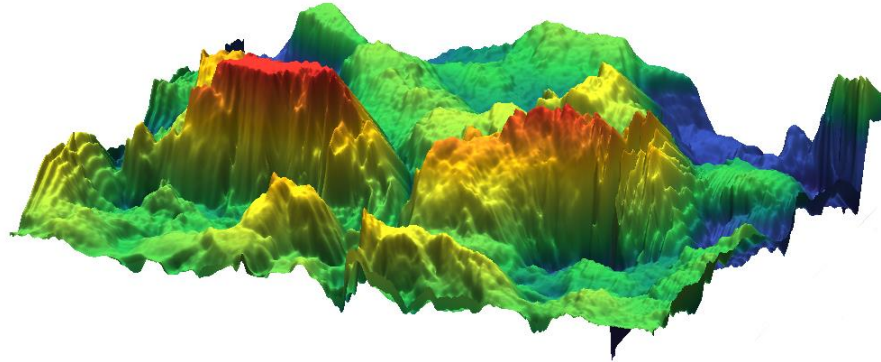


Potential Connection of Borrelia Infection and Breast Cancer



EVA SAPI Ph.D.
UNIVERSITY OF NEW HAVEN



Borrelia burgdorferi the spirochete that causes Lyme disease

- In 1982, the etiologic agent of Lyme disease was discovered by Willy Burgdorfer who isolated spirochetes belonging to the genus *Borrelia* from the mid-guts of *Ixodes* ticks.
- He showed that these spirochetes reacted with immune serum from patients that had been diagnosed with Lyme disease. Subsequently, the etiologic agent was given the name *Borrelia burgdorferi*.



Borrelia burgdorferi , FA stain (CDC)

Let's look at *Borrelia* exposed to penicillin



Youtube video

The most recognized forms of *Borrelia burgdorferi*



- Spirochetes
- Round bodies (cysts, granules)
- Attached biofilm

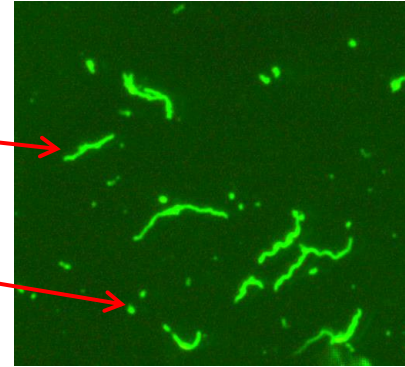
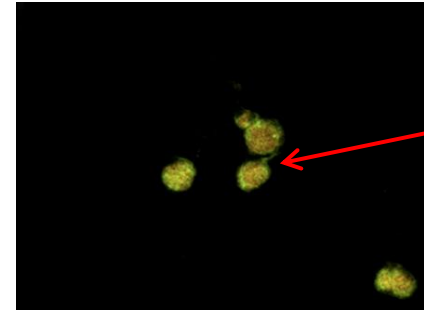
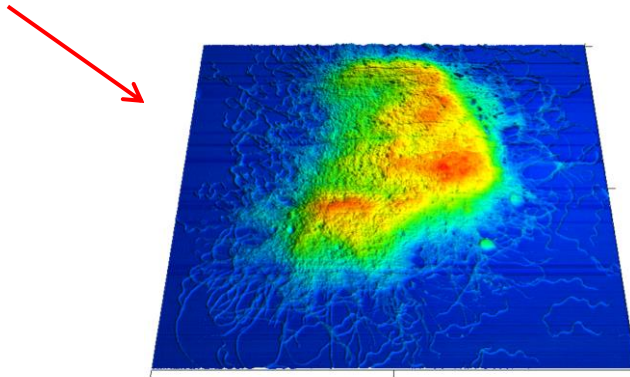


Photo by Pabbati N MS



Persisters

Photo by Socarras K MS

Photo by Luecke DF MS

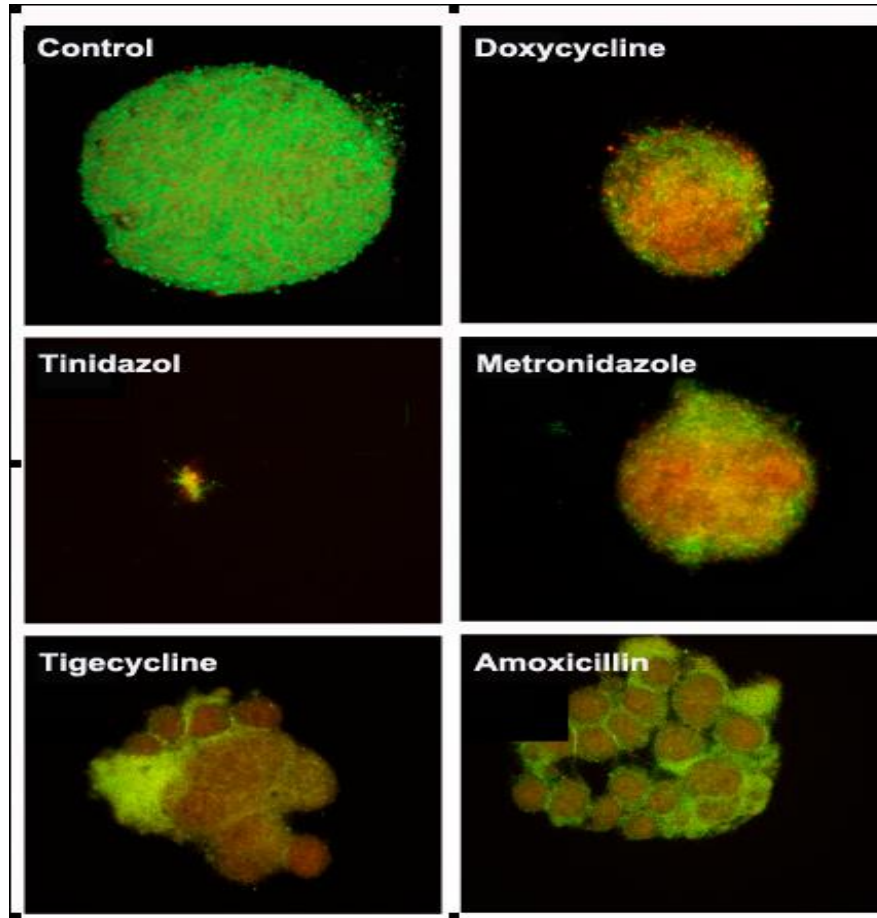
Evaluation of in-vitro antibiotic susceptibility of different morphological forms of *Borrelia burgdorferi*

Sapi et al

Dovepress

Table I MIC and MBC determination by different methods^a

| Antibiotics | Microdilution method/literature data (MIC) µg/mL | Our data (MIC) µg/mL | | | Microdilution method/literature data (MBC) µg/mL | Our data (MBC) µg/mL | | |
|---------------|--|----------------------|----------------------|--------------------|--|----------------------|----------------------|--------------------|
| | | Microdilution method | Direct cell counting | BacLight™ staining | | Microdilution method | Direct cell counting | BacLight™ staining |
| Doxycycline | 0.06–2.00 | 0.4 | >25 | >25 | 0.25–6.40 | 25 | >200 | >200 |
| Tigecycline | 0.006 | 0.015 | >5 | >5 | 0.05 | 0.125 | >10 | >10 |
| Amoxicillin | 0.03–2.00 | 0.3 | >100 | >100 | <0.03–32.00 | 5 | >200 | >200 |
| Metronidazole | 0.06–32.00 | 0.3 | >250 | >250 | >4 | 10 | >500 | >500 |
| Tinidazole | – | 0.09 | >62.5 | >62.5 | >128 | 10 | >250 | >250 |



Red stain: Dead
Green stain: Viable

Effect of antibiotics on the aggregates of Borrelia measured LIVE/DEAD staining
Sapi E et al 2011

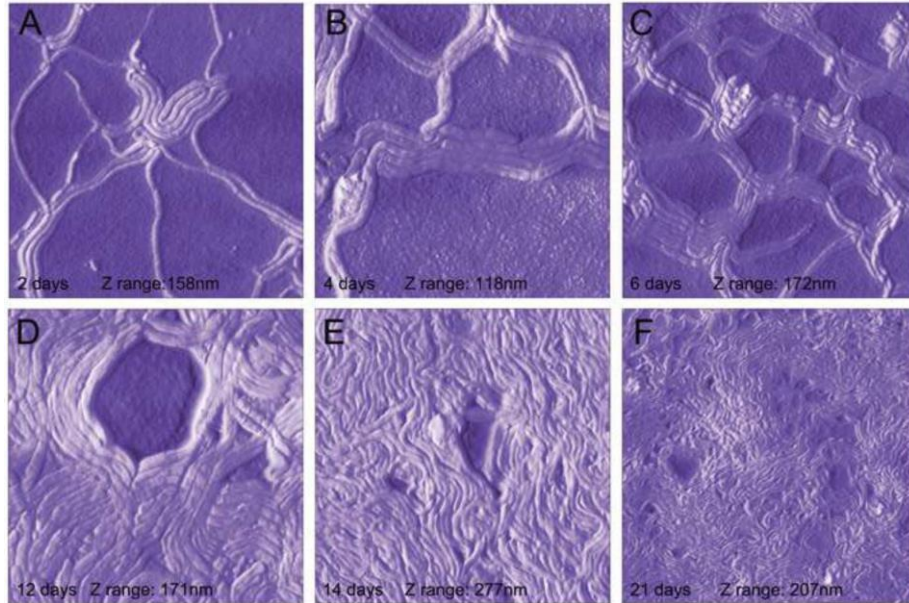
What is biofilm?

- **collections of microorganisms** (bacteria, yeasts or protozoa) that form on a hard surface (exception floating biofilms)
- examples: **plaque** that forms on teeth and the slime that forms on surfaces in watery areas (shower)
- surrounded by **slimy secretions**: mucoid polysaccharide structure which attaches the community to a surface
- estimated that over **90% of bacteria live in biofilm** (late Costerton WJ)

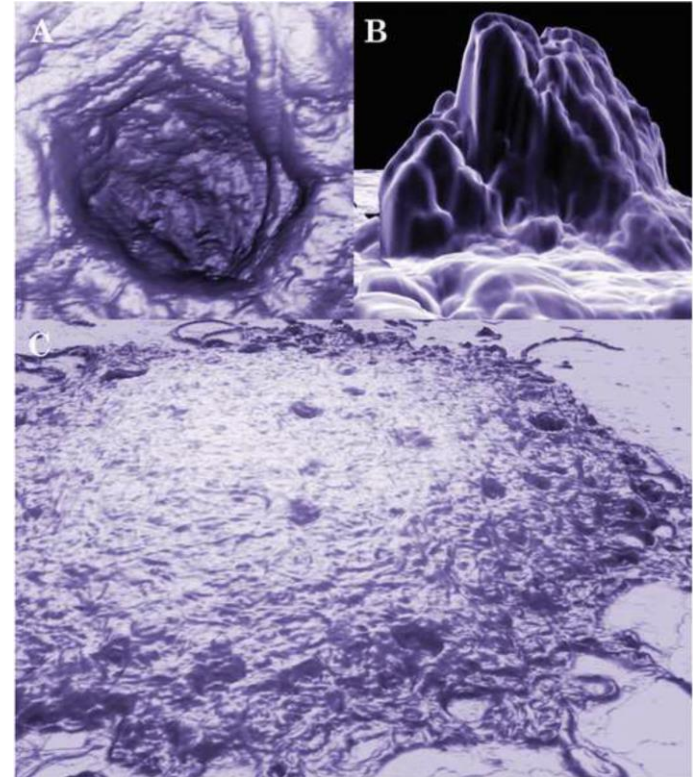
Problems caused by biofilms

- Can form almost anywhere that water is present, including catheters, kitchen counters, showers, water pipes etc.
- Damage to industrial equipment
- Contamination of food, pharmaceutical and medical products
- Energy loss through inefficient energy transfer
- Medical infections and super-resistant to antibiotics

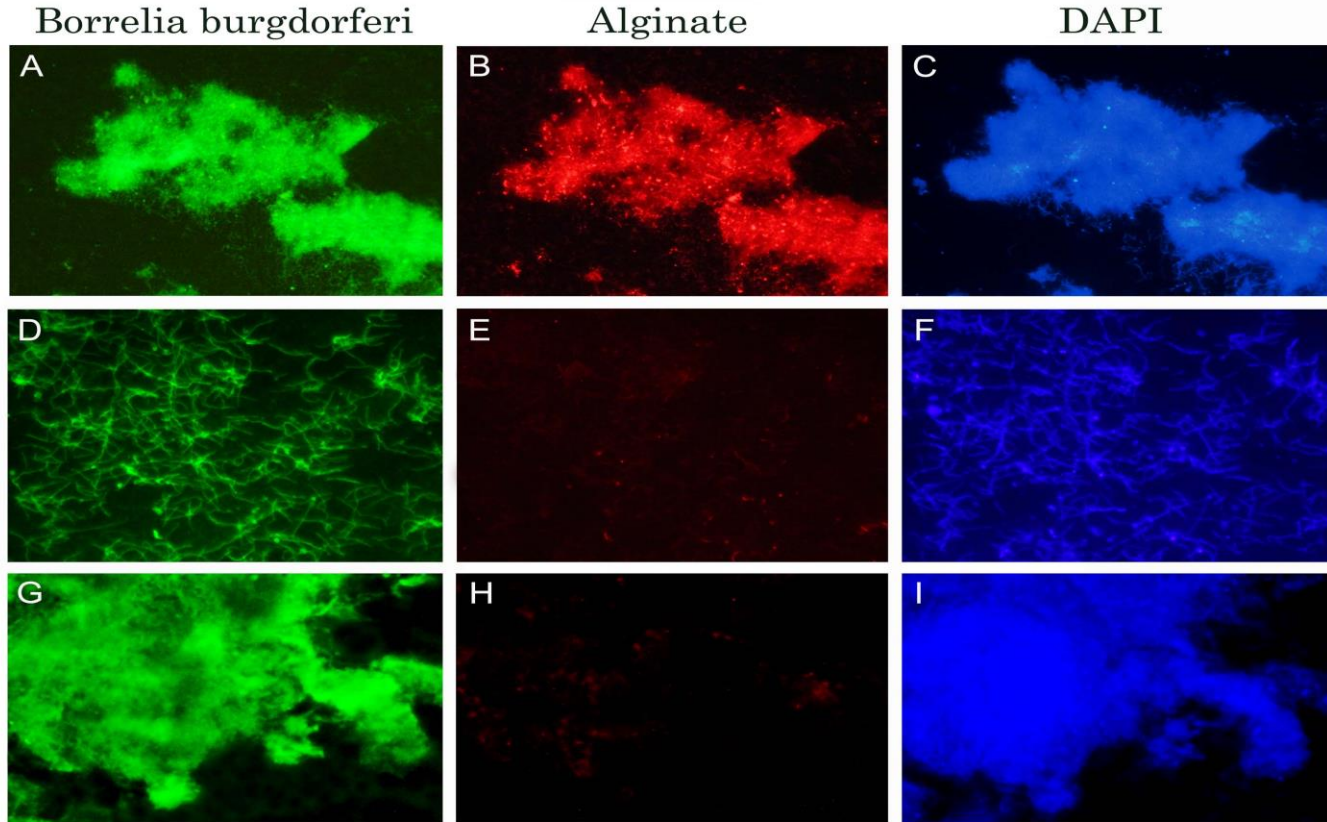
Borrelia biofilm



Sapi E et al PLoS ONE 2012



Alginate on the surfaces of *Borrelia burgdorferi* aggregates



Can *Borrelia* biofilms exist in vivo?

- *Double immunohistochemical staining with *Borrelia* and biofilm specific markers*
- *In situ hybridization confirmation experiments*
- *PCR/Whole genome sequencing confirmation*

Samples:

- *Skin biopsies* from *Borrelia* infected skin lesions
 - (Dr. B. Zelger)
- *Autopsy tissues* from a Lyme disease patient
 - (Dr. K. Leigner and Dr J. Goldman)



EVIDENCE OF *IN VIVO* EXISTENCE OF *BORRELIA* BIOFILM IN BORRELIAL LYMPHOCYTOMAS

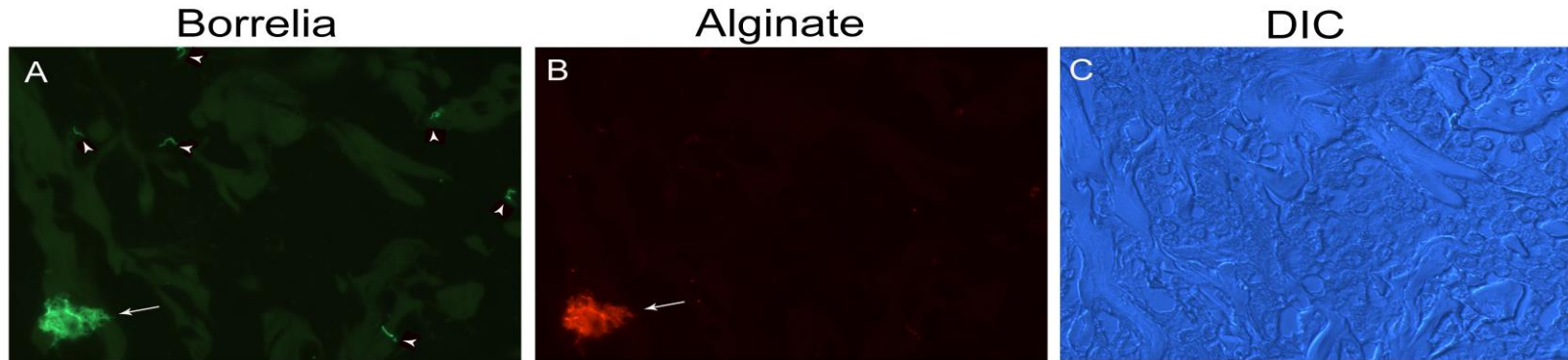
**E. Sapi^{1,*}, K. Balasubramanian¹, A. Poruri¹, J. S. Maghsoudlou¹, K. M. Socarras¹, A. V. Timmaraju¹,
K. R. Filush¹, K. Gupta¹, S. Shaikh¹, P. A. S. Theophilus¹, D. F. Luecke¹, A. MacDonald¹, B. Zelger²**

¹Department of Biology and Environmental Science, University of New Haven, West Haven, CT 06516, USA

²Department of Dermatology and Venereology, Medical University Innsbruck, Innsbruck, Austria

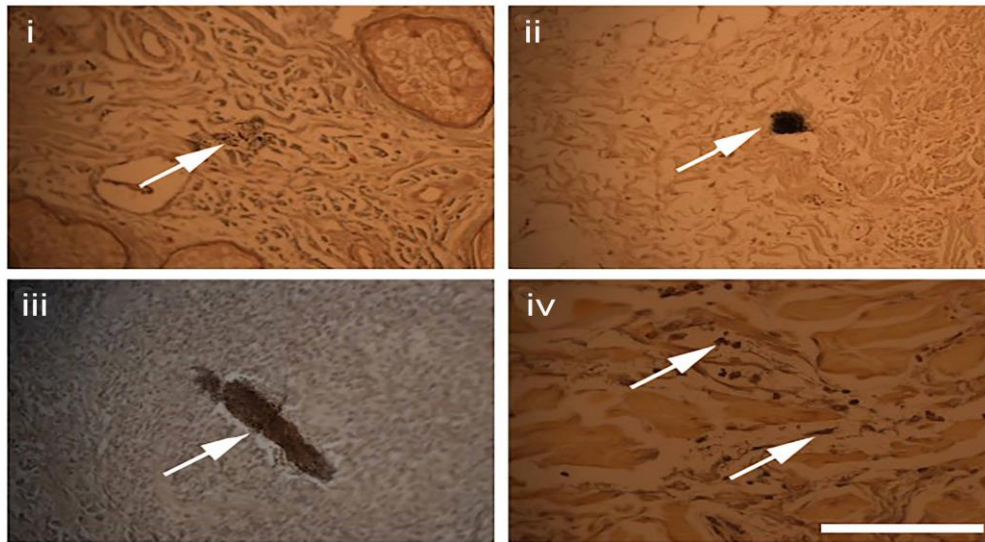


Double immunostaining: **Borrelia** and **alginate** **antibody** from Skin biopsies from **Borrelia** Lymphocytoma

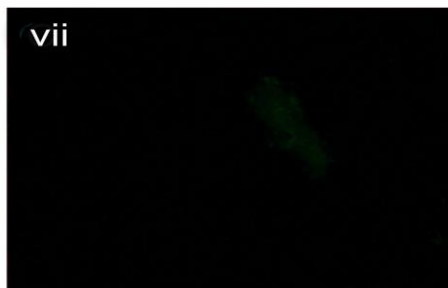
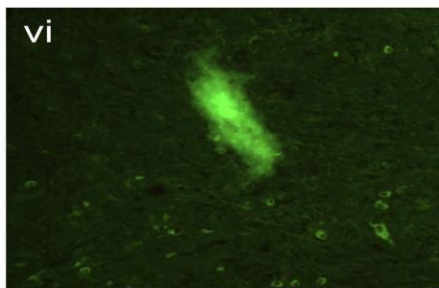


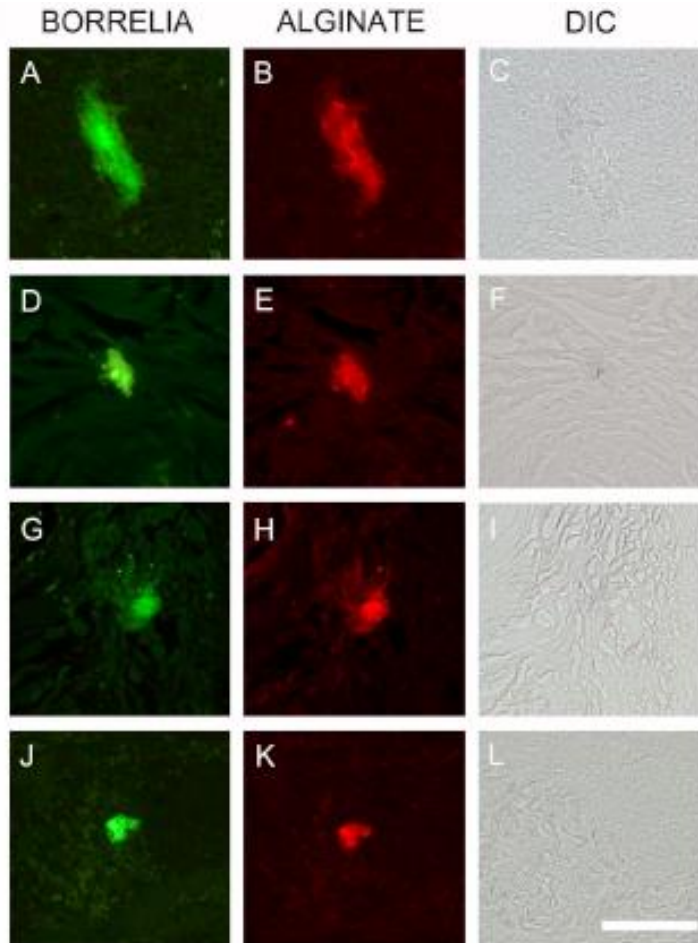
Sapi E et al 2016



A**DIETERLE SILVER STAINING**

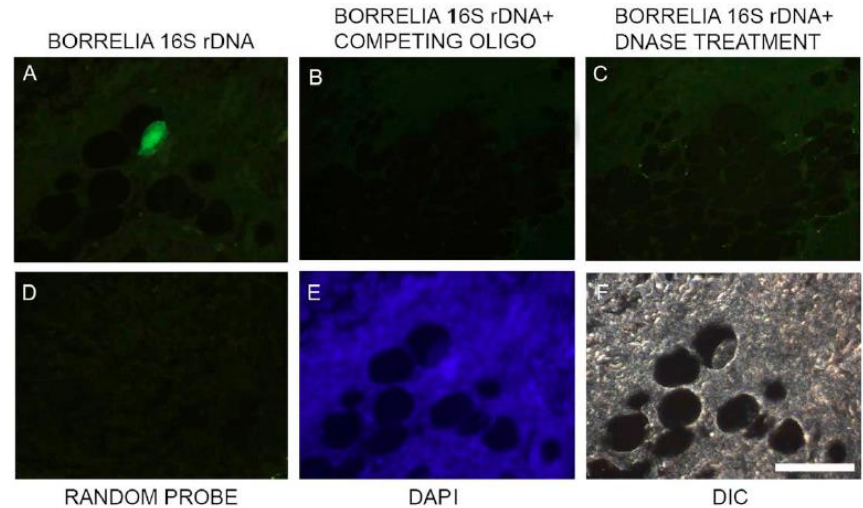
Sapi E et al 2016

B**SILVER****BORRELIA****NO ANTIBODY****DIC**



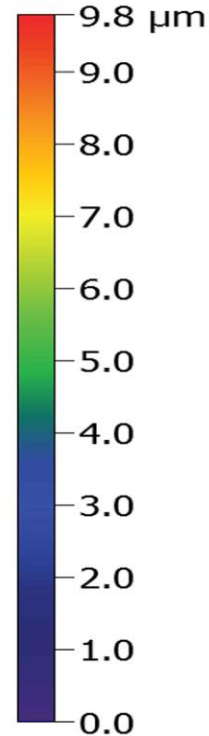
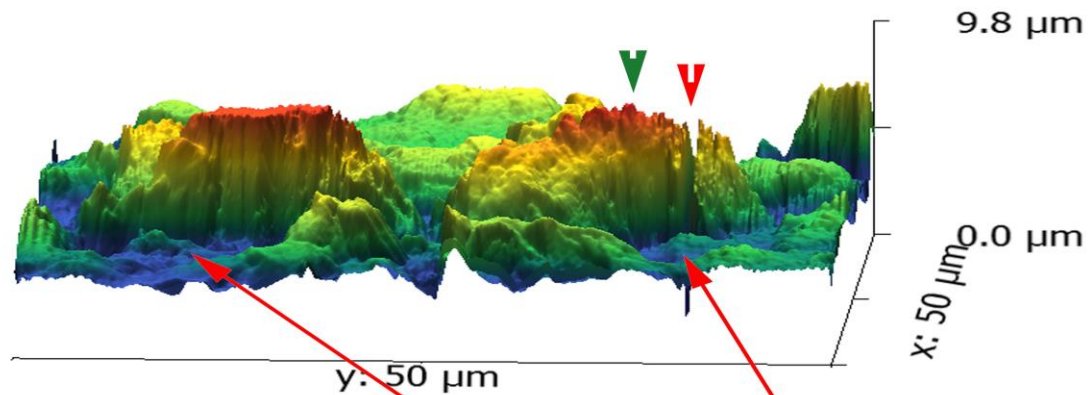
Additional IHC and
in situ hybridization data

Sapi E et al 2016

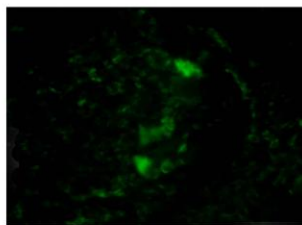




A



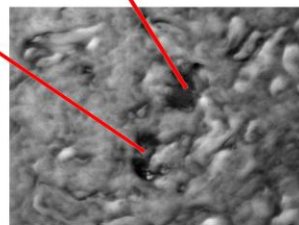
B



Borrelia



Alginate



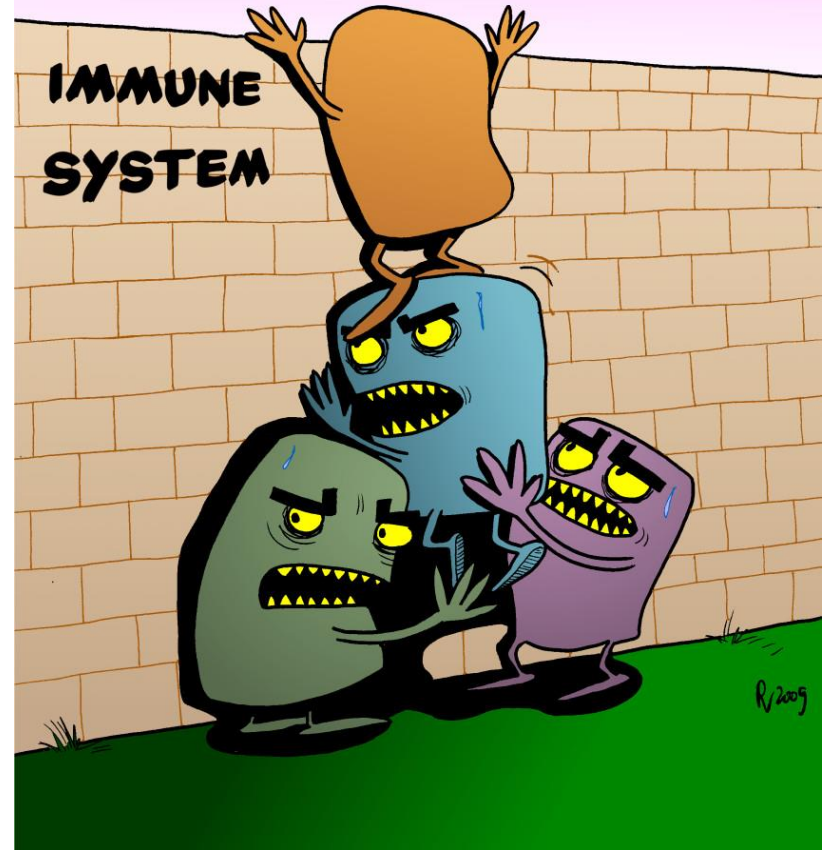
DIC

Sapi E et al 2016

So could other pathogens
exist in these tissues?

Velica, DevianArt

Pathogen cooperation...



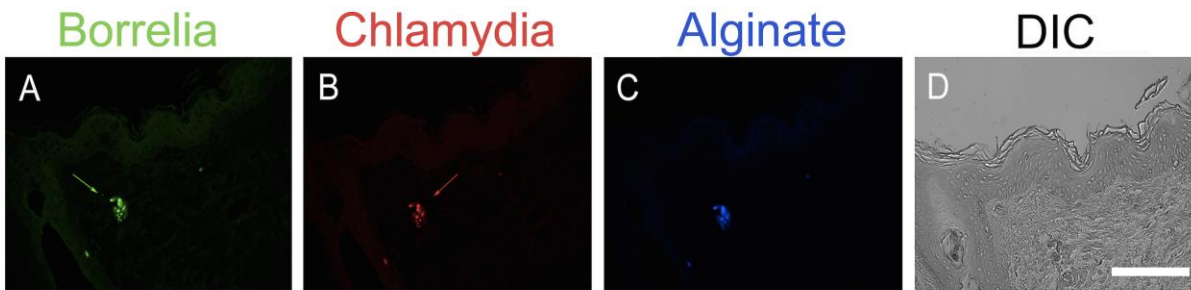
Metagenomic analyses for *Borrelia Lymphocytoma* Tissues

Reads *Borrelia burgdorferi sensu lato*,
Reads for *Chlamydia spp*

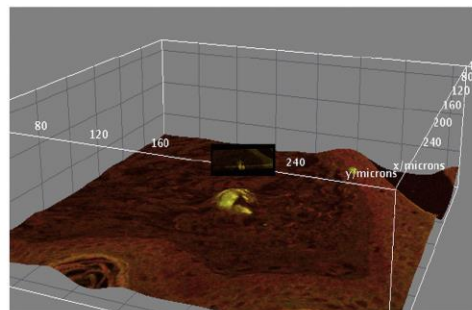
Genomic DNA analyzed by whole genome sequencing method (Perkin Elmer). Reads were aligned to human reference sequences using Burrows-Wheeler aligner software (<http://bio-bwa.sourceforge.net>)

After Sequence Alignment/Map (<http://samtools.sourceforge.net>) tool was used to filter out reads not mapped to human. The remaining reads were aligned to bacterial reference genomes using Blast program (NCBI).

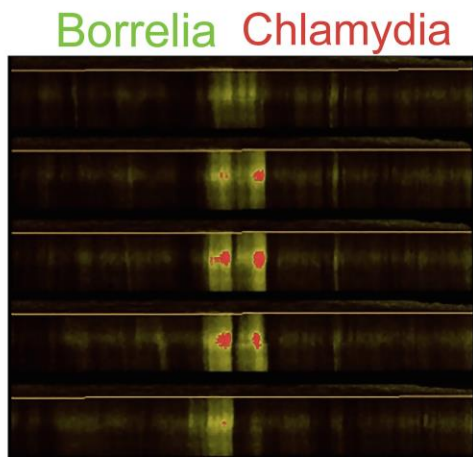




Immunohistochemistry



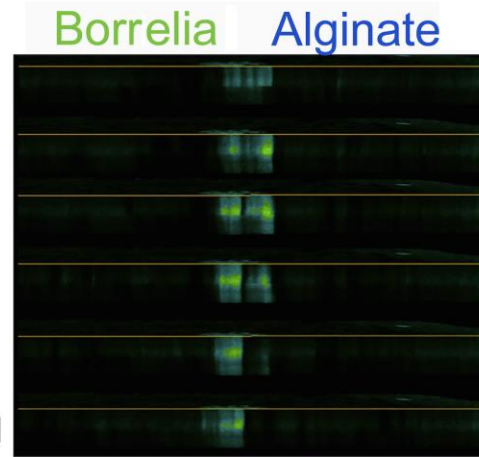
CONFOCAL MICROSCOPY



Z STACKS

TOP

BOTTOM



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Mixed Biofilm in Other Infected Skin Tissues



healthcare



Article

Mixed *Borrelia burgdorferi* and *Helicobacter pylori* Biofilms in Morgellons Disease Dermatological Specimens [†]

Marianne J. Middelveen ¹, Katherine R. Filush ², Cheryl Bandoski ², Rumanah S. Kasliwala ², Anthony Melillo ², Raphael B. Stricker ^{3,*} and Eva Sapi ²

Mixed Biofilm in Infected Skin Tissues

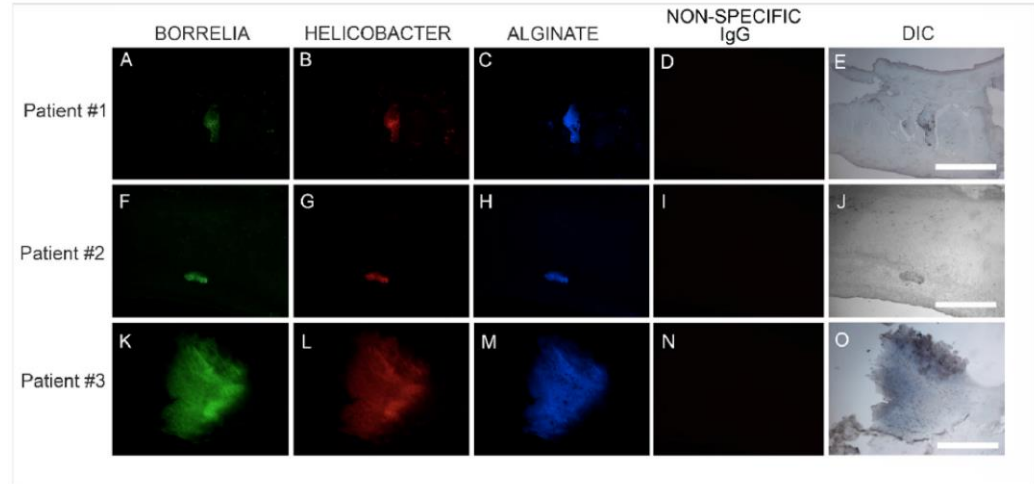


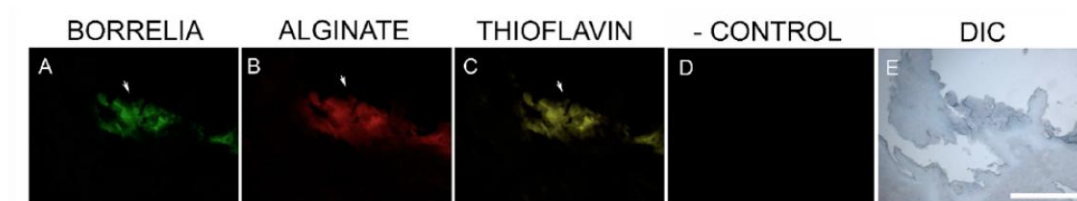
Figure 4. Representative IHC images showing biofilm aggregates in MD skin sections stained for *Borrelia*, *Helicobacter* and alginate. IHC detection was performed as described in the Methods section. Panels A, F and K show skin sections treated with anti-*Borrelia* monoclonal antibody (green). Panels B, G and L show skin sections treated with anti-*Helicobacter* antibody (red). Panels C, H and M show skin sections treated with anti-alginate antibody (blue). Panels D, I and N show negative control sections treated with non-specific IgG. Panels E, J and O show sections imaged with DIC. Images were taken at 200× magnification. Scale bar = 100 μ m.



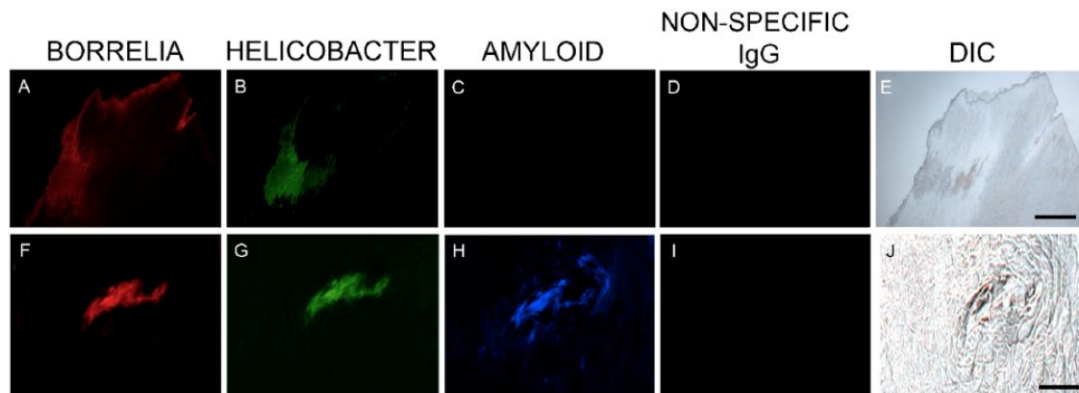
Amyloid Changes in Mixed *Borrelia* Biofilms

Healthcare 2019, 7, 70

13 of 24



(A)



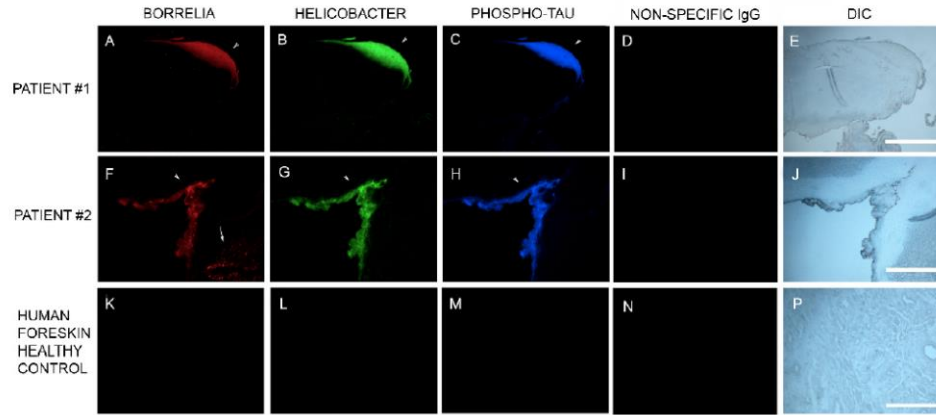
(B)



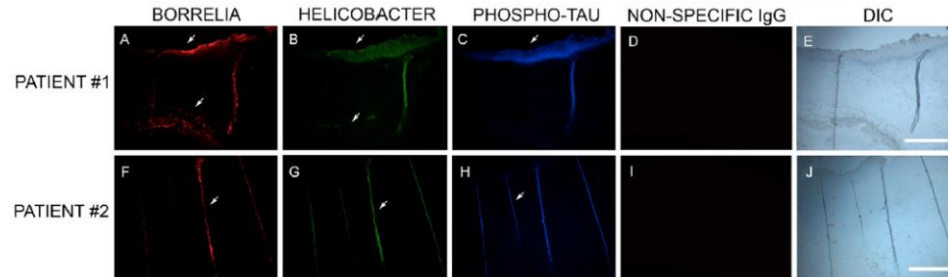
Mixed Biofilm and Phospho-Tau in Infected Skin Tissues

Healthcare 2019, 7, 70

14 of 24



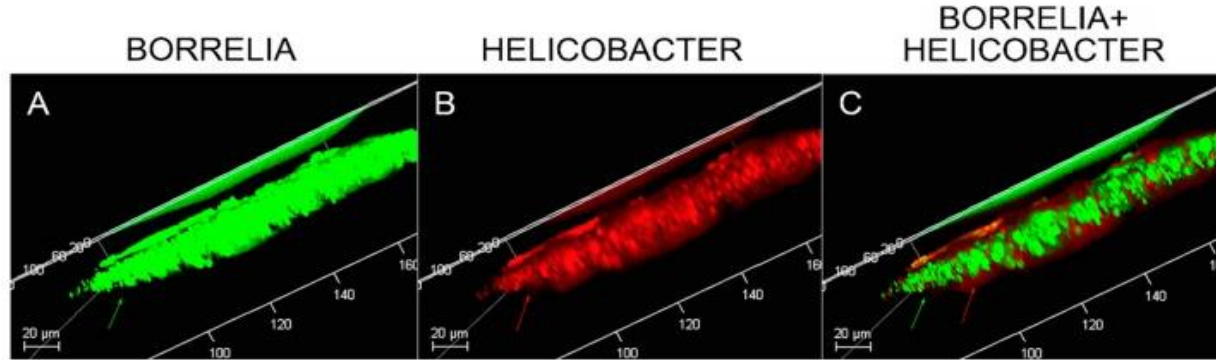
(A)



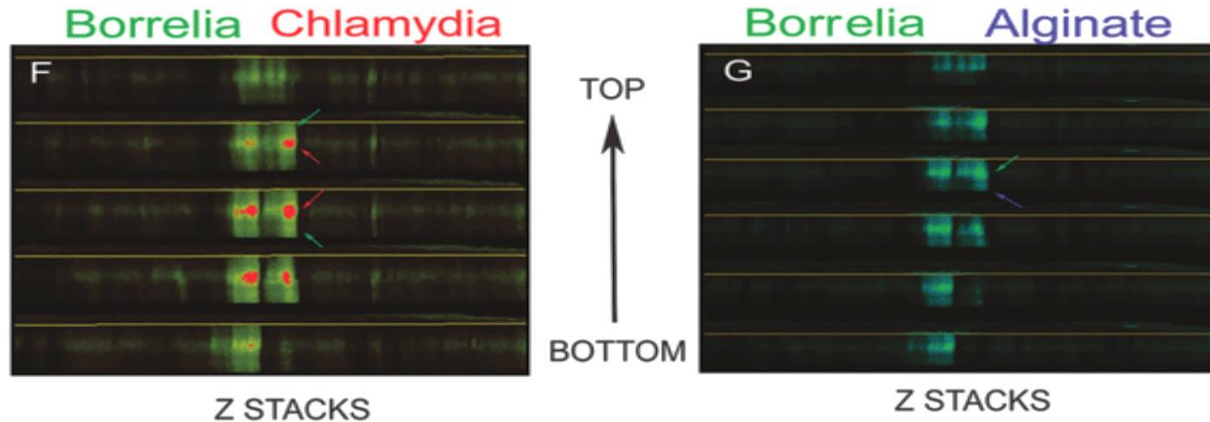
(B)



Borrelia in Mixed Biofilms

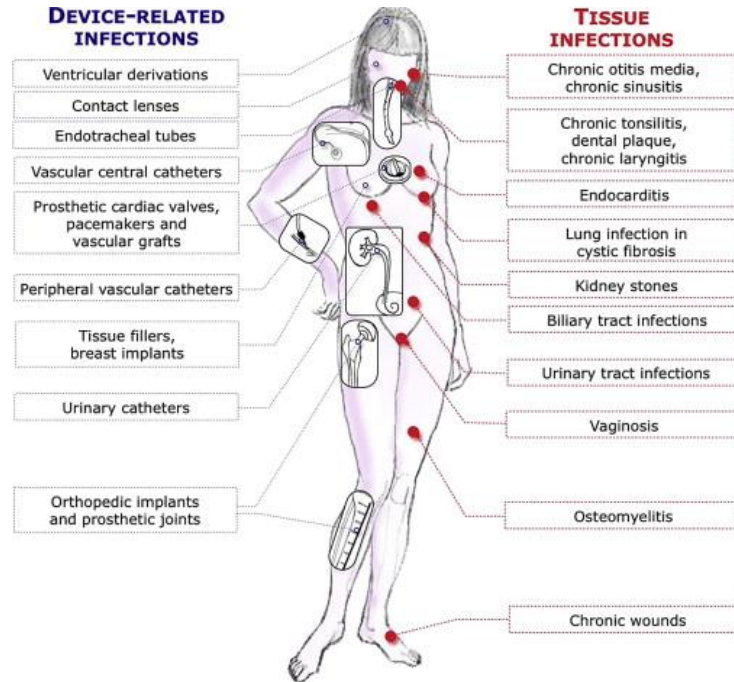


Middelveen MJ, Filush KR, Bandoski C, et al. Mixed *Borrelia burgdorferi* and *Helicobacter pylori* Biofilms in Morgellons Disease Dermatological Specimens. *Healthcare (Basel)*. 2019;7(2):70. Published 2019 May 17. doi:10.3390/healthcare7020070

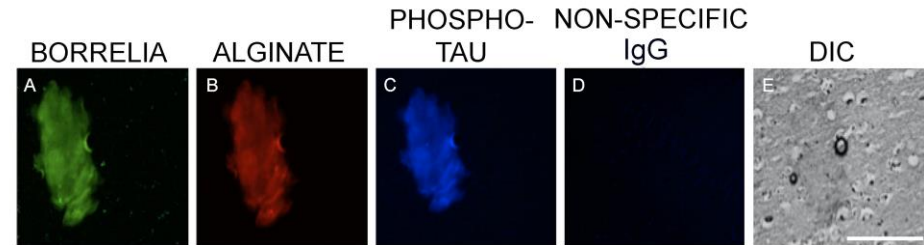
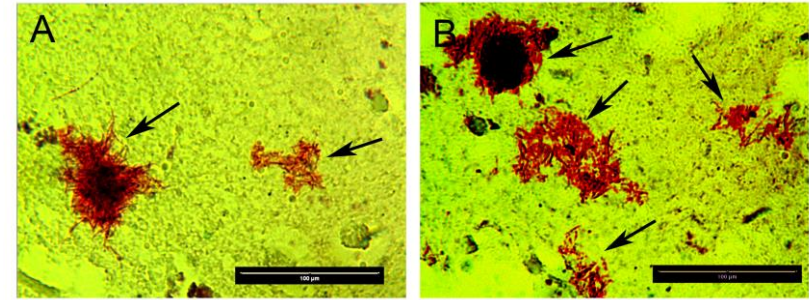
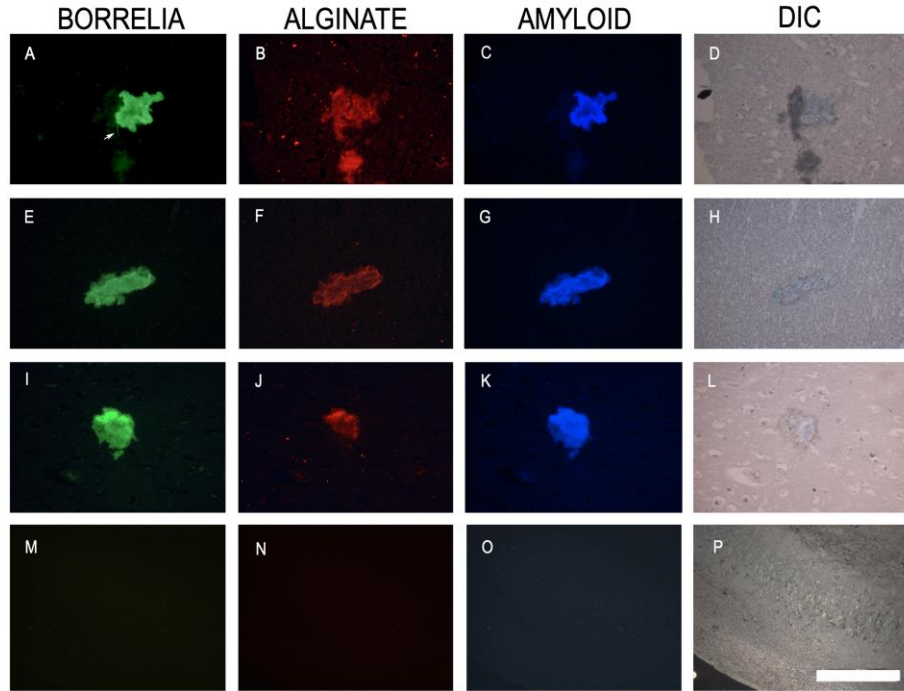


Sapi E, Gupta K, Wawrzyniak K, et al. *Borrelia* and *Chlamydia* Can Form Mixed Biofilms in Infected Human Skin Tissues. *Eur J Microbiol Immunol (Bp)*. 2019;9(2):46–55. Published 2019 Apr 11. doi:10.1556/1886.2019.00003

Where else you can find *Borrelia* biofilm in the human body?



Borrelia Biofilm in Human Brain from in a Lyme disease /Alzheimer patient Correlates with Amyloid Changes





Autopsy tissues from Columbia University

Dr. James Goldman and Dr. Kenneth Liegner

- A 53-year-old woman with a 16-year history of Lyme disease who initially presented with headaches, fevers, fatigue, memory loss, cranial nerve palsies and gait disturbance and later developed progressive spastic quadraparesis and died despite the multiple antibiotic treatment
- Autopsy tissues from liver, heart, brain, kidney, lung, lymph node arrived from Columbia and the study was funded by:



GLOBAL LYME ALLIANCE
Conquering Tick-borne Disease through Research & Education

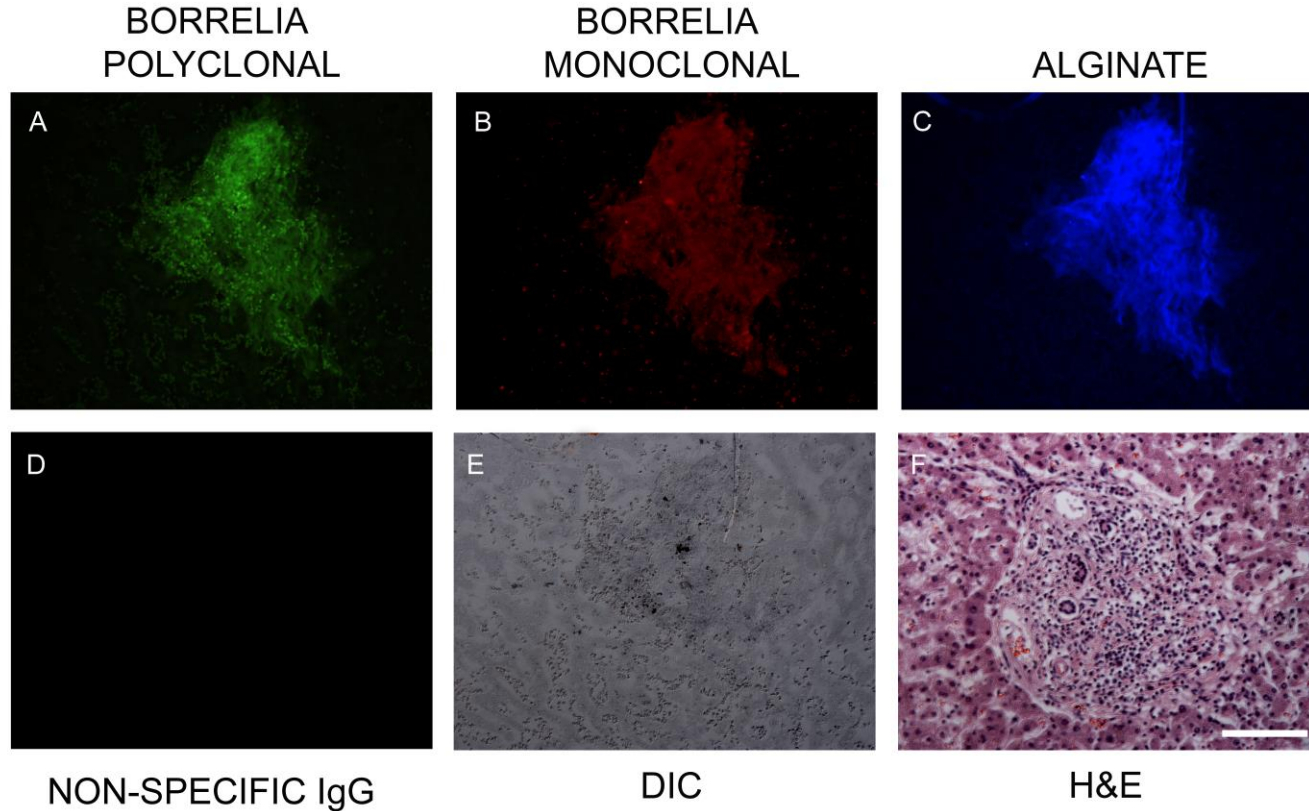
CULTURE-CONFIRMED TREATMENT FAILURE OF CEFOTAXIME AND MINOCYCLINE IN A CASE OF LYME MENINGOENCEPHALOMYELITIS IN THE UNITED STATES.

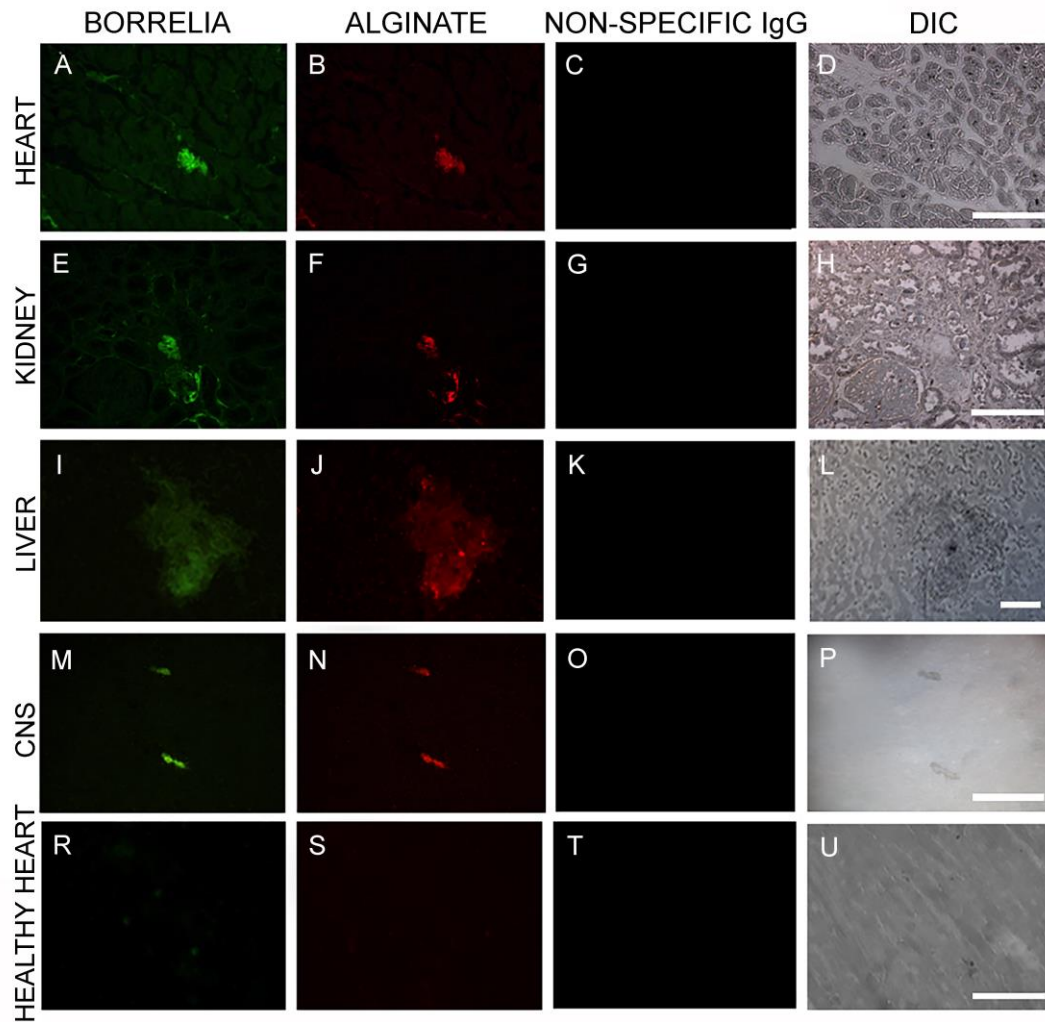
Kenneth B. Liegner, Carl E. Rosenkilde, Grant L. Campbell*, Thomas J. Quan, and David T. Dennis, Armonk, NY, USA, Mount Kisco, NY, USA, and Centers for Disease Control, Fort Collins, CO, USA.

In 1987, a 37-year-old woman living in Westchester County, NY, developed spastic paraparesis, bilateral Babinski reflexes, and cranial nerve and bulbar dysfunction characterized by dysphagia, dysphonia, diplopia, absent gag reflex, and dysfunction of bowel and bladder control. CSF contained 19 WBC/mm³ (86% lymphs). A test for antibodies to *Borrelia burgdorferi* (*Bb*) in serum was negative. No etiology was established despite an extensive workup. Symptoms and signs reportedly worsened gradually from 1988 to present. There was a past history of splenectomy for idiopathic thrombocytopenic purpura diagnosed in 1975. In 1989, the right frontal region and right basal ganglia were abnormal on brain MRI. In January 1990, CSF contained 6 WBC/mm³ (93% lymphs), but no oligoclonal bands or myelin basic protein. Paired CSF and serum tests for antibodies to *Bb*, and PCR for *Bb*-specific oligonucleotides in CSF, were negative. An empiric 21-day course of cefotaxime (3 g/12 hr i.v.) was given in January, 1990 with no clear clinical benefit. Following treatment, CSF contained 9 WBC/mm³ (93% lymphs). Four months of minocycline (200 mg/day p.o.) begun in November, 1990 also yielded no clear clinical benefit. In December, 1990 a T-cell stimulation test with *Bb* antigens was strongly positive. In December, 1991 CSF contained 6 WBC/mm³ (89% lymphs) and elevated IgG. Paired serum and CSF samples were strongly positive for antibodies to *Bb*, with a CSF-to-serum index of 1.04. Culture of this CSF specimen in BSK-II yielded a strain of *Bb*. Culture-confirmed treatment failures have been previously reported for three Lyme neuroborreliosis cases in Europe. The present case apparently is the first of this type to be reported from the United States.

5th International
Conference
of Lyme Borreliosis –
1992 Arlington VA

Liver autopsy tissues examined for Borrelia





Immunohistochemical analyses
of four different organs for *Borrelia*
and for alginate (biofilm marker)
Heart, Kidney, Liver, CNS

Sapi E et al 2019



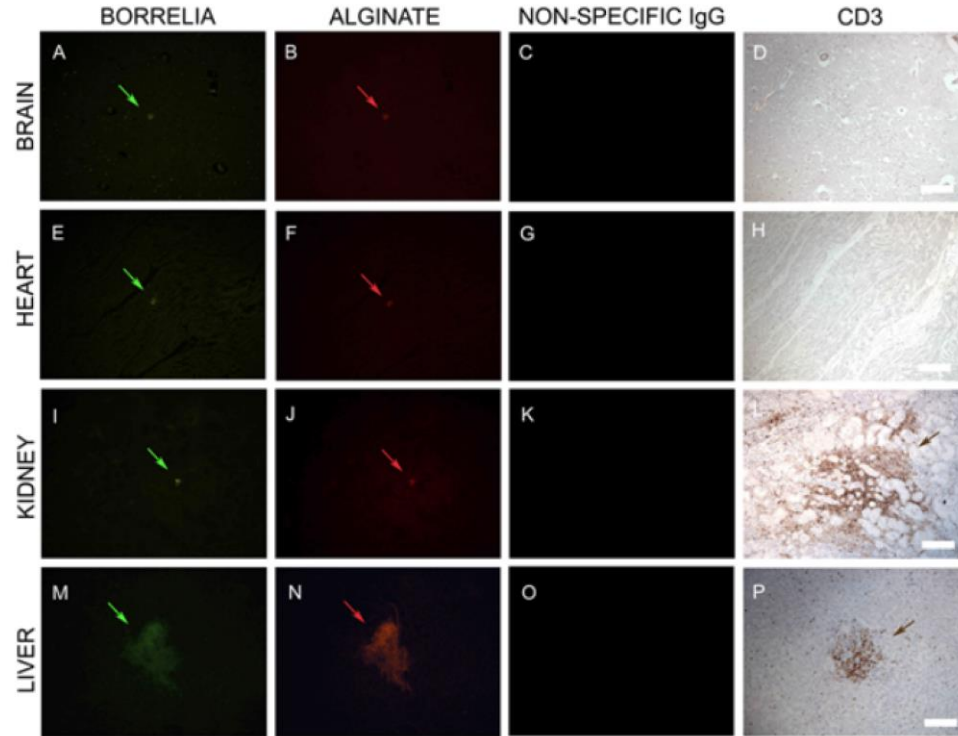
Quantitative Analysis of *Borrelia* biofilms in liver, brain, heart and kidney tissues

| Tissue | # Slides | # Biofilms/Slide | Size (μM) |
|--------|----------|------------------|------------------------|
| Liver | 150 | 0-6 | 20-300 |
| Brain | 210 | 0-3 | 20-100 |
| Heart | 130 | 0-4 | 20-100 |
| Kidney | 145 | 0-4 | 20-100 |

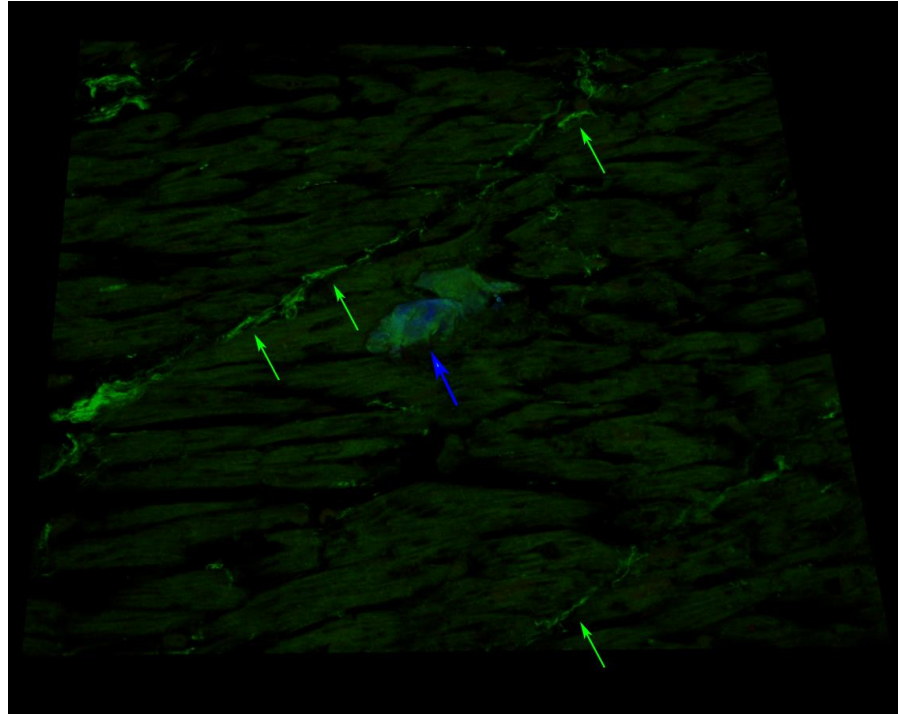
Sapi E, Goldman J, Liegner K 2019



Borrelia biofilm presence corresponded with infiltrating T cells in kidney and liver tissues – inflammation response?



Borrelia Spirochetes and Biofilms in the Heart



Sapi E, Goldman J, Liegner K 2019

Borrelia biofilm presence corresponded with inflammatory markers

Table 2 Expression of inflammatory mediators present in brain, heart, kidney and liver tissues (+++: high expression, ++: medium expression, +: minimal expression, -: no expression).

| | CD3 | CD8 | CD20 | CRP | CXCL-9 | CXCL13 | MMP-9 |
|---------------|-----|-----|------|-----|--------|--------|-------|
| Brain | - | - | - | ++ | ++ | - | ++ |
| Heart | - | ++ | ++ | +++ | ++ | - | ++ |
| Kidney | +++ | - | - | + | - | - | +++ |
| Liver | +++ | +++ | - | ++ | + | +++ | +++ |

*Sapi E, Goldman J, Liegner K 2019
unpublished data*

Effect of *Borrelia burgdorferi* Outer Membrane Vesicles on Host Oxidative Stress Response

by  Keith Wawrzeniak ,  Gauri Gaur ,  Eva Sapi  and  Alireza G. Senejani ^{*} 

Department of Biology and Environmental Science, University of New Haven, West Haven, CT 06516, USA

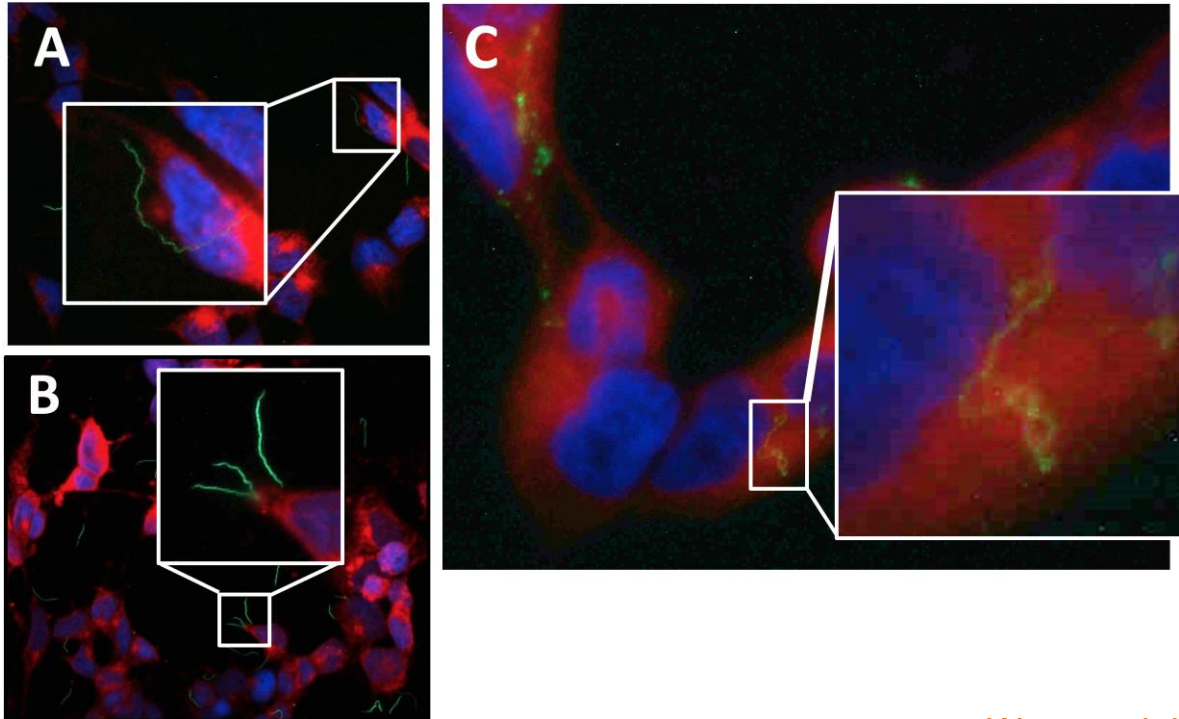
^{*} Author to whom correspondence should be addressed.

Antibiotics **2020**, *9*(5), 275; <https://doi.org/10.3390/antibiotics9050275>

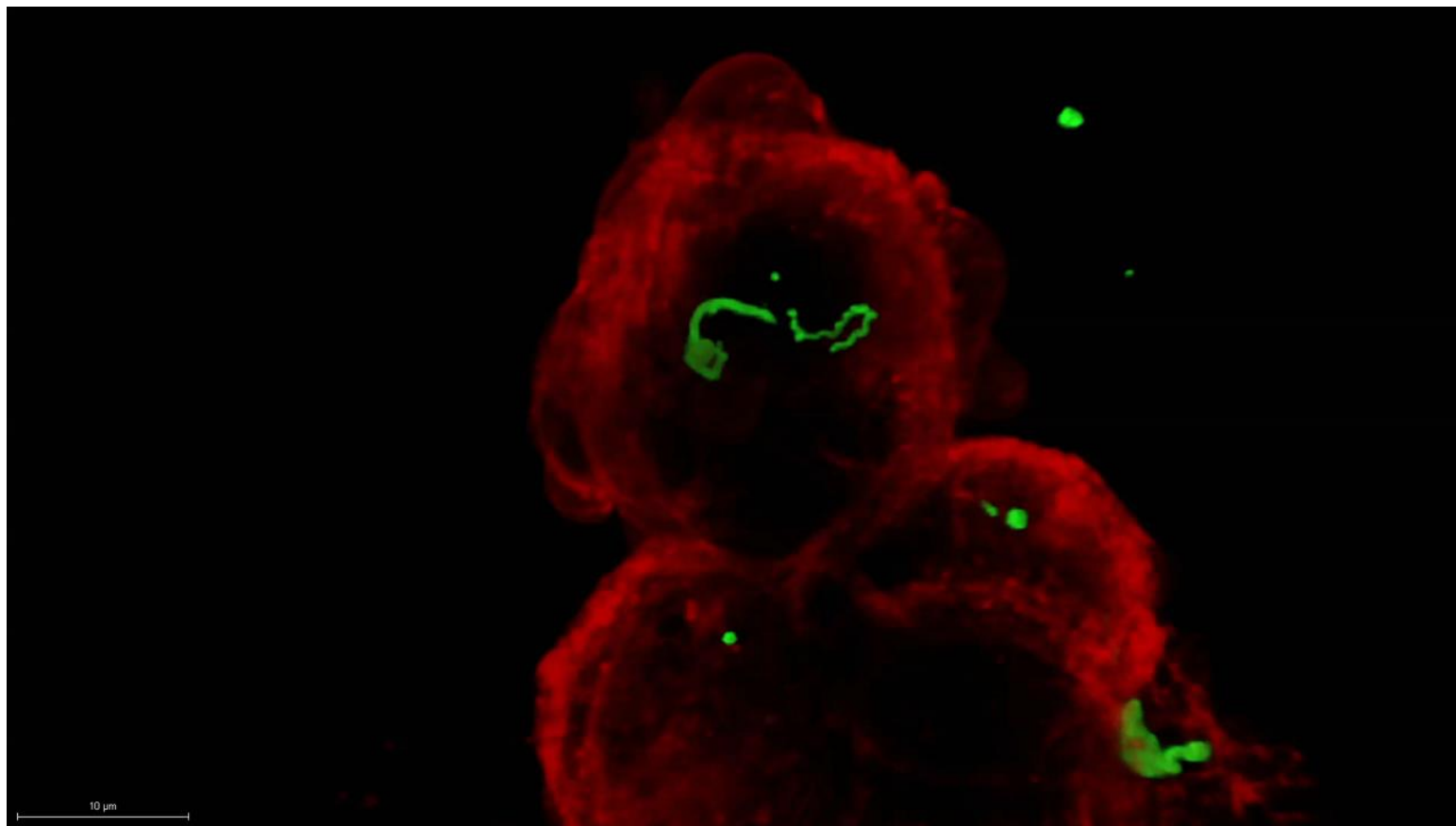
*“In summary, these results show that *Borrelia burgdorferi* OMVs serve to directly counter superoxide production in BE2C neurons, thereby ‘priming’ the host environment to support *B. burgdorferi* colonization”*



Borrelia-host interaction



- Neuron cells (BE2C) infected with Borrelia at
- 30 min (A),
- 2-hour (B), and
- 24-hour (C) time points.



Where else can we find
Borrelia –
Neoplastic Tissues?



Breast cancer pilot study



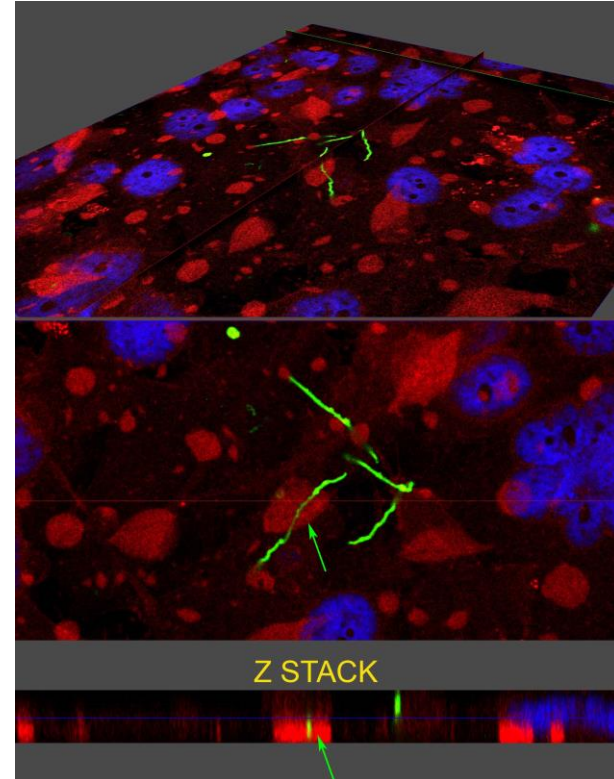
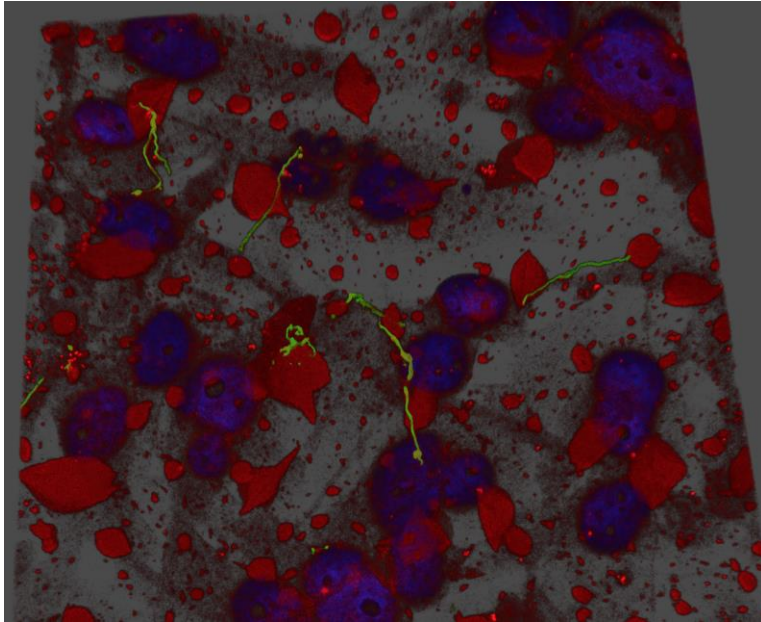
- **OBJECTIVE:** 200 invasive breast cancer samples and 20 from healthy breast tissues evaluated for *Borrelia burgdorferi* presence by immunohistochemical methods
- **RESULTS:** Significant numbers of invasive breast ductal carcinoma and lobular invasive breasts carcinomas were positive for the presence of *Borrelia burgdorferi* spirochetes and biofilms while all of the fibroadenomas or the healthy control tissues were negative.



Gauri G and Sapi E 2021 unpublished data

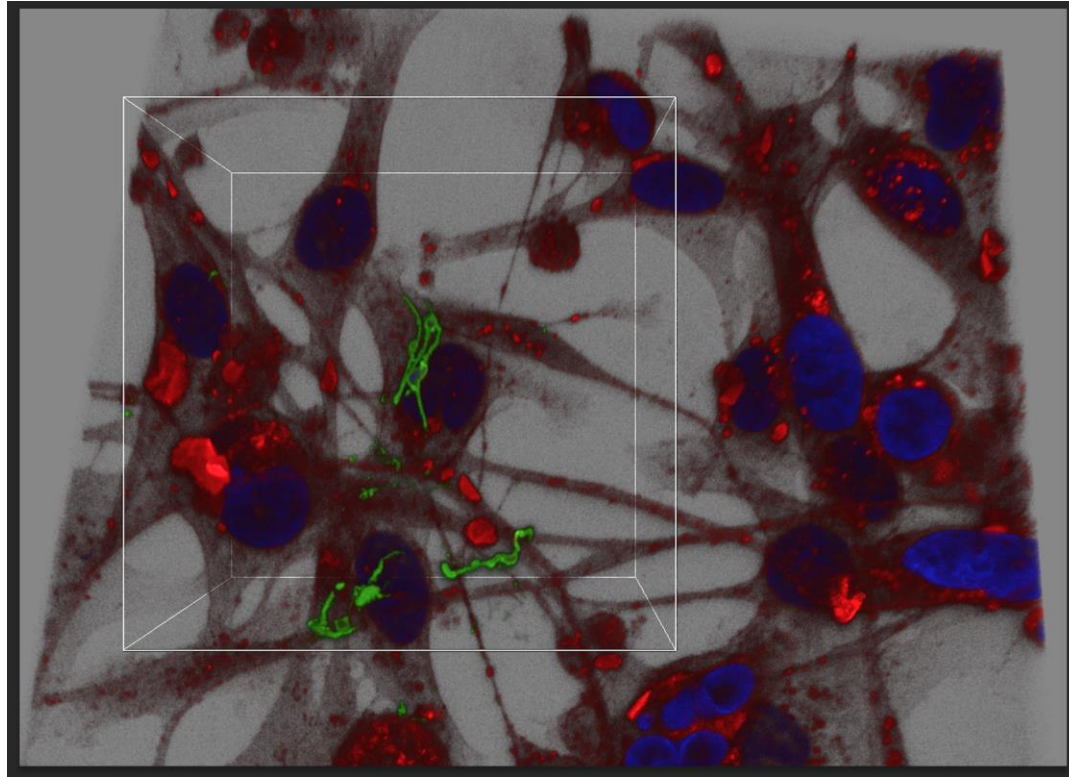


Borrelia inside of breast cancer cells?

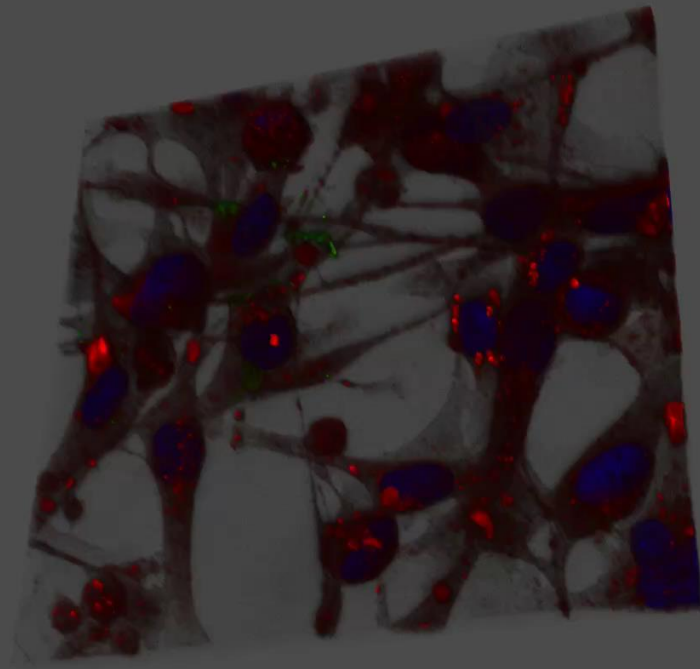


Gauri G and Sapi E 2020 unpublished data

Borrelia inside of breast cancer cells



Gauri G and Sapi E 2022



50 μ m

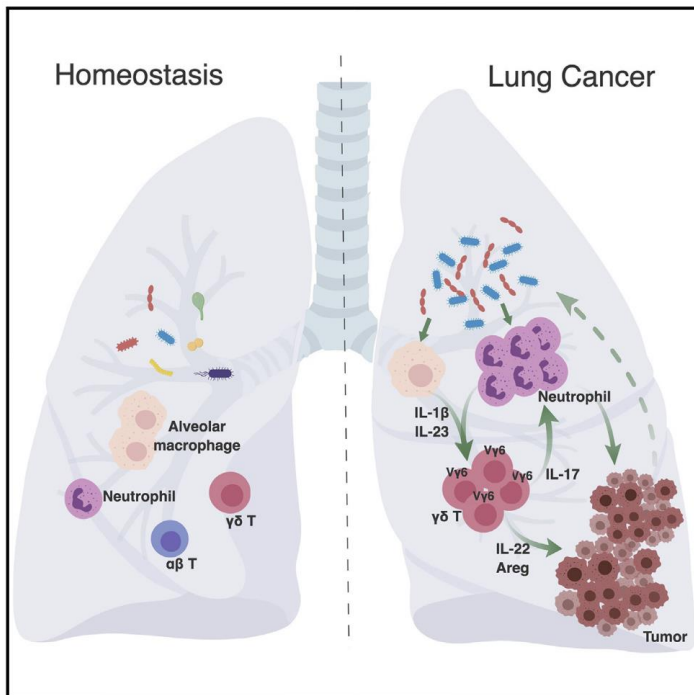


According to American Cancer Society - 15%
of all cancers are caused by infectious agents

- *Helicobacter pylori* - Gastric cancer
- *Streptococcus bovis/gallolyticus* - Colorectal carcinoma
- *Salmonella typhi* – Gallbladder carcinoma
- *Chlamydia pneumonia*, *Mycoplasma sp.* - Lung cancer
- *C. pneumonia*, *C. trachomatis*, *C. psittaci* - Pulmonary Mucosa-Associated Lymphoid Tissue (MALT) lymphoma
- *Mycoplasma sp.*, *C. trachomatis* - Ovarian cancer
- *Staphylococcus epidermidis*, *Escherichia sp.* *Bartonella sp.* – prevalent in breast cancer

Commensal Microbiota Promote Lung Cancer Development via $\gamma\delta$ T Cells

Graphical Abstract



Authors

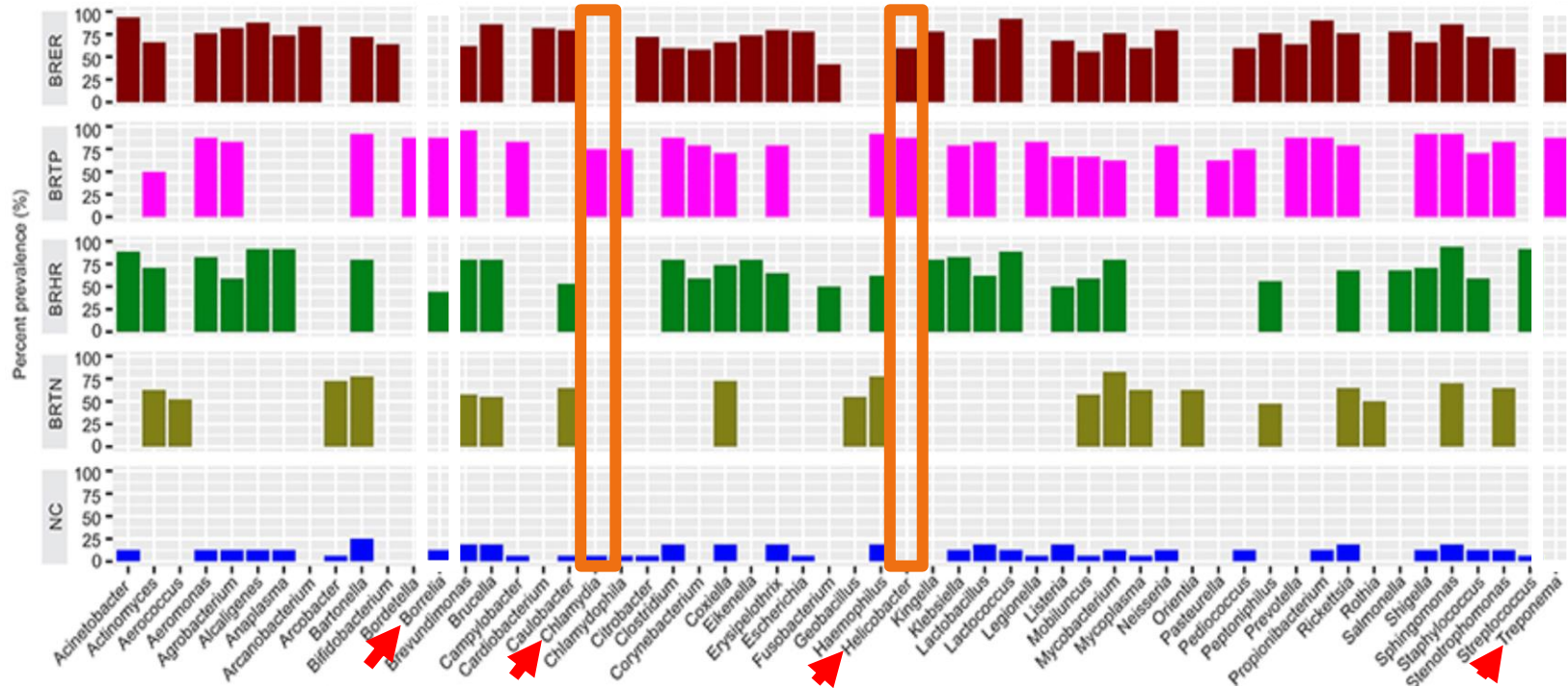
Chengcheng Jin, Georgia K. Lagoudas, Chen Zhao, ..., Paul C. Blainey, James G. Fox, Tyler Jacks

Correspondence

tjacks@mit.edu

In Brief

Lung cancer development is associated with increased bacterial burden and altered bacterial composition in the lung. Depletion of microbiota or blockade of the downstream cellular or molecular immune mediators significantly suppress lung tumor growth.



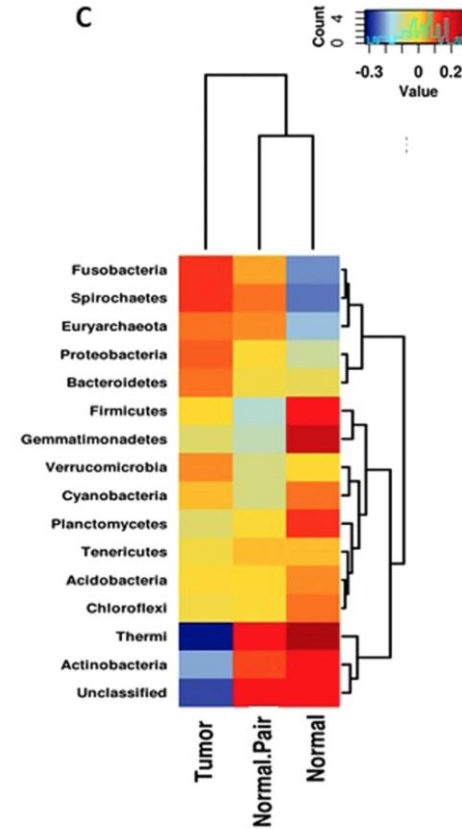
Banerjee S., Tian T., Wei Z., Shih N., Feldman M.D., Peck K.N., DeMichele A.M., Alwine J.C., Robertson E.S. Distinct microbial signatures associated with different breast cancer types. *Front. Microbiol.* 2018;9:951. doi: 10.3389/fmicb.2018.00951

► *Spirochete bacteria like Borrelia and Treponema associated with different types of breast cancer*

Distinct microbial communities that differ by race, stage, or breast-tumor subtype in breast tissues of non-Hispanic Black and non-Hispanic White women

[Alana Smith](#), [Joseph F. Pierre](#), [Liza Makowski](#), [Elizabeth Tolley](#), [Beverly Lyn-Cook](#), [Lu Lu](#), [Gregory Vidal](#) & [Athena Starlard-Davenport](#) 

[Scientific Reports](#) 9, Article number: 11940 (2019) | [Cite this article](#)

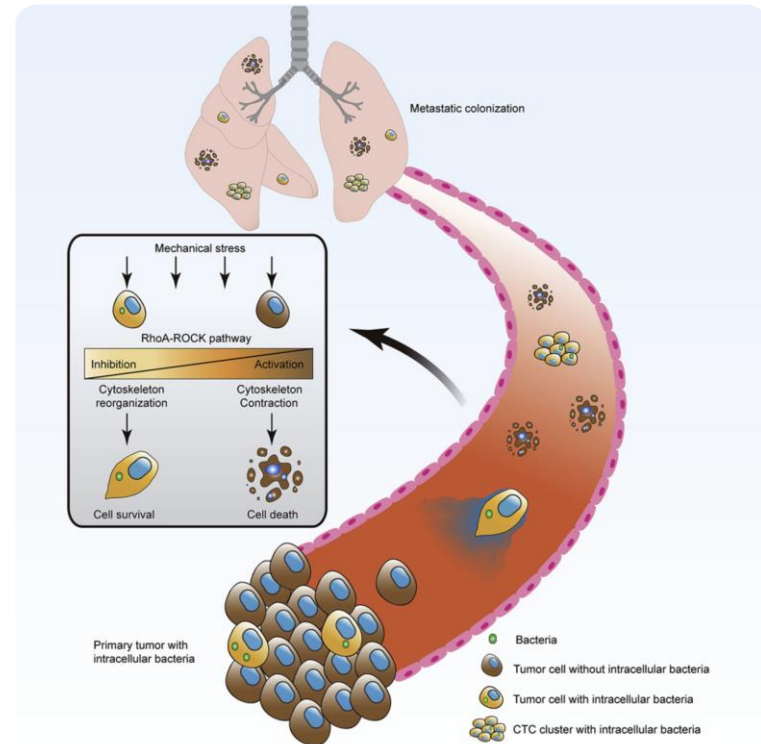


Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer

Fu A et al - Cell 185, 1356–1372, 2022

Bacteria living inside tumor cells promote cancer metastasis by helping cancerous host cells against mechanical stress in the bloodstream, in turn promoting cell survival during tumor progression.

Antibiotics treatment reduced tumor metastasis more than 3-fold!



THE RELATION OF SPIROCHETES TO CANCER IN MICE

HARVEY R. GAYLORD, M.D.

In Volume 4, No. 2, of The Journal of Infectious Diseases, 1907, we published an article entitled "A Spirochete in Primary and Transplanted Carcinoma of the Breast in Mice," which dealt with the results of the examination of ten primary cancers of the breast in mice and sixteen transplanted cancers of the breast in mice, derived from three different strains, in all of which, in the connective tissue surrounding the tumors were found numbers of a characteristic spirochete. The final conclusion of this article was as follows:

"Our observations do not as yet establish an etiological relation between this organism and cancer of the breast in mice, but the presence of the organism in primary mouse cancers with which it is regularly transplanted through many generations, greatly increasing in number as the tumors increase in virulence, instead of interfering with, and finally preventing, transplantation, as do bacteria, is suggestive."

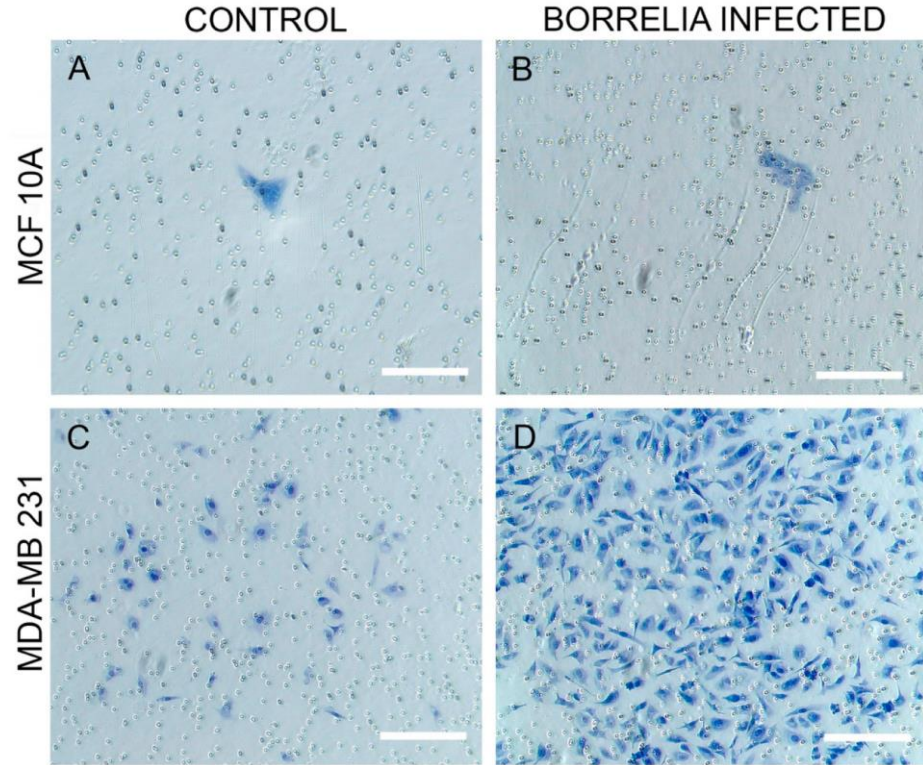
- Gaylord, 1907 – *in vivo* study suggests a potential connection between spirochetes and primary breast cancer in mice

HYPOTHESIS

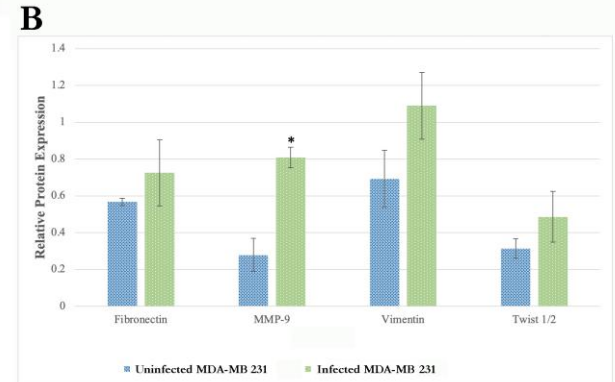
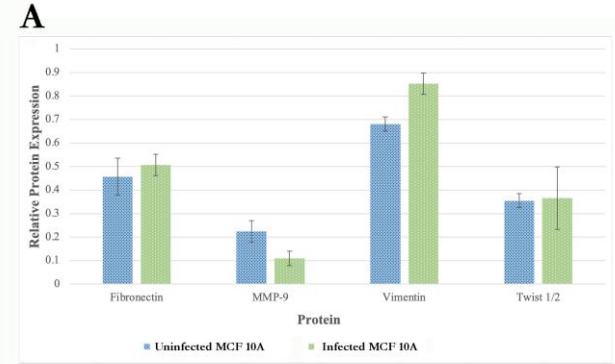
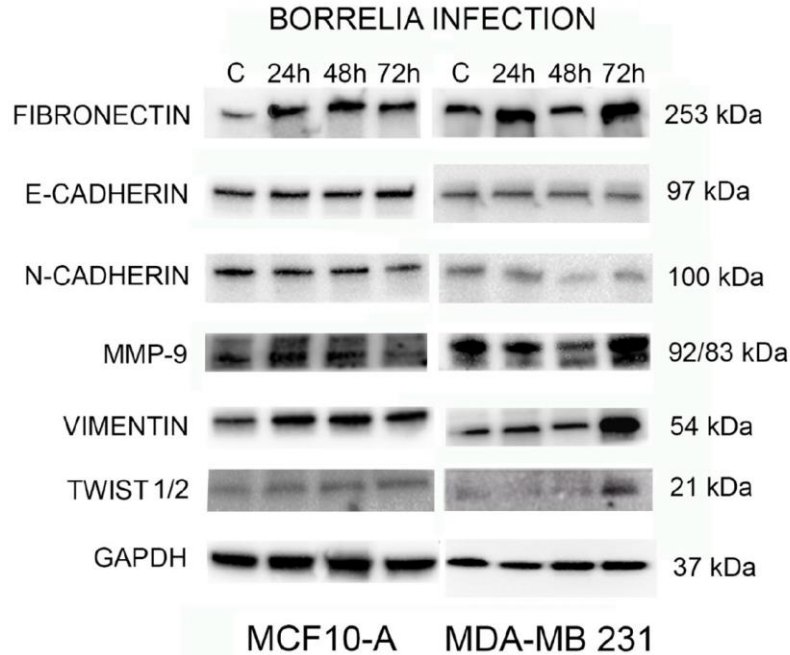
*Borrelia burgdorferi plays a role in tumorigenic
changes in breast epithelial cells*

Effect of Borrelia on normal and breast cancer cells invasion

- *Gauri G et al 2021*



Effect of Borrelia infection on Breast Cancer Markers



Gauri G et al 2021



Current Projects:
Identifying pathways and searching for markers in Borrelia infected cells

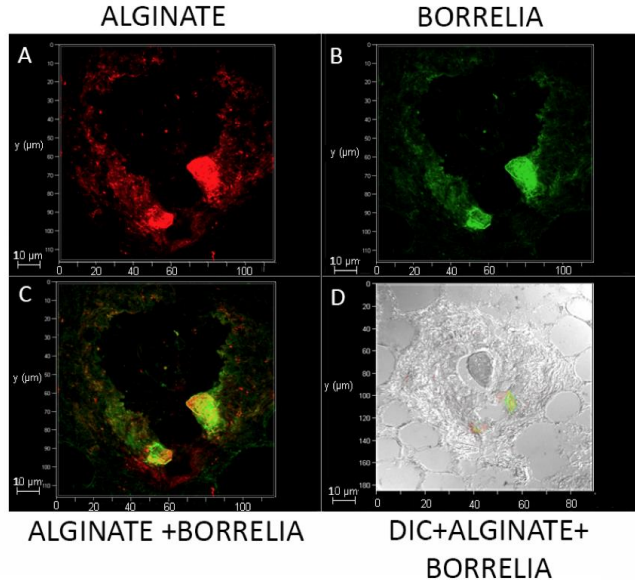
- Molecular analyses of Infected Breast Cancer and Normal Epithelial Cells
 - *RNAseq and tumor panel analyses*
 - *MicroRNA analyses*
 - *Tissue remodeling factors*
 - *Chemotherapeutic resistance markers*



Current Projects: New models for antibiotic studies



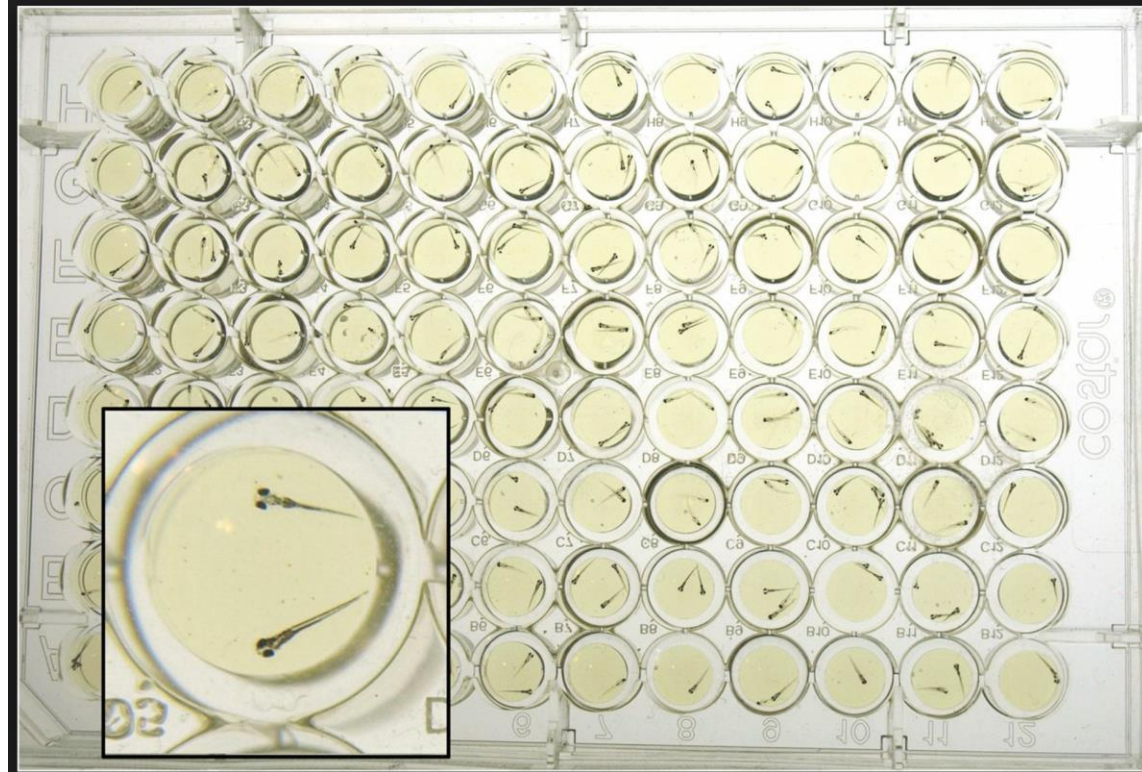
Ex vivo culture systems:
skin biopsies (see left)
heart valve cultures



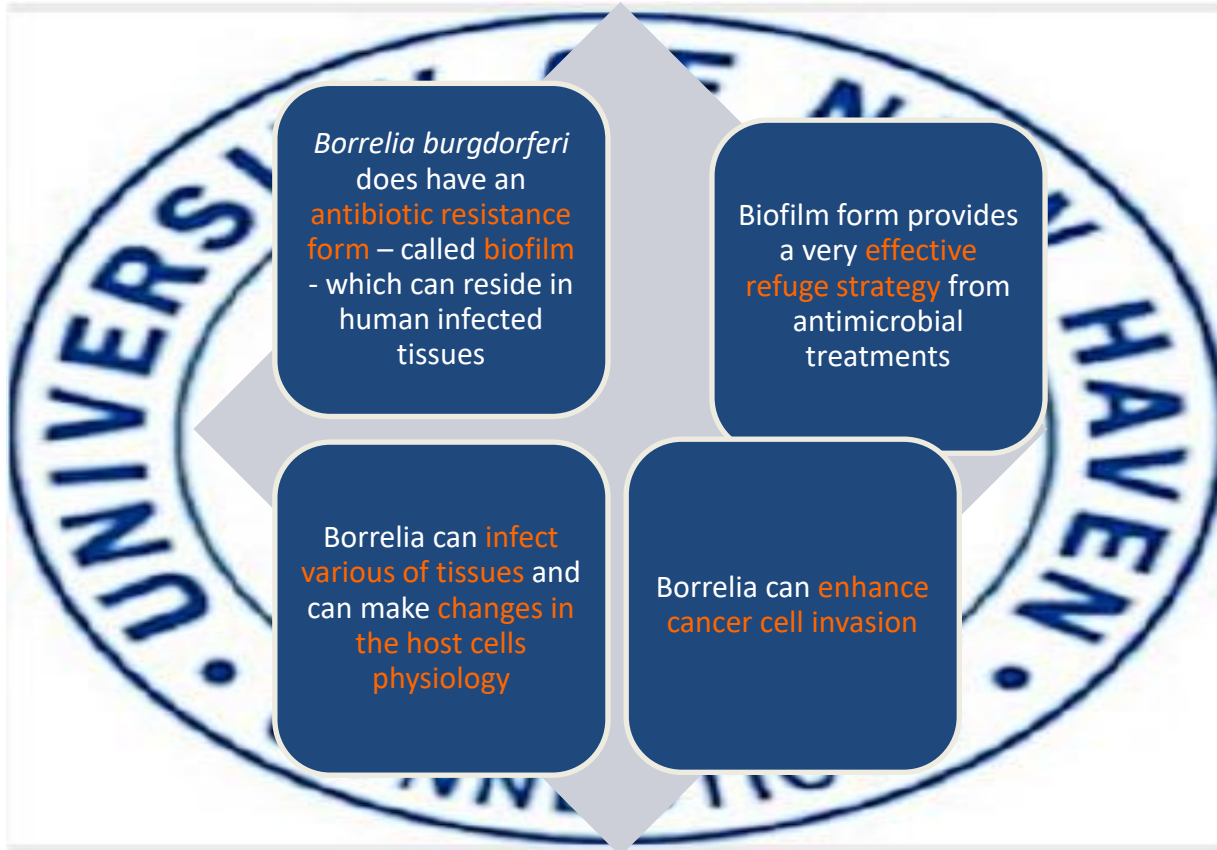
Torres J, Sapi E 2019

Zebrafish model

Antimicrobial Testing in Zebrafish



Summary



UNH Lyme and Breast Cancer Disease Research Group



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Question?



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