

In Vitro Effect of Peruvian Antimicrobial Agents on *Borrelia burgdorferi*

by

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Lyme Disease – The fastest growing epidemic

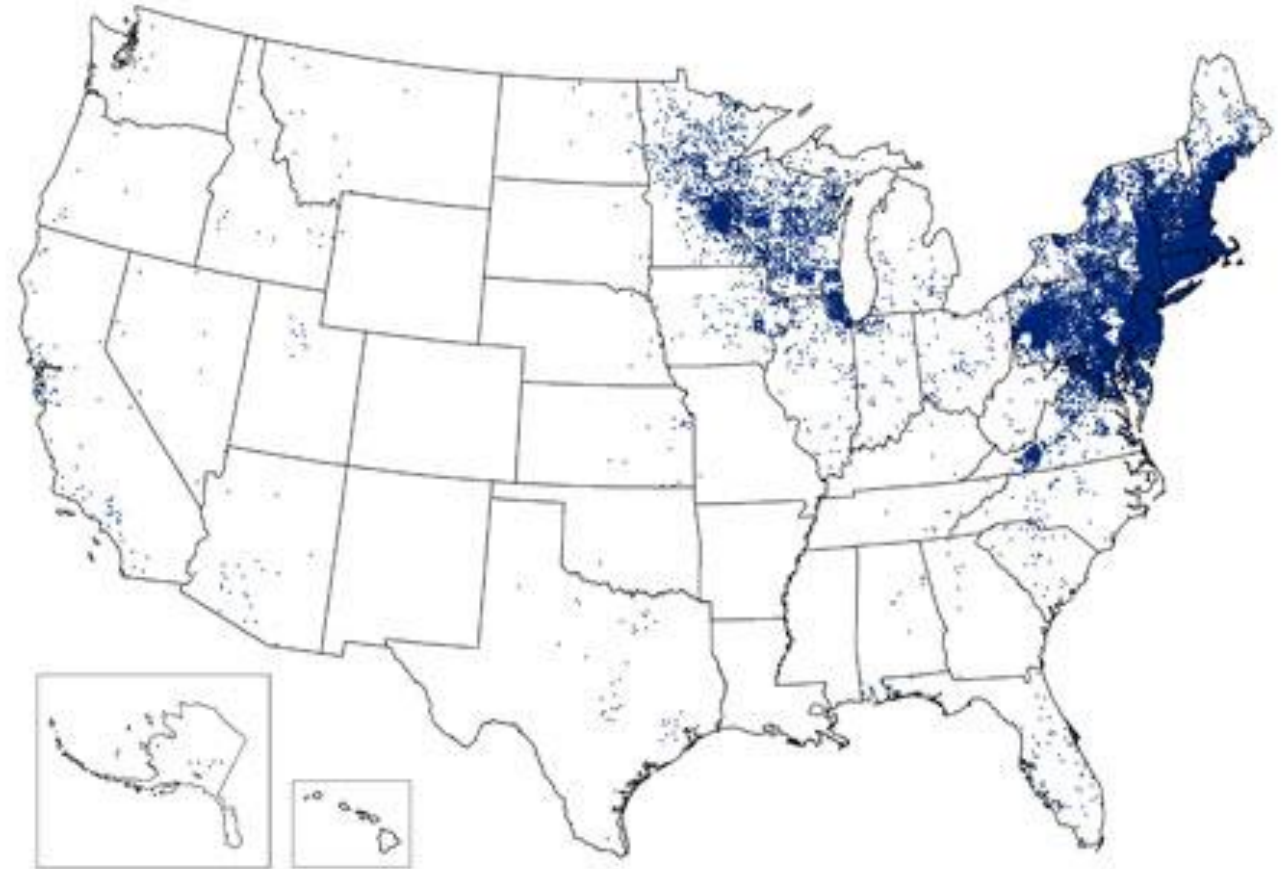


Lyme Disease – The most common vector-borne infectious disease in the Northern United States

Number of people diagnosed with Lyme Disease each year in the US ≈300,000

*Rapidly spreading throughout Europe and northeast Asia
(The Centers for Disease Control and Prevention)*

Reported Cases of Lyme Disease -- United States, 2013



1 dot placed randomly within county of residence for each confirmed case

How long did we know about Lyme disease?



The autopsy of Ötzi the Iceman (5,300-year-old mummy) revealed the presence of DNA sequences of *Borrelia*



New discoveries of ticks fossilized in amber show that the bacteria which cause it may have been lurking around for 15 million years



Borrelia burgdorferi

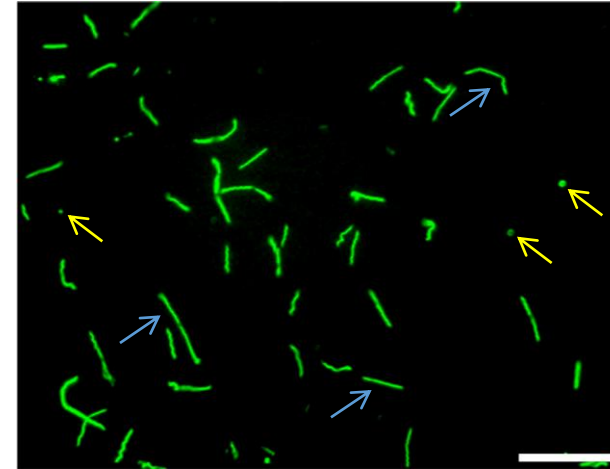
Exists in different morphological forms

Round body and biofilm form under stressful conditions

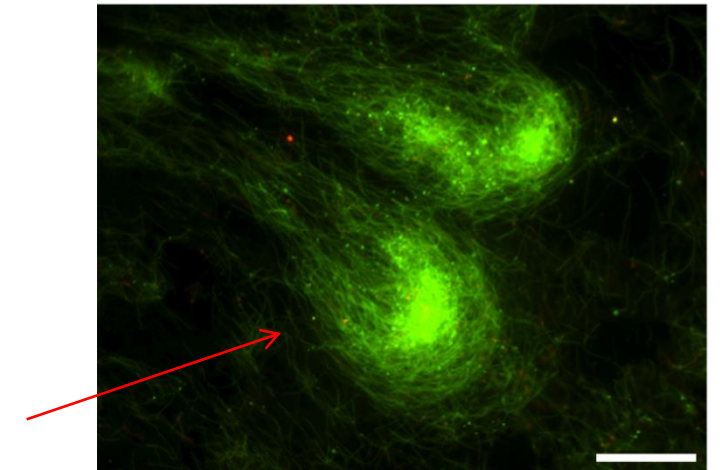
(Sapi E *et al*; 2011)

In favorable conditions, round body form revert back to spirochetes

Round body and biofilm forms show higher resistance to antimicrobials (Sapi E *et al*; 2012)



Spirochetes and Round body forms



Biofilm

Conventional Antibiotics – Advantage/Disadvantage



Doxycycline - Choice of Lyme disease treatment

Doxycycline failed to cure infected mice (Moody K. D *et al*; 1994)

Spirochetes are capable of persisting in doxycycline treated mice and nonhuman primate hosts (Hodzic E *et al*; 2014) (Embers M. E *et al*; 2012)

Our research group has proven that all forms have different sensitivities to antimicrobial agents (Sapi E *et al*; 2011)

Borrelia frequently becomes resistant to antibiotic treatment

Known to have unfavorable side effects (Chang E.T *et al*; 2005)



Alternative therapies for Lyme disease



Enzyme therapy

Homeopathics

Herbal antimicrobial agents

Bee venom therapy

Chi machine/light beam generator

Hyperbaric oxygen therapy

Detox therapy

Photon therapy

Ozone therapy



Clinical study on Peruvian Herbal extracts



Utilizes several herbal extracts from the Cowden support program designed to eliminate microbes in advanced Lyme disease patients

(Dr. Richard Horowitz, ILADS, 2010)

Study conducted on **over 100 Lyme disease patients who did not respond to standard antibiotic treatment**

Samento and Banderol plus 8 other natural products used for the first 78 days

Later, 4 other antimicrobial agents (Enula, Mora, Cumanda, Houttuynia) are used in rotation along with Samento and Banderol

Effective for **more than 80%** of the patients



Another independent European clinical study

Dr. Armin Schwarzbach – Study conducted on 20 advanced Lyme disease patients
(1st Ireland Lyme Disease conference, 2012)

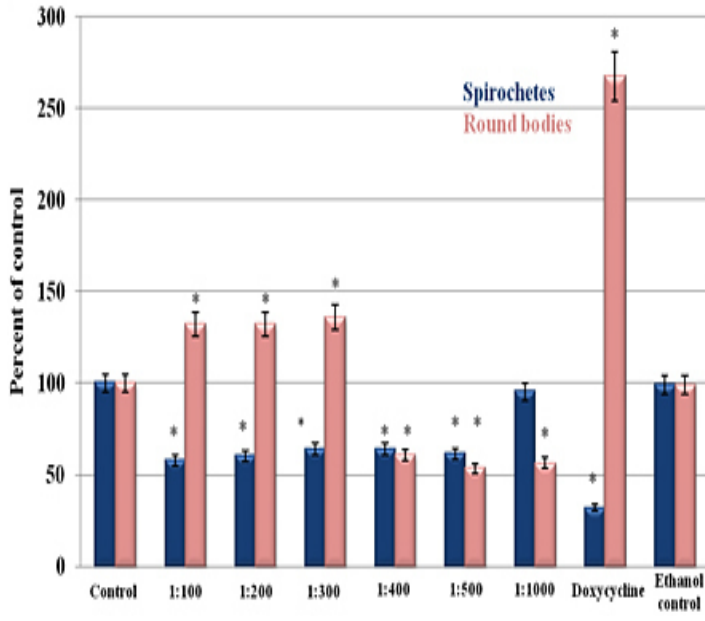
The herbal extracts from the Cowden support program markedly improved symptoms
in **80% of the patients**

Improved laboratory test results in **90% of the patients**

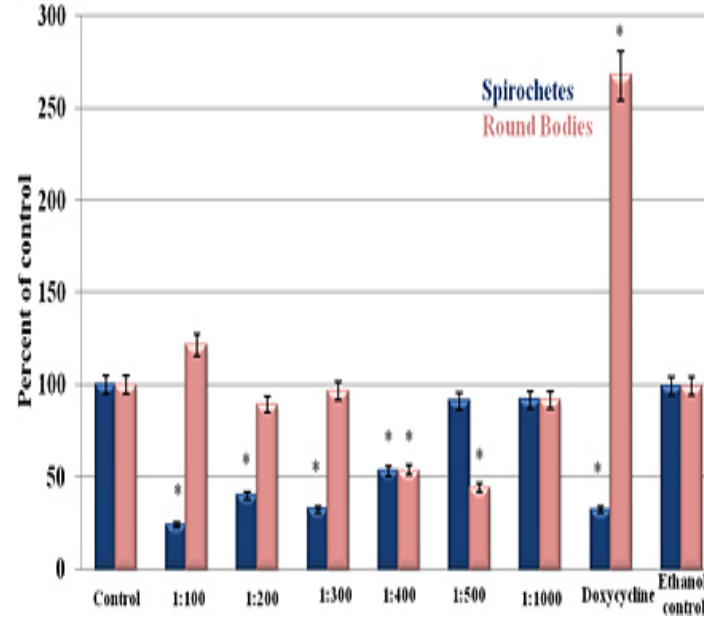
The doses were equivalent to thousands of times the recommended doses for
humans

Caused no acute or chronic organ damage or any adverse effects

Samento and Banderol (Datar A *et al*; 2010)

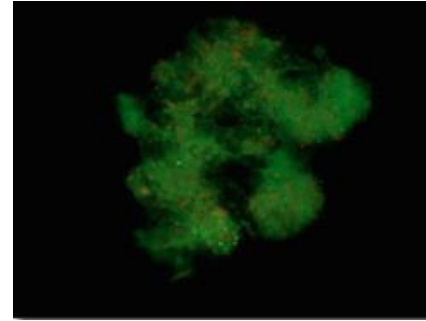


Samento

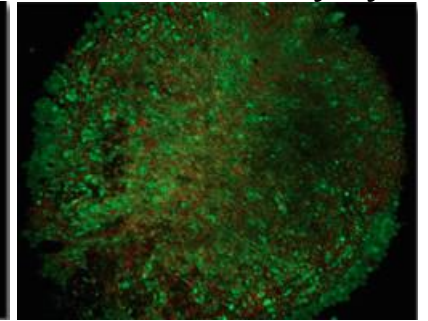


Banderol

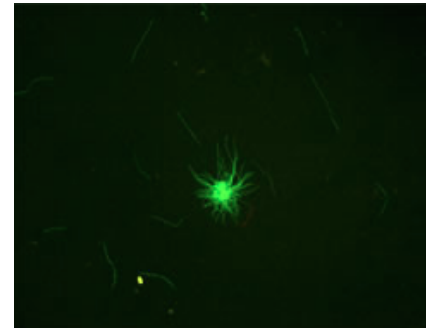
Control



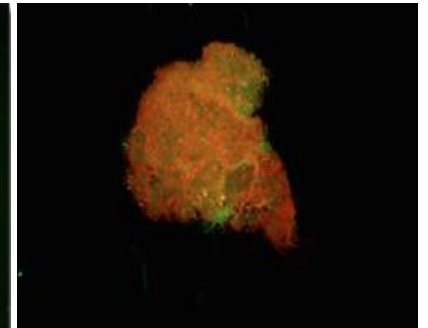
Doxycycline



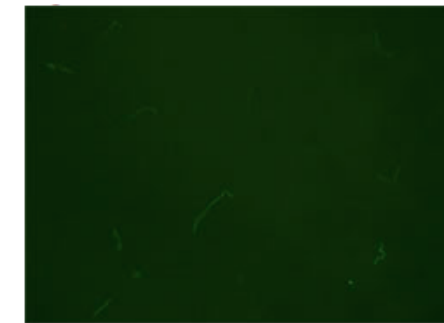
Samento



Banderol



Samento + Banderol



Research question



Can we find additional effective antimicrobial agents from the list of Peruvian herbs for *Borrelia burgdorferi* ?

Peruvian Medicinal Antimicrobial Agents - Benefits



Have significant antimicrobial, antiprotozoal and antiviral properties

Safer to use

Cost effective

Might provide effective therapeutic options



Name	Species	Activity of the agent	Reported effectiveness against the following pathogens
Mora	Premix of 3 plant extract: <i>Rubus fruticosus</i> , <i>Achillea millefolium</i> and <i>Calycophyllum spruceanum</i>	Antibacterial	<i>Staphylococcus aureus</i> ²⁴ , <i>Streptococcus pneumoniae</i> and <i>Clostridium perfringens</i> ²⁵
Enula	<i>Inula helenium</i> , <i>Mirabilis jalapa</i> and <i>Vitis tiliifolia</i>	Antibacterial and antifungal	<i>Bacillus cereus</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> ^{26,27} , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> ^{20,27} and <i>Aspergillus parasiticus</i> ²⁸
Stevia	<i>Stevia rebaudiana</i>	Antimicrobial	<i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Vibrio cholera</i> ²⁹ , <i>Bacillus subtilis</i> , <i>Micrococcus luteus</i> , <i>Serratia marcescens</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus megaterium</i> , <i>Escherichia coli</i> and <i>Proteus vulgaris</i> ³⁰
Cumanda	<i>Campsiandra angustifolia</i>	Anti-parasitic, anti-fungal, anti-viral	<i>Plasmodium falciparum</i> ³¹
Houttuynia	<i>Houttuynia cordata</i>	Antibacterial	<i>Salmonella typhimurium</i> ³²
Samento	<i>Uncaria tomentosa</i>	Antibacterial	<i>Borrelia burgdorferi</i> ⁹ and <i>Pseudomonas aeruginosa</i> ³³
Banderol	<i>Otoba</i> species	Antibacterial	<i>Borrelia burgdorferi</i> ⁹
Takuna	<i>Cecropia strigosa</i>	Antibacterial	<i>Escherichia coli</i> and <i>Bacillus subtilis</i> ³⁴
Barberry	<i>Mahonia aquifolium</i>	Antimicrobial, antifungal	<i>Staphylococcus aureus</i> and <i>Candida</i> sp ³⁵
Lakato	<i>Echinacea angustifolia</i>	Antibacterial	<i>Escherichia coli</i> , <i>Proteus mirabilis</i> , <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i> ³⁶
Quina	<i>Cinchona calisaya</i>	Antimalarial	<i>Plasmodium falciparum</i> ³⁷

Experimental conditions



Borrelia burgdorferi B31 laboratory strain

The herbal extracts were prepared in alcohol and formulated by Nutramedix Inc®

Positive control - Doxycycline (10 µg/ml)

Direct counting method– Seeded a concentration of 1×10^5 cells for 5 days

Crystal violet assay- Seeded a concentration of 5×10^6 cells for 7 days

Treatment regime – Antimicrobial agents treated for every 24 hrs for subsequent 3 days

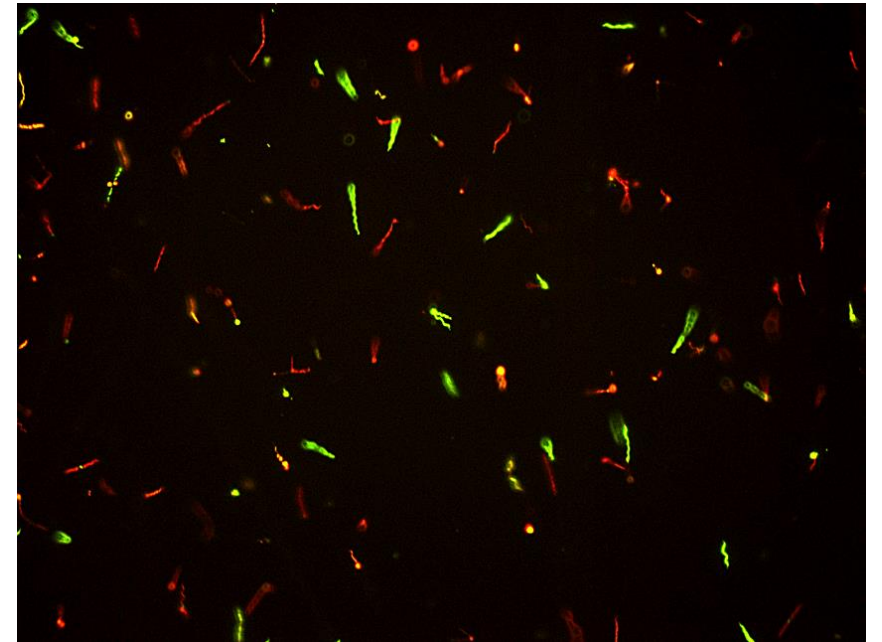
In vitro evaluation of antimicrobial agents against *Borrelia burgdorferi*



Direct counting method – Counting live spirochetes and round body forms stained with special dyes

Live cells – Green color

Dead cells – Red color



Spirochetes and Round body forms

Qualitative and quantitative analysis of the attached biofilm form after treatment with antimicrobial agents

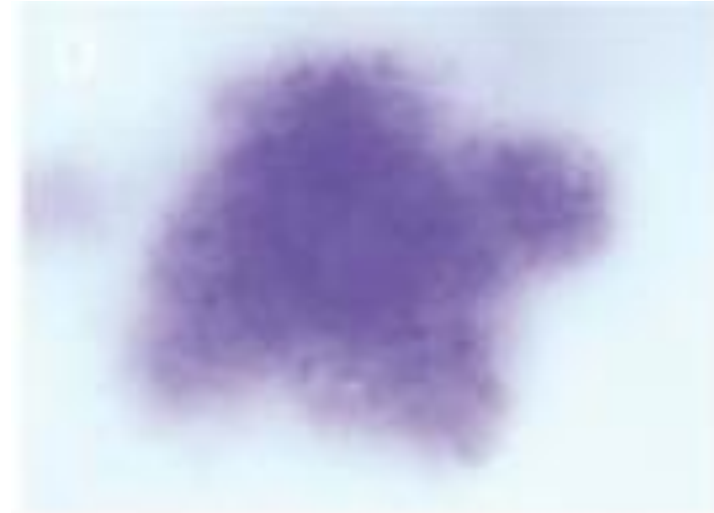


Crystal violet staining assay

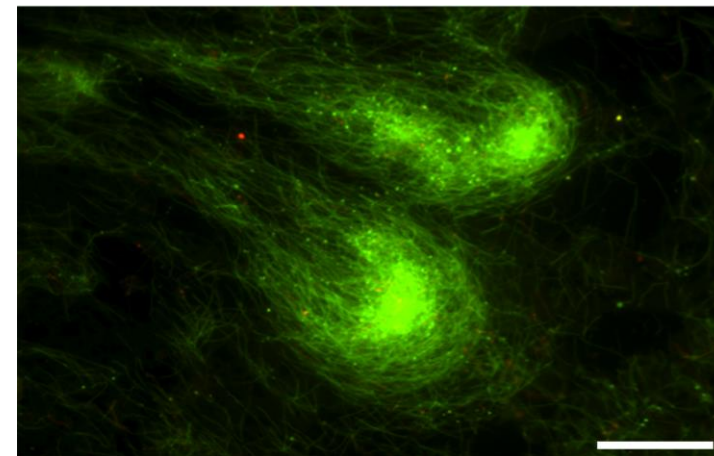
Live dead assay

Live cells – Green color

Dead cells – Red color



Crystal violet staining

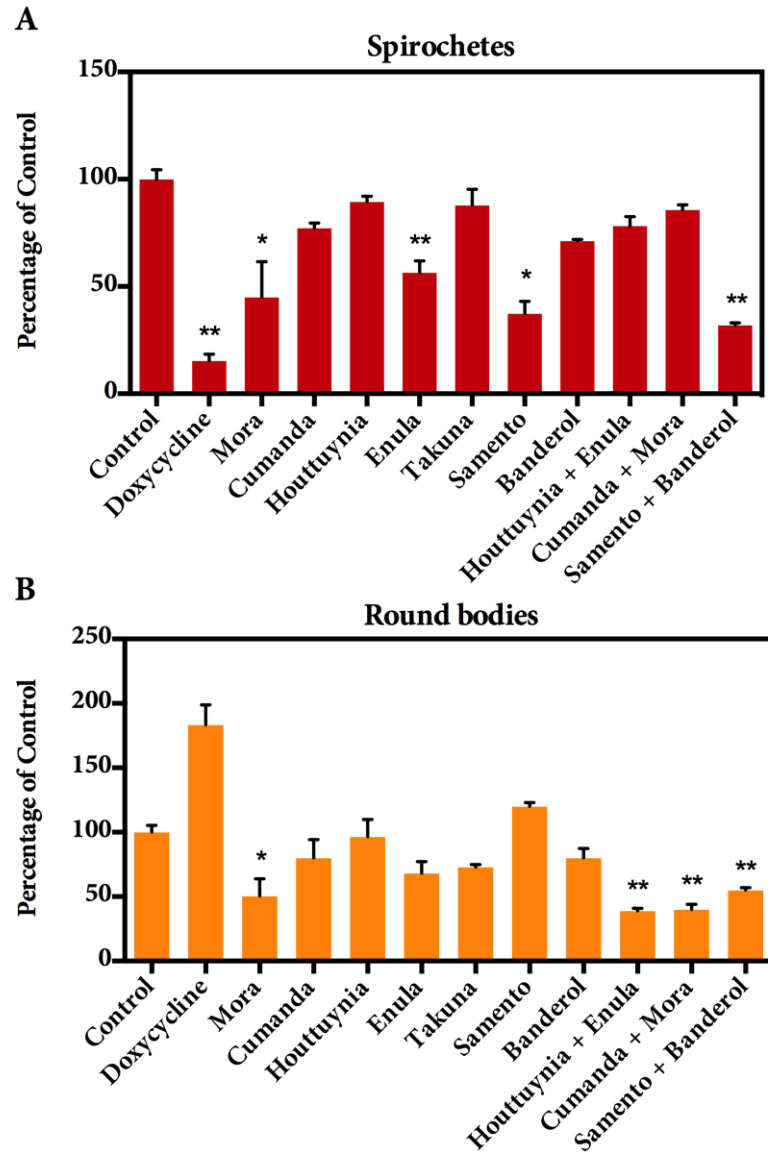


Live dead

RESULTS



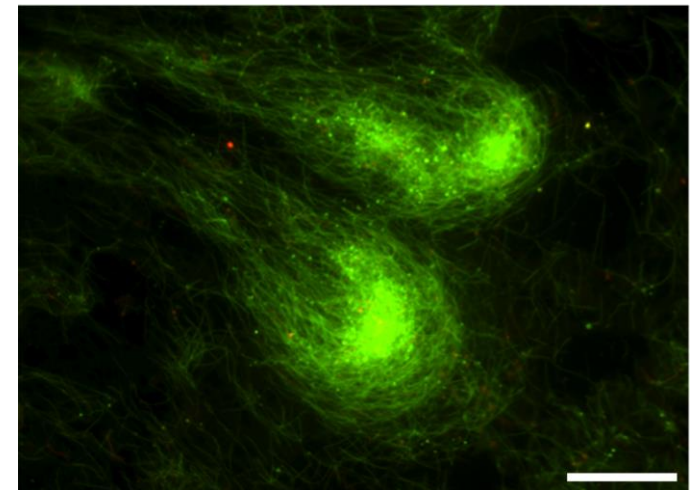
Direct counting method – Live spirochetes and round body forms



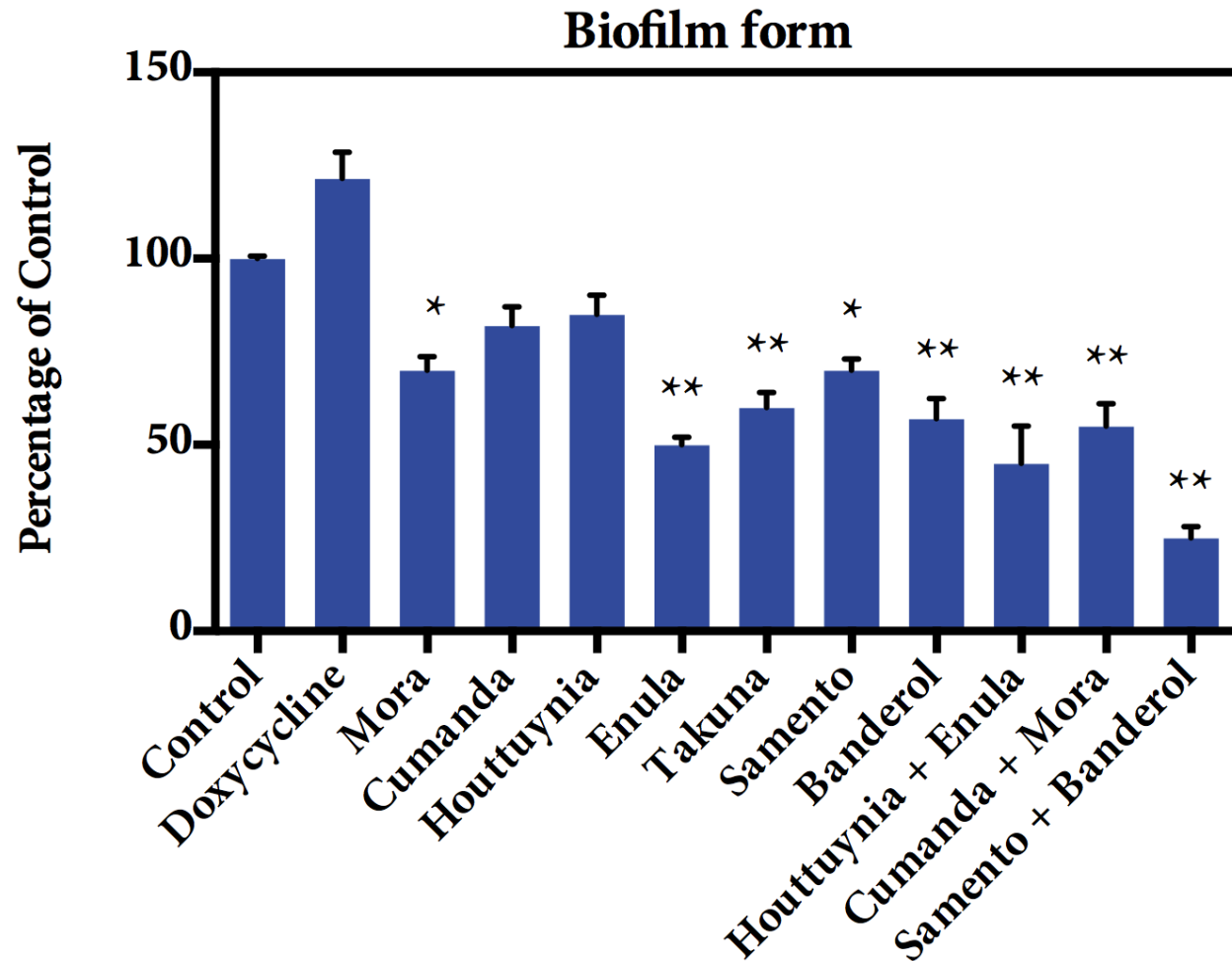
Antimicrobial agents	% reduction – Spirochetes	% reduction – Round body forms
Doxycycline (25 µg/ml)	85%	Increase
Mora	55%	50%
Cumanda	23%	20%
Houttuynia	11%	4%
Enula	44%	32%
Takuna	12%	27%
Samento	63%	Increase
Banderol	29%	20%
Houttuynia + Enula	22%	61%
Cumanda + Mora	14%	60%
Samento + Banderol	68%	45%

Effectiveness of antimicrobial agents on the spirochete and round body forms evaluated using direct counting method. (A) Counting live *Borrelia* spirochetes after treatment with antimicrobial agents (B) Counting live round body forms of *Borrelia* after treatment with antimicrobial agents (n=3 ± SD, *p≤ 0.05, **p≤ 0.01)

Do the antimicrobial agents also work on biofilms?



Quantitating attached biofilms by standard crystal violet staining method



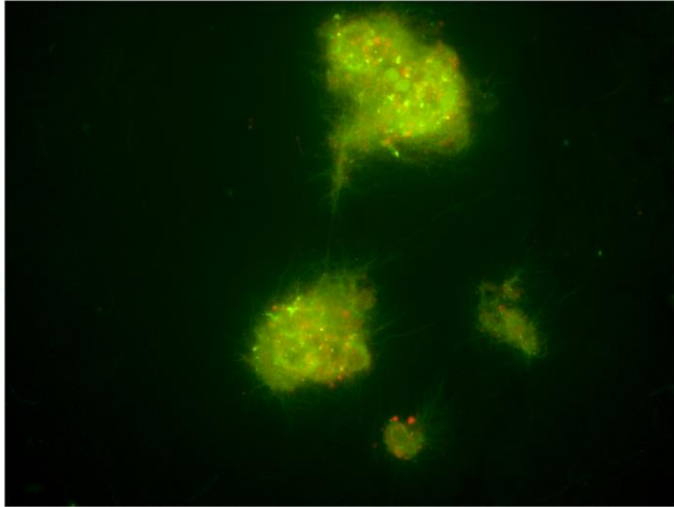
Antimicrobial agents	% reduction in biomass after 72 hr treatment
Doxycycline (25 µg/ml)	Increase
Mora	30%
Cumanda	18%
Houttuynia	15%
Enula	50%
Takuna	40%
Samento	30%
Banderol	43%
Houttuynia + Enula	55%
Cumanda + Mora	45%
Samento + Banderol	75%

Quantitative analysis measuring the total biomass after 72 h treatment measured by crystal violet staining technique. 25% grain alcohol (1:50 dilution) was used as a negative control. (n=3 ± SD, *p≤ 0.05, **p≤ 0.01)

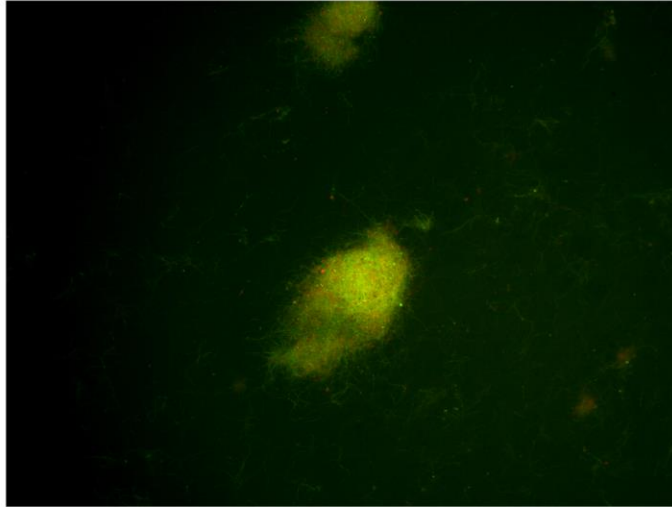
Antimicrobial treated *Borrelia* biofilms stained using BacLight viability kit



Doxycycline



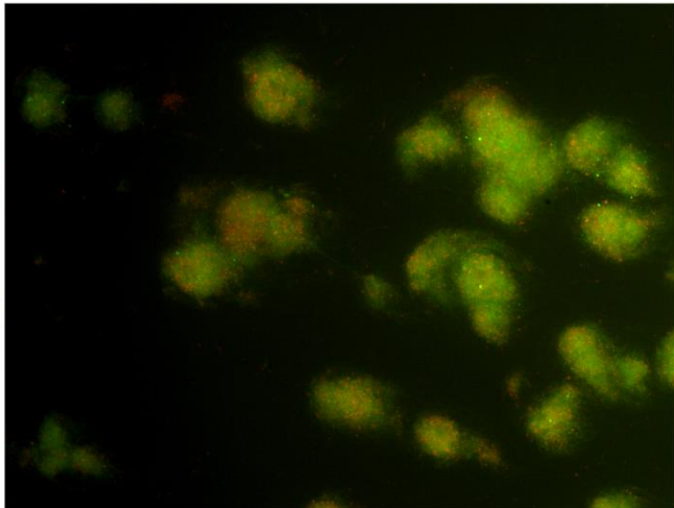
Houttuynia + Enula



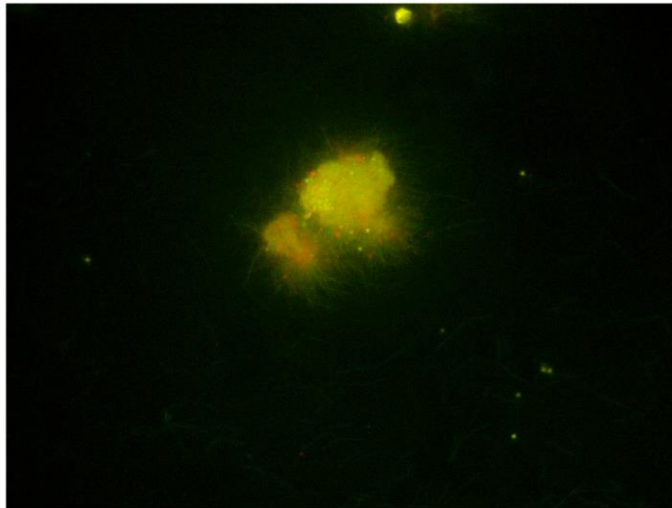
The micrographs represent the biofilm form of *Borrelia burgdorferi* to the antimicrobial agents after 72 h treatment measured by fluorescent microscopy using BacLight viability kit

Live cells – Green color
Dead cells – Red color

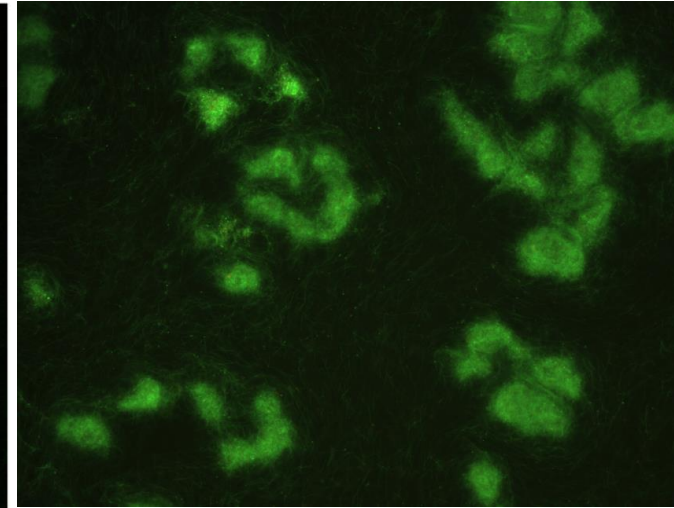
Cumanda + Mora



Samento + Banderol



Control (B31)



LiveDead

LiveDead

Conclusion



Agents effective against spirochetes
Mora
Enula
Samento + Banderol

Agents effective against round bodies
Mora
Houttuynia + Enula
Cumanda + Mora
Samento + Banderol

Agents effective against biofilms
Mora
Enula
Takuna
Banderol
Houttuynia + Enula
Cumanda + Mora
Samento + Banderol

- Clinical studies + *in vitro* studies show the effectiveness of the antimicrobial agents against *Borrelia burgdorferi*
- However the efficacy of these antimicrobial agents must be further reinforced by *in vivo* studies
- These antimicrobial agents could provide an effective therapeutic approach for Lyme disease patients

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Thank
You