENTS



Usually in nest fragments in capillary blood

16 yo female with 4 yr history of fatigue, anxiety, insomnia, suicidality, and cutting





On <u>atovaquone</u> had herxing, followed by improvements.
Bartonella antibody tests pending.

BARTONELLA AND *BABESIA* ENCEPHALITIS CHILDHOOD PSYCHIATRIC DISORDERS

Encephalitis: *Bartonella* and *Babesia* infest the brain's blood vessels→brain inflammation→neuropsychiatric disorders

Children are especially susceptible to infection—Playing outside, playing with pets.

***Bartonella* and *Babesia* can be passed transplacentally—from mother to fetus.**

Immature immune systems often do not react strongly to parasitic infections→Lack of acute signs and symptoms

The reported cases of congenital and childhood infection are the tip of the iceberg.

Zintl A et al. Possible mechanisms underlying age-related resistance to bovine babesiosis. *Parasite Immunol*. 2005 Apr;27(4):115-20. PMID: 15910419

Breitschwerdt EB et al. *Bartonella henselae* Bloodstream Infection in a Boy With Pediatric Acute-Onset Neuropsychiatric Syndrome. *J Cent Nerv Syst Dis*. 2019 Mar 18;11:1179573519832014. PMID: 30911227

Greenberg R. Infections and Childhood Psychiatric Disorders: Tick-Borne Illness and Bipolar Disorder in Youth. *Bipolar Disord.* 2017; 3: 113. doi: 10.4172/2472-1077.1000113

Breitschwerdt EB, Bradley JM, Maggi RG, Lashnits E, Reicherter P. *Bartonella* Associated Cutaneous Lesions (BACL) in People with Neuropsychiatric Symptoms. *Pathogens*. 2020 Dec 4;9(12):1023. PMID: 33291688

Breitschwerdt EB et al. Molecular evidence of perinatal transmission of *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* to a child. *J Clin Microbiol*. 2010 Jun;48(6):2289-93. PMID: 20392912

Rojas-Pirela M et al. Congenital Transmission of Apicomplexan Parasites: A Review. *Front Microbiol*. 2021 Sep 29;12:751648. PMID: 34659187

SPECIALIZED TESTING REQUIRED

***Bartonella* species: Galaxy Diagnostics (NC State Vector Borne Disease Diagnostic Lab—World center for *Bartonella* research)**

***Babesia odocoilei*: INVISIBLE TO DATE: No antibody test; Neg. venous blood smears**

Most Sensitive Test: Capillary blood smear

Only Labs: IGeneX—FISH, Immunoblot, TLab FISH; both often false-negative

BEST TESTS at BEST LABS routinely dismissed: "Not FDA-approved"—required only for testing technology and kits sold to other labs or consumers

IGeneX and Galaxy: CLIA-certified, properly validated tests, Licensed in PA

Galaxy Diagnostics, 6 Davis Drive, Suite 201, Research Triangle Park, NC 27709, https://www.galaxydx.com/

IGeneX Inc., 556 Gibraltar Dr, Milpitas, CA 95035, https://igenex.com/

Shah JS et al. Combined Immunofluorescence (IFA) and Fluorescence In Situ Hybridization (FISH) Assays for Diagnosing Babesios is…*Diagnostics (Basel)*. 2020 Sep 28;10(10):761. PMID: 32998244

Lindner HH, A sequestering *Babesia* species found in humans with exertional intolerance and encephalopathy. 2022 (under review)

A NEW INFECTIOUS DISEASE PARADIGM

Bartonella **and** ***Babesia*** **are not reportable in Pennsylvania: Incidence unknown**

Probable cause of most chronic illness after tick bites: "post-Lyme treatment syndrome"

Treatment works: BUT ***Babesia*** **species are 1000 times more resistant to antimalarials than malaria***

B. odocoilei **is sequestered—protected from antimicrobials; Nests also protect** ***Bartonella*** **(opinion)**

Prolonged, high-dose, multi-agent treatment is required + fibrinolytics to dissolve nests

*Abraham A et al. …*in vitro* culture of *Babesia duncani* in human erythrocytes reveals unusually high tolerance to recommended therapies. *J Biol Chem*. 2018;293(52):19974-19981. PMID: 30463941
Schetters T. Mechanisms Involved in the Persistence of *Babesia canis* Infection in Dogs. *Pathogens*. 2019;8(3):94. PMID: 31261942

RECOMMENDATIONS

Add Bartonellosis and Babesiosis to PA's list of reportable diseases

Train PA pathologists and health care providers to examine capillary blood for *Babesia*

Mandate that Insurers, Medicare, Medicaid cover antimicrobial therapy, including antimalarials for babesiosis at higher doses and for longer times than FDA-approved for malaria

Instruct pharmacists to fill providers' prescriptions for these infections, absent any other objection.