Persistent Lyme Disease: Supportive Evidence and Potential Solutions

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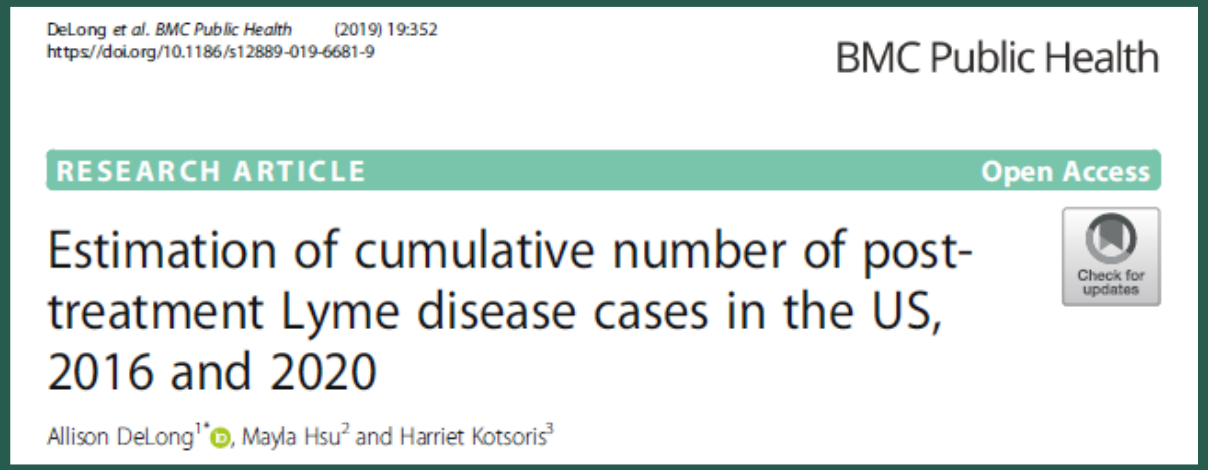

DeLong et al. BMC Public Health (2019) 19:352
https://doi.org/10.1186/s12889-019-6681-9

BMC Public Health

RESEARCH ARTICLE Open Access

Estimation of cumulative number of post-treatment Lyme disease cases in the US, 2016 and 2020

Allison DeLong[1*], Mayla Hsu[2] and Harriet Kotsoris[3]

"Although most patients are successfully treated by timely antibiotic therapy, it is broadly accepted that a sizeable number of patients experience treatment failure and continue to suffer long-term, debilitating symptoms, including pain, fatigue, cognitive dysfunction and other symptoms. This is known as post-treatment LD (PTLD), for which diagnosis is not standardized and treatment remains controversial."

TAKE HOME: Prevalence in 2020 is predicted to be higher than 2016, and may be as high as 1,944,189 (CI 1,619,988 to 2,304,147) cases.

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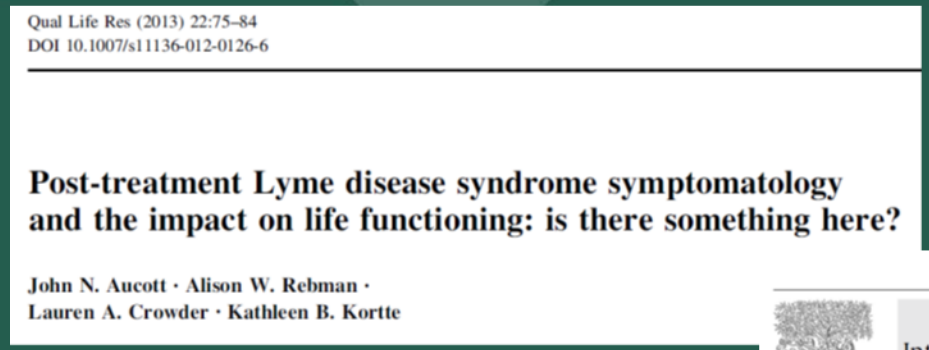
PTLD



Qual Life Res (2013) 22:75–84
DOI 10.1007/s11136-012-0126-6

Post-treatment Lyme disease syndrome symptomatology and the impact on life functioning: is there something here?

John N. Aucott · Alison W. Rebman · Lauren A. Crowder · Kathleen B. Kortte

Published by Oxford University Press on behalf of the International Epidemiological Association

International Journal of Epidemiology 2005;**34**:1340–1345
doi:10.1093/ije/dyi129

Post-Lyme borreliosis syndrome: a meta-analysis of reported symptoms

Victoria Cairns[1]* and Jon Godwin[2]

International Journal of Infectious Diseases 17 (2013) e443–e449

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Development of a foundation for a case definition of post-treatment Lyme disease syndrome

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Clinical case definitions

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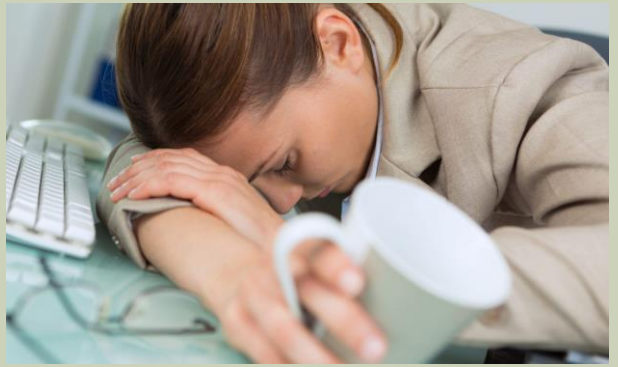
POST-TREATMENT LYME DISEASE (PTLD)

- **Potential causes include:**
  - **Induction of inflammatory responses by lingering dead spirochetes or spirochetal antigen**
  - **Continuation of active spirochetal infection**
  - **Irreversible sequelae from previous active infection (autoimmune)**



Antibiotic efficacy-considerations

- Doxycycline is microbiostatic; efficacy relies on immune clearance of static bacteria

- *B. burgdorferi* evades the immune response in many ways—persistence is the norm in immunocompetent hosts
- Dormant bacteria/persisters more tolerant of microbiostatic antibiotics (antibiotic resistance vs. tolerance)

- *B. burgdorferi* survives for many months inside ticks without nutrient replenishment or replication
- *B. burgdorferi* can be found deep in connective tissues and joints (tissue penetration of antibiotic?)

IDSA Lyme disease treatment guidelines

- **Prophylaxis:** For high-risk Ixodes spp. bites in all age groups, recommend the administration of a single 200 mg dose of oral doxycycline within 72 hours of tick removal
- **Early or early disseminated phase patients who do not have neurological involvement:** recommend that patients with erythema migrans be treated with either a 10-day course of doxycycline or a 14-day course of amoxicillin or cefuroxime axetil rather than longer treatment courses. **Comment:** If azithromycin is used, the indicated duration is 5–10 days, with a 7-day course preferred in the United States
- **Patients with disseminated disease/arthritis:** doxycycline or amoxicillin (same doses) for 28 days
- **Patients with clinically evident neurological and/or cardiac involvement:** ceftriaxone (2 g once a day intravenous) cefotaxime, penicillin G, or oral doxycycline for 14-21 days

- **The efficacy and accepted regimen of antibiotic treatment for human Borreliosis has been a very contentious issue.**

ILADS Guidelines

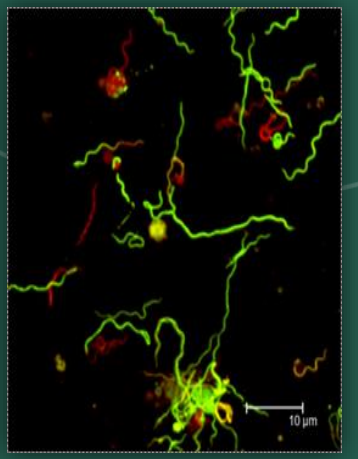
- recommends against the use of a single 200 mg dose of doxycycline for the prevention of Lyme disease.

- 4-6 weeks of doxycycline, amoxicillin or cefuroxime. A minimum of 21 days of azithromycin is also acceptable, especially in Europe. Pediatric dosing: amoxicillin 50 mg/kg/day in three divided doses, with a maximal daily dose of 1500 mg; cefuroxime 20–30 mg/kg/day in two divided doses, with a maximal daily dose of 1000 mg and azithromycin 10 mg/kg on day 1 then 5–10 mg/kg daily, with a maximal daily dose of 500 mg.

- Patients with persistent symptoms and signs of Lyme disease be evaluated for other potential causes before instituting additional antibiotic therapy.

- Antibiotic retreatment when a chronic Lyme infection is judged to be a possible cause of the ongoing manifestations and the patient has an impaired quality of life.

Antibiotic Efficacy against *B. burgdorferi*



- **Background**
  - Studies of the development of persister *B. burgdorferi* cells in the presence of antibiotic have been performed *in vitro*.
  - Lyme disease is most commonly treated with microbiostatic antibiotics, such as doxycycline.
  - All determinations of the MIC have been performed *in vitro*, despite the fact that the phenotype and growth of bacteria can be very different *in vivo*.

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- We have shown that *B. burgdorferi* spirochetes can persist following standard antibiotic treatment of disseminated infection in nonhuman primates (NHP).
- No specific mechanisms of resistance are apparent
- Antibiotic tolerance is achieved with slowed growth
- HOST ADAPTATION contributes to antibiotic tolerance
- The persisters are "viable, but non-culturable (VBNC)"

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

Manuscript in progress

GIVE
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Manuscript in progress

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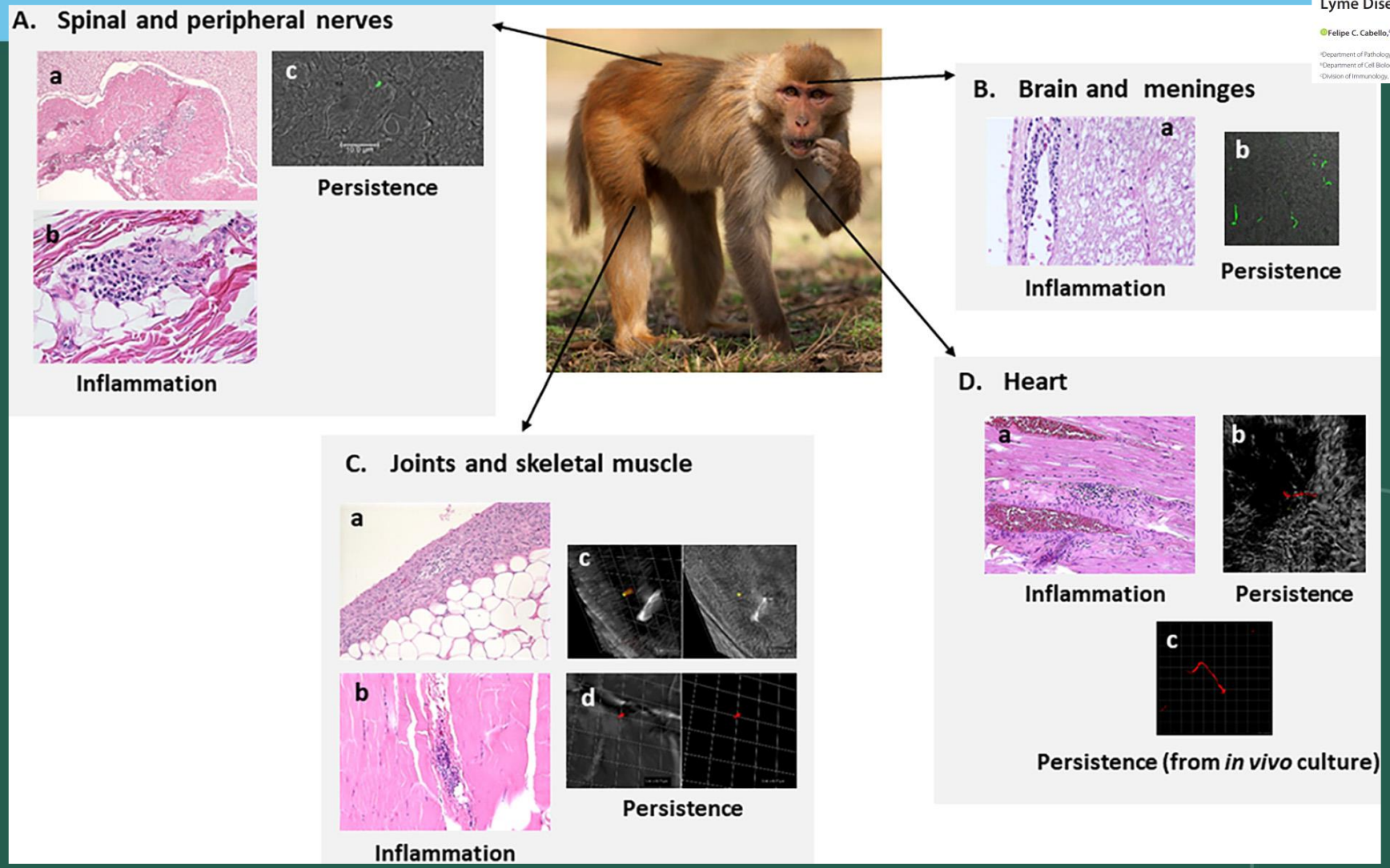
Inflammatory infiltrates and antimicrobial-tolerant persistent *B. burgdorferi* in tick-inoculated rhesus macaques 8 to 9 months after treatment with oral doxycycline



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frontiers in Neurology

ORIGINAL RESEARCH
published: 10 May 2021
doi: 10.3389/fneur.2021.628045

Check for updates

Detecting Borrelia Spirochetes: A Case Study With Validation Among Autopsy Specimens

Shiva Kumar Goud Gadila[1], *Gorazd Rosoklija*[2,3], *Andrew J. Dwork*[2,3,4,5], *Brian A. Fallon*[2*] *and Monica E. Embers*[1*]

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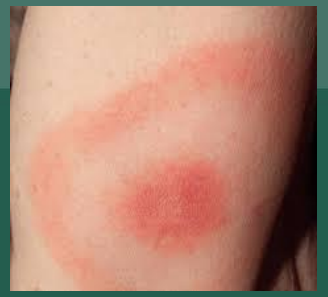
Case Study Description-Phase 1



- 69 year-old woman (Patient 12-577) contracted LD at age 54 with a well-documented EM
- convalescent serologies positive on ELISA and IgM and IgG WB.
- Treatment with doxycycline for 10 days led to symptom resolution.

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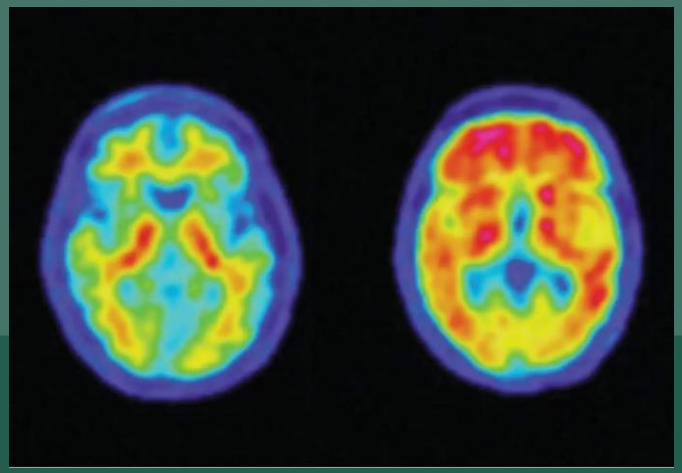
Case Study Description- Phase 2



- 2 yrs later, a sleep behavior disorder emerged.
- Four years later, cognitive problems (processing speed, mental tracking, and word-finding) emerged and gradually worsened.
- Other symptoms included photophobia, paresthesias, fasciculations, and myoclonic jerks.
- Neurocognitive testing revealed deficits in visuospatial skills and executive functions with preservation of verbal skills, suggesting a neurodegenerative process.
- MRI showed mild atrophy and non-specific scattered white matter hyperintensities without enhancement.
- Brain PET/CT scans showed decreased perfusion in the right posterior parietal and temporal lobes.

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Case Study Description-Phase 2, cont.

- PCR of blood for *Bartonella henselae, Babesia microti*, and *Borrelia burgdorferi* were negative.
- C6 ELISA was negative but Lyme IgG Western blot was positive with 9/10 bands.
- Tx with IV ceftriaxone at age 60 for 8 weeks led to 60% improvement in cognition and interpersonal engagement; oral amoxicillin 500mg three times daily was continued for 6 months after the IV treatment.

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Case Study Description-Phase 3

- The initial improvement was not sustained and subsequent antibiotic therapy with minocycline was of no clear benefit;
- Her visual spatial skills and executive functions deteriorated, and anxiety worsened.
- Serum IgG WB continued to be positive. At age 62, CSF showed 4 IgG bands by WB;
- A 2nd brain MRI showed periventricular and subcortical T2 hyperintensities possibly due to “small vessel ischemia or demyelinating disorders like Lyme disease.”
- FDG-PET scan showed “diffuse cortical hypometabolism, worse in the posterior parietal and temporal lobes, with sparing of the sensory motor cortex and visual cortex bilaterally—consistent with Alzheimer’s disease.

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Case Study Description-Phase 3

- Diagnoses of (1) a REM behavioral disorder with verbalizations and movements; and (2) a neurodegenerative dementia characterized by expressive aphasia, visual agnosia, anomia, deficits in executive function and calculation, and mild memory problems.
- Eventually, she developed severe oral dystonia, making feeding more difficult; she died 15 years after the initial infection with *B. burgdorferi*.
- Early and severe movement disorders, REM behavioral disorder, paranoia, and personality changes all favored a clinical diagnosis of dementia with Lewy bodies.

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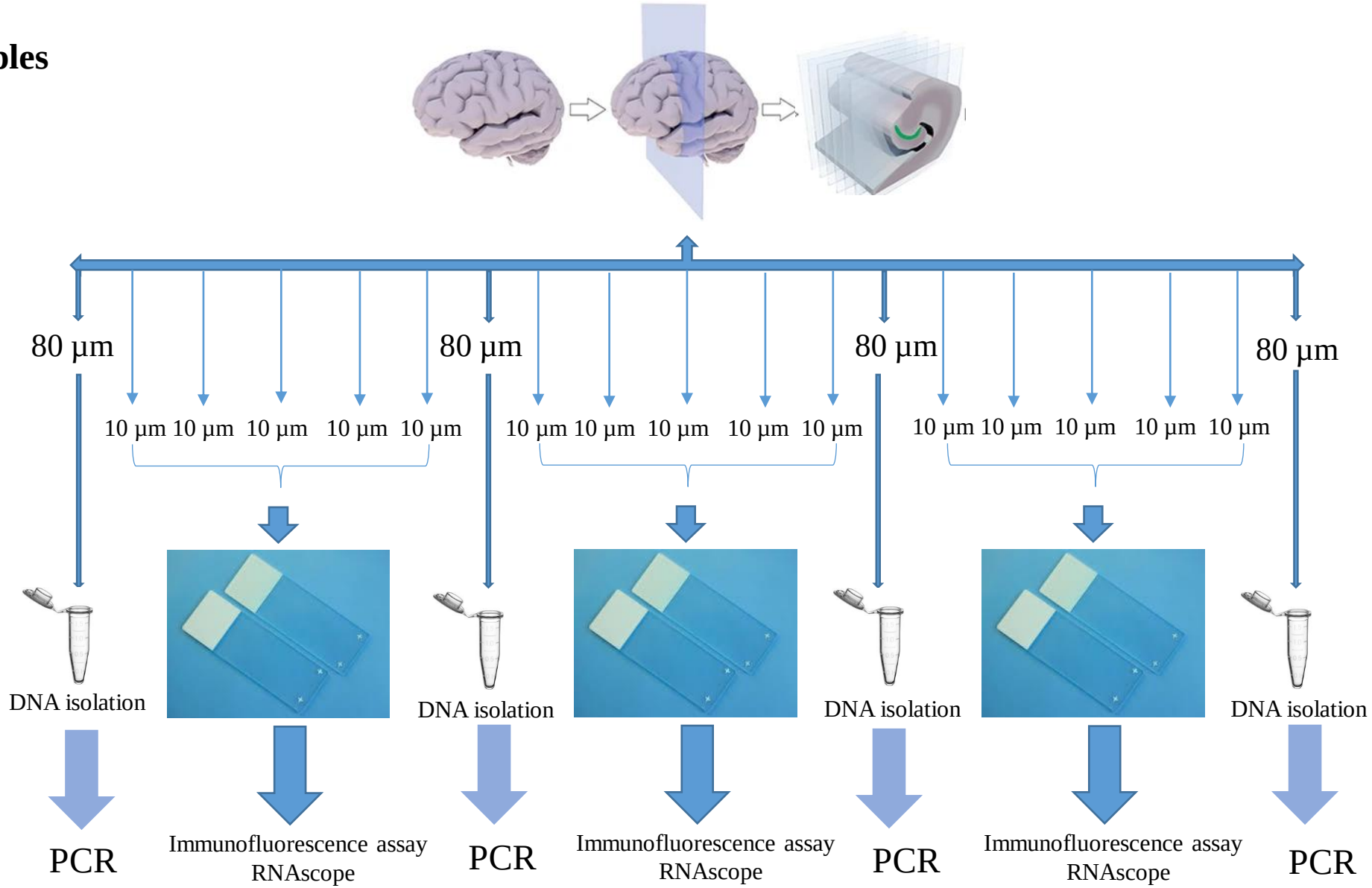
Human samples assay design

Figure 2



Histopathologic findings-Lewy bodies and Amyloid plaques

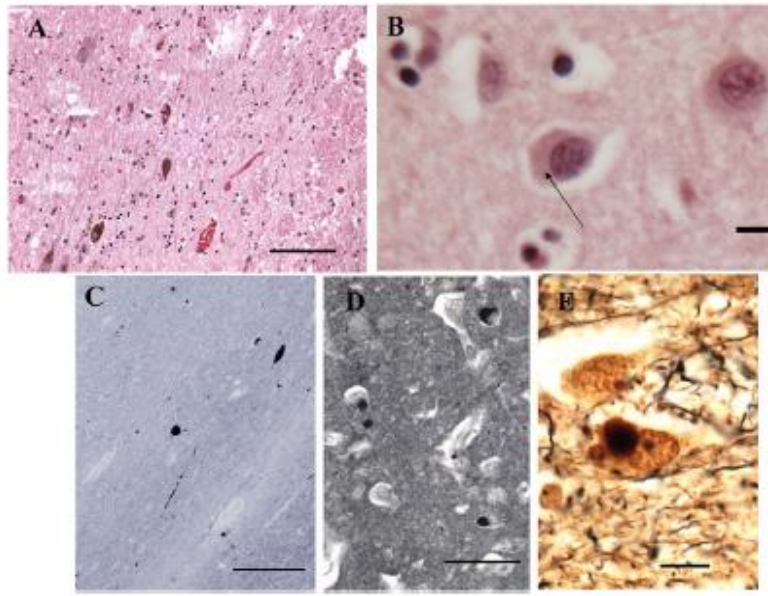


FIGURE 4 | Histopathological findings from the case study. **(A)** Substantia Nigra. H&E. Disintegration of pigmented neurons, with Lewy bodies in two of those remaining (center and lower left). Bar = 100 microns. **(B)** Frontal cortex. H&E. A Lewy body is seen in the central neuron as a perinuclear region of increased eosinophilia (arrow). Bar = 20 microns. **(C–E)** Immunohistochemistry for α-synuclein. **(C)** Substantia nigra pars compacta. Inclusions are seen in cell bodies and neurites. Bar= 200 microns. **(D)** Hilus (CA3) of hippocampus. Bar = 50 microns. **(E)** Bielschowsky stain, hippocampus. Central neuron contains a Lewy body. Bar = 10 microns.

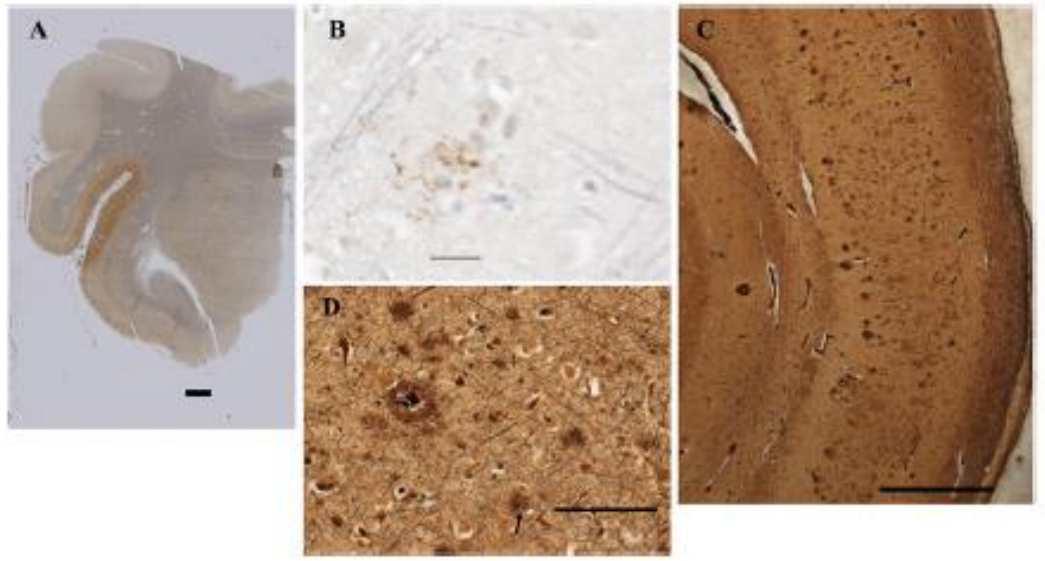
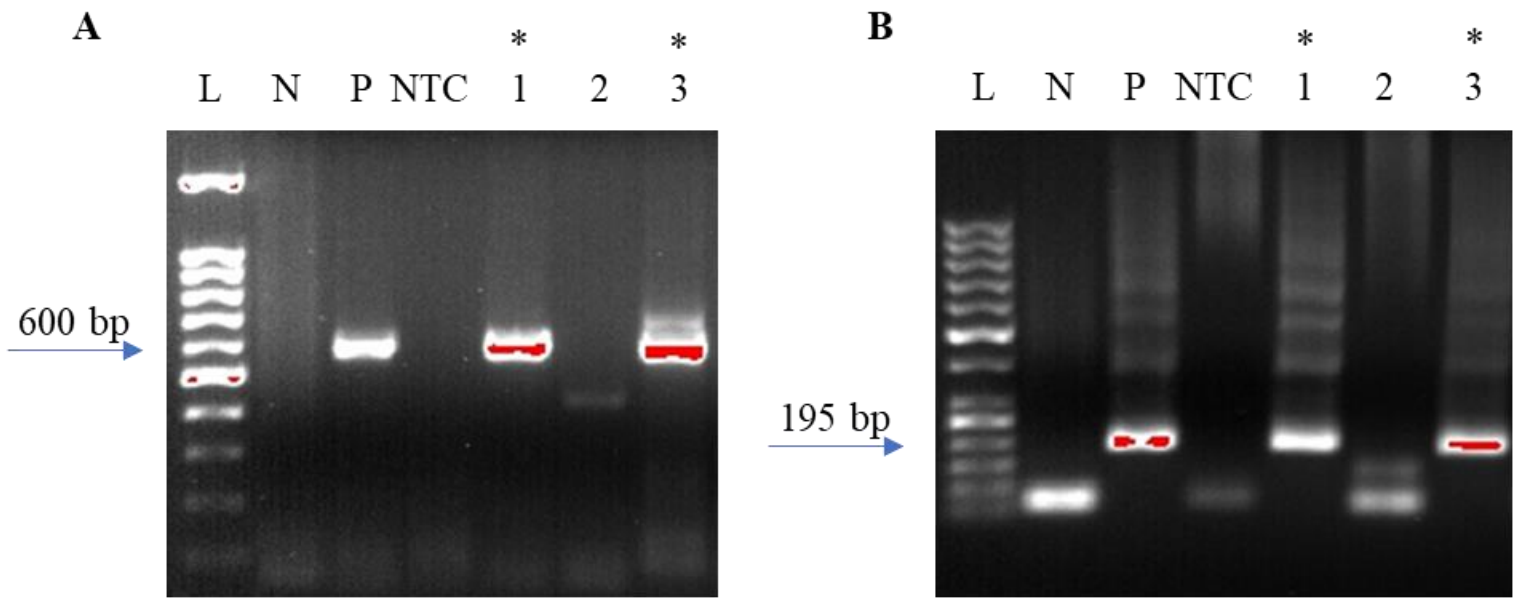
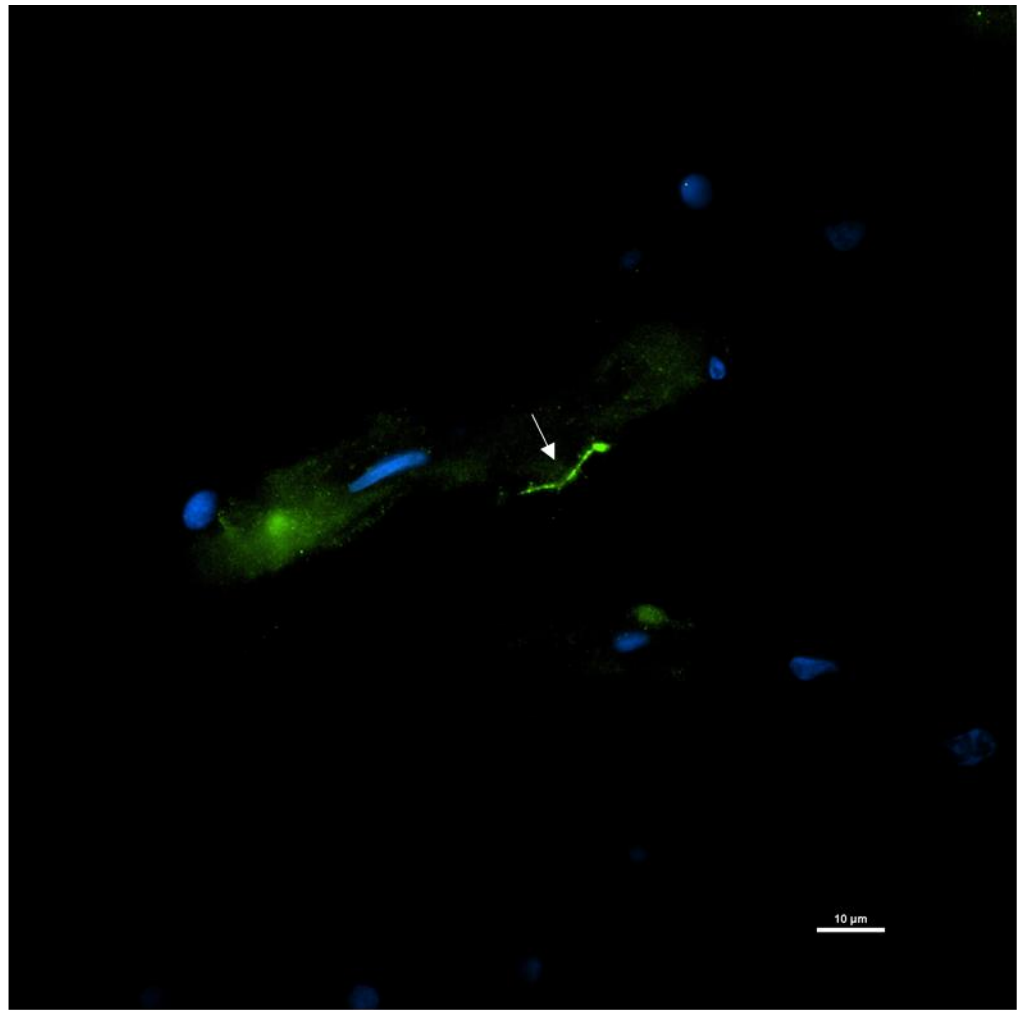


FIGURE 5 | Detection of amyloid plaques in the patient's CNS. **(A)** Amygdala and rostral hippocampus, entorhinal cortex, and transentorhinal cortex. AT8 immunohistochemistry with Verhoeff myelin counterstain. BAR = 2 mm. **(B)** Rare cortical neuritic plaque in middle frontal gyrus. AT8 with Verhoeff myelin counterstain. BAR = 20 microns. **(C)** Bielschowsky stain of CA1 and subiculum demonstrates numerous amyloid plaques. Bar = 1 mm. **(D)** Bielschowsky stain of frontal cortex. Amyloid plaques predominate; rare neuritic plaques are also present (arrow). Bar = 100 microns.

Detection of Borrelia DNA in the Amygdala and Pons

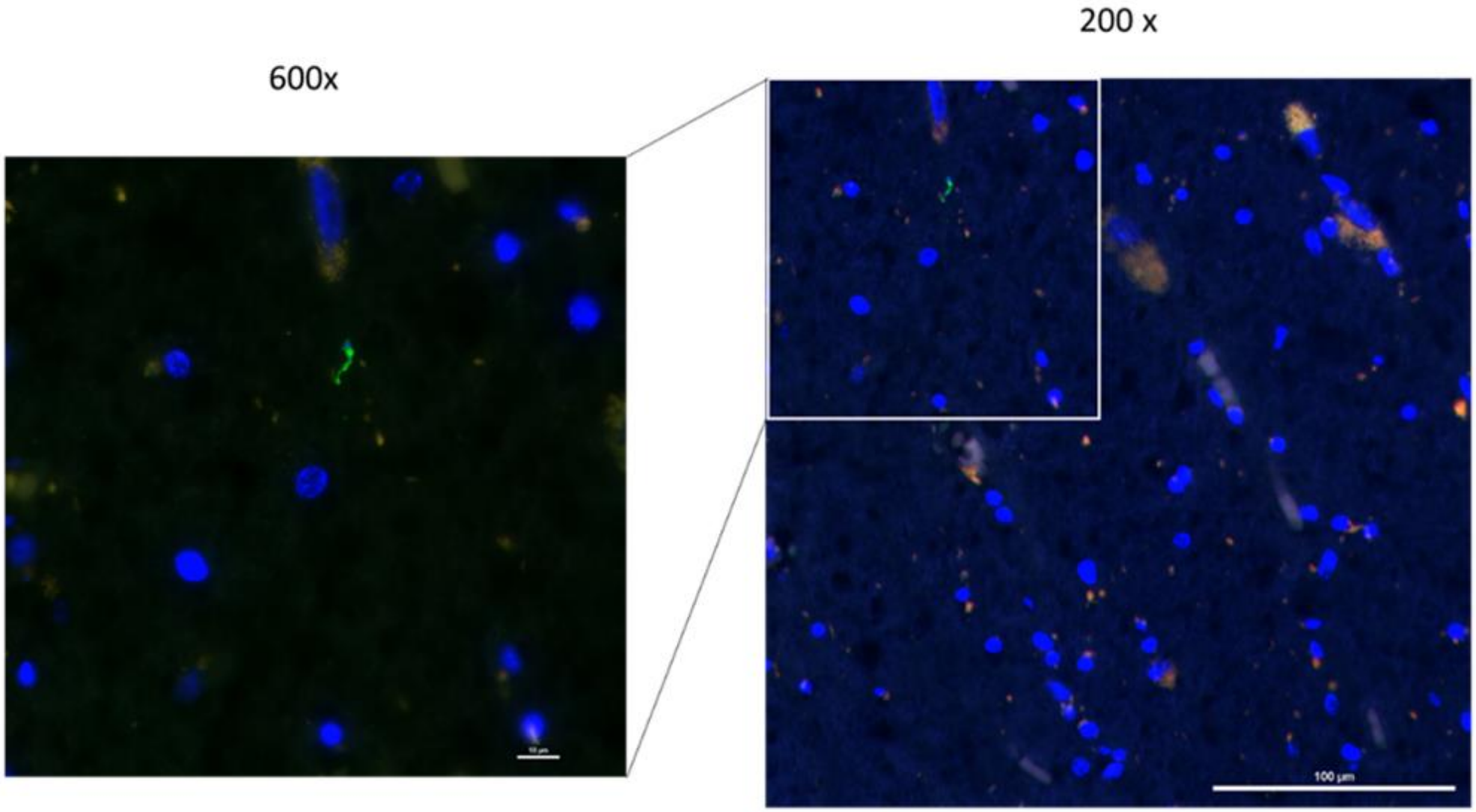


Primers amplify Borrelia 16S-23S internal transcribed spacer (ITS) region. Bobu primers generate an amplicon size of 600 bp. To increase the specificity bobu internal primers were designed to generate an amplicon size of 195 bp**. A:** External PCR: L-molecular weight marker of 100 bp, N-negative control, P-positive control, NTC-No Template Control, 1-12577 amygdala, 2-12577 pons, 3-12577 spinal cord **B:** Internal PCR: L-molecular weight marker of 50 bp, N-negative control, P-positive control, NTC-No Template Control, 1-12577 amygdala, 2-12577 pons, 3-12577 spinal cord. * indicates samples that were positive to borrelial DNA.

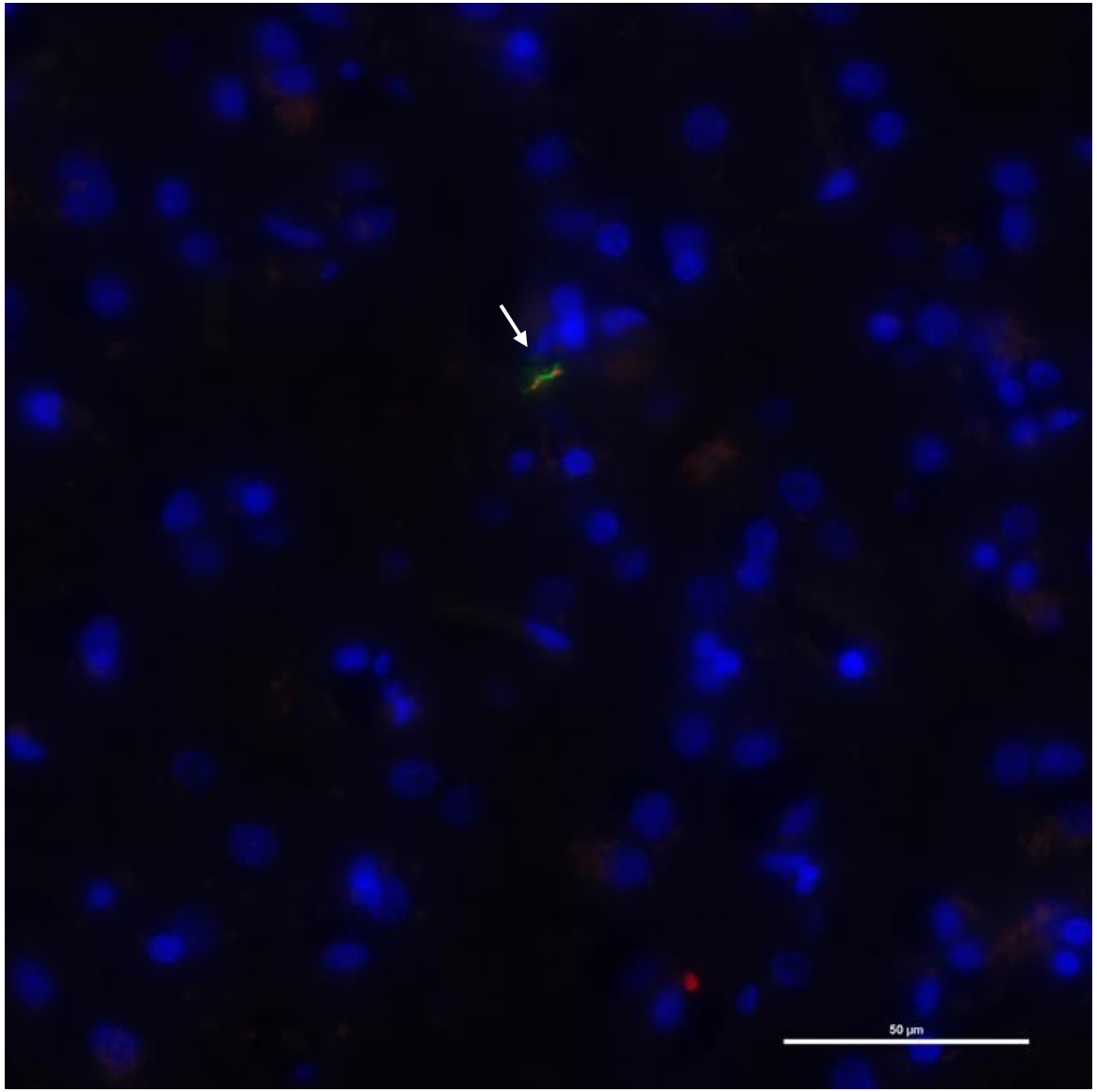


Detection of an intact borrelial spirochete in a fixed autopsy tissue using Immunofluorescence assay. 12577-Spinal cord autopsy tissue was immunostained with rabbit *B. burgdorferi* hyperimmunized polyclonal (primary) and goat anti-rabbit IgG Alexa Fluor 488 (secondary) antibodies. White arrow points towards a green fluorescence of morphologically intact spirochete. Scale bar - 10 µm. Image was acquired with a Nikon fluorescence microscope using a 40x objective.

Detection of Bb spirochete within human autopsy brain tissue



Detection of Bb spirochete within human autopsy brain tissue



Results/Conclusions on Borrelia Persistence

- Our studies, presented here and in animal models, indicate that the Lyme disease spirochete can persist after conventional antibiotic treatment.

- The case study presented here indicates that Borrelia may play a role in the development of dementia.

Strategy: combination therapy

Rationale: By combining antimicrobials which utilize different mechanisms to kill or inhibit growth of bacteria, persisters can be eliminated.

Antibiotics **2015**, *4*, 397-410; doi:10.3390/antibiotics4030397

OPEN ACCESS

antibiotics

ISSN 2079-6382

www.mdpi.com/journal/antibiotics

Article

Identification of Additional Anti-Persister Activity against *Borrelia burgdorferi* from an FDA Drug Library

Jie Feng, Megan Weitner, Wanliang Shi, Shuo Zhang, David Sullivan and Ying Zhang *

OPEN

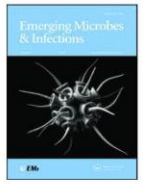
Emerging Microbes and Infections (2015) 4, e31; doi:10.1038/emi.2015.31

www.nature.com/emi

ORIGINAL ARTICLE

Identification of new compounds with high activity against stationary phase *Borrelia burgdorferi* from the NCI compound collection

Jie Feng, Wanliang Shi, Shuo Zhang and Ying Zhang



Emerging Microbes & Infections

ISSN: (Print) 2222-1751 (Online) Journal homepage: https://www.tandfonline.com/loi/temi20

Identification of novel activity against *Borrelia burgdorferi* persisters using an FDA approved drug library

Jie Feng, Ting Wang, Wanliang Shi, Shuo Zhang, David Sullivan, Paul G Auwaerter & Ying Zhang

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Strategy: combination therapy
In vitro studies, cont.

antibiotics

MDPI

Article

Evaluation of Disulfiram Drug Combinations and Identification of Other More Effective Combinations Against Stationary Phase *Borrelia burgdorferi*

Hector S. Alvarez-Manzo, Yumin Zhang, Wanliang Shi and Ying Zhang *,†

Summary: combination with two drugs (cefuroxime + clarithromycin/nitroxoline + furazolidone/nitazoxanide) inhibited the regrowth, while disulfiram combinations did not (cefuroxime + disulfiram + furazolidone/nitazoxanide).

Drug	Cntrl	DSF	Clari	NTX
——	85.8	46.3	41.1	37.5
CefU	36.8	41.7	25.9	27.5
FZD	57.1	37.7	34.2	21.4
NTZ	61.9	44.2	39.8	32.6
CefU + FZD	45.3	25.1	6.6 *	1.7 *
CefU + NTZ	38.3	23.9	5.0 *	11.0 ns

PLOS ONE

RESEARCH ARTICLE

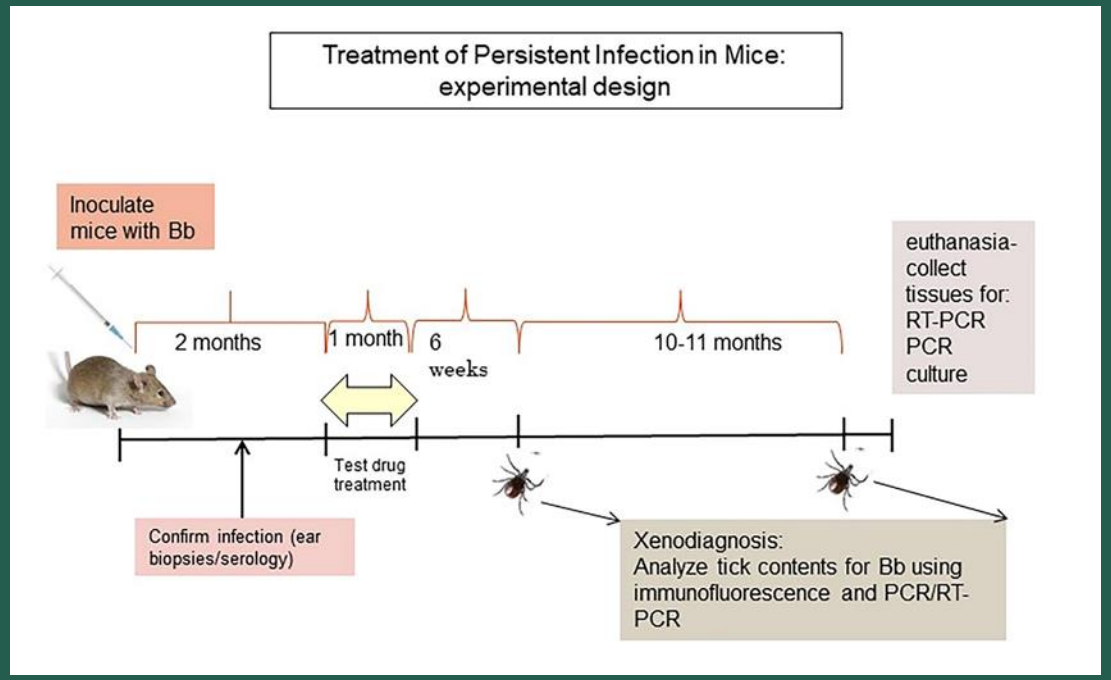
Drug Combinations against *Borrelia burgdorferi* Persisters *In Vitro*: Eradication Achieved by Using Daptomycin, Cefoperazone and Doxycycline

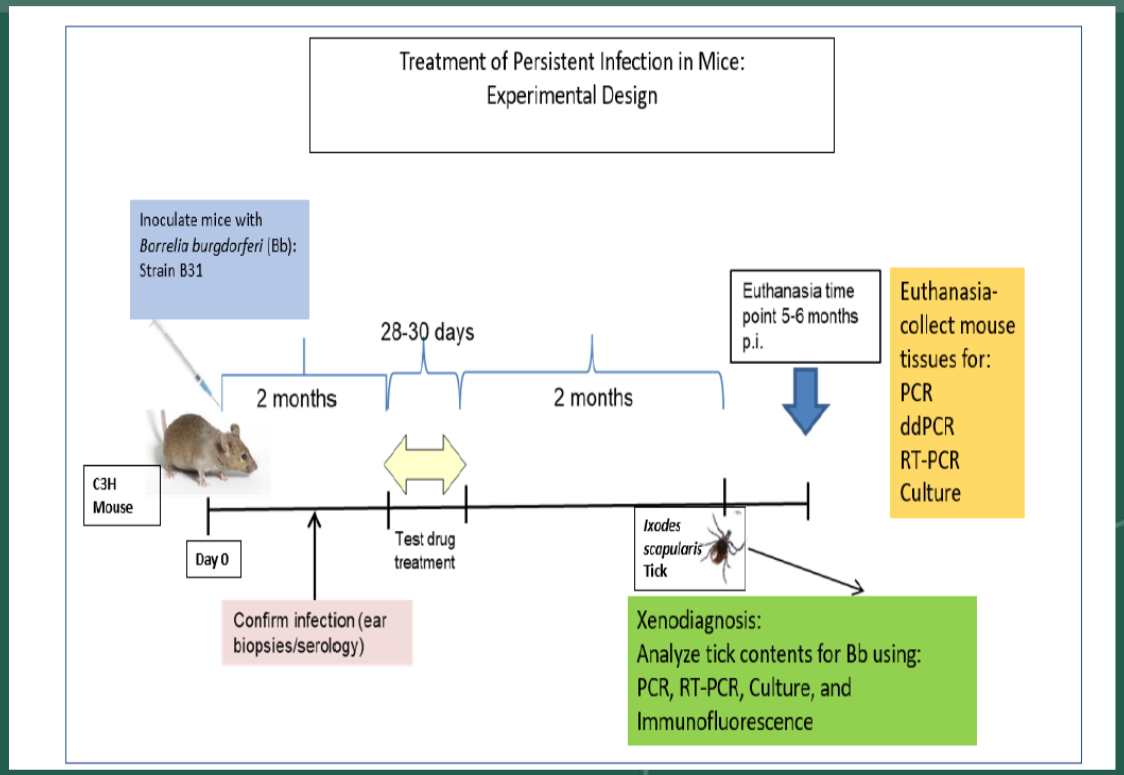
Jie Feng[1], Paul G. Auwaerter[2], Ying Zhang[1]*

Summary: Daptomycin was the common element in the most active regimens when combined with doxycycline plus either beta-lactams (cefoperazone or carbenicillin) or an energy inhibitor (clofazimine). Daptomycin plus doxycycline and cefoperazone eradicated the most resistant microcolony form of *B. burgdorferi* persisters and did not yield viable spirochetes upon subculturing, suggesting durable killing that was not achieved by any other two or three drug combinations.

Of, note, pulse-dosing has shown variable efficacy in vitro, but has not been directly tested in animal models.

Study Designs





Over 17 different drugs have been tested singly, and in combination, in mouse models.

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Results and Conclusions

- Doxycycline and cefuroxime in monotherapy do not clear the established infection
- In general, single drugs were not capable of clearing the infection, yet certain combinations show promise (in mice)
- Tested combos that worked well using rhesus macaque model (data analyses incomplete)

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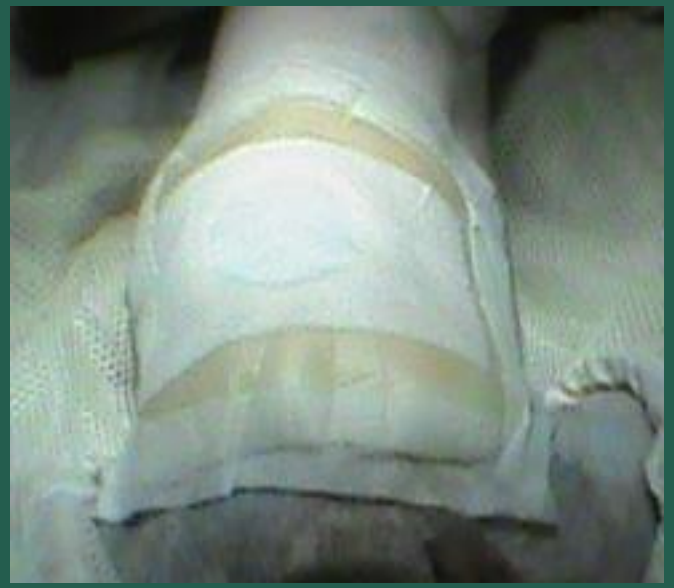
Lessons learned

- Ineffective treatment may result in very poor outcomes
- It is much easier to cure mice than to cure primates



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Lyme and Covid

Symptom	Long Covid	Persistent Lyme disease
Fatigue	X	X
Brain fog/cognitive impairment	X	X
Musculoskeletal pain	X	X
Peripheral Neuropathy		X
Shortness of Breath/Chest pain	X	
Loss of Smell and/or Taste	X	
Depression and Anxiety	X	X
Sleep Disorders	X	X
Post-exertional Malaise	X	X
Cardiac symptoms	X	X
Gastrointestinal disturbance	X	
Dizziness	X	X
Photophobia		X

- **Many similarities in the chronic phase**
- **Neurological manifestations are common**

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Lyme and Covid

- <u>Outstanding Questions:</u>
  - Can the suppression of immunity by Borrelia make SARS CoV-2 infection worse? Persist?
  - Does a dual infection exacerbate the pathology, esp. in the brain?
  - How is vaccine immunity affected by Lyme?

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Nonhuman primate models

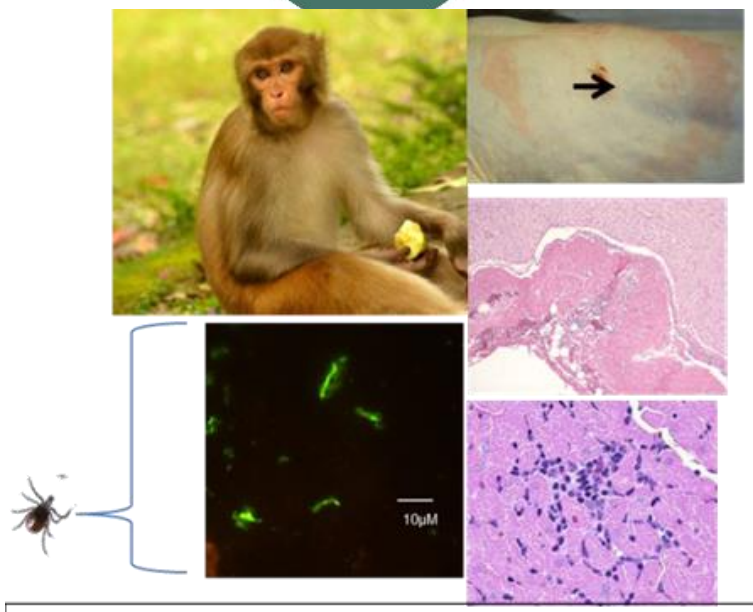

Figure 1. The nonhuman primate model of Lyme disease. Following tick-mediated infection, monkeys can develop erythema migrans (top, right) and characteristic inflammatory pathology in the tissue of the nervous system (middle, right) and myocardium (lower right). Recovery of persistent spirochetes by xenodiagnosis provides a definitive indication of *B. burgdorferi* persistence (lower left).

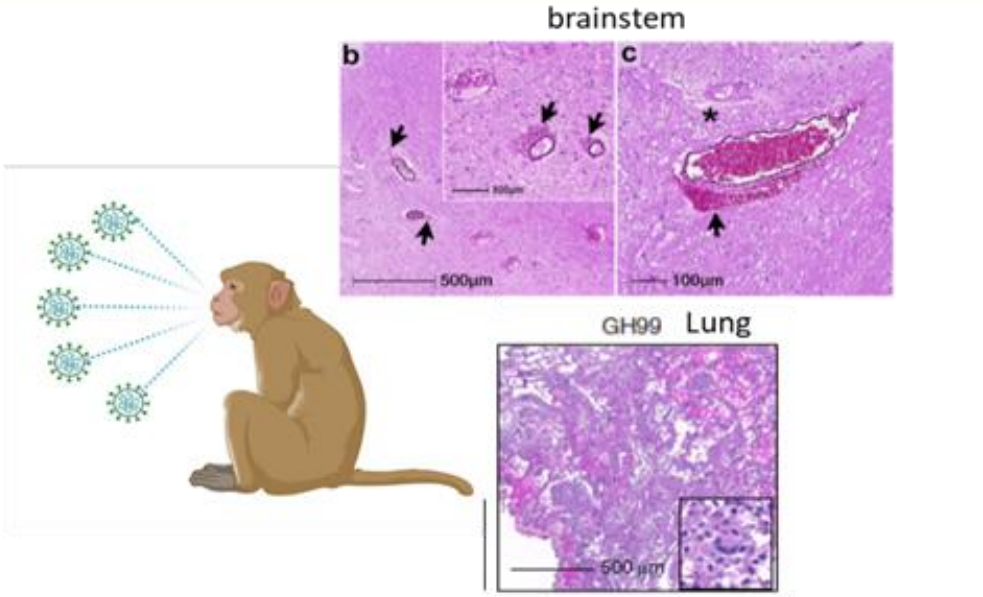

Figure 2. SARS-CoV-2 infection of rhesus macaques induces lung and CNS pathology. Monkeys exposed mucosally or by aerosol exhibit microhemorrhages (top image, brainstem), hypoxia, and microglial activation (Rutkai, et al 2022). The lower panel shows infiltration of activated macrophages in the lung tissue (Fahlberg, et al 2020).

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THANK YOU!

<u>Embers lab</u>
Nicole Hasenkampf, MPH
Amanda Tardo
Shiva Gadila, M.S.
Rebekah Bullard, Ph.D.
Marianne Hathaway, M.S.

Heather Adkison (BMS)
Remi Landry (TropMed)
Emily Olsen, M.S. (BMS)
Laura Lee (BMS)
Folasade Adekambi (BMS)
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Britton Grasperge, DVM, PhD
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Mercedes Cheslock, Ph.D. (BMS)
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Liz Didier, PhD

<u>Veterinary Medicine</u>
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