Vector Biology: Connecting human health, animal health and the environment

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1

Human tissue

- Late Lyme carditis

2

***Borrelia burgdorferi* and *B. bissettii and Leptospira* in wildlife**

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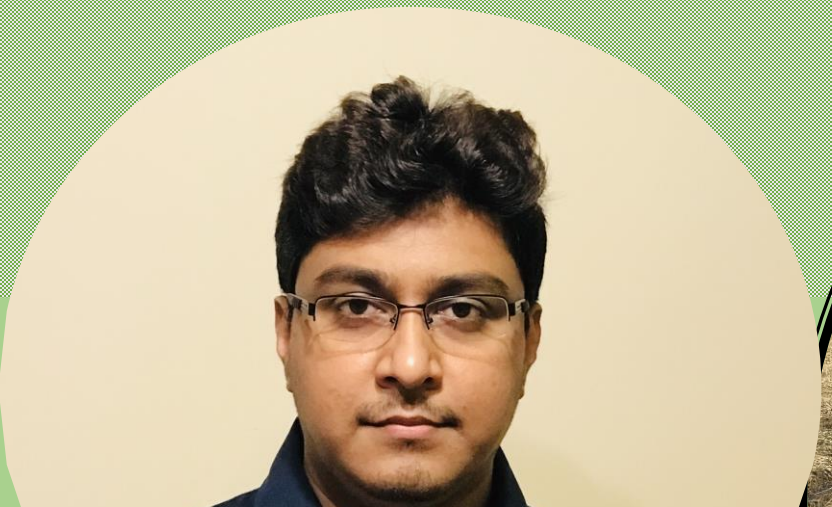
Borrelia burgdorferi* and *B. miyamotoi

Reservoir species

Tissue distribution and implications for congenital transmission

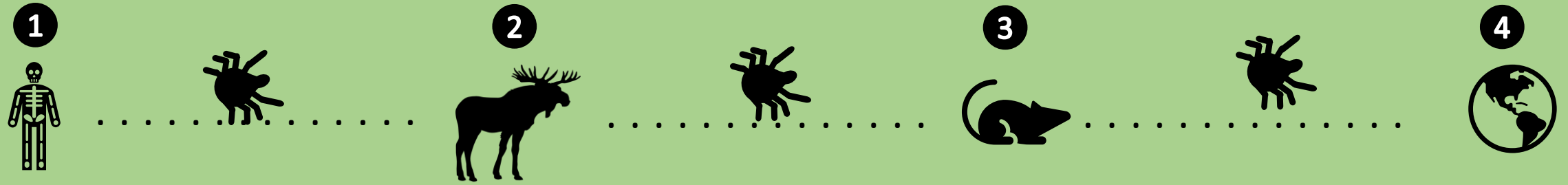
4

Implications

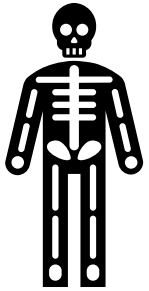


Molecular Tools for Diagnostics and Research

- Enables **direct** detection of pathogen
- Can be used to assess human seropositive cases, seronegative cases and cases with unknown serological status
- Can be used in humans, wildlife tissues, companion and agricultural animals
- Allows identification of *Borrelia* species

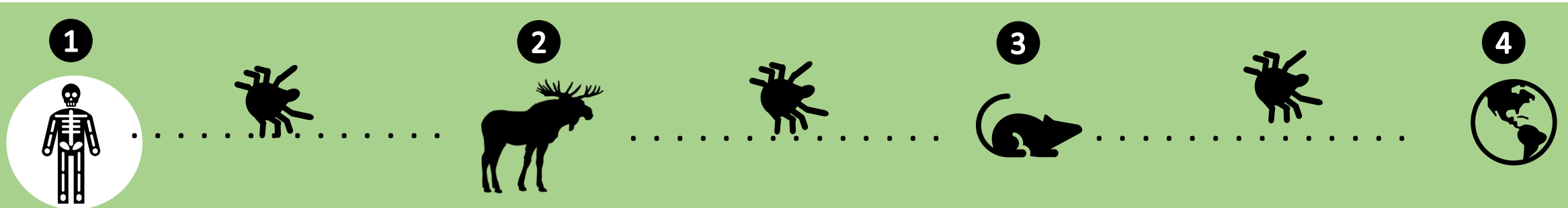


Cardiac involvement in late Lyme disease

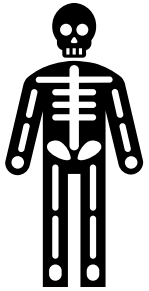


- In the course of infection, Lyme diseases infect multiple organs including heart
- Varies in nature in acute versus chronic disseminated infection
  - Acute phase
    - Atrioventricular block and pericarditis
  - Chronic phase
    - Dilated cardiomyopathy
- *Borrelia* association with cardiac dysfunction in late disease poorly studied
- Very few cases reported a possible relationship between Lyme endocarditis and cardiac valvular pathology

- The aim of this study was to assess the presence of *Borrelia* infection in cardiac tissue using molecular detection of *Borrelia* from biopsy and autopsy tissue



Methods



Autopsy

Biopsy

Necropsy

Organs/tissues

(cardiac/major vasculature

Ethanol-preserved specimens

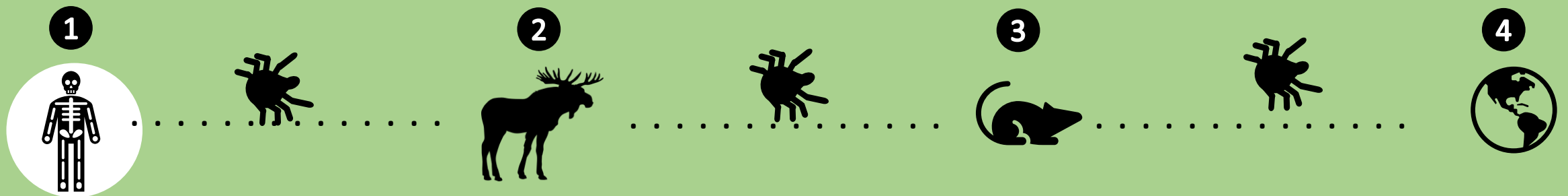
nPCR

DNA sequencing

Formalin-fixed paraffin embedded

FISH

Immunohistology



Methods

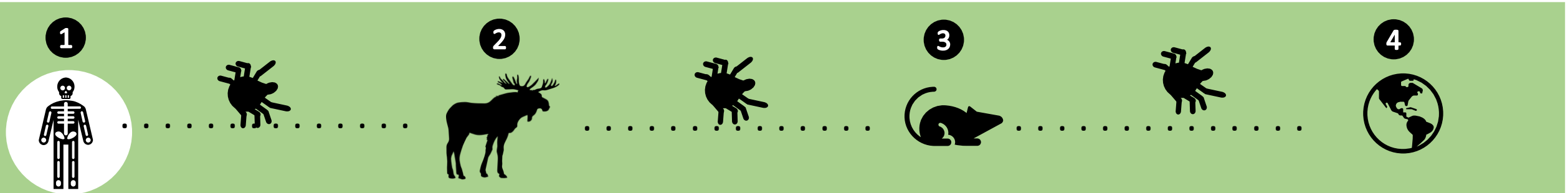
Donor 1

Long-term resident of Ontario, Canada
Recreational and residential exposure to ticks
Family members with Lyme disease
Lyme-like symptoms reported 9 years prior to aortic value replacement, and again 6 years later.
Clinical diagnosis of Lyme disease
Canadian ELISA serology negative, US WB equivocal
Aggressive long-term (7 years) antibiotic treatment
Aortic valve replacement surgery (2018) at age 70

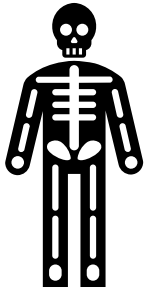
- Pericardium
- L. Internal thoracic artery
- Aortic valve

Donor 2

Resident of Nova Scotia, Canada
Province is considered endemic
Lyme-like symptoms and rashes at age 7 with acute cardiac symptoms
Lyme disease not considered at the time
Discharged when stabilized, steady deterioration over next 10 years with arthritis, fatigue,
Canadian serology negative, US positive
No Lyme disease treatment
Death in 2018 at age 17 from heart failure



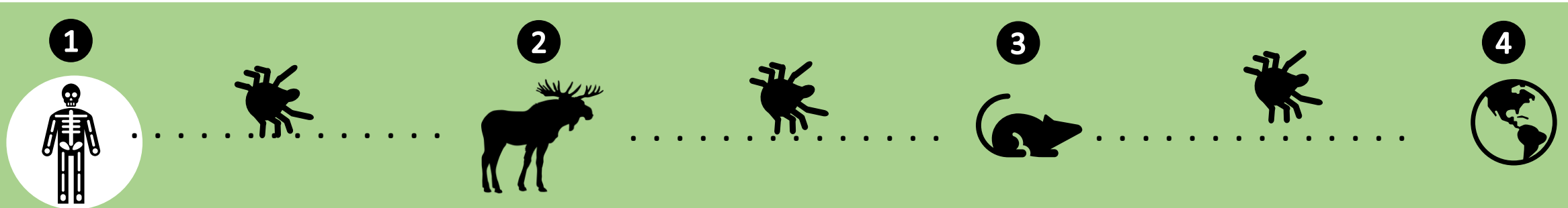
Methods



Donor 1 - Samples of pericardium, left internal thoracic artery and aortic valve were submitted as ethanol-submerged tissues

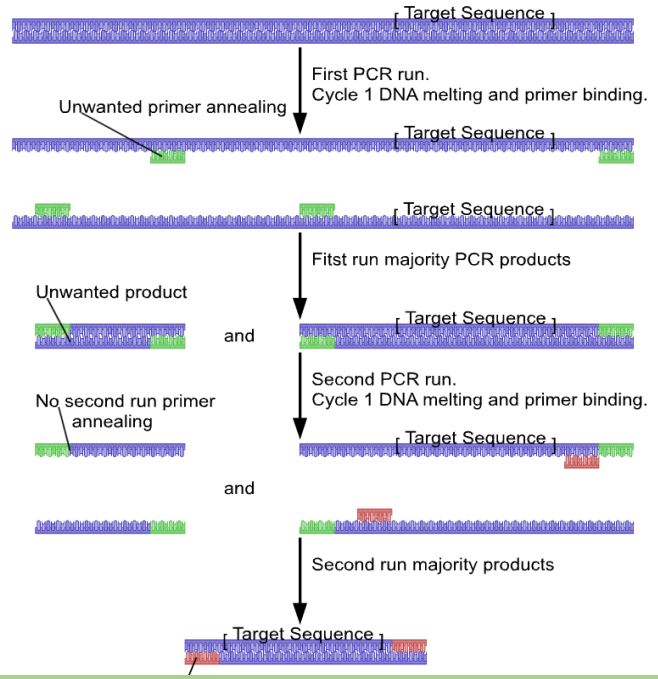
- Each of the three tissue samples was divided and a portion preserved in ethanol
  - DNA extraction
    - PCR and sequencing
- The remaining tissues were formalin-fixed and paraffin-embedded (FFPE) for fluorescent in situ hybridization (FISH) and immunohistology

Donor 2 – FFPE blocks for fluorescent in situ hybridization (FISH) and immunohistology

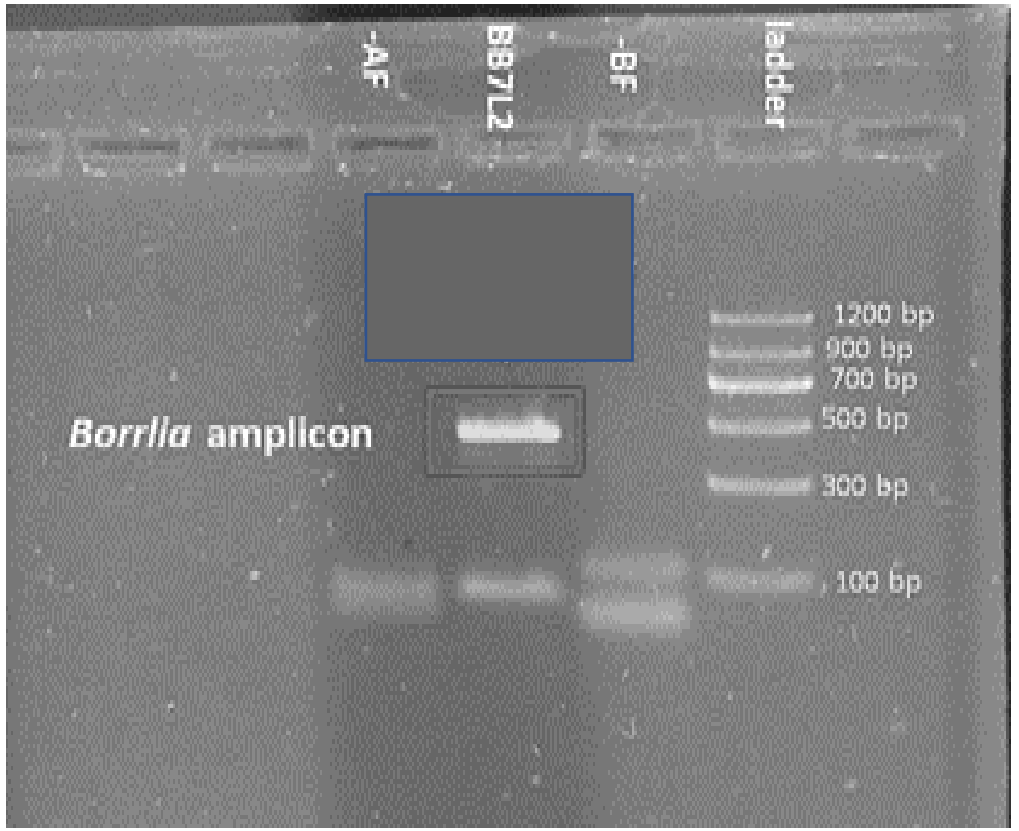


Results – Donor 1
Nested PCR (nPCR)

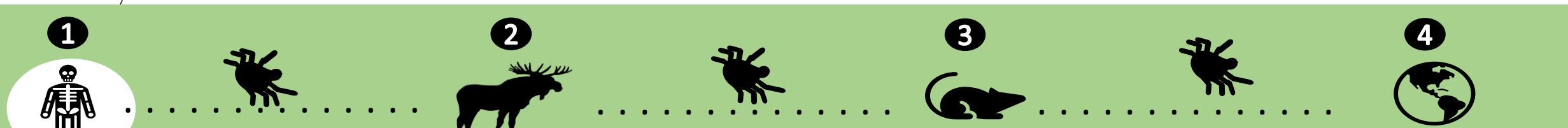
- Positive amplification for the left internal thoracic artery for *FlaB* gene
- The other two tissues did not demonstrate amplification
- Amplicons followed by nBLAST analysis of NCBI genbank showed strong sequence identity to *B. burgdorferi*



- **Sensitive and specific technique for amplifying low abundance DNA**

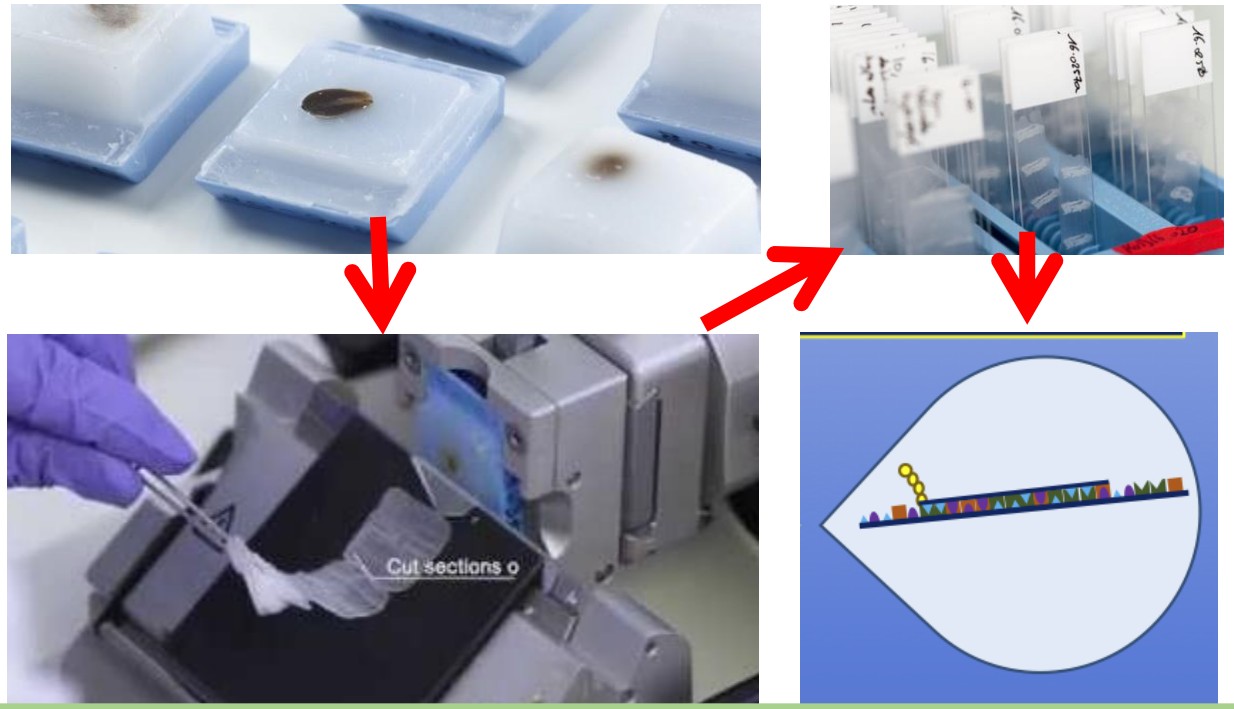


Detailed methods:
Detecting the Lyme Disease Spirochete, *Borrelia Burgdorferi*, in Ticks Using Nested PCR
Melanie K. B. Wills, Andrea M. Kirby, Vett K. Lloyd
https://www.jove.com/video/56471/detecting-lyme-disease-spirochete-borrelia-burgdorferi-ticks-using

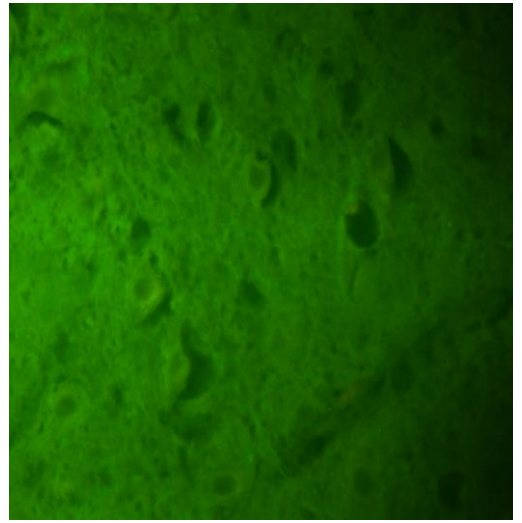


Results - Fluorescent in situ hybridization (FISH)

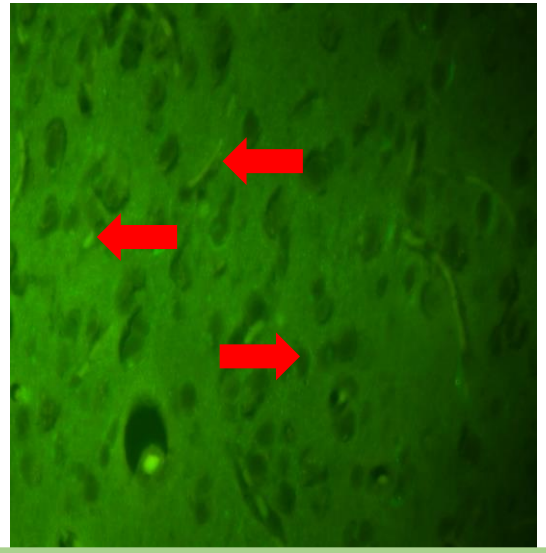
- Single-stranded oligonucleotide probes with fluorescent dye attached
- Complementary to a target sequence

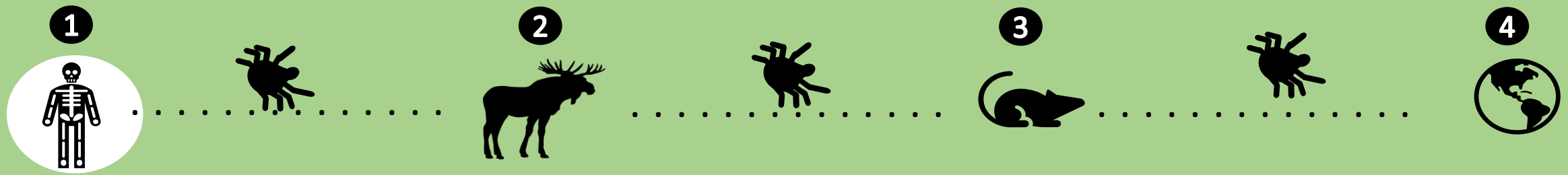


Negative control (mouse brain, uninfected



Positive control (mouse brain, infected)



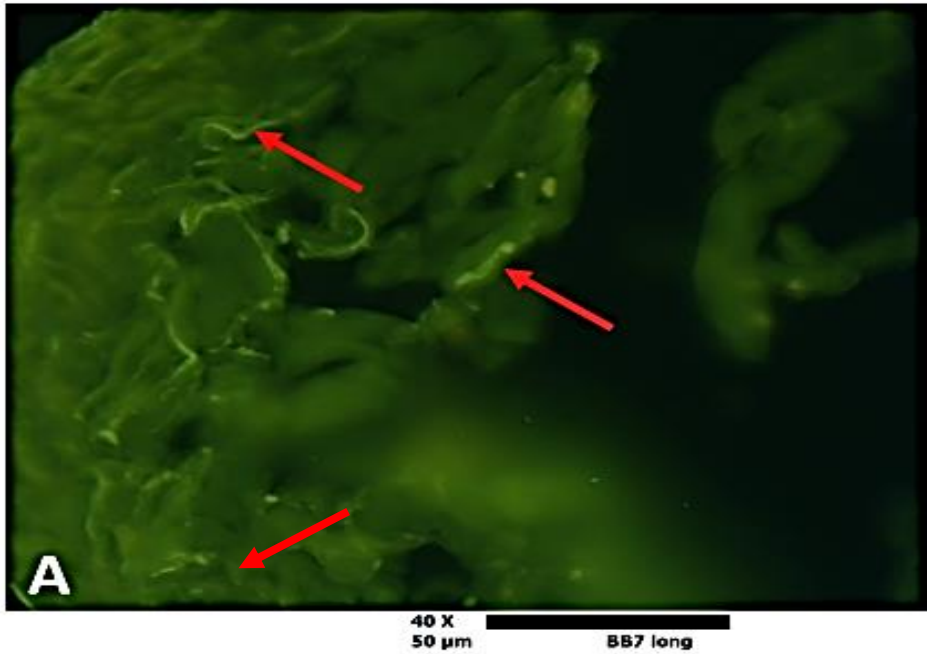


Results - FISH hybridization

Donor 1

- The *FlaB* probe appeared to hybridize and stain target DNA in the left internal thoracic artery sample only
- Stained structures appear to be both punctate and long structures
- Weak fluorescence seen in the pericardium sample, not clearly distinct from background, but none in the aortic valve

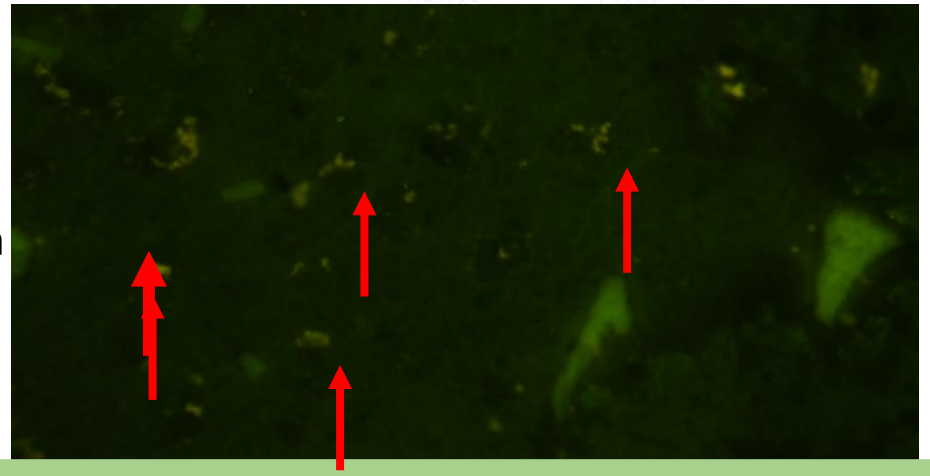
Donor 1
L thoracic artery

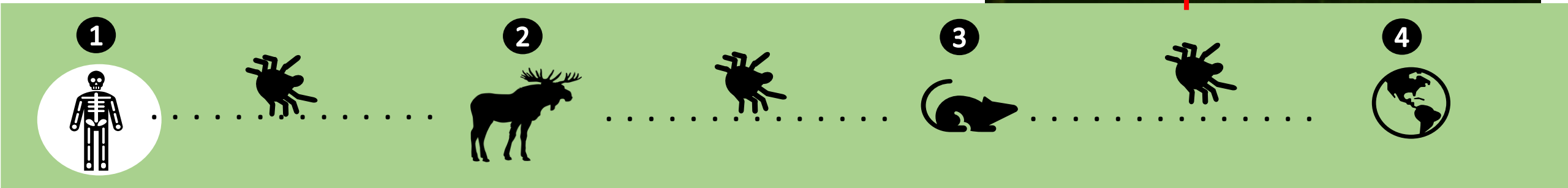


Donor 2

- The *FlaB* probe detected target DNA extensively in pericardium
- Stained structures appear to be both punctate and long structures (distinguishable from lipid and RBC autofluorescence)
- Other tissues not yet tested

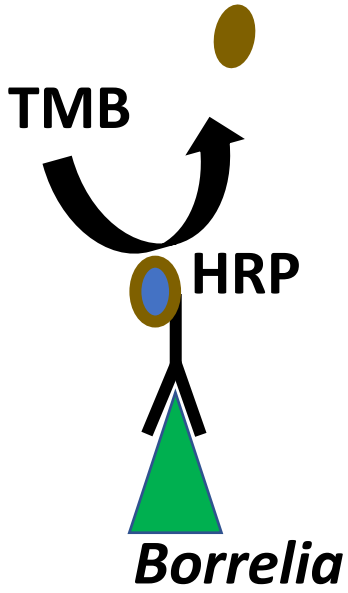
Donor 2
pericardium

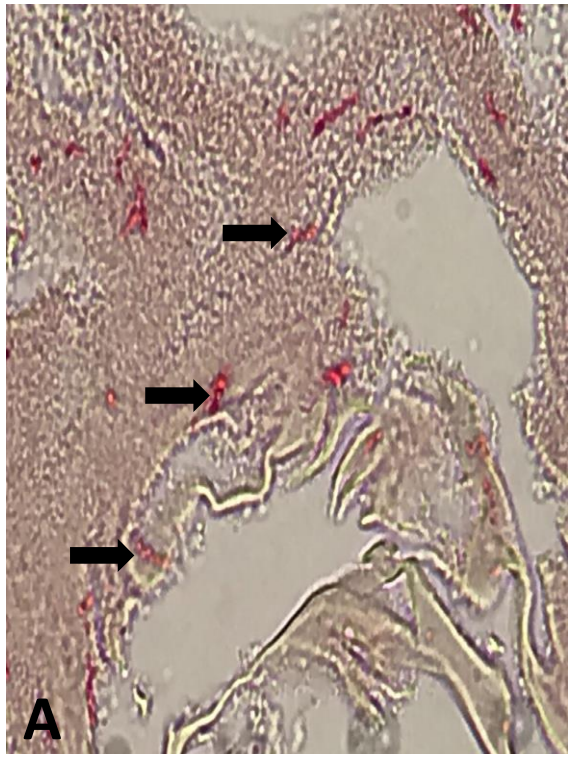




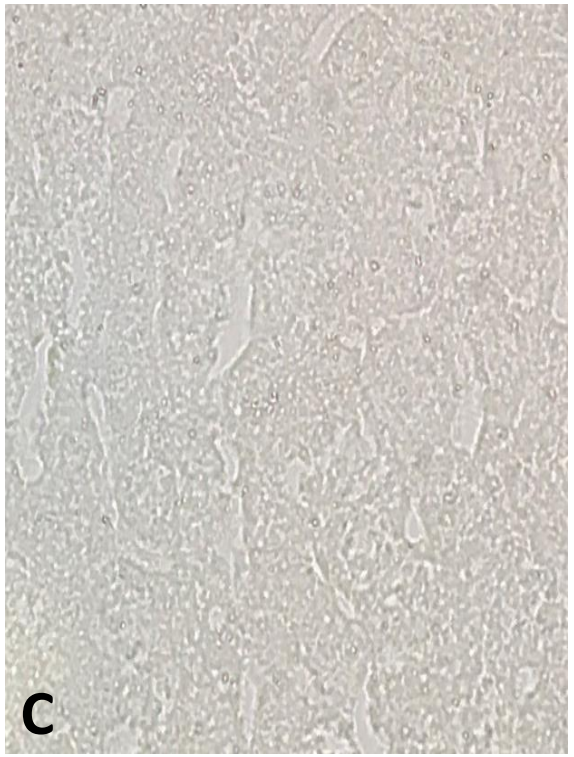
Results - Immunohistology

Anti-*Borrelia burgdorferi* B31 polyclonal antibody (Abcam) binds target proteins

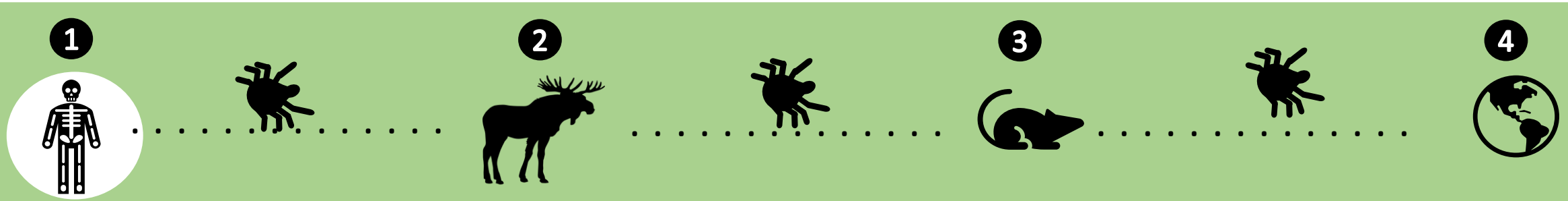




Positive control (infected mouse liver)



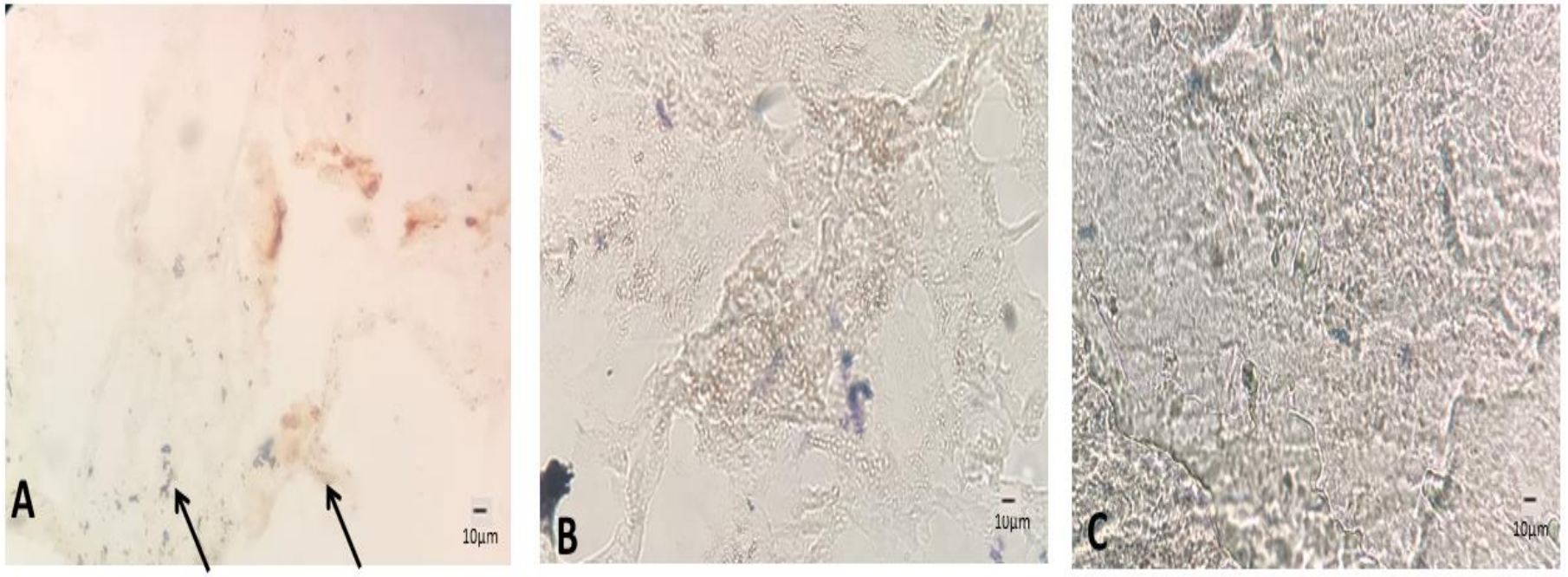
Negative control (brain, uninfected mouse)

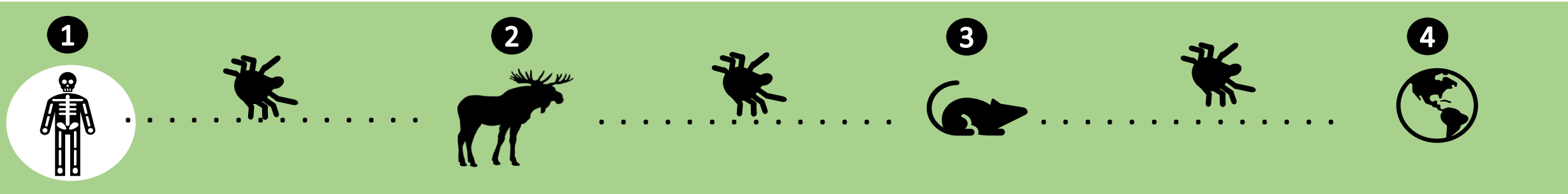


Results - Immunohistology

Donor 1

- Immunohistology on left internal thoracic artery showed immune-positive structures
- Pericardium and aortic valve tissues did not show immune-positive structures
- The immuno reactive structures showed a variety of forms, but not the typical spirochetal form





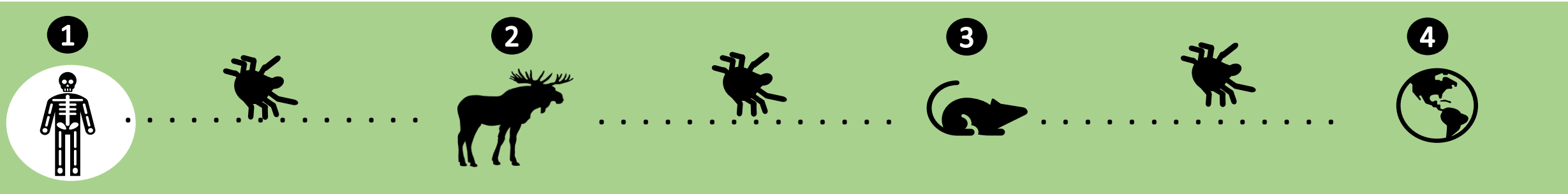
Conclusions:

Donor 1

- Borrelia was detected by nPCR, FISH and protein was detected by immunohistology in biopsied thoracic artery tissue. It was not detected in the other cardiac tissues.
- *Borrelia burgdorferi* DNA present only at low abundance in connective tissue
- These results validate the clinical diagnosis of Lyme disease in this individual, US serology and tick exposure
- Limited detection is consistent with aggressive treatment, although Borrelia DNA was detected. Viability cannot be assessed by these methods.
- Round body more common than long/spriocheatal forms
- The individual is still well, active and healthy

Donor 2

- Abundant Borrelia was detected by FISH in the pericardium
- Other tissues still to be tested
- These results are consistent with tick exposure and US WB but not Canadian serology
- Round body more common than long/spriocheatal forms
- Involvement of Borrelia infection in donor 2's heart failure is an important question for the family and for all individuals living in endemic areas



Significance

- This study demonstrates that *Borrelia* DNA can be detected in human tissues using molecular methods

- This approach offers the opportunity to address research questions such as *Borrelia* tissue distribution, amount and correlation with tissue damage