NEGLECTED INFECTIONS AND GASTROINTESTINAL ISSUES IN PATIENTS WITH LATE / PERSISTENT / CHRONIC VECTOR-BORNE INFECTIONS

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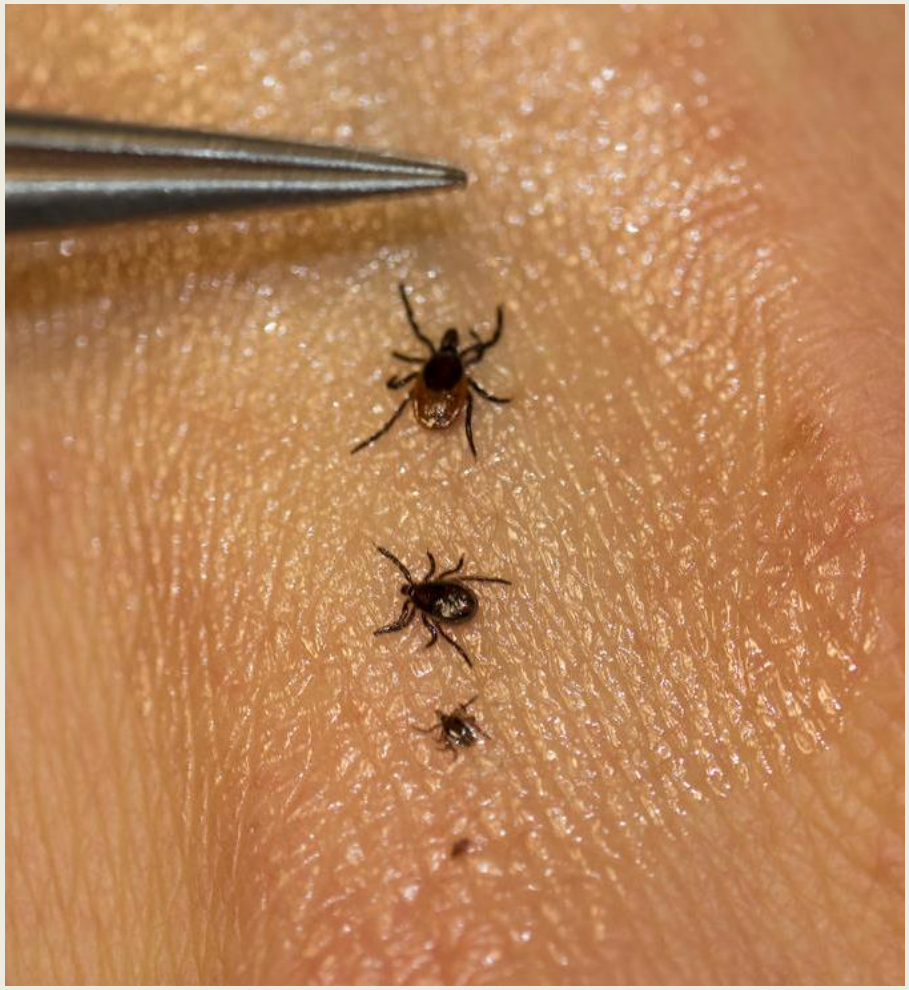
Tick-borne Infections



- Tick-borne diseases, which afflict humans and other animals, are caused by infectious agents transmitted by tick bites.
- Tick-borne illnesses are caused by infection with a variety of pathogens.
- Tick-borne infections are increasing globally - Lyme disease is among the most prevalent vector borne infection in the U.S. and Europe and is reaching epidemic levels (Kugeler et al. 2015; Sykes et al. 2014).

➔ **Whenever possible, get the tick tested**

Tick-borne Infections

- Lyme disease is the most widely known tick-borne disease and is caused by bacteria of the genus *Borrelia.*

- Because individual ticks can harbor more than one disease-causing agent, patients can be infected with more than one pathogen at the same time, compounding the difficulty in diagnosis and treatment.

- Among neglected infections, there are some that deserve more attention and investigations, like **Borrelia Relapsing fever group, Borrelia Miyamotoi**, **Tularemia**, **Yersinia**, **Mycoplasma, Chlamydia**, **Epstein-Barr virus and Herpesviruses**. Our data show that prevalence of these infections are not negligible and they should be more investigated.

Remember

- It is important to bear in mind that TBD can be acute or late stage / persistent / chronic; this is important given the diagnostic and treatment approaches might be different in these two situations.

- **Lyme disease exhibits a variety of symptoms that may be confused with immune and inflammatory disorders.**

- If an individual has any chronic health condition, ranging from arthritis to chronic fatigue syndrome to fibromyalgia, it is important to rule out or diagnose tick-borne disease(s). It is apparent that many cases of fibromyalgia and chronic fatigue syndrome are actually TBD in disguise

- **Chronic patients have complex clinical picture with multiple afflictions needing thus an integrative approach with multiple testing and careful interpretation of testing results.**

Tick-borne Diseases Testing

- **Importance of specialized medical advice and guidance**

- **Importance of novel testing approaches**

  The overall high failure rate of tick-borne infection (TBI)-related testing underscores the necessity for novel approaches to be applied widely, i.e. not relying on serology and two-tier testing.

- **Importance to enlarge borreliosis-related testing targets (i.e. not testing only for B. burgdorferi sl)**

  The overall high expansion of undiagnosed Lyme disease cases worldwide might be linked to the screening choice focusing only on B. burgdorferi sl and only rarely testing for B. miyamotoi while the later one seems to be much more prevalent. Searching for actual bacterial presence using phage - based testing might pacify the debate and controversies on testing choices and late/chronic stage patients.

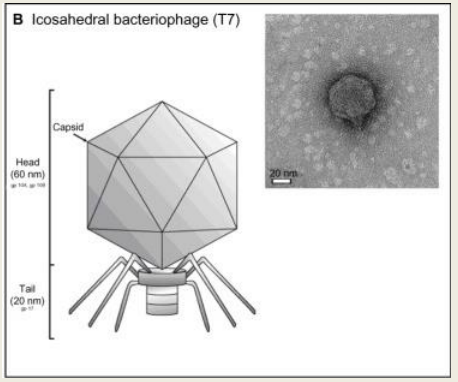
Novel Testing Approaches – Phelix Phage-based Test

- Bacteriophages could become a diagnostic tool (Patent WO2018083491A1) based on the principle that **if there are phages it is because there are living bacteria;** hence a phage-based test is a direct proof of an active infection.
- Phelix Charity together with Leicester University microbiology department have recently developed a Borrelia Phage-based PCR test searching for 3 major Borrelia groups:

  Borrelia burgdorferi sl (including B. burgdorferi ss, B. afzelii, B. garinii, B. spielmanii, etc)

  Borrelia miyamotoi and

  Relapsing fever group (B. recurrentis, B. hermsii, etc).
- This method is efficiently used to assess both human samples and ticks.
- Highly sensitive and specific.
- Do not generate positive signal against other bacterial strains.
- False positive are ruled out by sequencing.



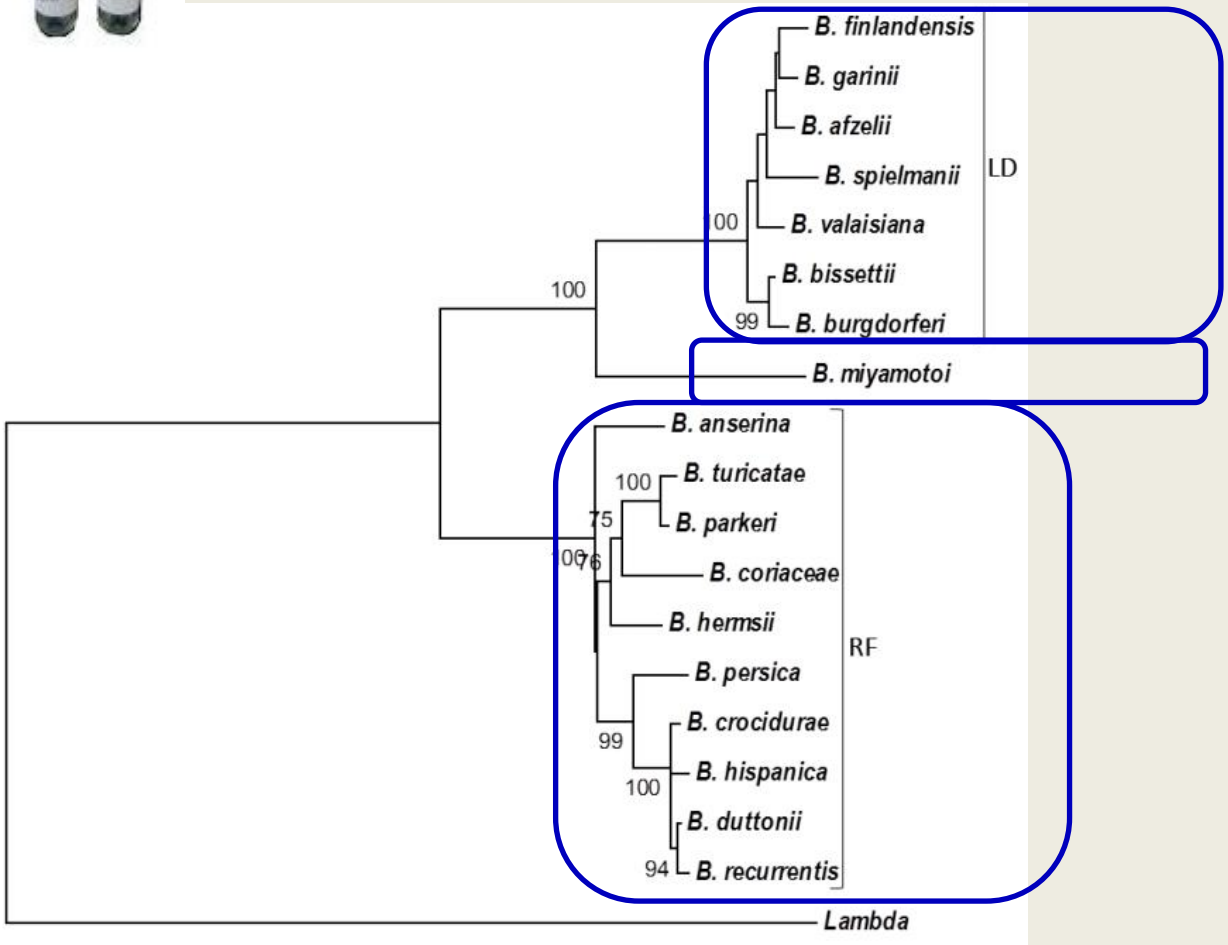
Phelix Phage Borrelia Test – How it works

Manual DNA Phenol / Chloroform extraction

QCs

- **Extraction control PCR** *quality of extracted DNA*
- **IAC control PCR** *absence of PCR inhibitors*

3 different real-time PCRs, each sample in 4 replicates per target



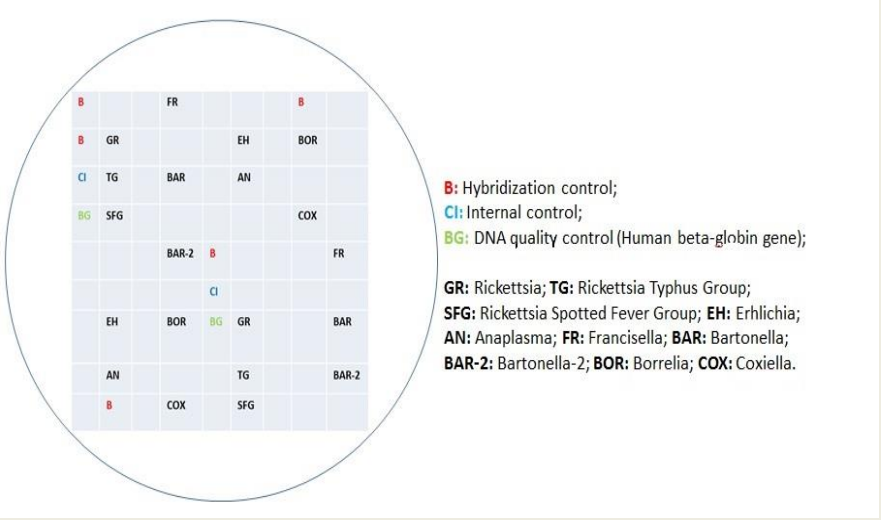
Confirmatory sequencing for positive-like samples

Results of the Tests Done on Ticks

- 40% of analysed ticks were negative (no detected pathogens)
- 60% of analysed ticks were positive for at least 1 pathogen

HYBRISPOT TICK-BORNE BACTERIA FLOW CHIP

Simultaneous detection of 7 tick-borne bacteria genera:



PHELIX PHAGE BORRELIA TEST

Pathogens found in positive ticks:

Borrelia burgdorferi sl:17% pos
Borrelia miyamotoi : 60% pos
Borrelia Relapsing Fever Group 23% pos

25% of positive ticks were for 2 pathogens

➔Unexpected high rate of B. miyamotoi and Relapsing Fever Borrelia Phages in tested ticks

Unexpected Results?

- 60% of analysed ticks were positive for at least 1 pathogen
- Among them, only 17% were positive for B. burgdorferi sl
- Among positive ticks, 60% were for B. miyamotoi!

- This result is in line with those obtained on human samples:

  - Since July 2019, over 2100 results from patients originating various countries have been obtained.

  - Testing included mainly late stage / chronic patients and the aggregated data are showing 30 % negative results and 70% positive among which over 60 % indicated the presence of specific Borrelia miyamotoi phages.

- ➔ *For analysis of some clinical data, please see the lecture from Dr Louis Teulières*

- With respect to the obtained results, a question raised: ***are we searching for the wrong culprit with Lyme-disease testing?***

Tularemia

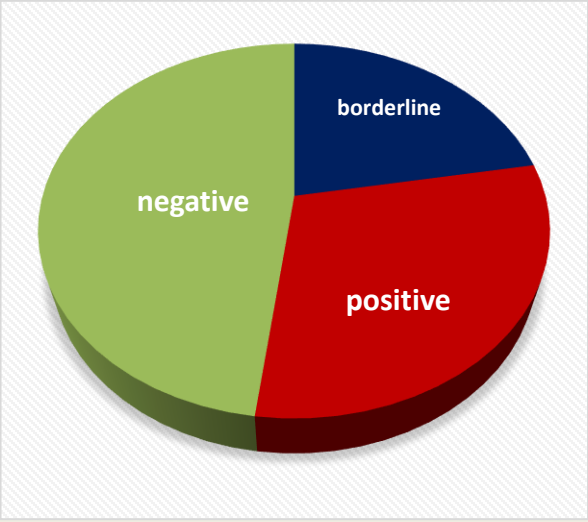
- Tularemia, also known as rabbit fever, is an infectious disease caused by the bacterium *Francisella tularensis*
- People can become infected in several ways, including:
  - Tick and deer fly bites
  - Skin contact with infected animals
  - Drinking contaminated water
  - Inhaling contaminated aerosols or agricultural and landscaping dust: can occur during farming or landscaping activities, especially when machinery (e.g. tractors or mowers) runs over infected animals or carcasses
  - Laboratory exposure
  - People could be exposed as a result of bioterrorism.
- Tularemia is not known to be spread from person to person. People who have tularemia do not need to be isolated.
- The diagnosis of tularemia is often delayed. It may take a significant length of time to diagnose and the condition and the disease may become complicated.
- Some persons present mild symptoms thus difficult to uncover.

Tularemia – testing results

- Testing period : 4 years

- Screening test : immunochromatography on serum
  - Total tested: 1769 samples
  - 392 (= 22.16%) were found positive and undergo confirmatory testing

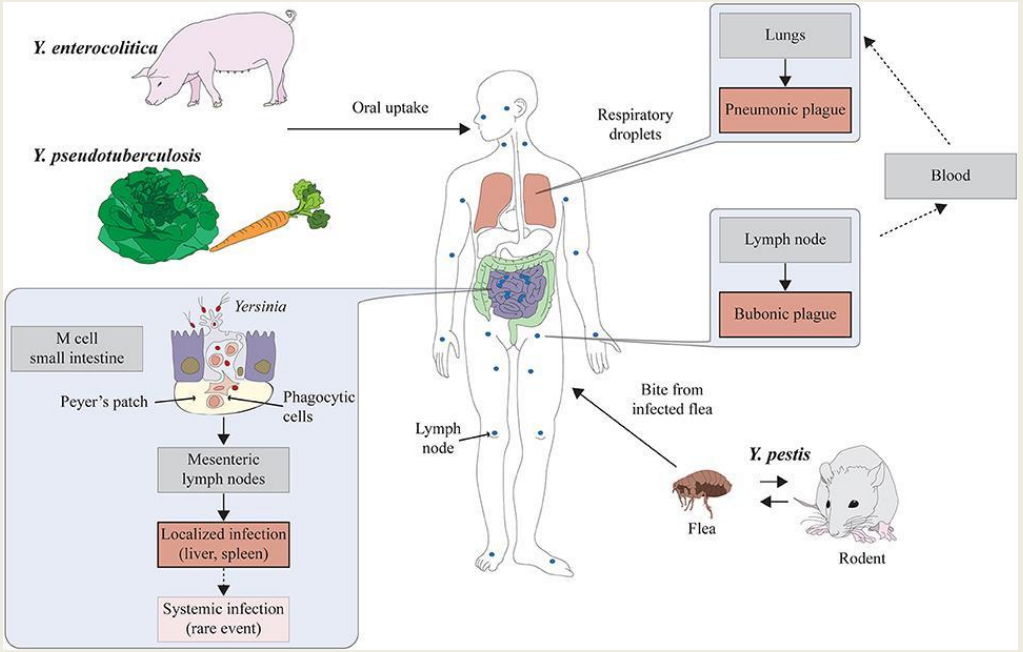
- Confirmatory test: IVD Tularemia IgM ELISA
  - 392 went to confirmatory testing
  - 87 were found borderline (22,2% among those that went for confirmatory testing, 5 % of total tested samples)
  - 117 were found positive (29,8% among those that went for confirmatory testing, 6,6% of total tested samples)



Yersinia

- The bacteria from the genus Yersinia are Gram-negative enterobacteria. From the 17 described species there are 3 know to be human pathogens:
  - ***Yersinia pestis*** causes bubonic and pneumonic plague. Bubonic plague is transmitted by the bite of infected rat fleas. Swollen, blackened lymph nodes (buboes) develop, followed by septicemia and hemorrhagic pneumonia and death. The pneumonic form spreads directly from human to human via respiratory droplets. Outbreaks are explosive in nature, and invariably lethal.
  - ***Yersinia enterocolitica*** causes severe diarrhea and local abscesses
  - ***Yersinia pseudotuberculosis*** causes severe enterocolitis.

- The most common source of Y. enterocolitica infection in humans is pork (raw or undercooked) and also contaminated water, meat, or milk.



The importance of testing for Yersinia

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Microbiota-dependent sequelae of acute infection compromise tissue-specific immunity

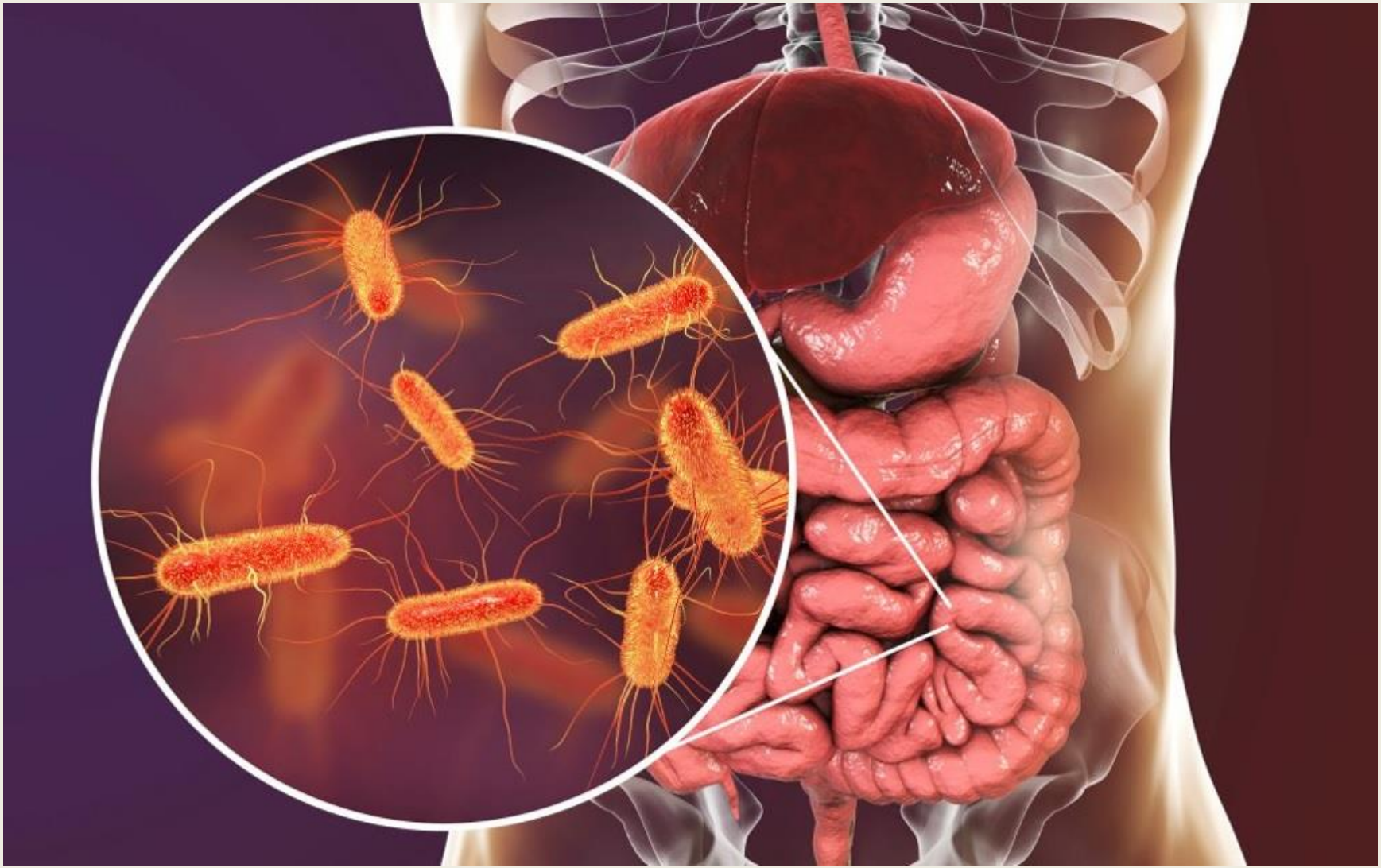
Denise Morais da Fonseca[1,2,3,*], **Timothy W. Hand**[1,2,*,#], **Seong-Ji Han**[1], **Michael Y. Gerner**[4], **Arielle Glatman Zaretsky**[1,2], **Allyson L. Byrd**[1,5,6], **Oliver J. Harrison**[1,2], **Alexandra M. Ortiz**[7], **Mariam Quinones**[8], **Giorgio Trinchieri**[9], **Jason M. Brenchley**[7], **Igor E. Brodsky**[10], **Ronald N. Germain**[4], **Gwendalyn J. Randolph**[11], and **Yasmine Belkaid**[1,2]

- "These repeated and unregulated inflammatory challenges may profoundly remodel the immune system and thereby contribute to the increased burden of autoimmune and inflammatory disorders.
- Together, this study provides a framework to understand how previously encountered infections can induce a breakdown of tissue immune homeostasis, thereby contributing to disease later in life. Thus, in order to fully comprehend the etiology of complex diseases, it may be necessary to look beyond a patient's genetic susceptibilities and concurrent environmental stressors and examine whether immunological scarring associated with previous infections may have 'set the stage' for chronic inflammation."
- These **repeated and unregulated inflammatory challenges may profoundly remodel the immune system and thereby contribute to the increased burden of autoimmune and inflammatory disorders**."

Yersinia – testing results

- Yersinia immunoblot tests make it possible to detect past Yersinia infections, and are thus ideally suited for identification of Yersinia-induced immunopathological complications and chronic yersiniosis. Detection of IgG and IgA antibodies can be a very useful diagnostic tool if Yersinia-induced arthritis is suspected.
- An immunoblot for the detection of IgG and IgA antibodies against all pathogenic Yersinia by means of Yersinia outer proteins (YOPs). Serological differentiation of Y. enterocolitica and Y. pseudotuberculosis infections is possible for the first time with the use of new species-specific Yersinia antigens (PsaA, MyfA).
- Testing period : 4 years
- Immunoblot on serum
  - Total tested: 2396 samples
  - IgA positive: 412 (➔ 17.2%)
  - IgG positive: 946 (➔ 39.5%)
    - IgG, no differentiation: 624 positives (➔ 66% of IgG positives; 26.1% of all tested
    - IgG Y. pseudotuberculosis: 202 positives (➔ 21.4% of IgG positives; 28.4% of all tested
    - IgG Y. enterocolitica: 120 positives (➔ 12.6% of IgG positives; 5% of all tested

Intestinal dysfunctions



TBI and gastrointestinal disorders

Signs and symptoms related to the gastrointestinal tract and liver may provide important clues for the diagnosis of various tickborne diseases

Manifestation	Lyme disease	Ehrlichiosis	RMSF	Tularemia	Colorado tick fever	TBRF	Q fever	Babesiosis
Anorexia	+	++	+	+	+	+	+	+
Nausea	+	++	++	++	++	+++	++	+
Vomiting	+	++	++	++	++	+++	++	+
Abdominal pain	+	++	++ to +++	++	+	++	+	+
Diarrhea	+	++	++	++ to +++	+	+ to ++	++	+
Hepatomegaly	R	+ to ++	+	+ to ++	R	+	+	+
Splenomegaly	+	+ to ++	+	+ to ++	R	R to +	+	+
Jaundice	+	+++	+	+	+	+	+	+ to ++
Elevated bilirubin level	+	+++	+ to ++	+	+	+	+ to ++	++ to +++
Elevated ALT level	++	++++	++ to +++	++	+	++	++[a]	+

NOTE. ALT, alanine aminotransferase; R, rare; RMSF, Rocky Mountain spotted fever; TBRF, tickborne relapsing fever; +, uncommon; ++ common; +++, very common; ++++, almost always present.

[a] Elevated alkaline phosphatase level is the predominant abnormality.

From: Gastrointestinal and Hepatic Manifestations of Tickborne Diseases in the United States
Syed Ali Zaidi & Carol Singer, Clin Infect Dis. 2002;34(9):1206-1212. doi:10.1086/339871

Lyme disease and gastrointestinal disorders

- **Patients with Lyme and TBDs may present primarily with GI manifestations.**
- **2015 ILADS conference, Dr. Farshid Rahbar: These patients may have complex or persistent GI symptoms involving upper, mid, or lower GI tract and have already been treated for GI issues**

Bloating/Gas: in 76% of patients
Abdominal Pain: in 48% of patients
Constipation: in 42% of patients
Food Intolerance: in 42% of patients
Irregular Bowel Movements: in 37% of patients

- **The number of patients presenting with such symptoms is probably reaching epidemic proportions.**
- **Testing for gastrointestinal problems need to be included**
- **Useful assays to investigate intestinal dysfunctions:**
  - **BLOOD-BASED Tests: IgA/IgM against intestinal bacteria, Lactase deficiency assay, D-lactate, sCD14**
  - **BIOPSY-BASED Tests: PCR-based detection of viral and bacterial infections**
  - **STOOL-BASED Tests:**
    - **Intestinal Inflammation: sIgA, Beta-2 Defensin, EPX / EDN, Inflammation markers in stool samples**
      - ! Gut inflammation contributes to increased bacterial translocation.
    - **Intestinal Infections : immunochromatography antigenic testing for intestinal infections**
    - **Leaky gut: ZONULIN ELISA test in stool samples**
    - **Dysbiosis: MSA assay (metagenomic stool test)**

Consequences of the leaky gut – Chronic activation (inflammation) of the immune system

- Leaky gut testing (Zonulin in stool) : 3 years testing period, **1301 samples** **63.87% patients with increased levels!!**
- Lipopolysaccharide (LPS) - bacterial compound that can easily make its way to the blood.
- Present in the bloodstream LPS will induce a strong pro-inflammatory response in monocytes and macrophages, involving recognition by a receptor (Toll-like receptor-4) and the subsequent secretion of cytokines such as IL-1, IL-6, TNF-alpha.
- LPS also induces the NK-kB-mediated production of nitric oxide. Because NO is increased, NK function is inhibited and opportunistic infections such as mycoplasma infections are often observed.
- Herpesviruses, which tend to reactivate in a context of immune activation, will also be frequently detected.

Example - 1

Prevotella: strong hydrogen sulfide (H_2S) producers. In excess, H_2S acts as a

PHYLUM	FAMILY	GENUS	Jan 2018 % of total	Oct 2018 % of total	Jan 2019 % of total	Apr 2019 % of total
		Streptococcus	18.35	4.75	1.12	0.93
	Leuconostoc	Leuconostoc	0.19	0.01	0	0.06
Bacteroidetes (gram-)	Bacteroidaceae	Bacteroides	4.42	8.53	7.3	4.41
	Rikenellaceae	Alistipes	0.11	0.65	1.19	0.69
	Porphyromonadaceae	Barnesiella	0.11	0.95	1.3	0.67
		Odoribacter	0.01	0.15	0.13	0.06
		Parabacteroides	0.23	1.77	0.66	0.36
	Prevotellaceae	Prevotella	42.02	26.07	15.61	8.96
		Xylanibacter	0.03	0	0	0.01
	Bifidobacteriaceae	Bifidobacterium	0	0.01	0	0

Example - 2

1st visit April 2017:

PHYLUM	FAMILY	GENUS	Apr 2017 % of total	Dec 2017 % of total	Dec 2018 % of total
	Lachnospiraceae	Anaerostipes	0.05	0.03	0,01
		Coprococcus	19.75	13.52	8,83
		Dorea	3.96	9.5	5,38
		Moryella	0	0	0
		Roseburia	8.02	0.97	13,26
		Sporobacterium	0	0	0
		Syntrophococcus	0.01	0	0
	Ruminococcaceae	Acetanaerobacterium	0	0	0
		Acetivibrio	0	0	0
		Ethanoligenens	0	0.01	0,01
		Faecalibacterium	2.96	18.87	25,32
		Papillibacter	0	0	0
		Ruminococcus	0.01	8.06	0,19
		Sporobacter	0.01	0	0
		Subdoligranulum	0.01	0.01	0,02

PHYLUM	FAMILY	GENUS	Apr 2017 % of total	Dec 2017 % of total	Dec 2018 % of total
	Oscillospiraceae	Oscillibacter	0.98	3.65	0,53
	Staphylococcus	Staphylococcus	0	0	0,01
Bacteroidetes (gram-)	*Bacteroidaceae*	Bacteroides	13.8	20.21	6,88
	Rikenellaceae	Alistipes	1.66	0.55	0,23
	Porphyromonadaceae	Barnesiella	0.25	0.27	0,04
		Odoribacter	0.1	0.17	0,02
		Parabacteroides	2.49	0.67	0,3
	Prevotellaceae	Prevotella	21.71	3.85	3,77
		Xylanibacter	0	0	0
	Bifidobacteriaceae	Bifidobacterium	2.21	0.11	0

		Slackia	0.58	0.28	0,19
Proteobacteria (gram-)	*Enterobacteriaceae*	Escherichia/Shigella	0.05	0	0
		Klebsiella	0	0	0
	Sutterellaceae	Sutterella	0.81	0	0
	Desulfovibrionaceae	Lawsonia	0	0	0

CONCLUSIONS (1)

- Seen a high prevalence of B. miyamotoi in tested ticks, further supported by similar percentages found in tested patients, one can hypothesize that the high failure rate of current two-tier screening testing, searching for B. burgdorferi sl only, might be due to the wrong testing target.

- In other words, the overall high expansion of undiagnosed Lyme disease cases worldwide might be linked to the screening choice focusing only on B. burgdorferi sl and only rarely testing for B. miyamotoi while the later one seems to be much more prevalent.

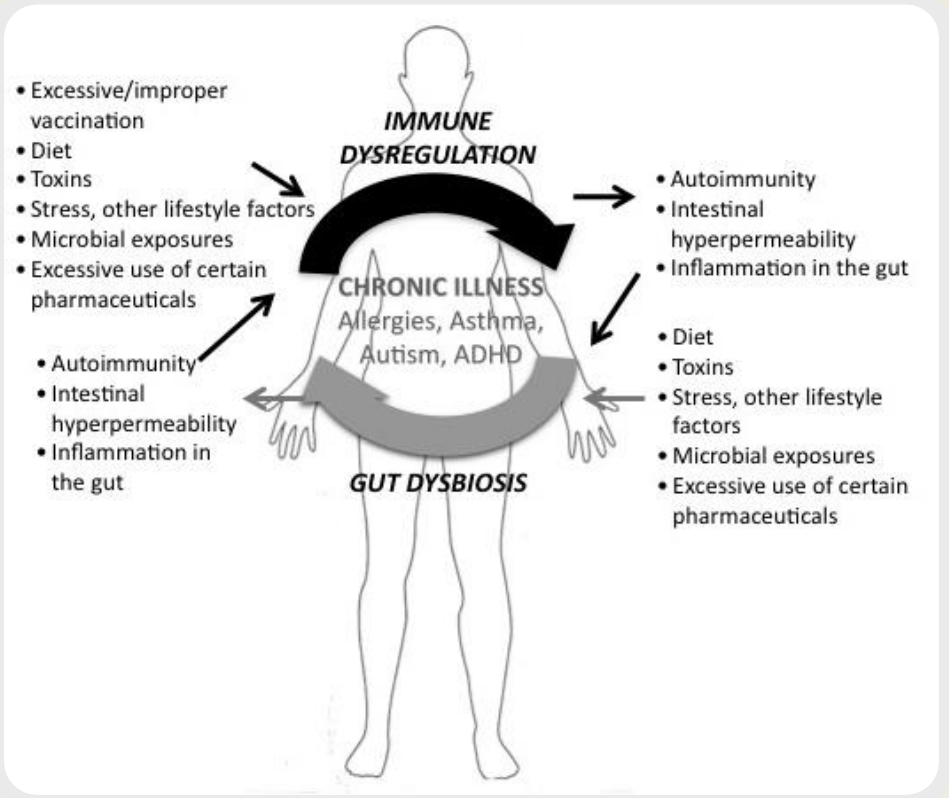
- Further accumulation of data both from the patients and ticks should bring the answer to the question ***are we searching for a wrong culprit.***

- Searching for actual bacterial presence using phage-based testing might pacify the debate and controversies on testing choices and late/chronic stage patients.

CONCLUSIONS (2)

- The overall high failure rate of therapies for vector-borne infections, especially in late/persistent/chronic patients, underscores the necessity to fully investigate different concurrent infections along with resulting gastrointestinal and immune dysregulations.

- It is important to investigate different "co-infections" (i.e. tick-borne infections) but also other opportunistic infections (viral, bacterial, parasitic).



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THANK YOU

- **Material available on the website (www.redlabs.com)**
- Check regularly our website (www.redlabs.com) for the updates
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