Demystifying TBD: Borrelia, Bartonella & Babesia

IGENEX Webinar
February, 2025

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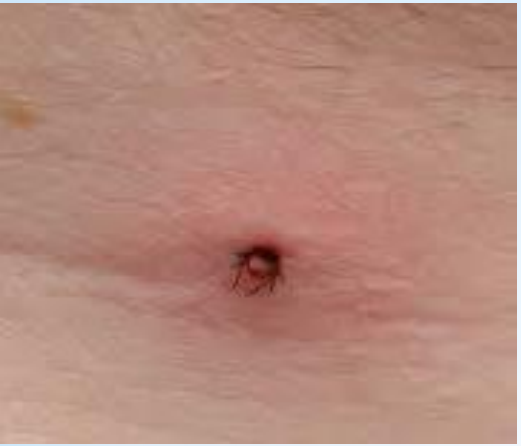
* Disclaimers & Learning Objectives

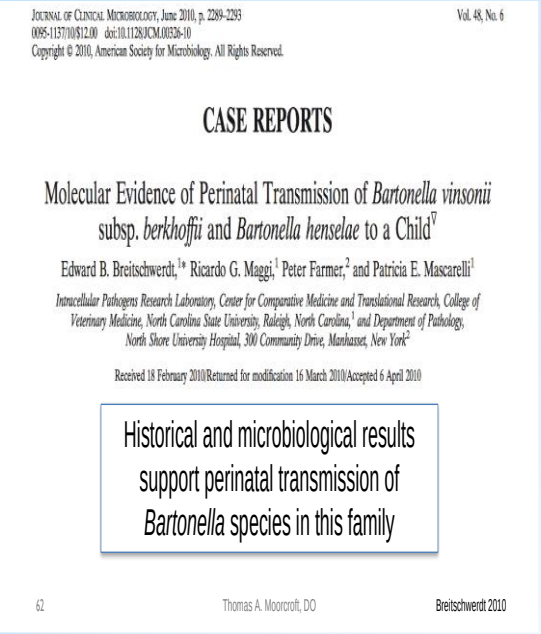
- I am on the medical advisory board of Designs For Health
- I have no other financial disclosures

- The participant will be able to discuss the background, prevention and differential diagnosis of vector borne diseases.
- The participant will be able to outline the assessment of Borrelia, Bartonella, Babesia.
- The participant will be able to outline the treatment of Borrelia, Bartonella, Babesia.



* Etiology and Epidemiology

- The main route of dissemination for *B. burgdorferi* is through the tick species *Ixodes scapularis*, more commonly known as the black-legged tick
- *B. burgdorferi* infection occurs in northeastern and midwestern regions.
- *B. burgdorferi* is one of five main species *(Borrelia afzelii, Borrelia garinii, Borrelia spielmanii,* and *Borrelia bavariensis)*
- *Borrelia Mayonii found in Midwest of US in 2016*
- *Borrelia Miyamotoi first discovered in Japan in 1995; now in US from ixodes scapularis and pacificus*



JOURNAL OF CLINICAL MICROBIOLOGY, June 2010, p. 2289–2293 Vol. 48, No. 6
0095-1137/10/$12.00 doi:10.1128/JCM.00526-10

CASE REPORTS

Molecular Evidence of Perinatal Transmission of *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* to a Child[▽]

Edward B. Breitschwerdt,[1]* Ricardo G. Maggi,[1] Peter Farmer,[2] and Patricia E. Mascarelli[1]

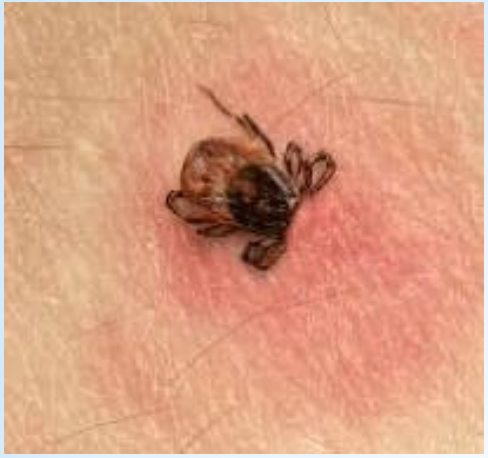
Intracellular Pathogens Research Laboratory, Center for Comparative Medicine and Translational Research, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina,[1] and Department of Pathology, North Shore University Hospital, 300 Community Drive, Manhasset, New York[2]

Received 18 February 2010/Returned for modification 16 March 2010/Accepted 6 April 2010

Historical and microbiological results support perinatal transmission of *Bartonella* species in this family

- Understand what ticks carry what spirochetes
  - (www.tickencounter.org)
- > 22 species of borrelia
  - (burgdorferi, afzelli, garinii, miyamotoi…)
- Ixodes transmitted tick borne diseases (TBD)
  - Borrelia burgdorferi, miyamotoi, mayonii
  - Babesia, Bartonella- most common species in Northeast
- Ixodes ticks bite a variety of hosts
  - Mice, rats, squirrels, shrew
  - Incidental hosts – humans, domesticated animals, lizards
  - Gestational
- Tick dispersion over wider distances
  - Many species of songbirds
  - Deer

ASSESSMENT



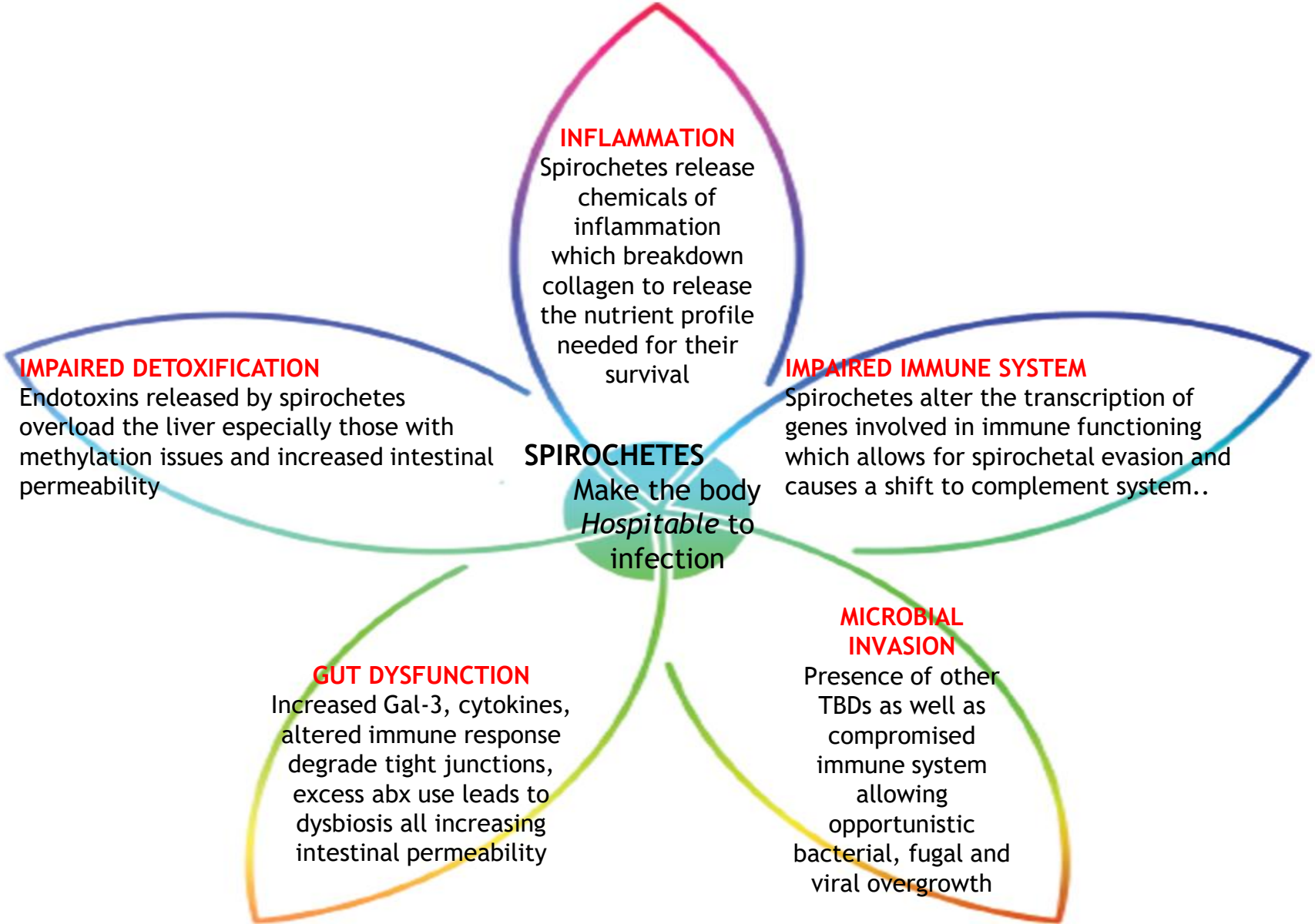
* Prevention

The best defense against Lyme disease is to guard against tick bites. Measures include:

- DO NOT go into heavily wooded or grassy areas that may be infested with ticks.
- Wear long-sleeved shirts and long pants.
- Apply insect repellent. Use permethrin for your cloths and apply PICARIDIN repellant to the exposed skin.
- Carefully check for ticks after you have been outdoors.
- If you find a tick, remove it with tweezers, making sure to remove the head as well as the body. Do not use hot matches, petroleum jelly, nail polish, or other substances to remove the tick.
- Send to tickreport.com or local health department checking for 3Bs or those TBD most prevalent in your area.

* How to think about Tick Borne Diseases (TBD)

1. Background check – no this is not the FBI or CIA but rather looking at root issues in nutrient deficiencies, the gut, the diet, the hormonal balance, sleep, relationships and lifestyle
2. Band-Aids – minimize symptoms
3. Block inflammation with dietary changes, nutraceuticals, herbals and medications
4. Buffer the Autonomic Nervous System to improve dysautonomia
5. Balance immune system
6. Build Gut
7. Break down biofilms
8. Bolster detoxification
9. Bind toxins and treat herx heimer reactions
10. Blast bugs

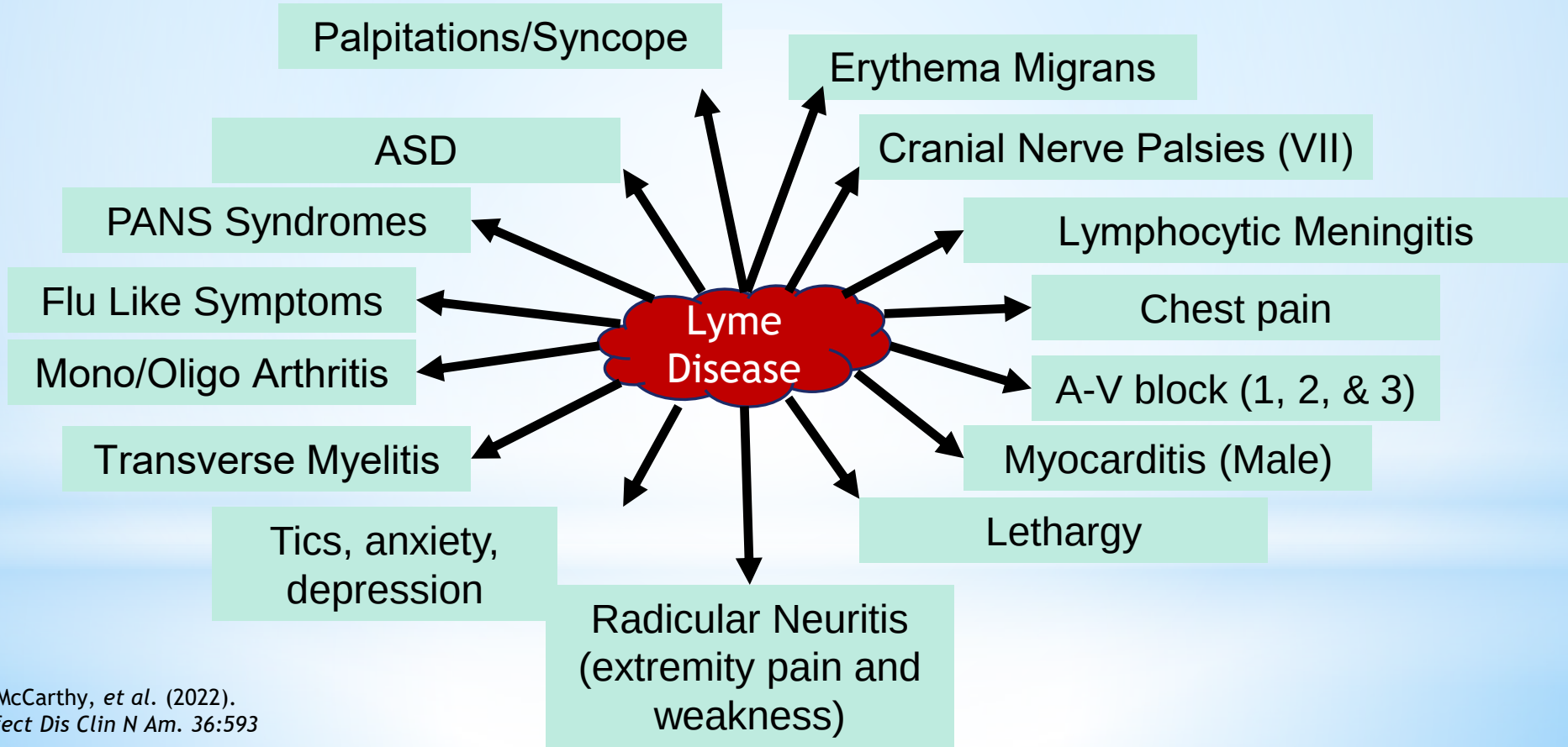

INFLAMMATION
Spirochetes release chemicals of inflammation which breakdown collagen to release the nutrient profile needed for their survival
IMPAIRED DETOXIFICATION
Endotoxins released by spirochetes overload the liver especially those with methylation issues and increased intestinal permeability
SPIROCHETES
Make the body *Hospitable* to infection
IMPAIRED IMMUNE SYSTEM
Spirochetes alter the transcription of genes involved in immune functioning which allows for spirochetal evasion and causes a shift to complement system..
GUT DYSFUNCTION
Increased Gal-3, cytokines, altered immune response degrade tight junctions, excess abx use leads to dysbiosis all increasing intestinal permeability
MICROBIAL INVASION
Presence of other TBDs as well as compromised immune system allowing opportunistic bacterial, fugal and viral overgrowth

* Differential Diagnosis of Tick/Vector Borne Diseases (TBD/VBD)

- Bell's palsy and cranial nerve palsies (PMID: **36116837**)
- Chronic Fatigue Syndrome
- Fibromyalgia
- Arthritis
- Myocarditis
- Long COVID
- Neuropsychiatric Disease
- Autism?
- If you are thinking of any of these, think Vector Borne Diseases!

Testing for Lyme disease is highly problematic.

* Presentations of Borreliosis



McCarthy, *et al.* (2022).
Infect Dis Clin N Am. 36:593

* Flu-like or GI symptoms
* Neck pain
* Joint pain
* Nerve pain
* Peripheral neuropathy
* Vertigo
* Headaches
* Cardiac symptoms
* Brain Fog
* Anxiety and Depression
* Cognitive Impairments

* Lyme Disease - Borreliosis

Review > Psychiatr Clin North Am. 1998 Sep;21(3):693-703, viii.
doi: 10.1016/s0193-953x(05)70032-0.

The underdiagnosis of neuropsychiatric Lyme disease in children and adults

B A Fallon [1], J M Kochevar, A Gaito, J A Nields

Affiliations + expand

PMID: 9774805 DOI: 10.1016/s0193-953x(05)70032-0

Free article

Abstract

Lyme Disease has been called "The New Great Imitator," a replacement for that old "great imitator" neurosyphilis. This article reviews the numerous psychiatric and neurologic presentations found in adults and children. It then reviews the features of Lyme Disease, which makes it almost uniquely hard to diagnose, including the complexity and unreliability of serologic tests. Clinical examples follow that illustrate those presentations of this disease that mimic attention deficit hyperactivity disorder (ADHD), depression, and multiple sclerosis.

* Neuropsychiatric Borreliosis

Front Psychiatry. 2021; 12: 505941. PMCID: PMC7884317

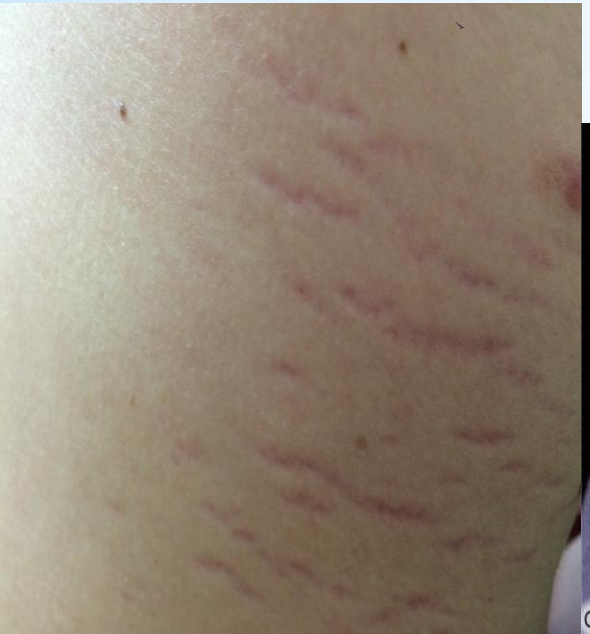
Published online 2021 Feb 2. doi: 10.3389/fpsyt.2021.505941 PMID: 33603684

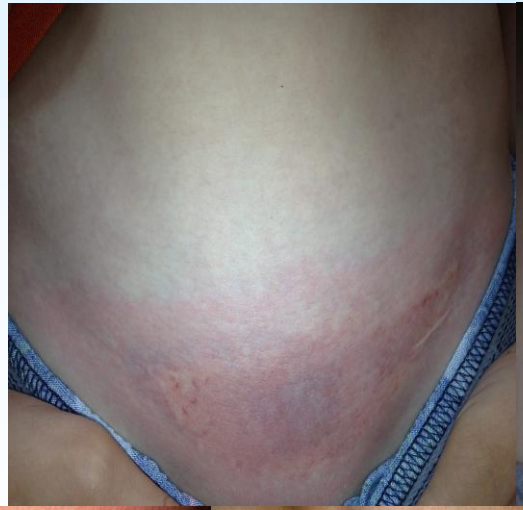
Case Report: PANDAS and Persistent Lyme Disease With Neuropsychiatric Symptoms: Treatment, Resolution, and Recovery

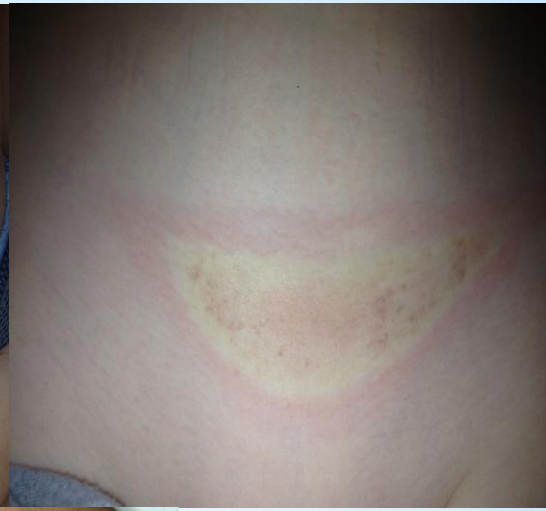
Amy Cross,[1] Denis Bouboulis,[2] Craig Shimasaki,[1] and Charles Ray Jones[3,*]

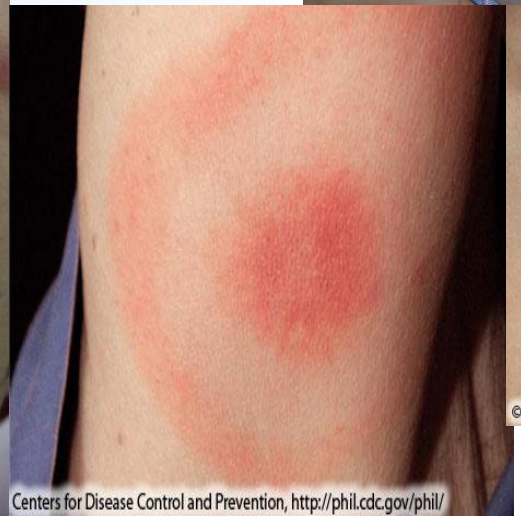
* Physical Exam Clues

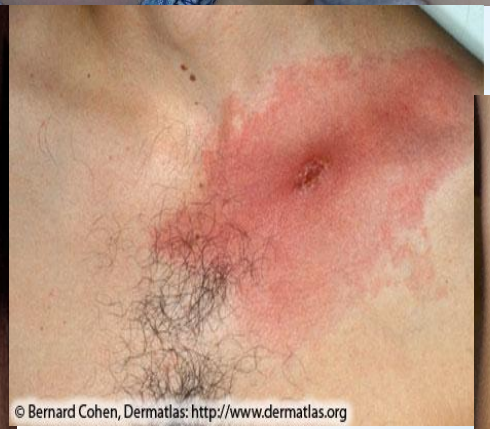
- Erythema Migrans
- There may be a rash,
- but in children,
- the numbers are at most 36%
- doi.org/10.1186/s12887-018-1163-2

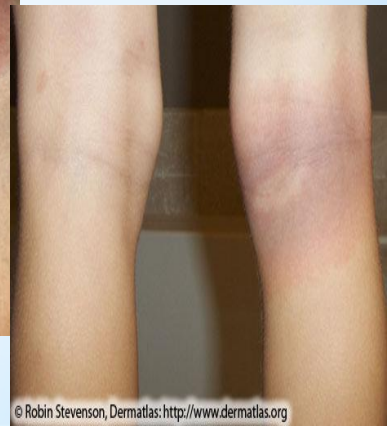










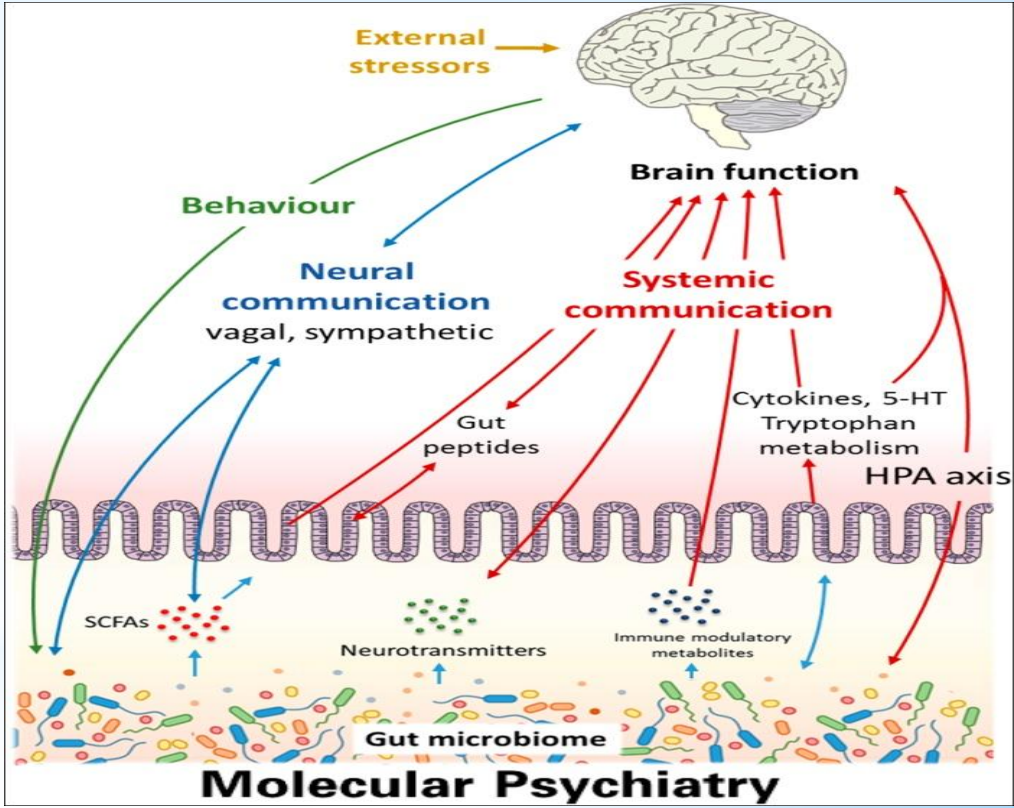


* Some research on Borrelia

- Tick Borne Diseases (TBD) are clinical diagnoses! Research since the 1990s has shown that Lyme disease, particularly neuropsychiatric Lyme, is underdiagnosed. PMID: 9774805
- Rates of infection are highest among children 5-15 and those over 50 years of age. PMID: 36116831
- **ELISA testing is only 53% positive and the 2 tier testing of ELISA and western blot is insensitive and PCR has sensitivity of < 30%. Of most importance, the more ill a person is, the weaker their serologic response meaning that the most sensitive tests are needed for example, the immunoblot! PMID: 27314832**
- Using Denmark's National Register, Fallon et al found that in patients with LYME DISEASE there was a 28% higher rate of any mental disorder, 42% higher rate of affective disorder, a 2-fold higher rate of suicide attempts, and a **75% higher rate of death by suicide.** https://doi.org/10.1176/appi.ajp.2021.20091347
- In a 2018 study, Greenberg reported that 80% of children with psychiatric symptoms tested positive for Tick Borne Diseases (TBD). PMID: 29799538
- **There may be a rash, but in children, the numbers are at most 36%. https://doi.org/10.1186/s12887-018-1163-2**

* "Co-infections" - Bartonella & Babesia

* Polymicrobial infections are common
* Increase severity of illness
* May make diagnosis more difficult
* Clinical presentation is more complex
* Lab diagnostics even less reliable
* Higher risk of long-term failure



Dunn, 2014; Adelson, 2004; Sapi 2009

Infectious and Autoimmune Causes of Encephalitis in Children

Timothy A. Erickson, PhD, MSPH,[a,b,f] Eyal Muscal, MD, MS,[g,f] Flor M. Munoz, MD,[c] Timothy Lotze, MD,[d] Rodrigo Hasbun, MD,[e] Eric Brown, PhD,[b] Kristy O. Murray, DVM, PhD[a,f]

abstract

BACKGROUND AND OBJECTIVES: Encephalitis can result in neurologic morbidity and mortality in children. Newly recognized infectious and noninfectious causes of encephalitis have become increasingly important over the past decade.

METHODS: We retrospectively reviewed medical records from pediatric patients in Houston diagnosed with encephalitis in both an urban and rural catchment area between 2010 and 2017. We conducted an investigation to understand the etiology, clinical characteristics, and diagnostic testing practices in this population.

RESULTS: We evaluated 231 patients who met the case definition of encephalitis, among which 42% had no recognized etiology. Among those with an identified etiology, the most common were infectious (73; 31%), including viral (n = 51; 22%), with the most frequent being West Nile virus (WNV; n = 12), and bacterial (n = 19; 8%), with the most frequent being *Bartonella henselae* (n = 7). Among cases of autoimmune encephalitis (n = 60; 26%), the most frequent cause was anti–N-methyl-D-aspartate receptor (NMDAR) encephalitis (n = 31). Autoimmune causes were seen more commonly in female ($P < .01$) patients. Testing for herpes simplex virus and enterovirus was nearly universal; testing for anti-NMDAR encephalitis, WNV, and *Bartonella* was less common.

CONCLUSIONS: WNV was the most common infectious cause of encephalitis in our pediatric population despite lower testing frequency for WNV than herpes simplex virus or enterovirus. Increasing testing for anti-NMDAR encephalitis resulted in frequent identification of cases. Increased awareness and testing for WNV and *Bartonella* would likely result in more identified causes of pediatric encephalitis. Earlier etiologic diagnosis of encephalitides may lead to improve clinical outcomes.

* Bartonella & AE/BGE

J Cent Nerv Syst Dis. 2019; 11: 1179573519832014. PMCID: PMC6423671

Published online 2019 Mar 18. doi: 10.1177/1179573519832014 PMID: 30911227

Bartonella henselae Bloodstream Infection in a Boy With Pediatric Acute-Onset Neuropsychiatric Syndrome

Edward B Breitschwerdt,[1] Rosalie Greenberg,[2] Ricardo G Maggi,[1] B Robert Mozayeni,[3] Allen Lewis,[4] and Julie M Bradley[1]

* How is Bartonella transmitted

- Bite or scratch wounds of cats (saliva, claws)
- Cat fleas; lice (*B. quintana*)
  - Englisch CK, Wear DJ, Margileth AM, Lissner CR, Walsh GP. Cat-scratch disease.Isolation and culture of the bacterial agent. *JAMA*. 1988;259(9):1347–52.
- Tick bite & More:
  - Dust mites
  - Flea bites, flea feces (oral infection)
  - Contact with cats, contact with dogs (paws, saliva, lice)
    - Rolain JM, Foucault C, Guieu R, La Scola B, Brouqui P, Raoult D. Bartonella quintana in human erythrocytes. *Lancet*. 2002;360(9328):226–8.
  - Flies, gadflies
  - Blood transmission
  - Mother-child transmission
    - Schaller DJ. *Bartonella Diagnosis and Treatment*. Tampa, Florida: Hope Academic Press; 2008.

* Bartonella Species

At least 37 distinct species

- *B henselae*
  - Reservoirs: Cats
  - Vectors: Fleas (cat or dog), ticks
  - Cat Scratch Disease, Endocarditis, Neuroretinitis, Encephalitis
- *B quintana*
  - Reservoirs: Cats, humans
  - Vectors: Lice
  - Trench fever.
- B bacilliformis
  - Reservoirs: Humans
  - Vectors: Sand flies
  - Carrions disease (Peruvian warts)

* Bartonella Species Continued

- *B. elizabethae*
  - Reservoirs: Rats
  - Vectors: Fleas
  - Endocarditis
- *B alsatica* (rabbit)
- *B clarridgeiae* (cat)
- *B mayotimonensis* (bat)
- *B grahamii* (mouse)
- *B vinsonii* (mouse, cat, dog)
- *B washoensis* (squirrel)
- *B koehlerae*

Edward Bealmear Breitschwerdt; Bartonellosis: One Health Perspectives for an Emerging Infectious Disease, ILAR Journal, Volume 55, Issue 1, 1 January 2014, Pages 46–58, https://doi.org/10.1093/ilar/ilu015

* Bartonella Symptoms

- Muscle and Joint pains
- Headaches
- Fatigue & Brain Fog
  - Impaired executive function
  - Slow processing speed
- Migratory neuropathy/fasciculations
- Eye findings/neuroretinitis
- Rage and Aggression
- Constant and daily “flare”
- Anxiety, depression, OCD
- Foot/heel pain
- Striae
  - Do not follow dermal lines
  - Blanch

Berghoff W. Chronic Lyme Disease and Co-infections: Differential Diagnosis. Open Neurol J. 2012;6:158-78. doi: 10.2174/1874205X01206010158. Epub 2012 Dec 28. PMID: 23400696

Bartonella & Eye Disease

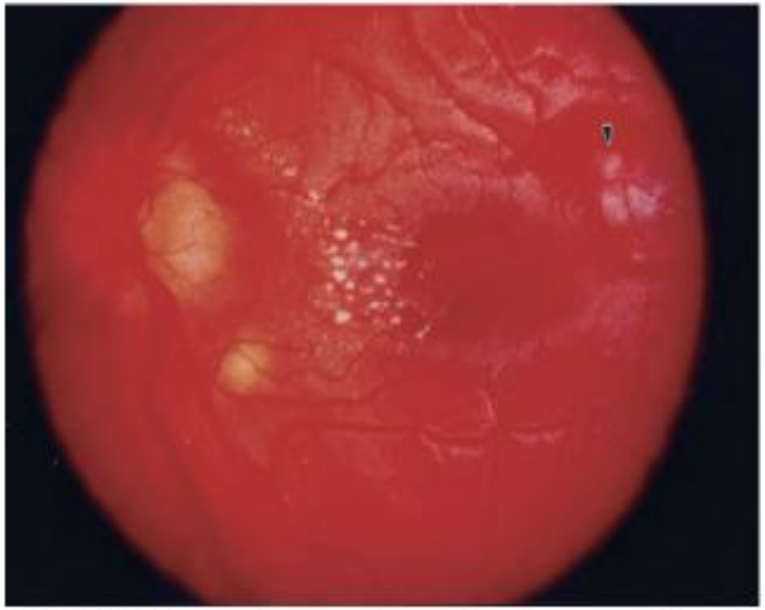


FIGURE 6. *Bartonella henselae*–associated neuroretinitis. An edematous optic disk, partial macular star, and two separate foci of retinochoroiditis, one inferotemporal to the optic disk and a second superotemporal to the fovea, which has produced a small branch artery occlusion (arrowhead). Reproduced with permission from *Retina*.[61]

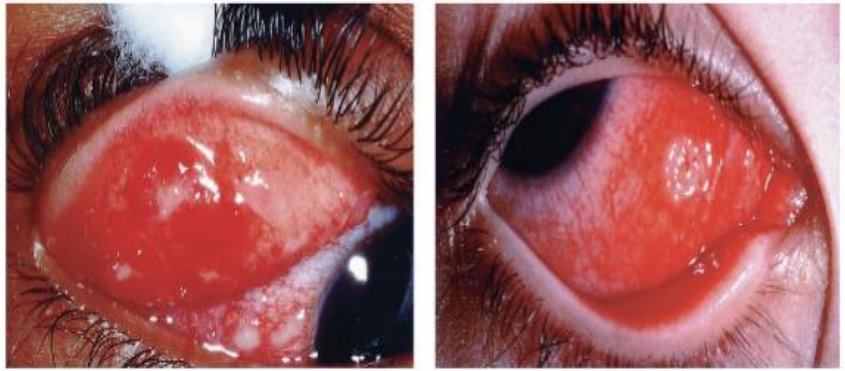


FIGURE 5. *Bartonella henselae*–associated conjunctivitis. Granulomatous nodules on (Left) the upper tarsal and bulbar conjunctiva of a 10-year-old boy, and (Right) the bulbar conjunctiva of a 12-year-old-girl (courtesy of Dr John P. Whitcher and Dr Bruce E. Silverstein). Note the necrotic center and ulcerated epithelium over the bulbar lesion (right). Anti–*B. henselae* serum antibody titers were markedly elevated in each patient.

- Uveitis
- Conjunctivitis
  - Granulomatous nodules
  - Ulcerated epithelium
- Spirochetes in vitreous
- Otherwise negative TBD testing

Tey, MY., Govindasamy, G. & Vendargon, F.M. The clinical spectrum of ocular bartonellosis: a retrospective study at a tertiary centre in Malaysia. *J Ophthal Inflamm Infect* **10**, 31 (2020). https://doi.org/10.1186/s12348-020-00224-0

* Babesiosis Basics

- Most common Lyme disease co-infection
- Similar to malaria in symptoms and treatment
- Acute Babesiosis – From CDC
  - Many people who are infected with *Babesia microti* have NO symptoms.
  - Some people develop flu-like symptoms (fever, chills, sweats, headache, body aches, loss of appetite, nausea, or fatigue)
  - *Babesia* parasites infect red blood cells and can cause hemolytic anemia (from the destruction of red blood cells).
  - Babesiosis can be a severe, life-threatening disease, particularly in people who
    - Do not have a spleen;
    - Have a weak immune system for other reasons (such as cancer, lymphoma, or HIV); what about autoimmune disease, PANS, PANDAS, ASD?
    - Have other serious health conditions (such as liver or kidney disease); or
    - Are elderly.

Vannier E, Gewurz BE, Krause PJ. Human Babesiosis. Infect Dis Clin North Am. 2008;22(3):469-488,ix. doi:10.1016/j.idc.2008.03.010

* Babesiosis

- Chronic Symptoms
  - Unrelenting headache (especially head pressure)
  - Paresthesias and dysautonomia
  - Night sweats
  - Rib and bone pain
  - Cough and Air Hunger
  - Encephalopathy, anxiety
  - Myalgias and arthralgias
  - Brain fog, depression, insomnia
  - Gastrointestinal symptoms

* Babesia Species

- More than 100 species have been identified globally (group of tick-borne protozoal parasites under the order *Piroplasmida)*
  - Babesiosis (*Babesia bigemina)* was first discovered in cattle in 1888
  - First human case (most likely *B. divergens*) was identified in 1957.
- According to CDC - Many different species (types) of *Babesia* in animals
  - *Babesia microti*—which usually infects white-footed mice and other small mammals—is the main species that has been found in people in the US.
  - Occasional cases caused by other *Babesia* species have been detected.
- *B duncani*
- *B. odocoilei* serologically cross-reacts with *Babesia duncani*.
- There are more than two *Babesia* spp. in North America that cause human babesiosis.

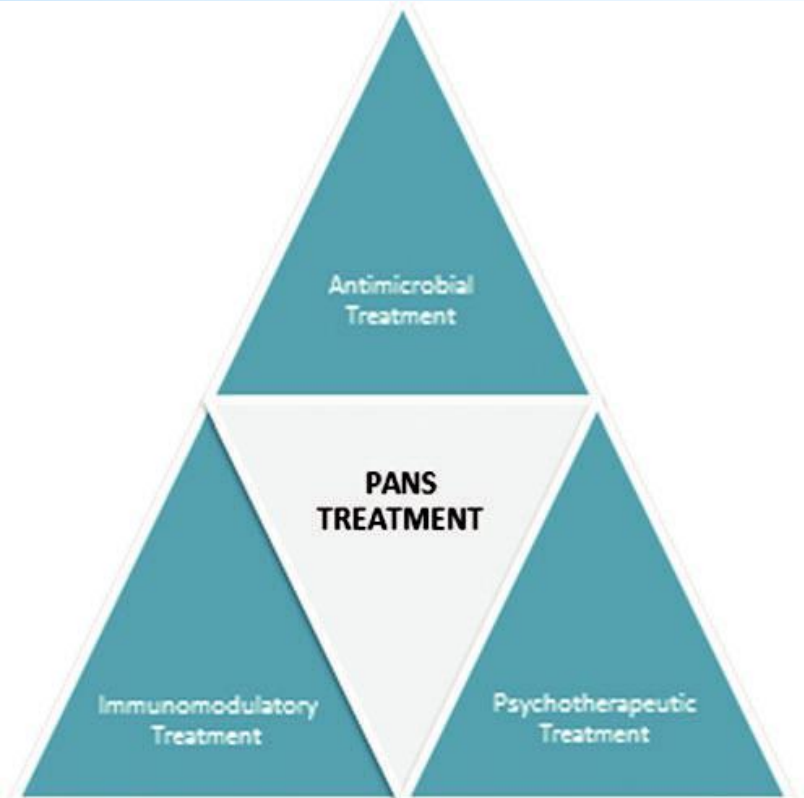
Lee, S. et al. (2022). Evolutionary analysis of *Babesia vulpes* and *Babesia microti*-like parasites. *Parasites & Vectors*, *15*, Article 404. https://doi.org/10.1186/s13071-022-05528-9
https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-022-05528-9

* Testing - Possible Choices & Pet Peeves

- **TBD**
  - IGeneX
    - * Immunoblot IgM and IgG
    - * FISH – Bartonella & Babesia
  - Quest for Borrelia miyamotoi (Imugen test)
  - Quest – Borrelia Immunoblot (relatively accurate)
- Galaxy for Bartonella
- Do NOT order a bunch of tests that you don't know how to interpret and open pandora's box
- Lone Star tick is mainly located in the southeastern and eastern U.S
  - Responsible for spreading **ehrlichiosis,** Heartland virus disease, southern tick-associated rash illness (STARI), and tularemia
  - Bites from the Lone Star tick can sometimes lead to alpha-gal syndrome
  - You can NOT miss a lonestar tick bite; Test if have known exposures

* Tests Worth Considering

- DNA testing
  - For acute tick bites or acute Lyme when antibody testing not indicated
    - * Quest (or similar local lab)
    - * Clongen (DNA tissue testing at birth for moms with TBD)
- MDL – ELISA & Western blot
- T Labs
  - Microscopic imaging methods & molecular probes
  - Researching causes, mechanisms, and tissue injury resulting from inflammation.
  - Tests in various stages of development to show how vector-born diseases contribute to inflammation
  - rRNA probes
    - * Higher sensitivity
    - * Reflect metabolic activity & demonstrate metabolically active infection



Three modes of intervention

* Treating the symptoms including supportive interventions (CBT, supplements, psychoactive medications)
* Removing the source of the inflammation – treating with antimicrobials
* Treating immune disturbances with immunomodulatory and/or anti-inflammatory interventions

Swedo et al, J Child Adol Psychopharm, 2017

* Tick Bite Treatment

- Tick bite considerations
  - NOT one dose DOXYCYCLINE
  - LEDUM 30c 1/day for 7-10 days

- For an acute tick bite get topical DMSO gel from a local pharmacy- add 4 drops CATS CLAW and 4 drops OTOBA BARK to 1-2 drops of the DMSO cream and rub it on the bite. Do this twice a day for 7 days.

- Consider the following for 1-3 months. DO at once, not one at a time:
  - Add CATS CLAW 10 drops per day 1-2x a day
  - Add OTOBA BARK same
  - Add CAMPSIANDRA same
  - Consider or STEVIA
  - GI & Detox support - Modified Citrus Pectin, Castor oil and/or EPSOM SALT

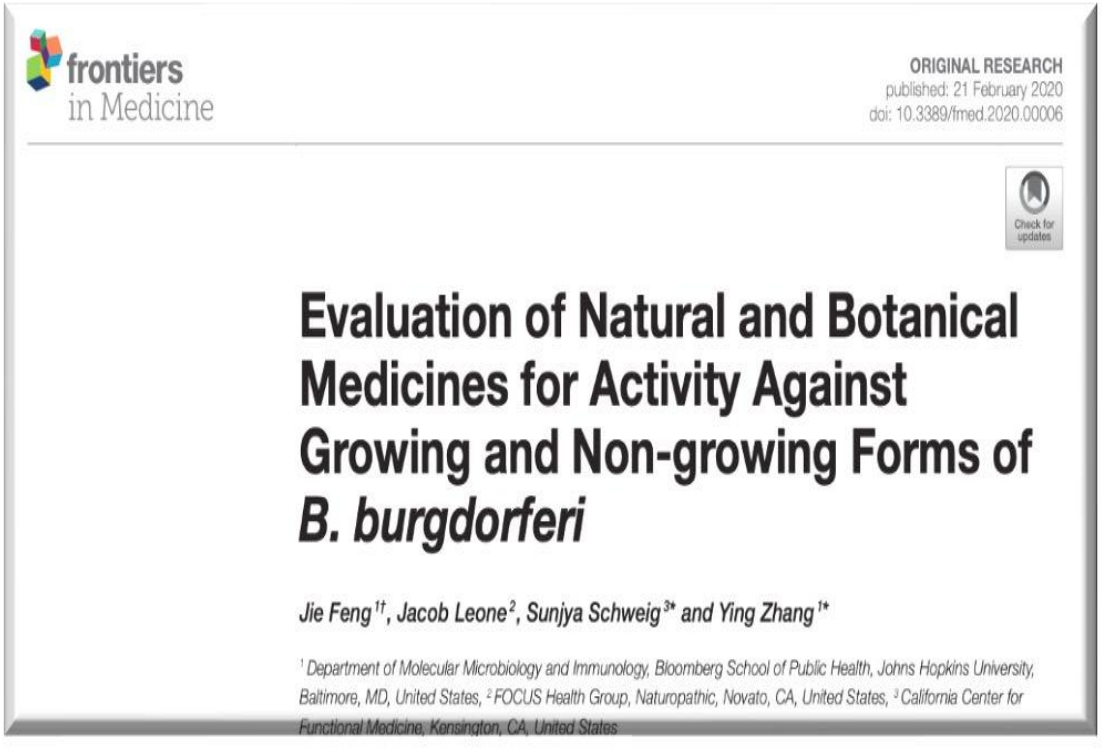
- Medications – tetracyclines – MINOCYCLINE or DOXYCYCLINE for 1-3 months
  - 200 mg if > 100 lbs
  - 4.4 mg/kg for children of any age weighing less than 45 kg
  - 40 - 100 pounds = 100 mg

- Antibiotics
- Herbals (Clarks Rule $(childs\ weightin\ pounds \div 150) \times 30)$
- LDA & LDI
- Essential Oils
- Immune Support
- Nutrition & Gut Healing
- Lifestyle

*Treatment

ANTIBIOTICS: SITES OF ACTIVITY

Cell wall	Intracellular	Cyst
Amoxicillin	Clarithromycin	Metronidazole
Amoxicillin-clavulanate	Azithromycin	Tinidazole
Penicillin G	Tetracycline	Hydroxychloroquine
Benzathine PCN	Doxycycline	Tigecycline
Cefuroxime	Minocycline	Daptomycin
Cefuroxime	Tigecycline	
Cefdinir	Fluoroquinolones	
Ceftriaxone	TMP/SMZ	
Cefotaxime	Rifampin	
Ceftibuten	Other Rifamycins	
Aminoglycosides		
Vancomycin		



frontiers in Medicine

ORIGINAL RESEARCH
published: 21 February 2020
doi: 10.3389/fmed.2020.00006

Check for updates

Evaluation of Natural and Botanical Medicines for Activity Against Growing and Non-growing Forms of *B. burgdorferi*

Jie Feng[1†], *Jacob Leone*[2], *Sunjya Schweig*[3*] *and Ying Zhang*[1*]

[1] Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [2] FOCUS Health Group, Naturopathic, Novato, CA, United States, [3] California Center for Functional Medicine, Kensington, CA, United States

Cryptolepis sanguinolenta**

Polygonum cuspidatum

Scutellaria baicalensis

Juglans nigra **(Stationary, Not Growing)**

Artemisia annua **(Stationary, Not Growing)**

Uncaria tomentosa **(Stationary, Not Growing)**

All of these herbs outperformed Doxycycline & Cefuroxime

Cryptolepis is only herb or medication tested to eradicate B. burgdorferi stationary forms in subculture

JAPANESE KNOTWEED

- Inhibits the cytokine cascade initiated by Bartonella
- Active against stationary phase non-growing *B. henselae* and against log phase growing *B. henselae*

BERBERINE

- High activity against stationary phase *B. henselae* *(Li et al., 2019)*

CRYPTOLEPIS

- Active against stationary phase non-growing *B. henselae* and against log phase growing *B. henselae*
  - *Ma et al., 2020*

ARTEMISIA

- Against stationary phase of Borrelia burgdorferi (Feng et al, 2020)
- Reduces memory impairment when combined with IV ceftriaxone in Lyme patients (Puri et al, 2017)

STEVIA

- Efficacy of leaf against all forms of *Borrelia burgdorferi and* ~40% efficacy in reducing attached biofilm mass *(Theophilus et al., 2015)*

Others:

* **Cat's Claw**
* **Houttynia**
* **Otoba Bark**
* **Campsiandra**

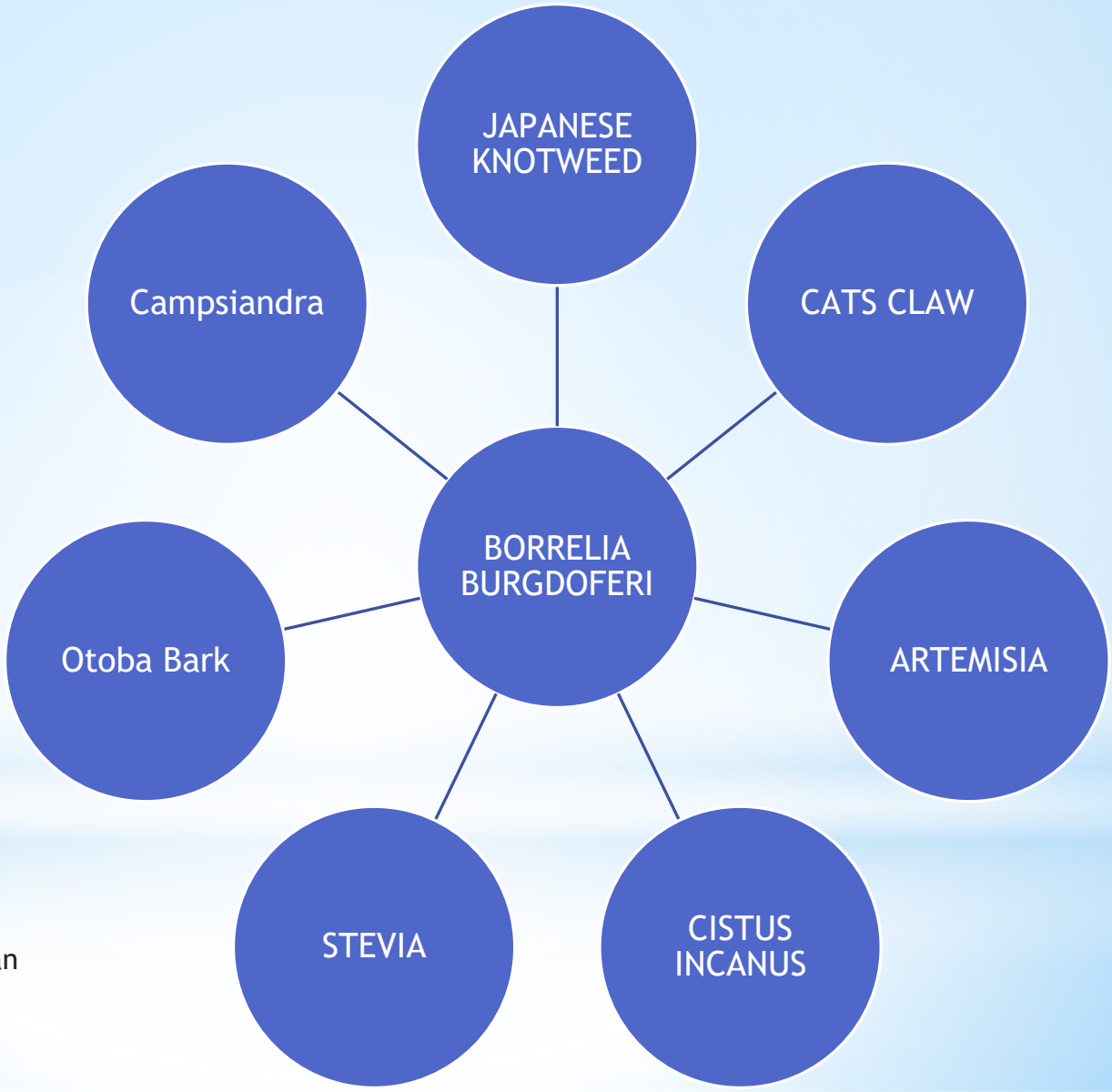
Clark's Rule (Wt in lbs/150)x30

* 2 week Rotation of:

Cat's Claw, Stevia & Otoba Bark

Then Cat's Claw, Campsiandra & Stevia

Take 1 ½ days (3 doses) off in between changes



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* Bartonella Treatment

Antibiotics

- Azithromycin
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- Clarithromycin
  - Rolain JM, Brouqui P, Koehler JE, Maguina C, Dolan MJ, Raoult D. Minireview. Recommendations for Treatment of Human Infections Caused by *Bartonella* Species. *Antimicrob Agents Chemother*. 2004:1921–33.
- Trimethoprim-Sulfamethoxazole
  - Florin TA, Zaoutis TE, Zaoutis LB. Beyond cat scratch disease: widening spectrum of *Bartonella henselae* infektion. *Pediatrics*. 2008;121:e1413.
- Rifampicin
  - Tsuneoka H, Yanagihara M, Nojima J, Ichihara K. Anitmicrobial susceptibility by Etest of Bartonella henselae isolated from cats and human in Japan. *J Infect Chemother*. 2010;16(6):446–8.
- Doxycycline/Minocycline
  - Foucault C, Raoult D, Brouqui P. Randomized open trial of gentamicin and doxycycline for eradication of Bartonella quintana from blood in patients with chronic bacteremia. *Antimicrob Agents Chemother*. 2003;47(7):2204–7.
- Ciprofloxacin
- Methylene Blue
- Clotrimazole & other azole antifungals

* Antibiotic Treatment of Bartonella

- Multiple Antibiotics
- Duration - Weeks? Months? … Longer!
- May require multiple courses of tx & Use of BIOFILM Busters

1. Macrolide and Tetracycline-Based Treatments

Take one of the following macrolides:

- Azithromycin 250-500 mg 1 pill/day; or Clarithromycin 500 mg 2x/day

Combine with one of the following tetracyclines:

- Minocycline 100 mg 1-2x/day; or Doxycycline 100 mg 1-2x/day

Combine with one of the following persister agents:

- Methylene blue 25-50 mg 1 pill 2x/day (compounded); or
- Oil of Oregano or GSE (1-2 pills/day)

Li T, Feng J, Xiao S, Shi W, Sullivan D, Zhang Y. Identification of FDA-approved drugs with activity against stationary phase Bartonella henselae. Antibiotics (Basel). 19;8(2):50. doi:10.3390/antibiotics8020050

* Antibiotic Combinations

2. Rifamycin-Based Treatments

- Rifampin 300 mg 1-2 pills 1-2x/day; or Rifabutin 150 mg 1-2 pills 1-2x/day.

Combine with one of the following macrolides, tetracyclines, or Bactrim:

- Azithromycin 500 mg 1 pill/day; or Clarithromycin 500 mg 1 pill 2x/day;
- Minocycline 100 mg 1 pill 1-2x/day; or Doxycycline 100 mg 1 pill 1-2x/day; or
- Bactrim DS 1 pill 1-2 times a day.

Combine with one of the following persister agents:

- Methylene blue 25-50 mg 1-2x/day (compounding pharmacy); or

3. Methylene Blue-Based Treatments

- Methylene blue 25-50 mg 1 pill 2x/day (compounding pharmacy).

Combine with one of the above macrolides or tetracyclines

Zheng X, Ma X, Li T, Shi W, Zhang Y. Effect of different drugs and drug combinations on killing stationary phase and biofilms recovered cells of Bartonella henselae in vitro. BMC Microbiol. 2020;20(1):87. doi:10.1186/s12866-020-01777-9

* Herbal Interventions

- Bartonella –
  - Houttuynia (50% success) partnered with Campsiandra (75% success)
  - Add Stevia
    - For biofilm and persistors
    - Taste
  - With drop to drop increases do not herx as badly
  - Start with 1 drop, increase by 1 drop per day to 30-60 drops/day or maximum as calculated by Clark's Rule (weight (lbs)/150) x 30
- If above does not work
  - Add Japanese Knotweed
  - Pull out Campsiandra and add Cryptolepis (Lymecore)
- If no significant improvement in symptoms or if relapse
- Add lumbrokinase to break apart Bartonella-fibrin nests
- Add Modified Citrus Pectin

Ma X, Leone J, Schweig S, Zhang Y. Botanical medicines with activity against stationary phase Bartonella henselae. Infect Microb Dis. 2021;3(3):158-167. doi:10.1097/IM9.0000000000000069

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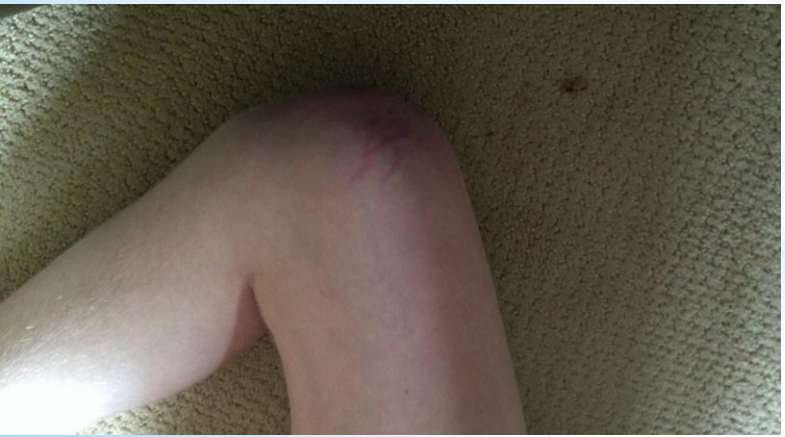
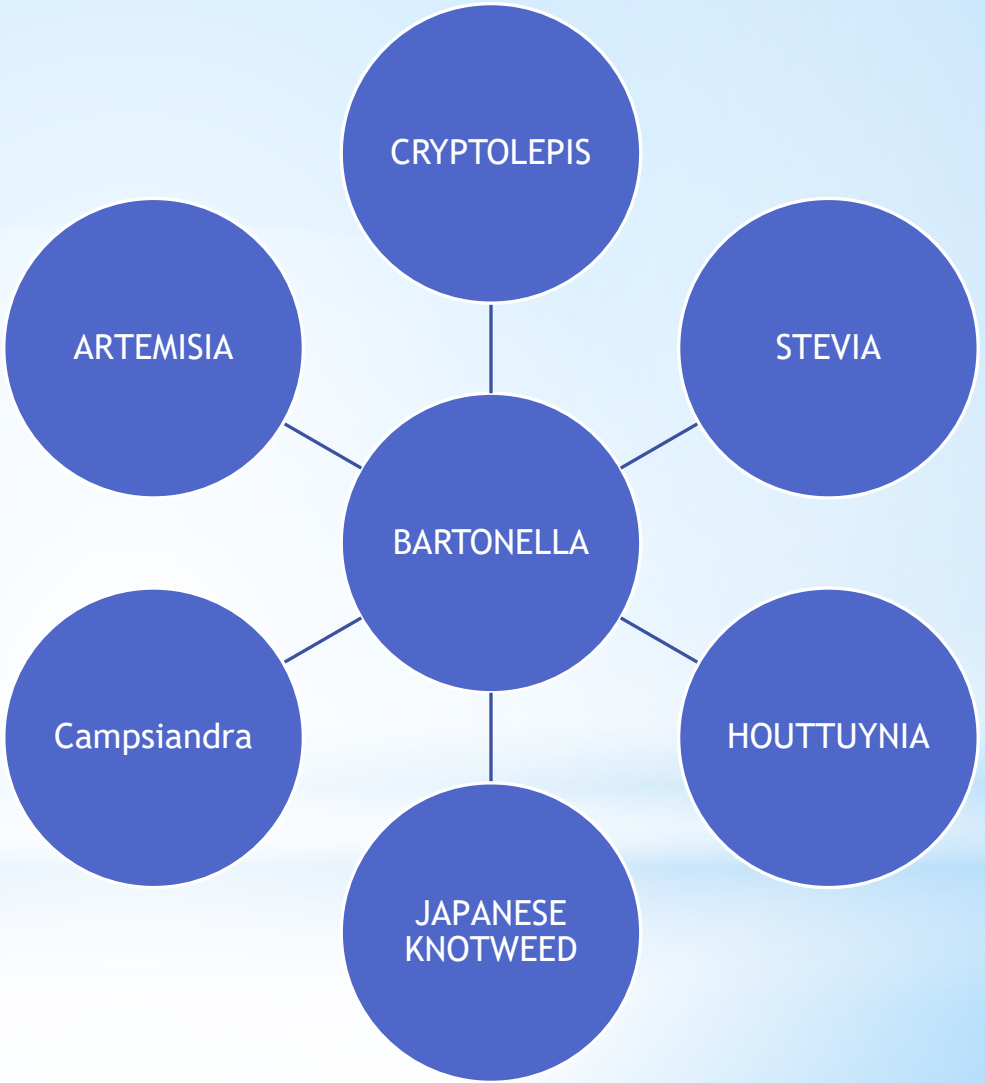
Cheslock MA, Embers ME. Human Bartonellosis: An Underappreciated Public Health Problem? Trop Med Infect Dis. 2019 Apr 19;4(2):69. doi: 10.3390/tropicalmed4020069. PMID: 31010191; PMCID: PMC6630881.





* Babesia Treatment

Antibiotics

- Atovaquone plus azithromycin is the preferred antimicrobial combination
- Clindamycin plus quinine is the alternative choice
- Duration of treatment - 7-10 days (extended when immunocompromised
- Atovaquone/Proguanil (Malarone) 250 mg/100 mg. 2 pills 2 times a day Atovaquone (Mepron) 750 mg/5 ml 10 ml 1-2 times a day. Combine with one of the following:
  - Azithromycin (Zithromax) 500 mg 1 time a day;
  - Clarithromycin (Biaxin) 500 mg 1 pill 2 times a day;
  - Doxycycline 100 mg 2 pills 2 times a day; or
  - Minocycline 100 mg 1 pill 2 times a day.

Be sure to take atovaquone with fat because this increases its absorption. Sources of fat include nuts, nut butter, butter, oils (like coconut oil or flaxseed oil), yogurt, cheeses, and avocado.

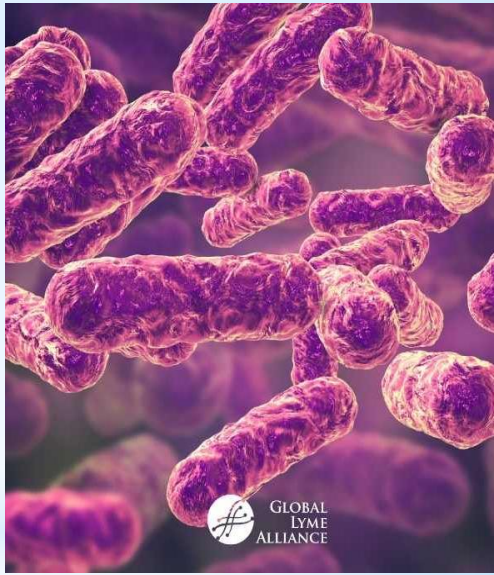
* Antibiotics Continued

Tafenoquine 150 mg (Krintafel) 2 pills 1 time a day every week.
Combine it with one of the following:

- Azithromycin (Zithromax) 500 mg 1 time a day;
- Clarithromycin (Biaxin) 500 mg 1 pill 2 times a day;
- Doxycycline 100 mg 2 pills 2 times a day; or
- Minocycline 100 mg 1 pill 2 times a day.

In addition, if these combinations are not working, add

- Atovaquone 750 mg/5 ml 2 times a day.



Dosing in Children

- 5-10 kg received 50 mg
  10-20 kg received 100 or 150 mg 20-35 kg received 200 mg
  >35 kg received 300 mg
- Check G6pd and be aware of risk of methemoglobinemia

* Herbal Interventions

- This study of babesia treatments
  - Bioactive compounds derived from *Cryptolepis sanguinolenta*, *Artemisia annua*, and Scutellaria baicalensis, had comparable or even better activity against B. duncani than the commonly used antimicrobial medications quinine and clindamycin.
- Cryptolepis is an herbal medicine originally used in Ghana to treat malaria. Works about 75 percent of the time.
- Artemisinin
  - Often it causes a worsening of the Babesia symptoms
  - Artemisinin resistance can occur
  - In malaria, it is shown that artemisinin works best by pulsing

Others - Sida Acuta/Alchornea

Botanical Medicines Cryptolepis sanguinolenta, Artemisia annua, Scutellaria baicalensis, Polygonum cuspidatum, and Alchornea cordifolia Demonstrate Inhibitory Activity Against Babesia duncani

*Yumin Zhang[1], Hector Alvarez-Manzo[1], Jacob Leone[2], Sunjya Schweig[3] and Ying Zhang[4]**

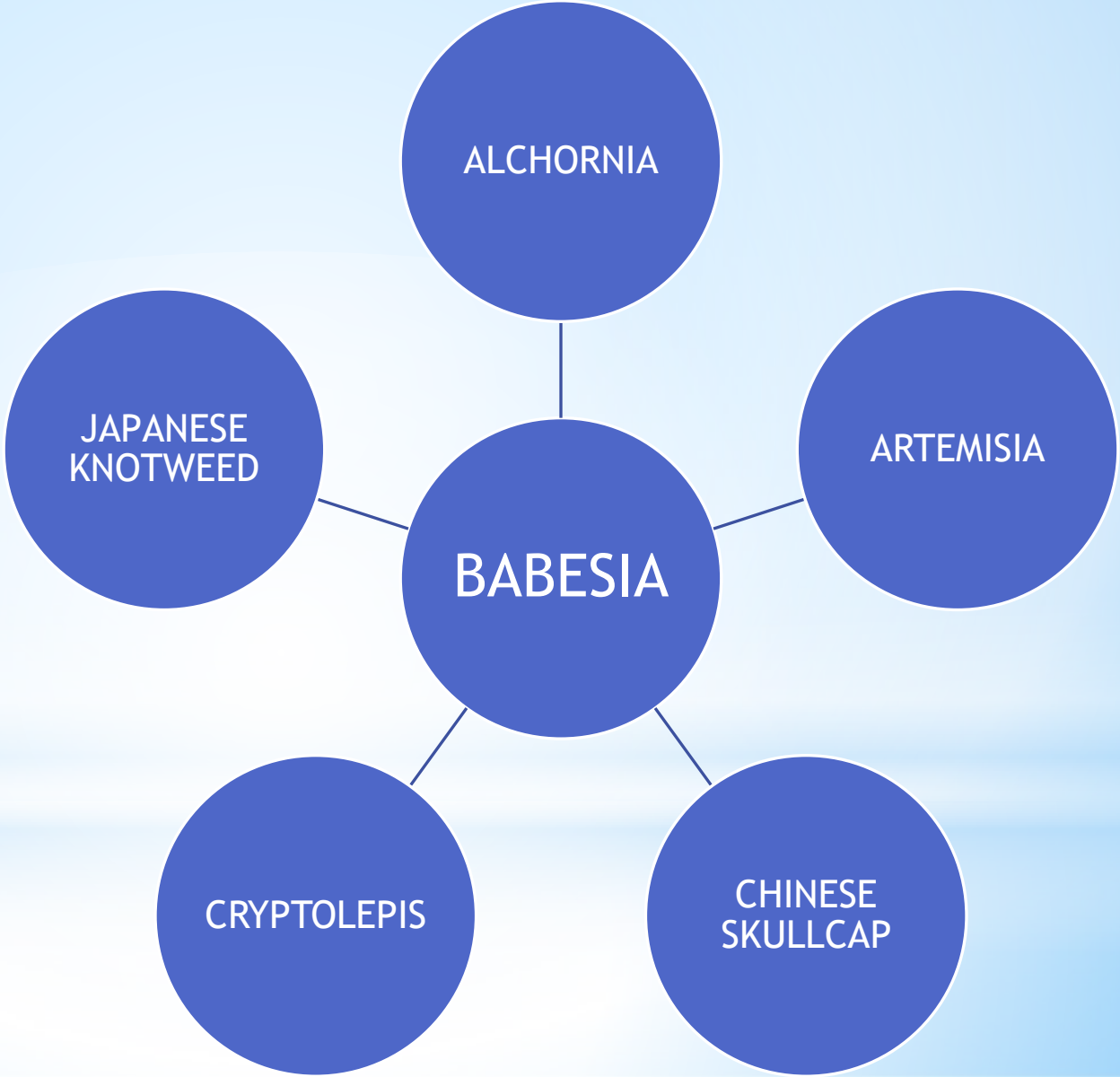
[1] Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [2] FOCUS Health Group, Naturopathic, Novato, CA, United States, [3] California Center for Functional Medicine, Kensington, CA, United States, [4] State Key Laboratory for the Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Zhang, Y., *et al.* (2021) Botanical Medicines Cryptolepis sanguinolenta, Artemisia annua, Scutellaria baicalensis, Polygonum cuspidatum, and Alchornea cordifolia Demonstrate Inhibitory Activity Against Babesia duncani. *Frontiers in Cellular and Infection Microbiology*. doi.org/10.3389/fcimb.2021.624745

* Atovaquone & Azithromycin

Cryptolepis and Cat's Claw

As well as Artemisia PULSING around new and full moons



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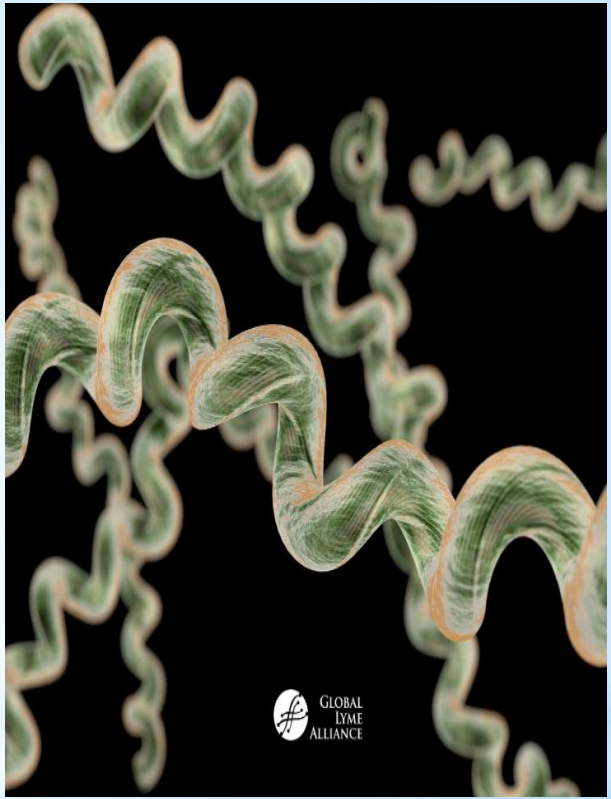
*Treatment Pitfalls

* **Not addressing Multi System nature of problem** - Merely treating the infection does not stop the autoimmune response that has already been triggered
* **Not using food-based binders like modified citrus pectin**
* **Not providing immunotherapy**
* **Not monitoring & treating yeast and other dysbiosis (especially with antibiotic use)**
* **Not treating mitochondrial, metabolic & nutrient deficiency issues**



ROADMAP

6. Re-check & Wean

- Once symptom free for 2 months, wean off protocol in the reverse order
- Remain on the basic nutrients still needed for optimal health
- Retest levels as warranted

4. Add Killers

- Antimicrobials
- Continue 1, 2 & 3

5. Monitor Progress

- Monitor progress every 8 weeks
- Look for 25% improvement by 90 days
- Treat til symptom free for 2 months
- Use rating scales
- Repeat abnormal labs as necessary (6-12 months) Treat the individual patient

3. Balance and rebuild

- Immune support
- Anti-inflammatory
- Binders

2. Remove & Replenish

- Gut healing (diet & nutrition)
- Food sensitivities
- Elimination (sweating and stooling)
- Sleep

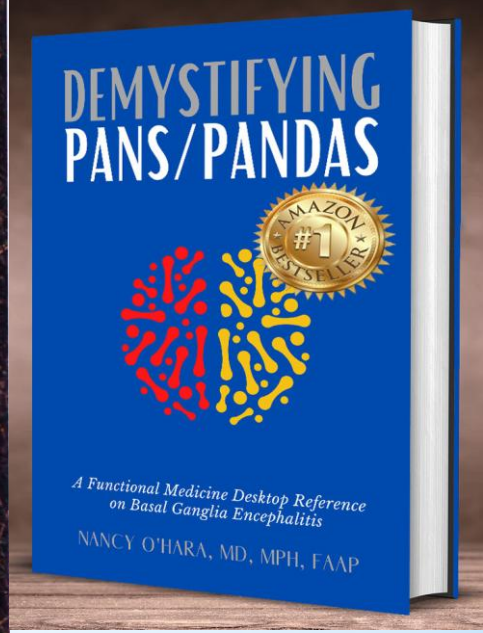
1.Test and Address Lifestyle

- Tick or Vector Borne Diseases
- CIRS - Mold & Mycotoxins
- Other Triggers - Viral, Bacterial, Fungal, etc
- Nutrient Deficiencies, MTHFR, SNPs
- Gut Function & Diet
- Inflammatory, Allergic and Immune markers
- Sleep, stress, exercise

* PANS Recap As It Applies to TBD/VBD

- **Clinical diagnosis** confirmed (or not) by laboratory evidence
- Complete medical & psychiatric history and physical exam
- Testing
  - Appropriate Borrelia, Bartonella, Babesia suspicion and testing
  - Immune markers (ANA, CRP, ESR, thyroid antibodies, immunoglobulins including subclasses, strep pneumococcal serotypes)
  - Mitochondrial, metabolic, antioxidant markers as warranted
- Treat the patient in front of you
- Treatment plan should include antimicrobial interventions, immunomodulatory interventions, and symptom support
- Be mindful of the sensitivity of your patients to determine dosage
- Anticipate & discuss Herxheimer reaction
- Treatment is a marathon and not a sprint, but things will get better

Do not go where the path may lead.
Go instead where there is no path
and leave a trail…
-Emerson



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Advanced Topics In Testing for Tick-borne Diseases

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February 2025

CONCURRENT INFECTIONS WITH TICK-BORNE PATHOGENS

Clinical "Lyme" is more than a Borrelia infection

A 2018 study of 10,000+ patient samples tested at IGeneX:

- 37.3% were positive for Babesia species
- 32.1% for Lyme Borrelia
- 27.7% for TBRF Borrelia
- 19.1% for Bartonella
- 16.7% for Anaplasma
- 12.8% for Rickettsia
- 6.9% for Ehrlichia

Concurrent infections

- 40% tested positive for two pathogens
- 15% tested positive for three
- 4.6% tested positive for four
- 0.7% tested positive for five

Note: These percentages reflect samples tested by IGeneX. As a reference lab, they often receive samples from more-ill patients so this may not reflect true USA incidence

Borrelia species known to infect humans

Lyme: B. Burgdorferi *senso lato* (Bb sl)

B. burgdorferi B31 (Bb ss)

B. burgdorferi 297

B. californiensis

B. mayonii

B. afzelii

B. garinii

B. spielmanii

B. valaisiana

B. bisettii

Others….

Tick-borne relapsing fever Borrelia (TBRF)

B. hermsii

B. miyamotoi

B. turcica

B. turicatae

B. coriaceae

B. parkeri

B. texasensis

B. anserina

B. lonestari

B. turicate

Others….

- Species in red represent those that large commercial labs test for
- IGeneX testing is capable of detecting these

Tick-borne relapsing fever (TBRF)

TBRF misinformation

- Was thought to be rare in the USA
  - **Not true!** Is found all over the USA and around the world
- Said to have a distinctive clinical presentation that could not be missed
  - Ha! **NOT TRUE**- can be EXACTLY like Lyme and therefore not correctly diagnosed
- Said to be only transmitted by “soft ticks”
  - Ha again!- **also not true**.

Clinical presentation of classic TBRF

- “Recurring febrile episodes that last ~3 days and are separated by afebrile periods of ~7 days duration.”
- “Each febrile episode involves a “crisis.” During the ‘chill phase’ of the crisis, patients develop very high fever (up to 106.7°F) and may become delirious, agitated, tachycardic and tachypneic. Duration is 10 to 30 minutes.”
- “This is followed by the ‘flush phase’, characterized by drenching sweats and a rapid decrease in body temperature, and patients may become hypotensive”.
- Patients who are not treated will experience several episodes of fever before illness resolves.”

But can also present like Lyme!

543 US patients with suspected Lyme:

- 29% were positive for Ab to TBRF (tested for just 2 species)

Cohort of 321 California residents with suspected Lyme:

- 38% were positive for Ab to TBRF (tested for just 2 species)

These patients did not have the “classic” acute TBRF presentation.

Seronegative for Lyme? Perhaps it is TBRF

- **NONE** of the available Lyme IFAs, ELISAs, western blots, PCRs or T-cell tests have been validated for any TBRF species

Always consider TBRF when testing for Lyme

- However, commercial TBRF serologic testing has only been validated against two species (hermsii and miyamotoi) and each test has to be ordered individually
- IGeneX TBRF testing is broadly sensitive to enable it to detect multiple TBRF species

TBRF- Important Facts

- Transmission within 15 seconds of tick bite!!!! (Soft ticks)
- Maternal-fetal passage well recognized and accepted
  - Spontaneous abortion, premature birth, and neonatal death
- Louse-borne RF (*B. recurrentis*) transmission via mucous membranes!!
- **A**cute **R**espiratory **D**istress **S**yndrome has been associated with *B. hermsii* (CDC)
- Some TBRF species are immune to complement-mediated killing, just like Lyme (immune evasion)
- Prolonged QT interval has been reported with TBRF infection- can lead to dangerous drug interactions
  - ***EKG is recommended at time of diagnosis, and before starting treatment***

Laboratory testing for TBRF- pitfalls

Standard TBRF serologies are not very good

- Large commercial labs- test for *B. hermsii* only (by IFA)
- Can get a GLP-protein based ELISA for *B. miyamotoi*
- GLP is the only one protein antigen in this test and not all miyamotoi express this- therefore prone to false negatives

Most TBRF species express some antigens found in Lyme

- Band p41 is common; band p23 occasionally
- Can get false positives on some ELISAs because of this
- Borderline ELISAs or a single band of 23 or 41 on a Lyme western blot may be a hint that TBRF is present and not Lyme

Solution is to use tests specific for TBRF:

- IGeneX recombinant-based serologies (**AcuDart, ImmunoBlot**), T-cell stimulation assays, PCRs and cultures

Babesia species known to infect humans (partial list)

More commonly found

- **B. microti**
- **B. duncani**
- B. MO-1
- B. odocoilei
- B. bigemina
- B. divergens
- B. venatorum

Also reported

- B. vogeli
- B. crassa
- B. vulpes
- B. ovata
- B. poelea

- Species in red represent those that large commercial labs test for
- IGeneX testing is capable of detecting these

The better known Bartonella species, their hosts, and their vectors (partial list- are over 45 species)

Bartonella Species	Host (s)	Vector(s)
B. henselae	Cat, human, dogs, horses	Fleas, lice, ticks, spiders
B. quintana	Humans, macaques, cats, dogs	Human body lice, fleas, bed bugs
B. bacilliformis	Humans	Sandflies, fleas
B. koehlerae	Cats, dogs, humans	Fleas
B. vinsonii ssp. berkhoffi	Dogs, horses, foxes, humans	Fleas, ticks
B. bovis	Cattle, cats, dogs, human	Biting flies, ticks
B. clarridgeiae	Cats, dogs	Fleas, ticks
B. rattimassiliensis	Rats	Fleas
B. tamiae	Rats, humans	Mites
B. tribocorum	Rats	Fleas
B. rousetii	Bats	Bat flies
B. schoenbuchensis	Cattle	Biting flies, ticks
B. chomelii	Cattle	Biting flies, ticks
B. doshiae	Rats, humans	Fleas
B. grahamii	Mice, humans	Fleas
B. birtlesii	Mice	Fleas
B. mayotimonensis	Bats, humans	Bat flies, fleas, ticks
B. elizabethae	Rats, humans, dogs	Fleas
B. washoensis	Dogs, humans	Fleas, ticks
B. rochalimae	Dogs, humans	Fleas, ticks
B. vinsonii ssp. arupensis	Dogs, humans	Fleas, ticks
B.melophagi	Sheep, humans	Sheep keds

- Species in red represent those that large commercial labs test for
- IGeneX testing is capable of detecting these

The table has been adapted from Breitschwerdt, 2017.

Rapid transmission of pathogens

- **Lyme disease**: W. Burgdorfer: “5-10% of ticks that are carrying Lyme Disease have a systemic infection and have the microbes in their saliva and can transmit it as soon as they bite.”
- **Relapsing fevers** can be transmitted in as little as one minute or less (soft-bodied ticks)
- **Bartonella** is found in tick saliva so rapid transmission is likely
- **Rickettsias** (Ehrlichia, Anaplasma, RMSF) and **arboviruses** are also present in tick saliva
- **Babesia**- “Invasion of the salivary gland by B. microti occurs before feeding of the nymph begins, and development of the parasite is further stimulated by feeding.”
  - Karakashian, S.J., Rudzinska, M.A., Spielman, A. et al. Ultrastructural studies on sporogony of Babesia microti in salivary gland cells of the tick Ixodes dammini . Cell Tissue Res. 231, 275–287 (1983). https://doi.org/10.1007/BF00222180

Also:

- **Improper tick removal**- grasp at skin surface and SLOWLY pull straight out
- **Infected tick feces**- is why antiseptics are needed after tick is removed

Available tests for TBDs

Serologies- are made up of protein antigens from the pathogen

- Standard: IFA, ELISA, Western blot; Microarrays (Vibrant)
- Advanced: LymeScreenAssay (LSA), Broad Coverage Assay (BCA), Recombinant ImmunoBlot- (IGeneX)
- Advanced: AcuDart (AcuDart Health)

T-cell mitogen stimulation assay- (IGeneX, Armin Labs)

Direct blood smear

- FISH (IGeneX, T-Lab)

Nucleic acid tests

- PCR, Culture-enhanced PCR (IGeneX), Droplet digital PCR- Galaxy

Direct antigen detection

- Urine antigen capture (Nanotrap- Galaxy; DotBlot-IGeneX)

Test method- IFA and ELISA (not recommended)

The test system contains antigens from the pathogen. Serum is added, and if antibodies to that specific antigen are present then you get a positive test

Reflects past exposure; single species only (lab strain)

IFAs and ELISA antigens come from either whole cell sonicates, or are made from one specific antigen- both have disadvantages

- Whole cell is too nonspecific and single antigen is too insensitive
  - **Example**- in Lyme, a sonicated whole Borrelia releases many non-specific proteins that can be present in other pathogens- can lead to false positives
  - **Example**- In Lyme, single antigen tests that use only p41 are not specific because most spirochetes and some other bacteria express this- again, false positives. And some pathogenic Borrelia do not express p41- false negatives
  - **Example**- In TBRF, the ELISA for B. miyamotoi targets only one antigen- GLpQ and since not every RFB expresses this, sensitivity suffers

Due to low sensitivity, these methods are not useful for early disease (<6 weeks) or for the chronic patient

Test method- Microarrays (not recommended)

Antigens are added to small wells and patient serum is added. Positive reactions (usually fluorescence) are recorded by machine

- Still subject to calibration issues- how much fluorescence is required to be called positive?
- Antigens- can be from lysed organisms or can be individual antigens
  - Source is critical- risk is of cross reactivity with antigens from other organisms and autoantibodies
- So far, published data shows Lyme sensitivity comparable to the CDC two-tier method (Vibrant Labs). No data for other pathogens
  - CDC Two-tier test for Lyme has only a 57% sensitivity
- Clinical experience indicates that specificity is low too

Interpreting IFA, ELISA and Microarrays

Results are framed around a statistical "cutoff"

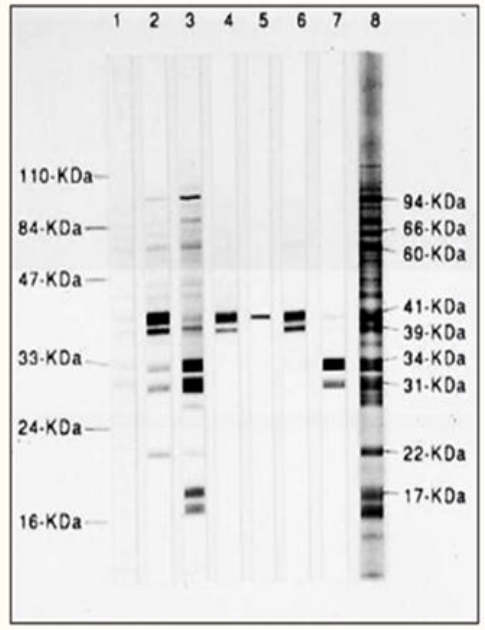
- For 95+% sensitivity, positive cutoff needs to be 3 SD above the negative control; some labs use 2 SD
- Borderline is usually 2 SD above negative control, some labs use 1 SD
- **Cutoff value is an artificial number**
- Tradeoff between sensitivity and specificity
  - **Borderline results** simply indicate uncertainty- increased chance of false positive if you rely on a borderline result
  - **A borderline result suggests you need a more specific test, or look for different pathogens, or autoimmunity**

Test method- Western Blots (not recommended)

Western Blot antigens also come from sonicated whole organisms

- Released antigens are separated by electrophoresis and placed on the test strip. Patient serum is added and if there are antibodies to any of the antigens on the strip, then after processing, a spot or “band” will appear
- The location of these bands are lined up with bands from a positive control- this is how bands are identified and named
- Many antigens are released that can co-migrate with those from other organisms and autoantibodies, causing false positives
- Quantity of both the antigens and the antibodies will affect band intensity and test interpretation
- Can detect only one species at a time

Result is poor sensitivity and poor specificity, plus limited species coverage



Significant Lyme disease antibodies detected on Western blot test, including 31- and 34-kilodalton bands. Courtesy of IGeneX, Inc.

Test method- Recombinant antigen assays

Used by IGeneX for their serologies: LSA, BCA, AcuDart and ImmunoBlots- *these replace the IFA, ELISA, WB and microarrays*

- **Individual antigens are lab-created** by reverse engineering- constructed from cloned gene sequences that produce the protein antigens
- Antigens are individually selected to be as specific as possible and to allow detection of multiple strains and species
- Antigens are **printed** on test strips- this allows for exact quantities and reliable identification. Far more exact and repeatable than the electrophoresis used in western blots

Clinical:

- **Are the most sensitive serological methods**- can be used in all stages of illness (early, established, chronic)- positives have been seen just two weeks from bite!
- Can be done while on treatment

Reasons why serologies can give false negatives

- Insensitive test or poor calibration of test cutoff
- Done too early (non-recombinant assays)
- Low levels of free antibody
  - Serologies only detect free antibody, but antibodies can be trapped in immune complexes during antigen excess- early disease, the chronic patient, poor immune response
  - Is why some seronegative patients seroconvert after treatment
- Cases of immune dysfunction
  - Can be pre-existing, or as a result of co-infections (Borrelia, Babesia and Bartonella are all potentially immunosuppressive)
- Testing for the wrong organism
  - Symptom overlap, especially with Lyme vs TBRF

Test method- T-cell mitogen stimulation assays

If antigens are present, T-cells activate. This test measures the activation

Reflects past exposure

- Patient's T-cells are kept viable in a cell culture and antigens of the organism to be tested are introduced into the cell culture
- Activation is assessed by liberation of interferons (ELISPOT method measures production of interferon-gamma)

Clinical features-

- **When sensitive: very early (2 weeks) and in late in chronic illness**
- Because T-cell responses are independent of B-cell responses, can be positive in seronegative patients
- Can be designed to offer genus-level detection (IGeneX)- broadens coverage.
- Sensitivity and specificity each are about 80% when tested *within its optimal time window*

Useful in very early and late chronic infections, and with B-cell dysfunction

Test method- FISH

FISH- fluorescent in-situ hybridization assay- Direct visualization of the organism on a blood smear

Reflects current, active infection

- Uses fluorescent-labeled RNA probes specific to the pathogen
- Can identify organisms even if embedded in a biofilm: **Babesia and Bartonella**
- Genus-level detection to broaden species coverage (IGeneX only)
- 100% specificity- *Will not cross react with the other TBDs*

Clinical-

- Pathogenemia is high early in the infection, before effective immunity develops - positives can appear **very early** in disease (days)
- Pathogen load also increases **very late** in the infection as immunity declines and the organisms adapt to the host- another time when this test can be very helpful
- **Highly specific**, so a positive result should not be dismissed, but a negative does not rule out infection

Test method- PCR (poorly sensitive)

PCR- detects the pathogen's nucleic acids (usually DNA) in a specimen

- **Very low sensitivity in blood** samples due to low numbers of organisms, PCR inhibitors, host DNA and other technical factors
- However is very highly specific
- Can also test other body fluids (CSF, urine, etc.) but these have inhibitors too
- Reasonable test for solid tissues (biopsies)- yield is higher than blood
- IGeneX PCRs have genus-level detection to broaden species coverage
  - IGeneX improves test sensitivity by testing both whole blood and serum, and for Borrelia, is designed to detect both chromosomal and plasmid DNA in each

Available for Lyme and TBRF Borrelia, Babesia, Bartonella, Anaplasma, Ehrlichia and the Rickettsias

PCR Enhancements

Droplet digital PCR

- Increases number of PCRs done on a sample by breaking it up into many droplets that are individually tested

Pre-culturing

- Blood sample is incubated for two weeks then PCR is performed on the culture
- Increases sensitivity over standard PCR by a factor of 10 or more
- Galaxy- Bartonella
- IGeneX- culture-enhanced PCR **(cePCR)**
  - Lyme, TBRF, Babesia, Bartonella, Rickettsias

Urine antigen capture

In Lyme, during an active infection, Borrelia antigens spill into the urine

This test is a direct assay (urine, CSF) to detect these antigens

- **Lyme disease only**
- **Lyme Dot-blot** (IGeneX)- sensitivity 70+%, specificity good if no UTI
  - Multispecies (Bb sl)
  - Multiple antigens (31, 34, 39, and 93 kDa)
- **Nanotrap** (Galaxy Labs)
  - Multiple species (Bb sl)
  - But only single antigen (31kDa)
- Extremely helpful when impractical to draw blood (poor access, **newborns**, **young children**, etc.)

Urine antigen capture

CLINICAL

- Antigen spillage is not constant- varies widely
- Spillage and therefore sensitivity track symptom severity- symptom flares, Herxheimers, menses
  - To increase sensitivity in untreated patients, some clinicians pre-treat with antibiotics to induce a Herxheimer- meds days 1-5, test on days 2, 4 and 6
  - Even for patients who have been treated, it is usual to collect samples on three different days to increase yield.
  - Specific as long as there is no UTI, so recommend doing a concurrent U/A and urine culture
  - **Is the one direct test that can be done while on treatment, but only during symptom flares**
- **Not all of the three samples may be positive; believe the positive ones**

FDA Clearance of the IGeneX Lyme ImmunoBlot

Finally, a modern and highly accurate test is FDA-cleared

CDC two-tier testing for Lyme

Background:

- The idea behind two tier testing is to begin with a very sensitive screening test (tier one)- very sensitive, therefore will not miss any cases, at the cost of some false positives
- If the first test is negative, then the whole test is called negative and the second tier will not be done
- If the screening test is positive, then follow it with a very specific second test to confirm true positives and exclude false ones
- **For this to work, the first tier must be 99% sensitive, and the second tier should be as sensitive but also 95% specific**

However, tier one had been the ELISA- 50% sensitivity

Tier two had been the western blot, or another insensitive ELISA, both of which are not only poorly specific but not much more sensitive than the first tier- ***Overall only 57% sensitivity***

CDC and the Lyme western blot

CDC interpretation criteria: specifies which antigens must be detected to have a positive test. *Antigens are reported as BANDS*

Problems:

- They specified which bands to be included on the WB
  - To be called positive, they require at least **5 out of the 10 specified bands** to be reactive on the IgG WB: 18-, 23-, 28-, 30-, 39-, 41-, 45-, 58-, 66-, and 93
  - *Not every patient's sample will have 5 positive bands*
  - 2 of 3 on the IgM WB: 23, 39, 41

Other problems:

- They included bands which are NOT specific to Lyme Borrelia- this can give rise to false positives (18, 28, 30, 41, 45, 58, 66) (**7 out of 10!!**)
- They EXCLUDED bands (31 and 34) that are very specific to Lyme Borrelia- this gives rise to false negatives (excluded over conflicts with the Lyme vaccine)

ONLY ONE STRAIN OF ONE SPECIES (Bb B31- a tick-derived lab strain)

FDA-cleared IgG Lyme ImmunoBlot (iDart)

IGeneX's Lyme IgG ImmunoBlot has been converted into the iDart Lyme IgG ImmunoBlot test kit and it has been cleared by the US Food and Drug Administration (FDA)

Sensitivity 90%+

- **Is a two-tier test!!** The kit includes the recombinant Lyme Screen Assay (LSA) to serve as tier-one, then the ImmunoBlot serves as tier-two
- Is the only FDA cleared test that **includes bands 31 and 34** → more sensitive
- **Does NOT use the nonspecific bands** specified for the CDC Lyme western blot (18, 28, 30, 41, 45, 58, 66): therefore more specific
- Interpreted using the **IGeneX 2-band criteria**, not the CDC/Dearborn 5 band criteria
- **Is multi-species capable!** Likely to detect all the important Lyme Borrelia species

Why the IGeneX Lyme IgG ImmunoBlot is so sensitive

Interpretation criteria

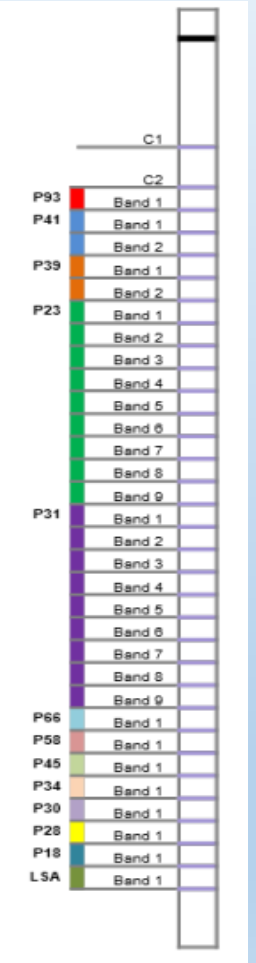
- One or more bands from the following **2 or more groups**: (P23, P31, P34, P39, P41 and P93) must be present on the ImmunoBlot

Notice these "groups"-

- **NINE different P23s**
- **NINE different P31s**
- **TWO P39s**
- **TWO P41s**
- **TWO different controls**

Total of THIRTY ONE bands plus the controls

Multiple specific antigens in multiple groups is why the ImmunoBlot can detect so many species and why it so much more accurate than all other serologies



What does FDA clearance mean?

Most commercial and hospital labs don't develop their own tests- they purchase test kits

- By law, all commercial test kits have to be FDA cleared
- Until now, all Lyme test kits, FDA-cleared and available to labs, had to follow the CDC Two-Tier scheme, which is not accurate and covers only one species
- FDA clearance means the ImmunoBlot kits can now be sold to other labs and hospitals to use

NOTE: only the IgG ImmunoBlot has been cleared- the IgM ImmunoBlot clearance is pending

The iDart is so accurate, it has forced the CDC to change its criteria for laboratory diagnosis of Lyme!

From the CDC website: “CDC recommends using antibody tests that have been cleared by the U.S. Food and Drug Administration (FDA) and follow a two-step process.”

- No longer is it listing all those nonspecific bands
- No longer is it specifying the need for 5 out of 10 bands on the IgG western blot
- No longer is it excluding bands 31 and 34

A BIG WIN for all of those who have been fighting the CDC for decades

AcuDart- at-home test kit

Highly accurate at-home or in-office blood test kit for the major tick-borne diseases

Can be gotten without a doctor's prescription!

Based upon the IGeneX Broad Coverage Assay

- Recombinant technology
- Multi-species
- Simple to interpret- clear yes-no result
- Does not name species or separately report IgM and IgG

SENSITIVITY 83%- The only tests that have a higher sensitivity than AcuDart are the ImmunoBlots from IGeneX.

- Lyme disease, Tick-Borne Relapsing Fever (TBRF), Babesiosis, Bartonellosis

AcuDart

Test kits can be gotten online, without a doctor's prescription, from www.acudarthealth.com

- Simple fingerstick- then send it to IGeneX for processing
- Practitioners can stock these in their offices
- Patients can order their own kit and do their own tests
  - No need to go to a lab or office to be tested
- Great for getting results while new patients are waiting for a visit, for testing between visits, for serial testing during treatment and for evaluating apparent recurrences of symptoms.
- Simplifies testing for telemedicine patients
- Family members can easily be screened
- Kids may prefer a fingerstick than going to a scary lab and getting a needle
- Positive results can then be confirmed and expanded upon by getting an ImmunoBlot

Optimizing testing using indirect tests

Indirect tests- Serologies and T-cell response assays

Key is to use these when immune response is expected to be highest

- Early disease- T-cell response assay, ImmunoBlot
- Disseminated but not chronic, with intact immunity:
  - Recombinant serology- ImmunoBlot, AcuDart, BCA
- Late, chronic infection: Immune-based tests are less sensitive
  - Recommendation is to do both the ImmunoBlot + T-cell response assay to document immune status, especially at baseline
  - And in most cases, best to add direct test(s)

Can test while on antibiotics

- **TIP:** After treating long enough to see a clinical response, can see a rise in antibody levels, making serologies more sensitive
  - Seronegatives convert to positive in 37%- Helpful if documenting a positive result is desired

Optimizing testing using direct blood tests

Direct blood tests: Culture, FISH, PCR

Key is to use these when pathogen load is expected to be highest

- Higher load early in the infection, before effective immunity develops
- Higher load during symptom flares
- Higher load at specific times of the day
  - Borrelia- early afternoon and during chill phase
  - Babesia- during chill phase
  - Bartonella- not known
- Antimicrobials can decrease test sensitivity
  - If on treatment, no meds for long enough for the organisms to re-emerge, but do NOT stop needed treatment just to do a test!!

Optimizing testing using urine antigen capture

Antigen spillage, and therefore sensitivity, tracks symptom severity- symptom flares, Herxheimers, menses

- To increase sensitivity in untreated patients, pre-treat with antibiotics to induce a Herxheimer- meds days 1-5, test on days 2, 4 and 6
- For patients who have been treated, best to collect samples on three different days, during a symptom flare, to increase yield.
- Specific as long as there is no UTI, so recommend doing a concurrent U/A and urine culture
- Lyme only

Not all of the three samples may be positive; believe the positive ones

Testing by stage of illness- early (<4 weeks)

- Urine Antigen capture- positives as soon as symptomatic (Lyme only)
- FISH-
  - Babesia- positive very early- as soon as symptoms begin
  - Bartonella- unknown but probably reasonably sensitive early on
- Serology: ImmunoBlots- begin to see positives during week 2
- T-cell stimulation assays- can respond in less than 2 weeks
- Culture-enhanced PCRs- No data, but probably sensitivity becomes useful soon after symptoms develop, but must wait 3 weeks for results!

Testing by stage of illness- disseminated but not chronic

- Recombinant serologies are the first choice
  - AcuDart and BCA- 83% overall sensitivity to blinded controls
  - ImmunoBlot- 97-99% overall sensitivity to blinded controls
- Urine antigen capture- OK to do while on treatment, but best results if done during symptom flares or resumption of Rx after a break
- Direct blood tests generally are less sensitive, especially if on treatment or if treatments ended recently
  - Still possible to get positive FISH
  - Less likely to get positive PCRs even with culture enhancement
  - However PCR of biopsies can be useful

Testing by stage of illness- chronic illness

Chronic- ill 6-12 months, especially if co-infected and are signs of increased disease severity or immune compromise

- Here, all blood testing is less sensitive:
  - **Serologies-** Depressed immunity or increased antigens with immune complexes impair results by binding up free antibody
  - **Direct tests**- In longstanding illness, there are relatively fewer pathogens in circulation due to tissue or blood vessel sequestration, making direct tests less sensitive
- However, Urine antigen capture still useful!
- **Recommendation** is to combine multiple different methods
  - Recombinant serology + T-cell stimulation assay: results can reflect not only exposure but also current status of immunity
  - Add FISH, culture-enhanced PCR, urine antigen capture

Testing by stage of illness- immune deficiency

Some patients have either pre-existing or acquired immunoglobulin deficiencies

- Logically this should favor doing direct tests over serologies
- However, I nevertheless recommend including an immunoblot to document antibody status
  - If the immunoglobulin deficiency is the result of the TBDs, then it is reasonable to do serial immunoblots to document immune recovery
- T-cell response is independent of B-cells/immunoglobulin levels, so T-cell response testing may be useful, especially as a baseline
  - T-cell response testing, while not always positive, can nevertheless track clinical status

Testing by stage of illness- gestational TBDs

Mother

- ImmunoBlots to confirm diagnosis during pregnancy
- Always test the placenta by histology (look for inclusions) and by PCR for the pathogens

Newborn

- Cord blood for culture enhanced PCR
- Urine antigen capture and urine PCR

Infant

- Monthly urine antigen screens for 6-12 months; consider urine PCR too

Testing Summary

Direct Testing **These tests look for evidence of the pathogen in the specimen**	**Indirect Testing** **These tests look for immune activity to the organism: specific antibodies or T-cell reactivity in the blood**
Why use direct tests: • Not every patient has detectable antibodies • Patients may be immune-suppressed • Antibodies may be bound in immune complexes • Some patients are challenging blood draws (i.e. young kids) so urine specimens can be tested instead	**Why use indirect tests:** • Pathogens not always in the bloodstream • Superior at detecting early and/or late stage disease • Certain tests can detect T-cell response, an early disease indicator and useful in B-cell immune deficiencies
Types of direct tests: • **Culture** (cePCR) • **FISH** • **Urine antigen capture** • **PCR**	**Types of indirect tests:** • **ImmunoBlot, LSA, BCA, AcuDart** • **T-cell response assay** • IFA, ELISA • Western Blot

Last slide- Testing Guide

TEST	METHOD	FEATURES	WHEN TO USE	ACCURACY
IFA, ELISA, WB	Serology	Single species	**Not recommended**	False negatives False positives
ImmunoBlot, LSA, BCA, AcuDart	Serology	Recombinant Ag's Multiple species	All stages	Maximal
T-cell response assay	Mitogen stimulation assay	Limited time window	Early and In B-cell dysfunction	Medium- depends on timing
PCR	DNA detection	Fluids and tissues	**Tissues only** if possible	Insensitive but very specific
Culture-enhanced PCR (cePCR- IGeneX) (Galaxy- Bartonella)	Culture with pathogen ID confirmed by PCR	Blood and CSF	All stages but not if on treatment	Maximal
FISH	RNA-stained blood slide	The best test if biofilms are present	All stages but not if on treatment	Good
Urine antigen capture	Direct antigen detection	Lyme only	All stages When avoiding needlesticks	Good


THANK YOU!!