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

by

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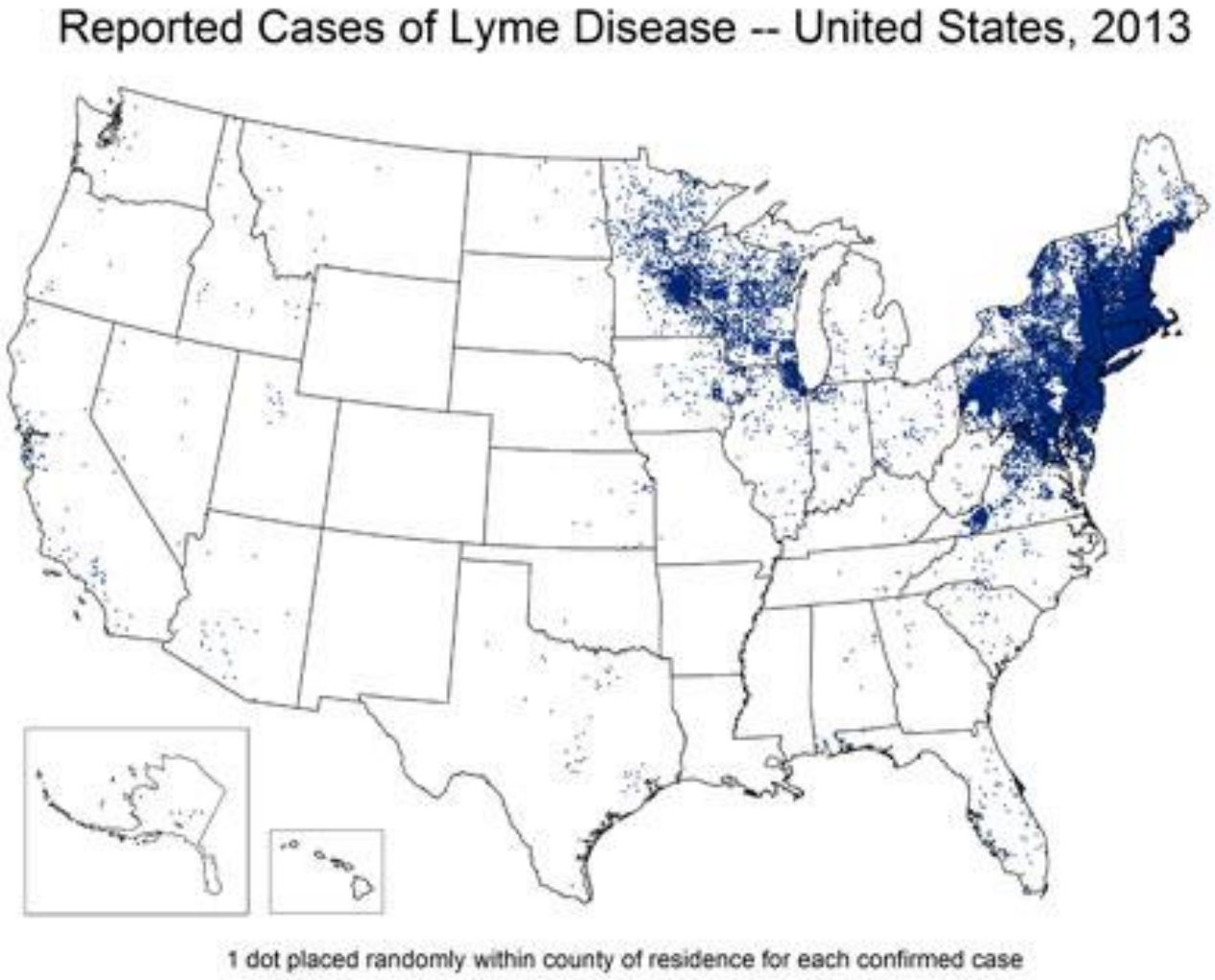
Lyme Disease – The fastest growing epidemic

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)



Source : Picture taken from the Centers for Disease Control and Prevention

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

Source : Picture taken from google image library

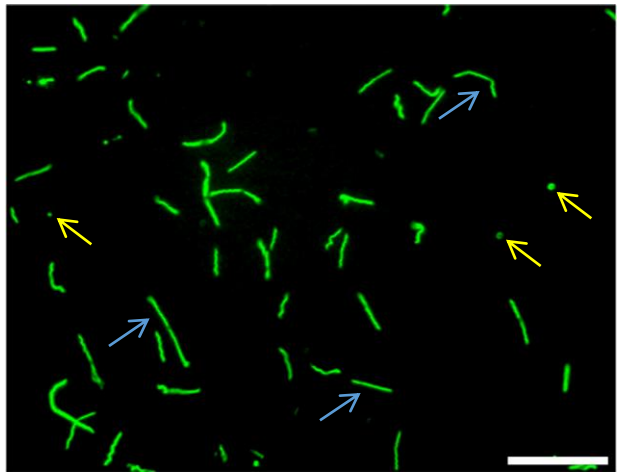
Borrelia burgdorferi

Exists in different morphological forms

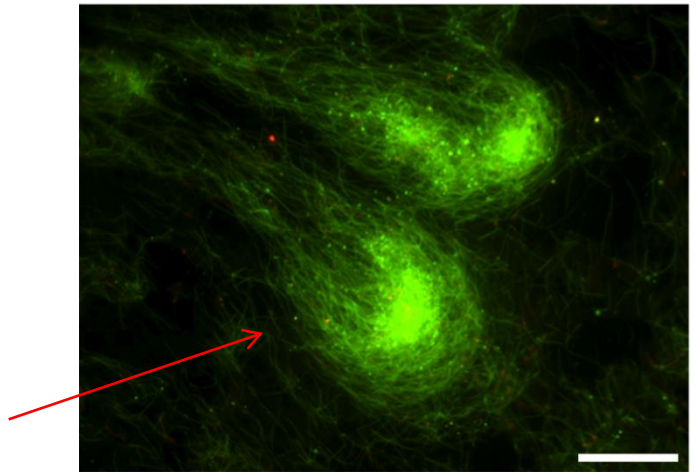
Round body and biofilm form under stressful conditions
(Sapi E *et al*; 2011)

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

Source : Picture taken from the UNH Lyme Disease Research Group. Scale bar-100 µm

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)

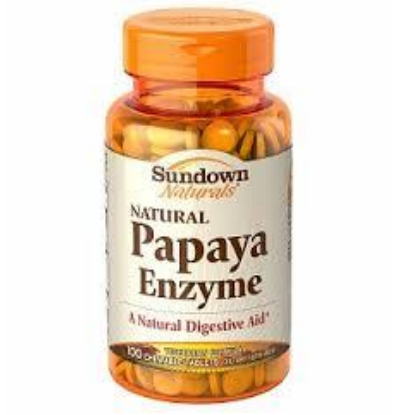
Borrelia frequently becomes resistant to antibiotic treatment

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

Source : Picture taken from google image library

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



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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)

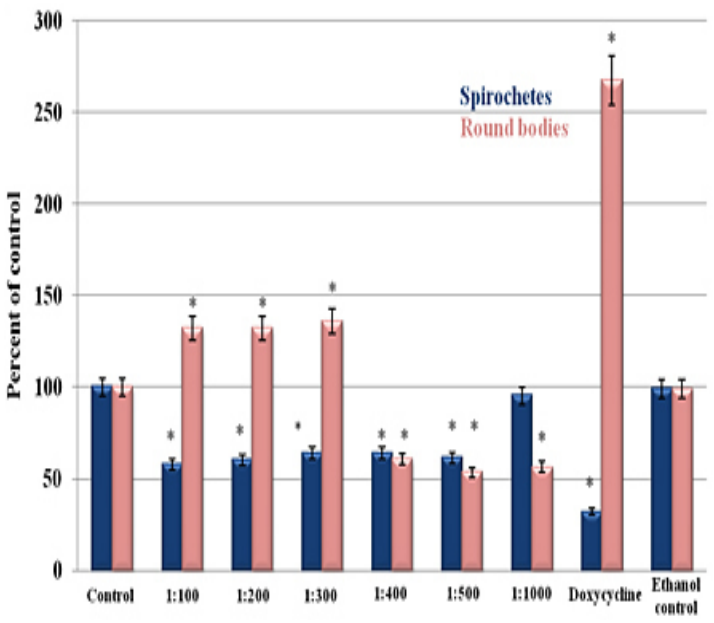
The herbal extracts from the Cowden support program markedly improved symptoms in **80% of the patients**

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

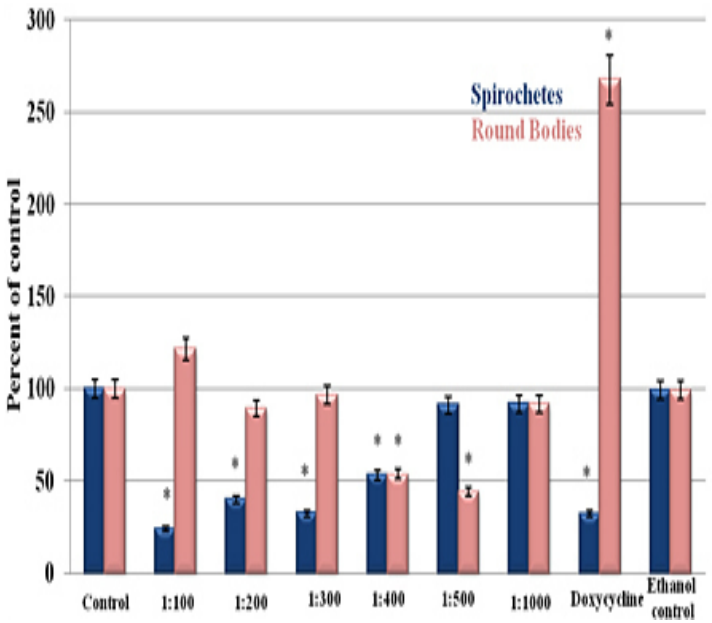
The doses were equivalent to thousands of times the recommended doses for humans

Caused no acute or chronic organ damage or any adverse effects

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

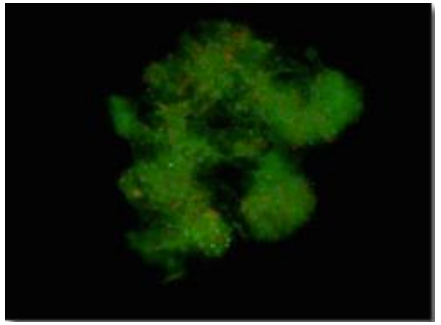


Samento

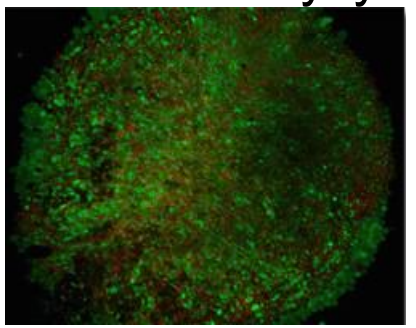


Banderol

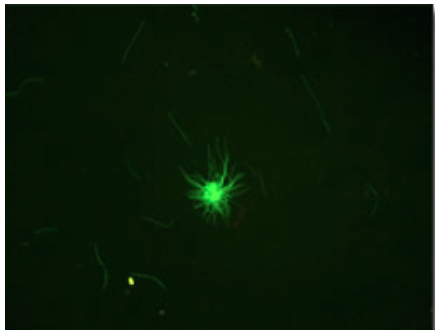
Control



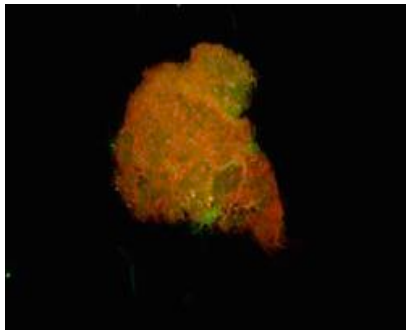
Doxycycline



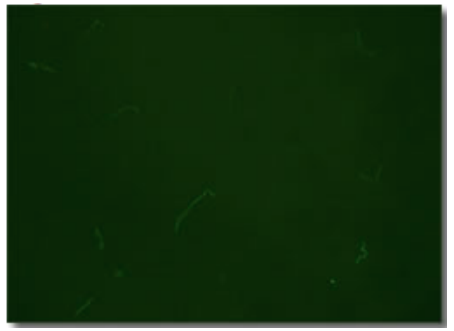
Samento



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

Source : Picture taken from Google image library

Name	Species	Activity of the agent	Reported effectiveness against the following pathogens
Mora	Premix of 3 plant extract: *Rubus fruticosus, Achillea millefolium* and *Calycophyllum spruceanum*	Antibacterial	*Staphylococcus aureus*[24], *Streptococcus pneumonia* and *Clostridium perfringens*[25]
Enula	*Inula helenium, Mirabilis jalapa* and *Vitis tiliifolia*	Antibacterial and antifungal	*Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus*[26,27], *Escherichia coli, Klebsiella pneumonia*[20,27] and *Aspergillus parasiticus*[28]
Stevia	*Stevia rebaudiana*	Antimicrobial	*Staphylococcus aureus, Bacillus subtilis, Vibrio cholera*[29], *Bacillus subtilis, Micrococcus luteus, Serratia marcenscens, Pseudomonas aeruginosa, Bacillus megaterium, Escherichia coli* and *Proteus vulgaris*[30]
Cumanda	*Campsiandra angustifolia*	Anti-parasitic, anti-fungal, anti-viral	*Plasmodium falciparum*[31]
Houttuynia	*Houttuynia cordata*	Antibacterial	*Salmonella typhimurium*[32]
Samento	*Uncaria tomentosa*	Antibacterial	*Borrelia burgdorferi*[9] *and Pseudomonas aeruginosa*[33]
Banderol	*Otoba* species	Antibacterial	*Borrelia burgdorferi*[9]
Takuna	*Cecropia strigosa*	Antibacterial	*Escherichia coli* and *Bacillus subtilis*[34]
Barberry	*Mahonia aquifolium*	Antimicrobial, antifungal	*Staphylococcus aureus* and *Candida* sp[35]
Lakato	*Echinacea angustifolia*	Antibacterial	*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa and Staphylococcus aureus*[36]
Quina	*Cinchona calisaya*	Antimalarial	*Plasmodium falciparum*[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 x 10^5 cells for 5 days

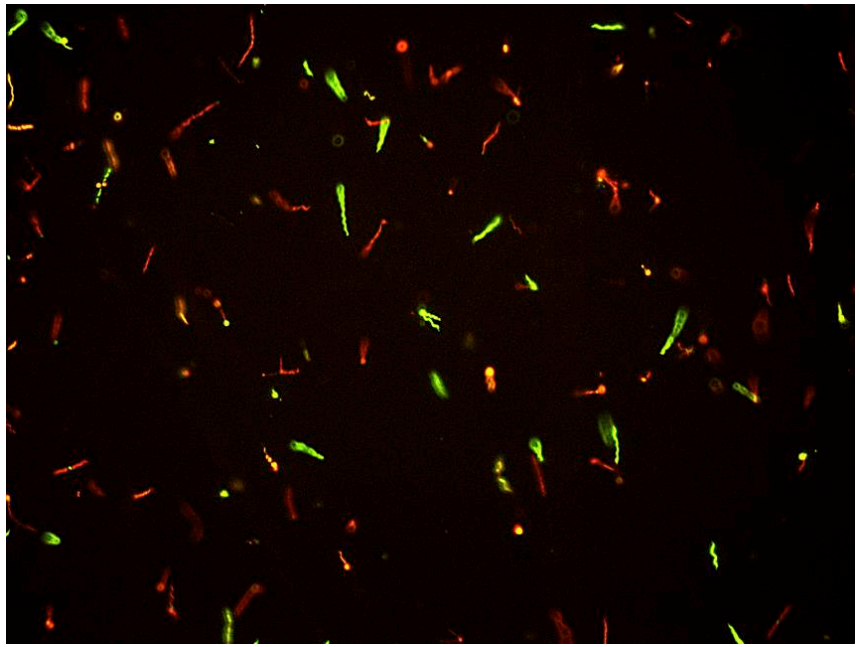
Crystal violet assay- Seeded a concentration of 5 x 10^6 cells for 7 days

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



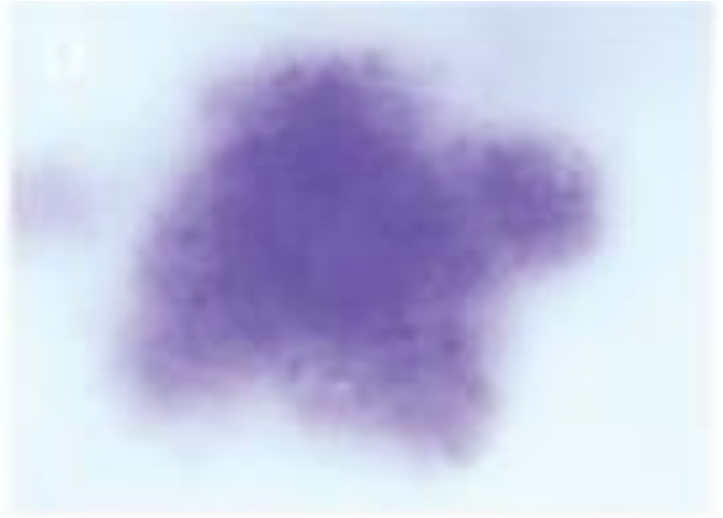
Spirochetes and Round body forms

Source : Picture taken from the UNH Lyme Disease Research Group. Scale bar-100 µm

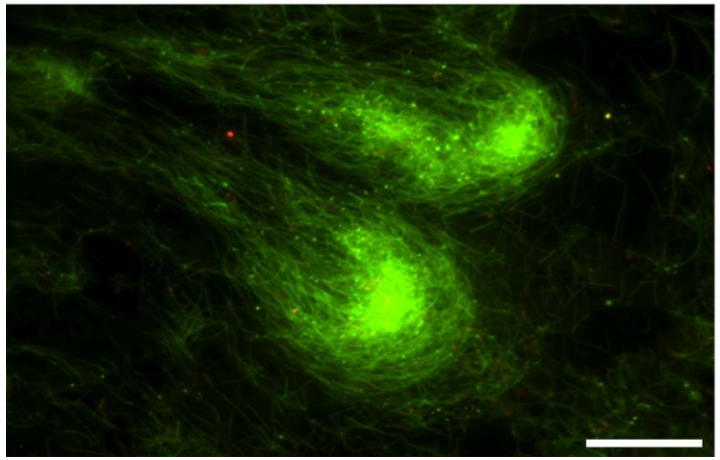
Qualitative and quantitative analysis of the attached biofilm form after treatment with antimicrobial agents

Crystal violet staining assay

Live dead assay
Live cells – Green color
Dead cells – Red color



Crystal violet staining

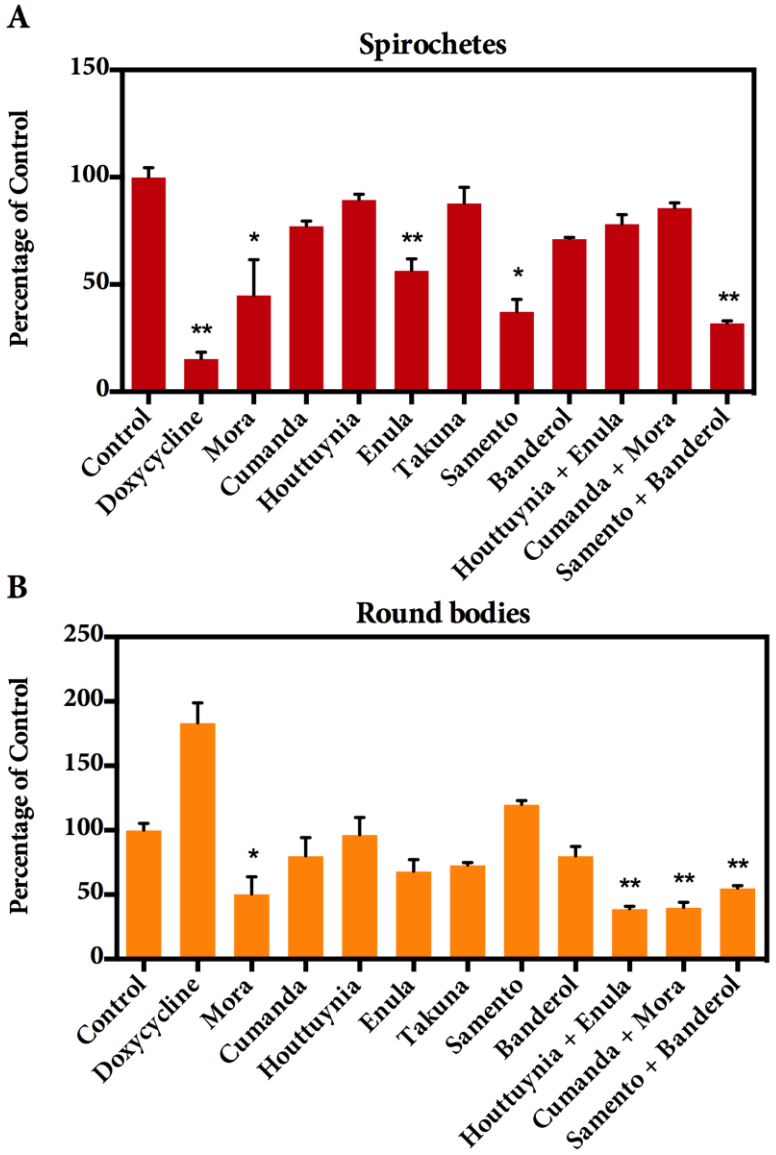


Live dead

Source : Picture taken from the UNH Lyme Disease Research Group. Scale bar-100 µm

RESULTS

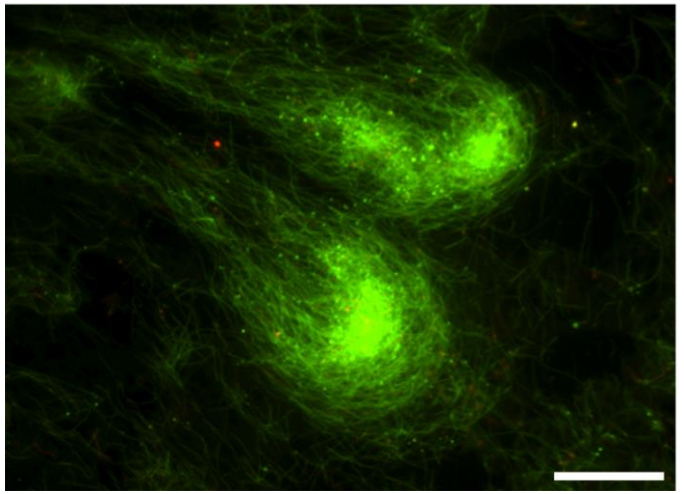
Direct counting method – Live spirochetes and round body forms



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%
Hottuynia + Enula	22%	61%
Cumanda + Mora	14%	60%
Samento + Banderol	68%	45%

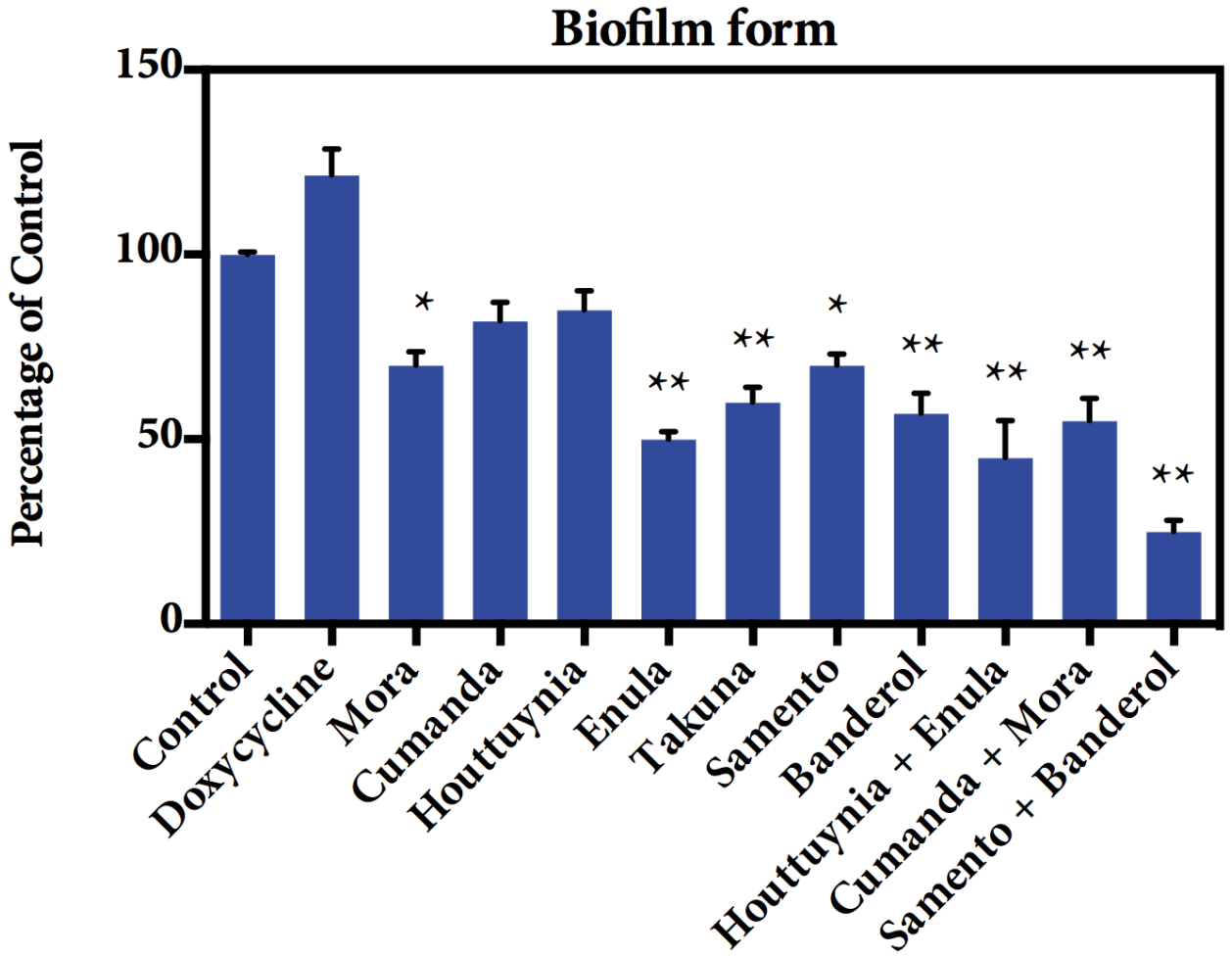
Effectiveness of antimicrobial agents on the spirochete and round body forms evaluated using direct counting method. (A) Counting live *Borrelia* spirochetes after treatment with antimicrobial agents (B) Counting live round body forms of *Borrelia* after treatment with antimicrobial agents (n=3 ± SD,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

Do the antimicrobial agents also work on biofilms?



Source : Picture taken from the UNH Lyme Disease Research Group. Scale bar-100 µm

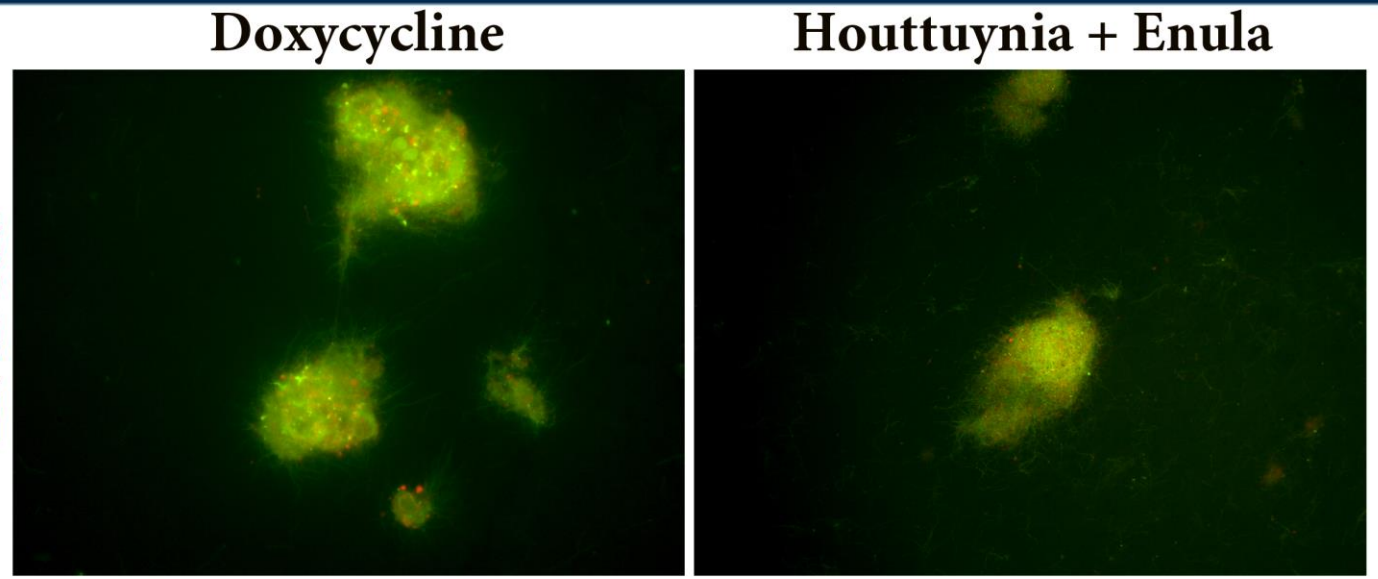
Quantitating attached biofilms by standard crystal violet staining method



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%
Hottuynia + Enula	55%
Cumanda + Mora	45%
Samento + Banderol	75%

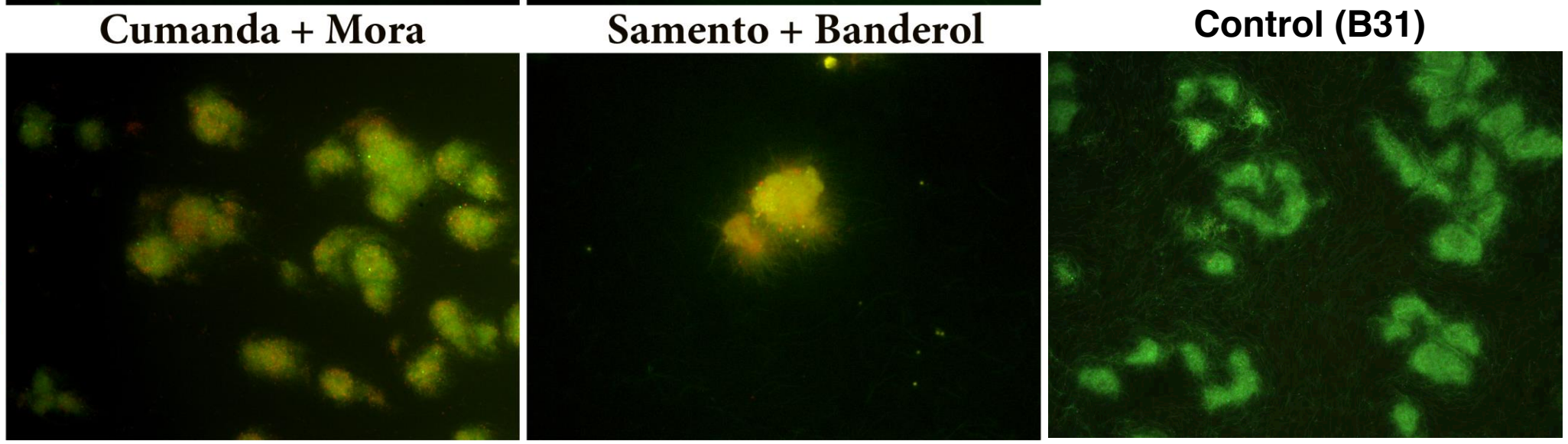
Quantitative analysis measuring the total biomass after 72 h treatment measured by crystal violet staining technique. 25% grain alcohol (1:50 dilution) was used as a negative control. (n=3 ± SD, *p≤ 0.05, **p≤ 0.01)

Antimicrobial treated *Borrelia* biofilms stained using BacLight viability kit



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



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