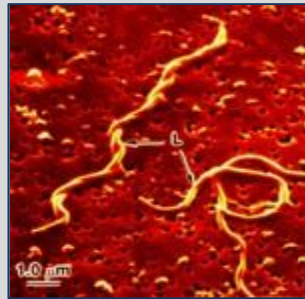
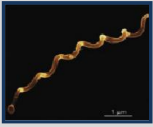


**Dr. Anna Goc**



**Webinar - 2014**



# Outline

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## 1. Introduction

- ◆ Lyme disease is a Health Concern
- ◆ Lyme disease is a Zoonosis
- ◆ Life cycle of Ticks
- ◆ Symptoms of Lyme disease
- ◆ Diagnostic of Lyme disease
- ◆ Current Therapies
- ◆ *Borrelia sp.* is a pathogenic factor
- ◆ *Borrelia sp.* exist in different forms
- ◆ Rounded forms are diverse
- ◆ What is Biofilm
- ◆ Pathogenesis of *Borrelia sp.*

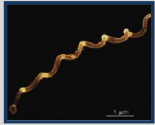
## 2. Methodology

- ◆ Aim of Project
- ◆ Plan of work
- ◆ Micronutrients Composition

## 3. Results

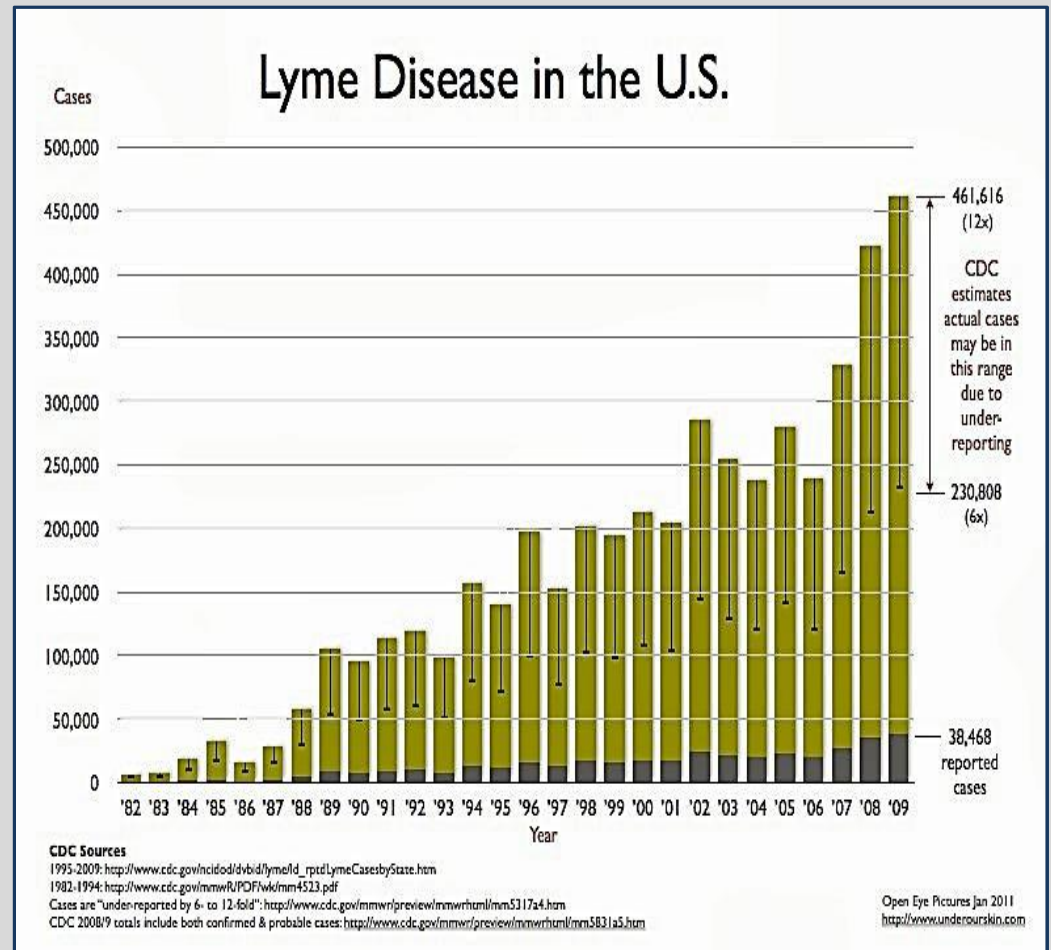
- ◆ Micronutrients can eliminate Spirochetes
- ◆ Micronutrients can eliminate Rounded Forms
- ◆ Micronutrients are effective against Biofilm
- ◆ Micronutrients do not induce Resistance

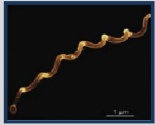
## 4. Conclusion



# Lyme disease is a Health Concern

- ◆ Zoonotic bacterial infection; after Old Lyme, CT (1975)
- ◆ The most common vector-borne bacterial disease:
  - USA (~30,000 /year)
  - Europe (~65-80,000/year)
  - Asia, Africa, Australia
- ◆ Highest rates in children (10-14 years) and adults (>35 years) and slightly in men than women





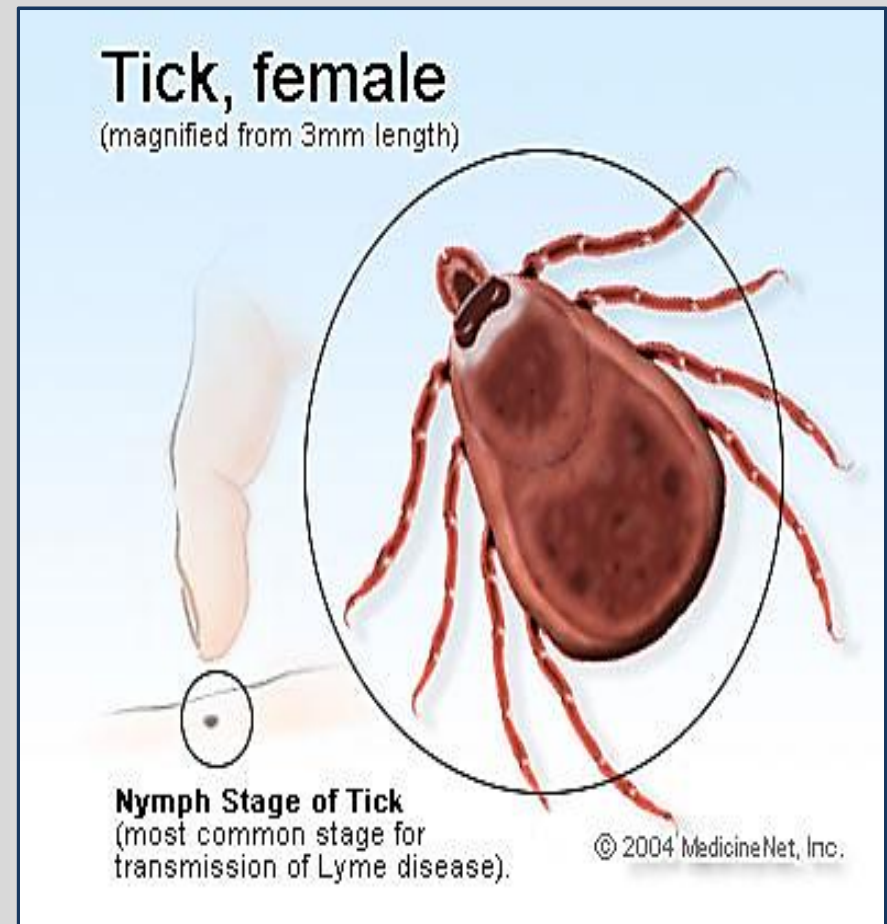
# Lyme disease is a Zoonosis

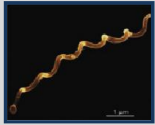
◆ Vector: ticks of the genus **Ixodes** during all phases of life cycle (egg -> **larval** -> **nymphal** -> imago)

- *I. scapularis* and *I. pacificus* in the Northern America

- *I. ricinus* and *I. persulcatus* in Europe and Asia

◆ Limited metabolic capabilities (rely on their host: **reptiles, birds, small and big mammals**)





# Symptoms of Lyme disease

Some patients may be asymptomatic and/or with non-specific symptoms

## ◆ Localized Early (Acute) Stage:

- erythema migrans
- fever
- headache
- swelling of the lymph glands near the bite



## ◆ Early Disseminated Stage:

- facial palsy, meningitis
- joint pain, stiffness
- changes in vision
- severe fatigue



## ◆ Late Stage:

- arthritis
- encephalomyelitis
- carditis
- chronic skin disorder



↓ Vitamin D3

↓ B12/folic acid

↓ CD57 count

↑ inflammatory markers

↑ rheumatoid factor and ANA

◆ Abnormal brain MRI and SPECT

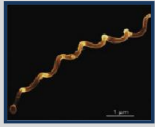


# Diagnostic of Lyme disease

Clinical diagnosis using your clinical history and symptoms  
Negative serology does not rule out Lyme disease (appears in ~50% of cases)

- ◆ Typically based on differential diagnosis backed by (serum, CSF):
  - Serological test (often called "Titer Test")
  - Western Blot
- ◆ Cultivation of the pathogen (CSF, skin biopsy)
- ◆ PCR (joint aspirates, synovial and endomyocardial biopsies)

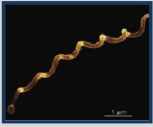
Patients with neurological symptoms need to have a spinal fluid tested  
(*patients may have negative blood tests and show positive spinal fluid test*)



# Current Therapies

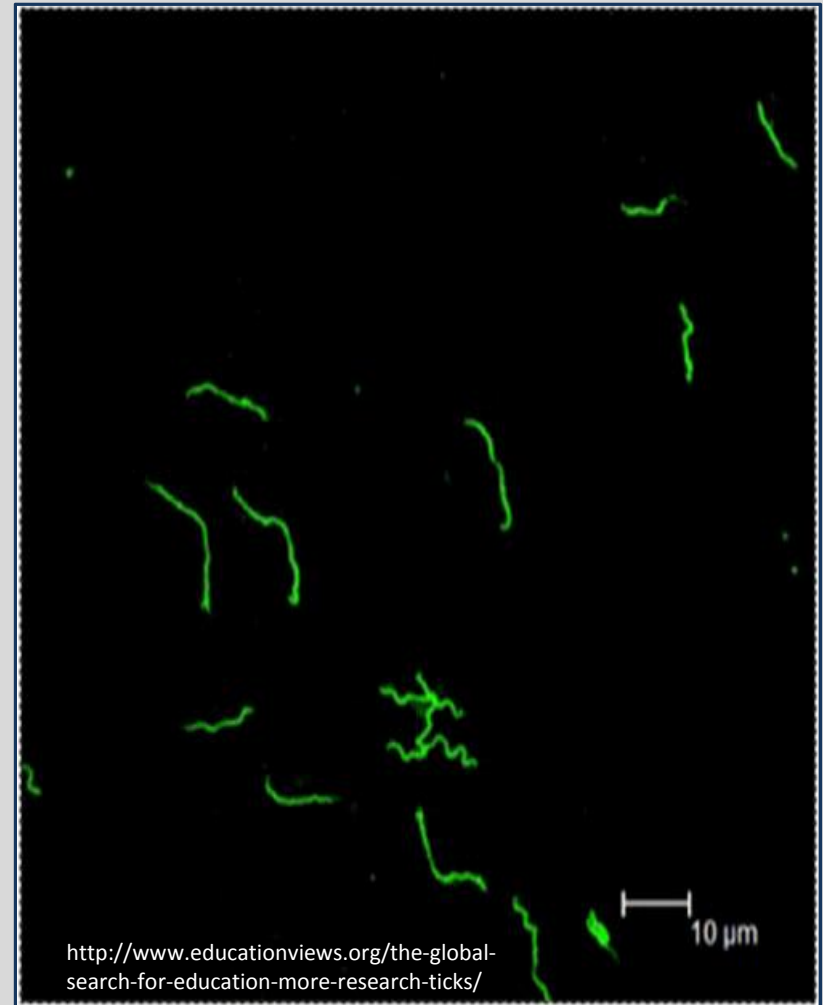
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- ◆ **Conventional treatments:** antibiotics  
(short term administration: 2-4 weeks)
  - Doxycycline, Amoxicillin, Cefuroxime ( **$\beta$ -lactam antibiotics**)
  - Ceftriaxone, Cefotaxime
  - Azithromycin, Clarithromycin, Erythromycin (**macrolide antibiotics**)
- ◆ **Alternative treatments:** plant extracts, enzymes, chelators, etc.  
(focus on symptoms not the cause)
- ◆ **Vaccines:** LYMErix – withdrawn (from 2002)  
LymeVax – available currently for dogs

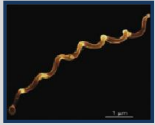


# *Borrelia sp.* is a pathogenic factor

- ◆ The first known existence of the *Borrelia* bacteria dates back to up to 20 million years ago
- ◆ 37 species with 12 species that are Lyme-related
- ◆ 1981 - *Borrelia burgdorferi* - host-dependent, tick-transmitted, invasive, nontoxicogenic, persistent pathogenic factor
  - ***B. garinii* and *B. afzelii*** (Europe)
  - ***B. burgdorferi ss*** (USA)
  - **Emerging genospecies** (10 of them)

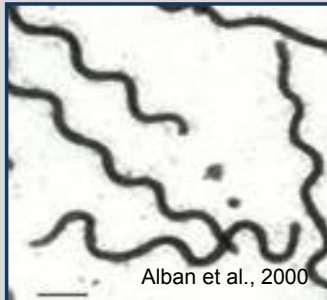






# *Borrelia* sp. exist in different forms

All forms are capable of producing injury in the human host

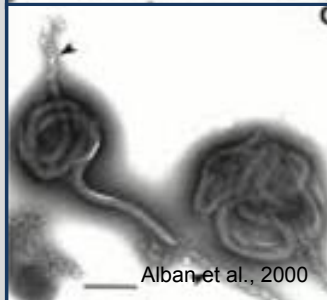


Alban et al., 2000



## Spirochetes (active form)

- extracellular and intracellular pathogen
- very mobile
- rapidly converts to dormant forms

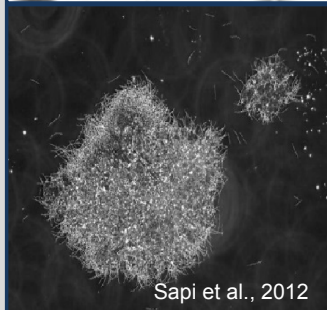


Alban et al., 2000



## Rounded forms (latent)

- survive antibiotics, starvation, etc.
- converts back to spirochetes
- cause of patient's relapse

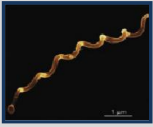


Sapi et al., 2012



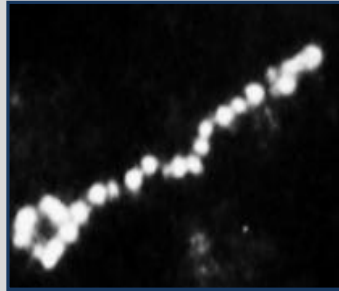
## Biofilm (latent, community)

- houses all morphological forms of *Borrelia* sp.
- even 1000x more resistant to antibiotics
- cause of patient's relapse

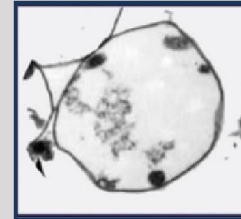
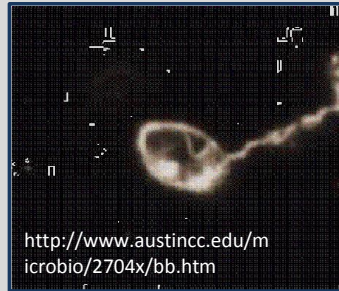


# Rounded forms are diverse

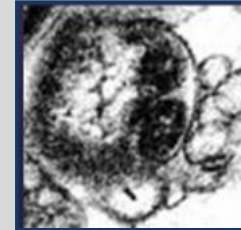
**Granular**  
(dot-like spirochetes)



**Cysts**  
(cystic spirochetes)



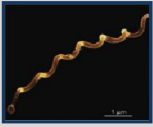
Young – small cylinders inside;  
regeneration up to 1 week



Aged – dense nucleoids inside;  
regeneration up to 6 weeks

**CWD**  
(bleb-like spirochetes)

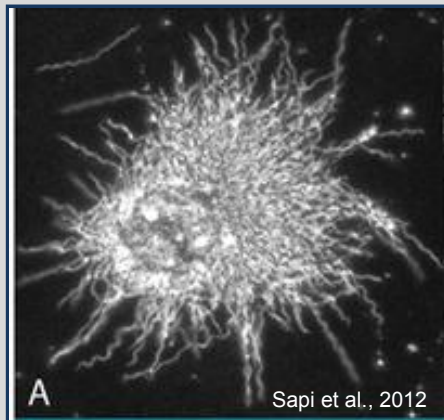




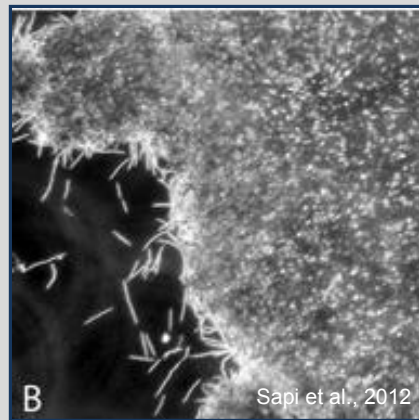
# What is Biofilm

Phagocytes can be found attached to biofilm but they are not able to eliminate it

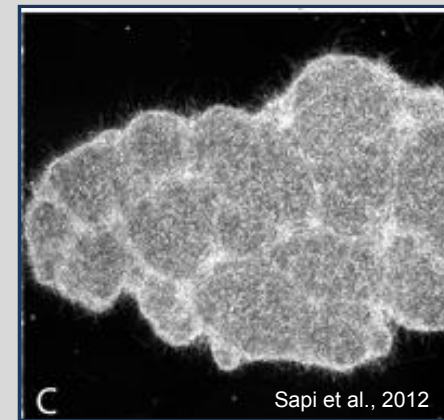
*0-2 DAYS*



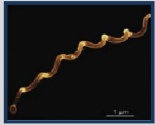
*3-6 DAYS*



*7-21 DAYS*



extracellular DNA  
polysaccharides  
phospholipids



# Survival strategies of *Borrelia sp.*

## Injection into host

Immune system suppression

Immune system evasion

### Innate:

- ◆ complement inhibition
- ◆ induction of anti-inflammatory cytokines
- ◆ tolerization of monocytes

### Adaptive:

- ◆ induction of anti-inflammatory cytokines
- ◆ tolerization of lymphocytes
- ◆ complement inhibition; plasminogen binding
- ◆ sequestration of antibodies in immune complexes

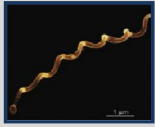
### Phase and antigenic variations:

- ◆ gene conversion
- ◆ mutation and recombination
- ◆ viable expression of antigens/lipoproteins

### Physical isolation (seclusion):

- ◆ intracellular: fibroblasts, ECs, neuronal cells, synovial cells, phagocytes, etc.
- ◆ extracellular: latent forms, immunologically privileged sites

Embers et al., 2004

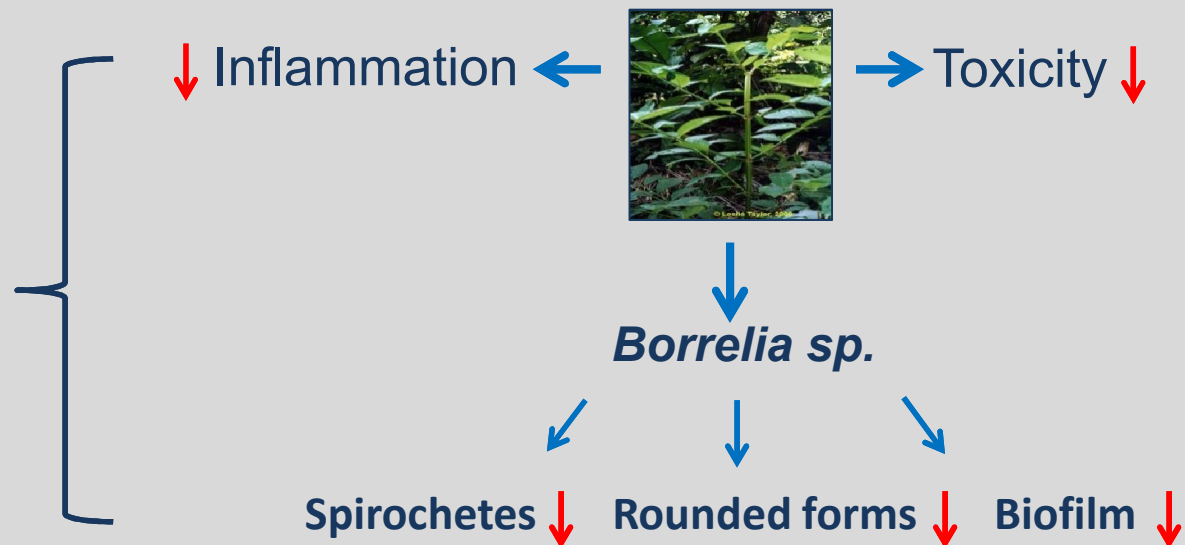


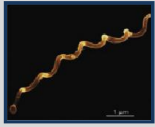
# Aim of Project

Develop effective treatment for Lyme disease based on active and naturally derived compounds in the aspects of prevention and cure

## Why this approach?

- ◆ high efficacy
- ◆ no side effects
- ◆ cost-efficient
- ◆ time-efficient





# Plan of work

Selection of naturally-derived compounds with the highest efficacy in “eliminating” all three forms of *Borrelia sp.*



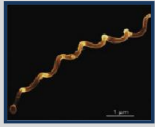
Selection of mixtures with the highest efficacy in “eliminating” all three forms of *Borrelia sp.*



**Growth Viability Biofilm formation Resistance**



**Spirochetes Rounded forms Biofilm**



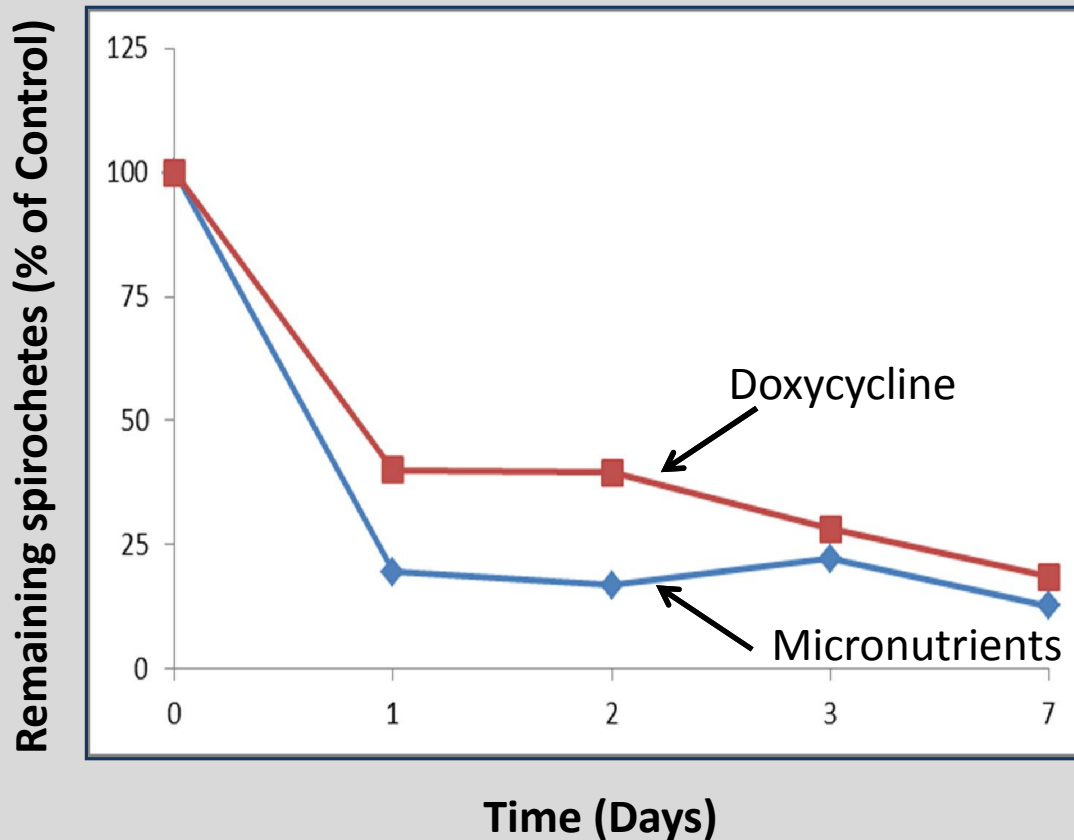
# Micronutrients Composition

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- ◆ **Vitamins:** Vitamin B-complex, Vitamin C, Vitamin D3
  
- ◆ **Specific natural phytochemicals from:**
  - Kelp (Iodine)
  - Royal Jelly (Cis-2-decenoic acid)
  - Coconut oil (Monolaurin)
  - *Terminalia chebula* (Luteolin)
  - Rosemary (Rosmarinic acid)
  - *Scutellaria baicalensis* (Baicalein)



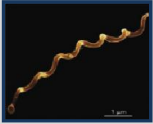
# Micronutrients can eliminate Spirochetes



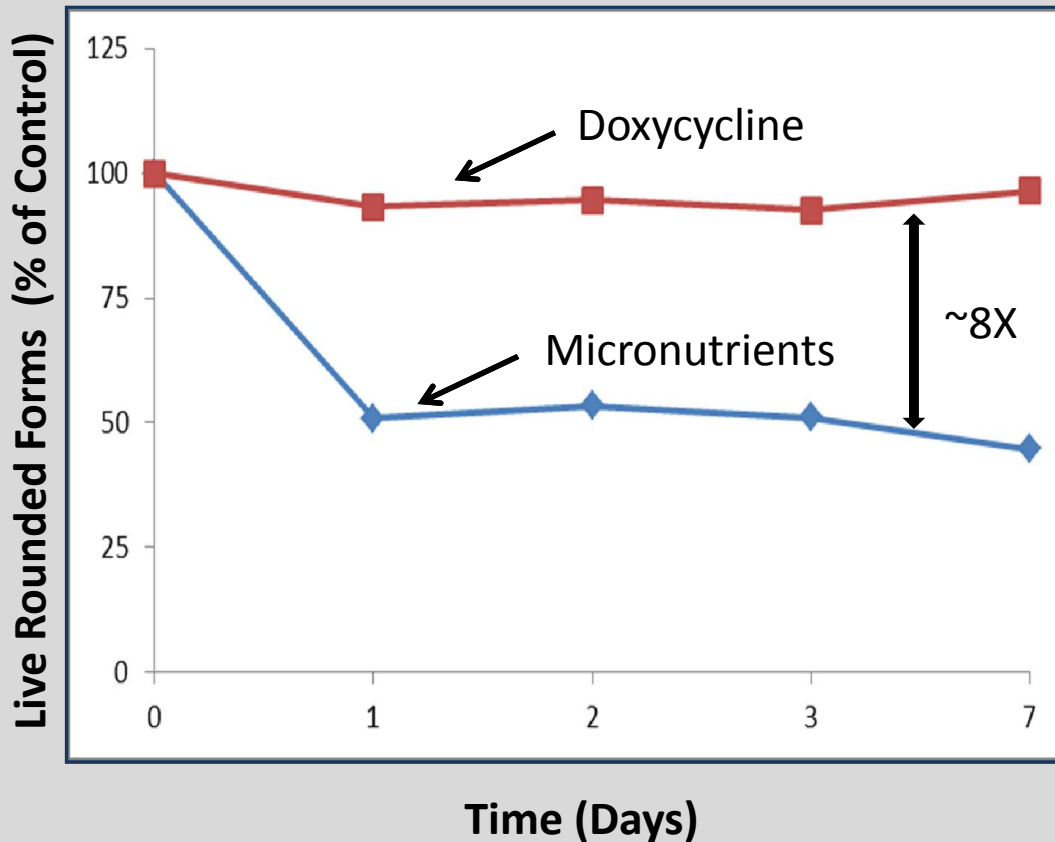
Micronutrients display similar efficacy to Doxycycline in eliminating *Borrelia* in its active form.

After 7 days of exposure ~75% of spirochetes become eliminated.



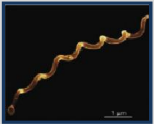


# Micronutrients can eliminate Rounded Forms



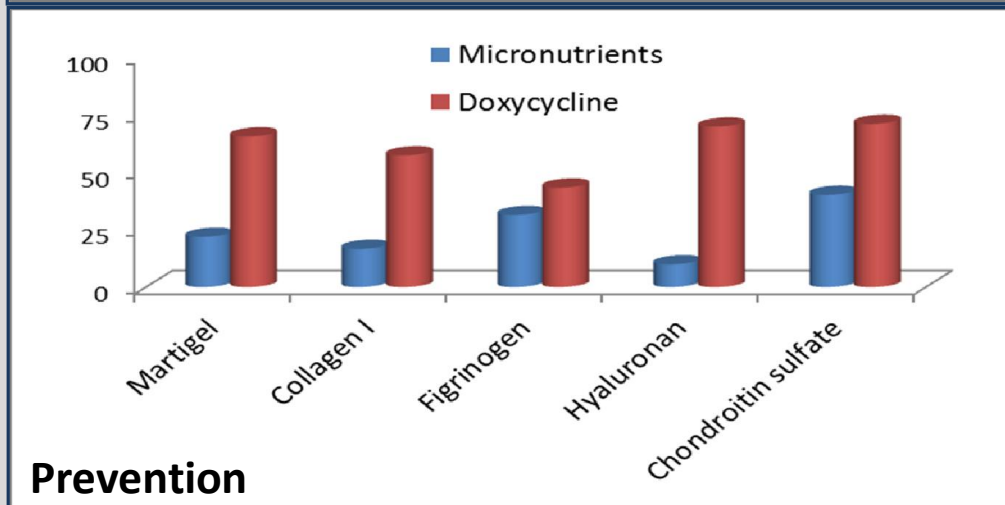
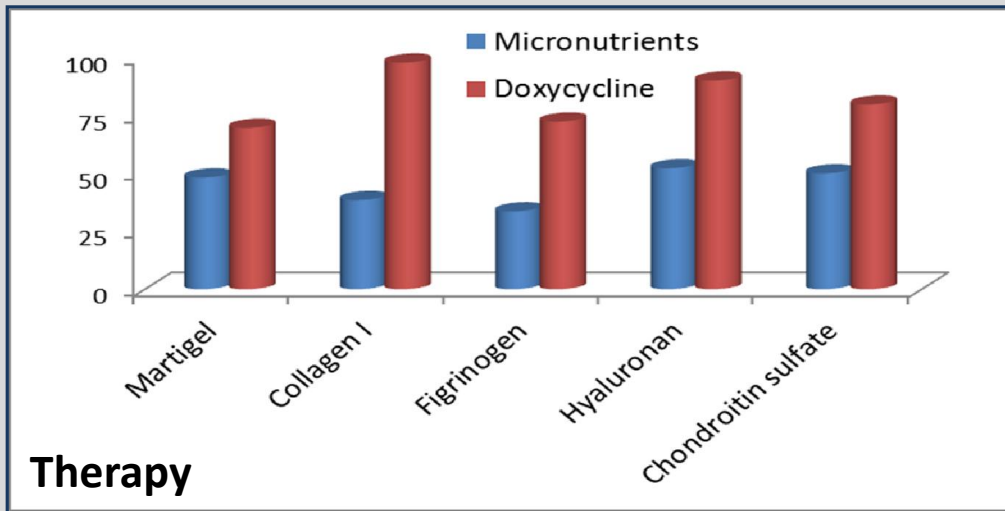
**Micronutrients are better than Doxycycline in eliminating *Borrelia* in its latent forms.**

**After 7 days of exposure ~50% of rounded forms become dead.**



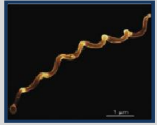
# Micronutrients are effective against Biofilm

Remaining biofilm (% of Control)



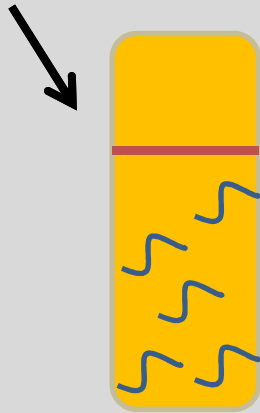
Micronutrients can eradicate the existing biofilm by ~50% and prevent from new biofilm formation by ~70%.

Doxycycline has a much lower efficacy (~25%).



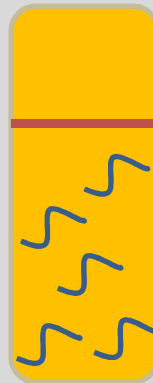
# Micronutrients do not induce Resistance

Micronutrients



1 or 5 months

Micronutrients



Remaining  
Spirochetes

↓  
~85%



Dead Rounded  
Bodies

↑  
~60%



# Conclusion

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Presented micronutrient mixture has demonstrated great potential in managing of Lyme disease. It was effective in:

- ◆ **decreasing** active (spiral) form of Borrelia
- ◆ **eliminating** latent (rounded) forms of Borrelia
- ◆ **preventing and eradicating** the biofilm of Borrelia
- ◆ **not developing resistance** of Borrelia to this natural treatment

## Lyme Research Laboratory



# Thank you



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Dr. Rath Research Institute, 2014