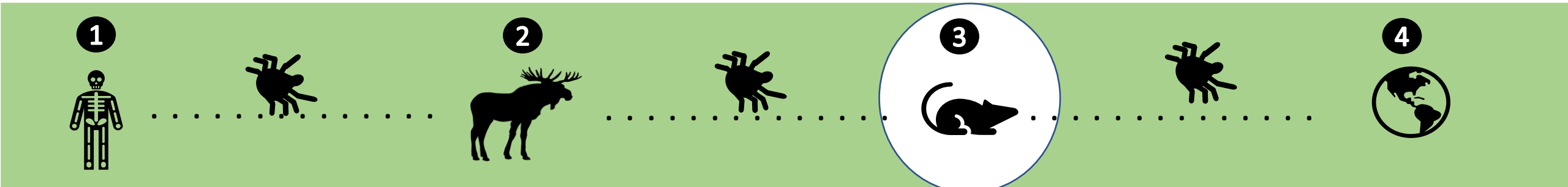
Wildlife *Borrelia* infection in Atlantic Canada:

Assessing the prevalence of *Borrelia* in wildlife hosts

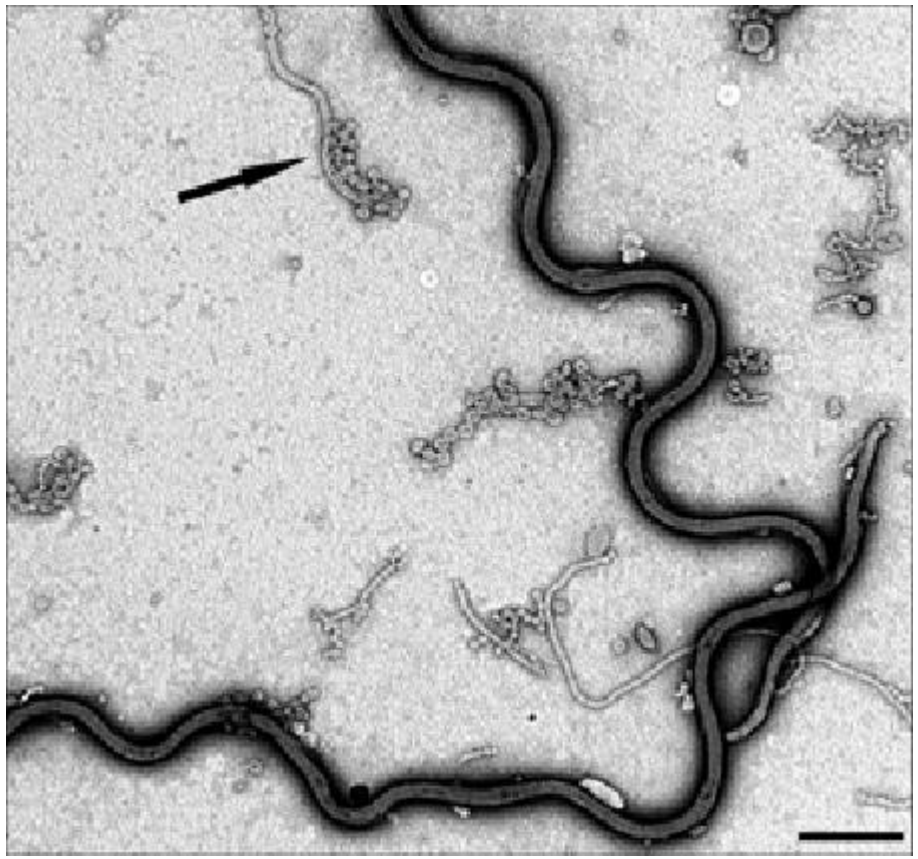
Christopher Zinck

Mount Allison University
Lyme Research Network

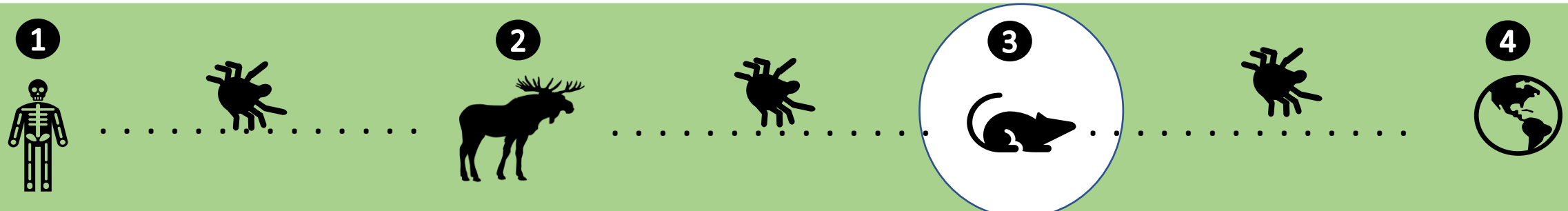


Background - Borrelia

- Two focal species
  - *Borrelia burgdorferi*
    - Zoonotic
    - Lyme Borrelia group
    - Vectored by Ixodid ticks
    - Discovered in 1981
  - *Borrelia miyamotoi*
    - Relapsing Fever group
    - One of few RF Borrelia vectored by Ixodid ticks
    - Discovered in 1995 in Japan
      - First recognised human case: Russia 2011
      - First recognised NA human case: 2013
    - Previously misidentified as *B. burgdorferi*

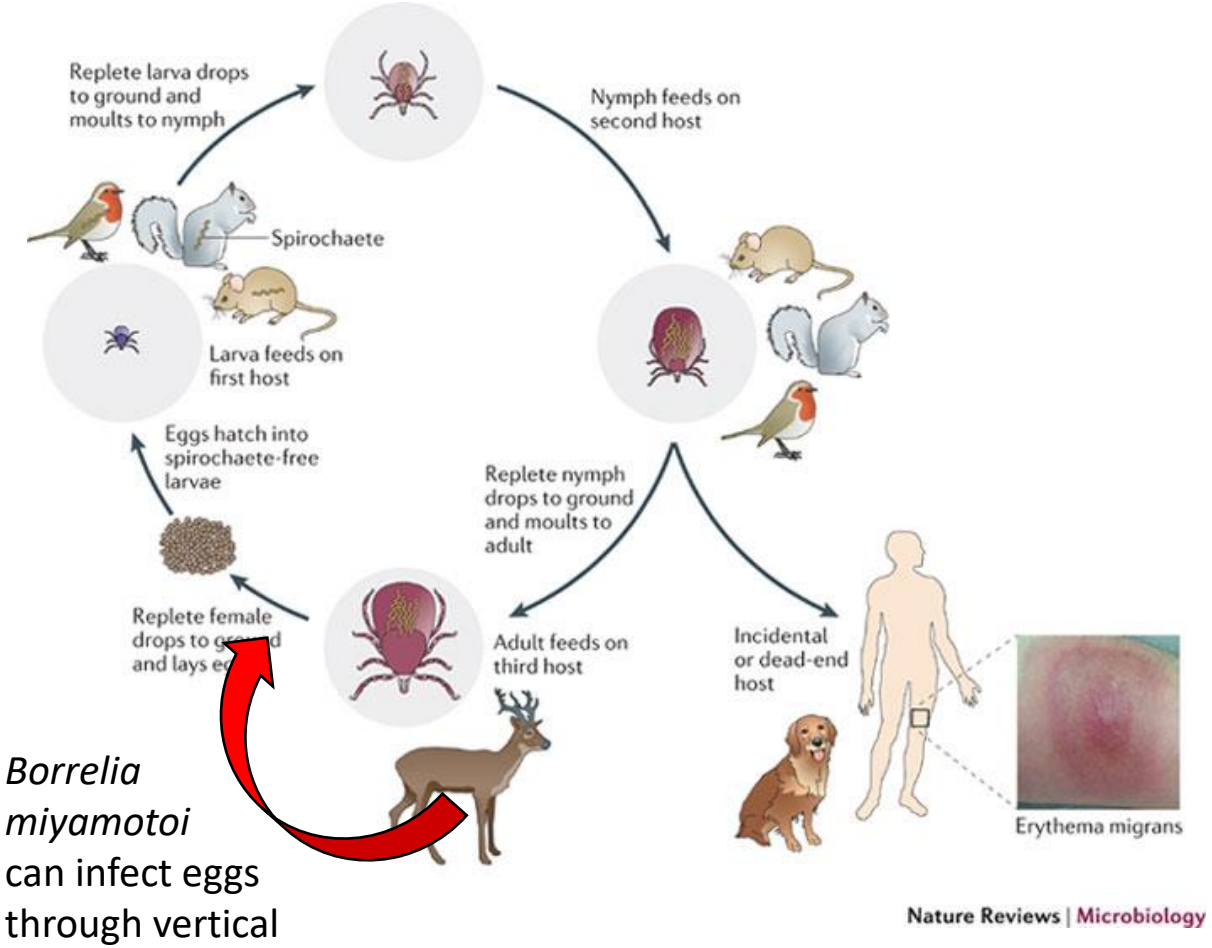


B. burgdorferi spirochetes, Grubhoffer et al. 2005

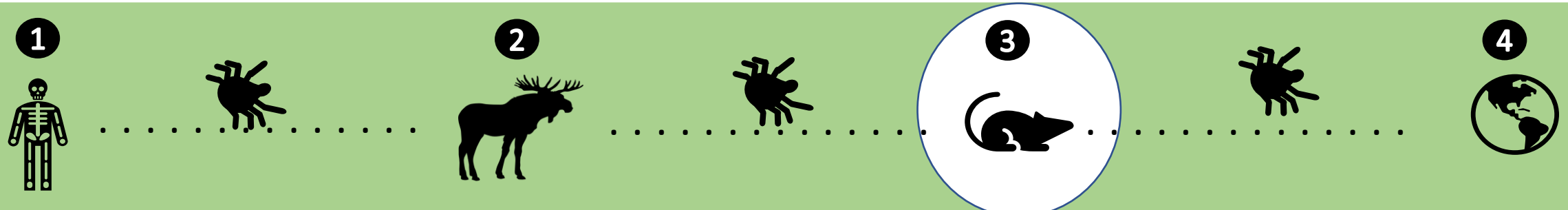


Background - Transmission

- Vector – Host transmission cycle
- Some wildlife species can be “reservoirs”
  - White-footed mouse, Eastern Grey Squirrel
  - Carry and transmit the infection readily
  - *Borrelia* is maintained and spread by tick and animal movement
- *B. burgdorferi* is not passed from adult ticks to eggs, *B. miyamotoi* can be
  - **<u>Vertical Transmission</u>**

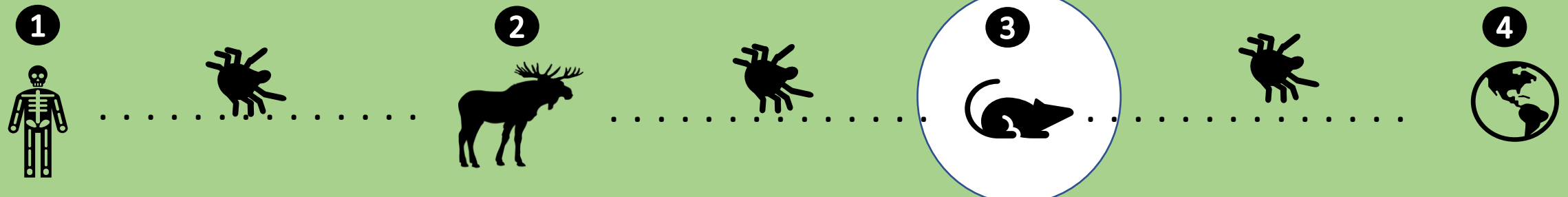


Borrelia miyamotoi can infect eggs through vertical transmission



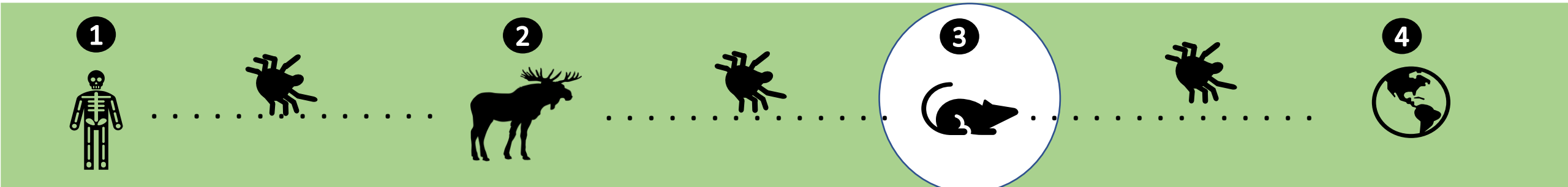
Objective 1 - Wildlife Surveillance

- In New Brunswick, no existing data on wildlife infection prevalence
- Many known reservoir competent species here, however their role (if any) in contributing to local *Borrelia* levels in ticks unknown
- As a first look, targeted small and medium animals to try and capture a diverse pool of species.
- Migratory birds, rodents, porcupines and rabbits etc. all included



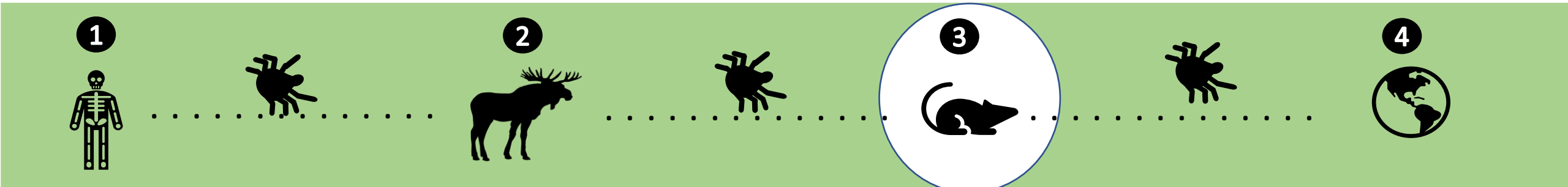
Methods

- Summer 2016 – roadkill collected for mid-sized animal samples
- 2016-2017 – deceased small animals (rodents, songbirds) collected by public donation
- Roadkill – bi-weekly, 5am start, June-August.
  - All specimens photographed, GPS coordinates recorded, assessed for condition, and then removed from the road
- Catkill – pet owners recorded date and address, and froze the specimens in sealed bags
  - All dissections done in lab, and whole specimens preserved for future use – Tissue bank
- Two tissues used in all downstream testing per animal
  - Liver and kidney preferentially targeted



Methods - nPCR

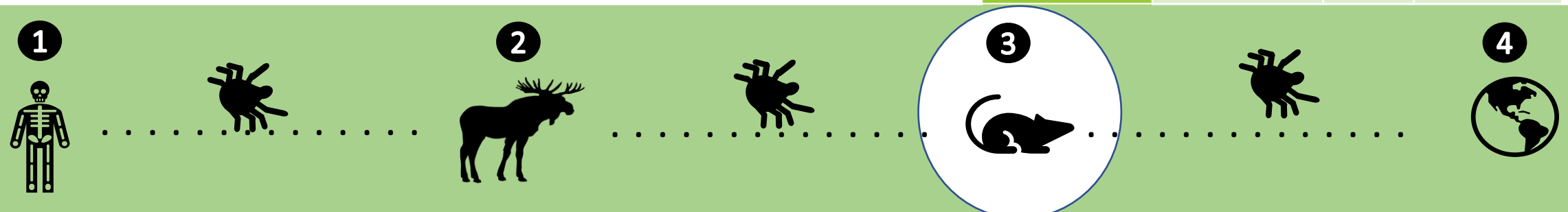
- 2016 surveillance standard, used with ticks
  - *OspA* and *FlaB:* NML tick testing procedure for *B. burgdorferi*
- *OspA* primers cross react with some mammalian species, porcupine, meadow vole etc.
- 16-23s IGS conserved region: a popular target for bacterial identification
- Dibernardo et al. 2014: Borrelia genus 23s primers
  - Validated in ticks, good starting point for wildlife
- Designed species specific inner primers
  - Tested for specificity and sensitivity



Prevalence

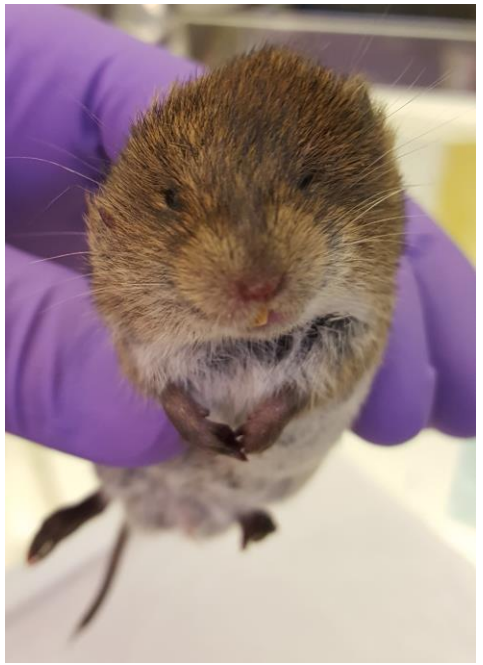
- 339 total animals tested, two tissues minimum per animal
- Twenty-nine tested species, highly variable amounts for each
- Jumping mouse highest *B. burgdorferi* and *B. miyamotoi* infections by percent (9.5% +/-6.4% & 14.3% +/-10.0%) excluding Eastern grey squirrel
  - 1 co-infection, also jumping mouse
- Meadow vole, shrew, eastern grey squirrel, deer mouse, known *B. burgdorferi* reservoirs in other regions
- *B. burgdorferi* more prevalent and in more species than *B. miyamotoi*
- *Spatial & statistical analyses performed, no significance*

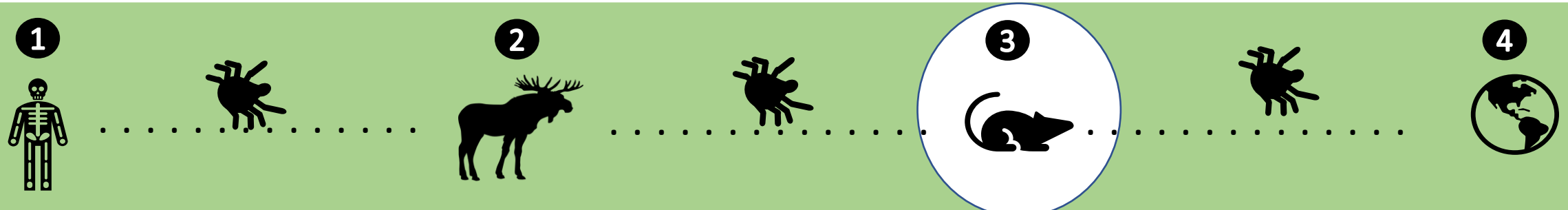
Species	Number sampled	B.burg	B. miya
meadow vole	146	4	1
deer mouse	34	2	1
Eastern grey squirrel	4	0	1
jumping mouse	21	2	3
Chipmunk	2	0	0
Bird	23	0	0
Shrew	28	2	0
Brown rat	9	0	0
short tailed weasel	1	0	0
raccoon	9	0	0
groundhog	4	0	0
snow shoe hare	5	0	0
Porcupine	21	2	0
American crow	11	1	0
Red fox	1	0	0
red squirrel	3	0	0
Muskrat	2	0	0



Making Predictions, Species Driven?

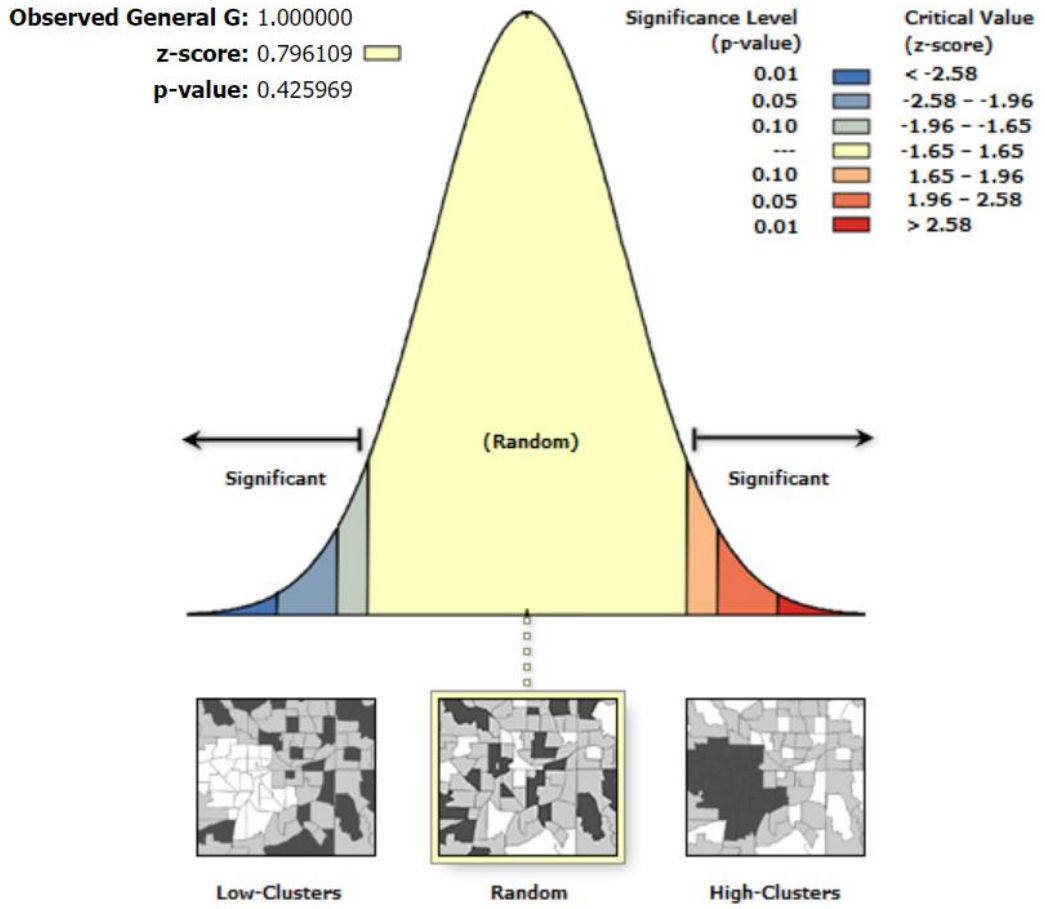
- If one species is significantly more likely to carry the infection, this can be used to predict the location of the bacteria based off that species
  - Done with logistic regression
- No significance found
  - Variation in sample size between species
  - No biologically relevant way to collapse them down to more even categories
  - *B. burgdorferi* and *B. miyamotoi* tested separately
    - No tests for coinfection due to no significance found at this level

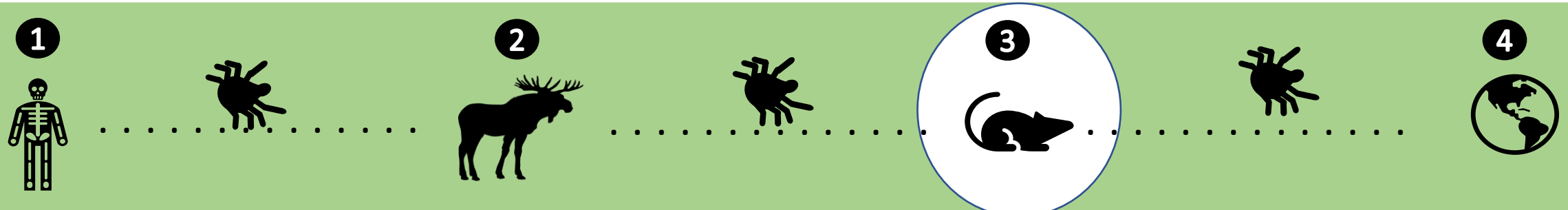


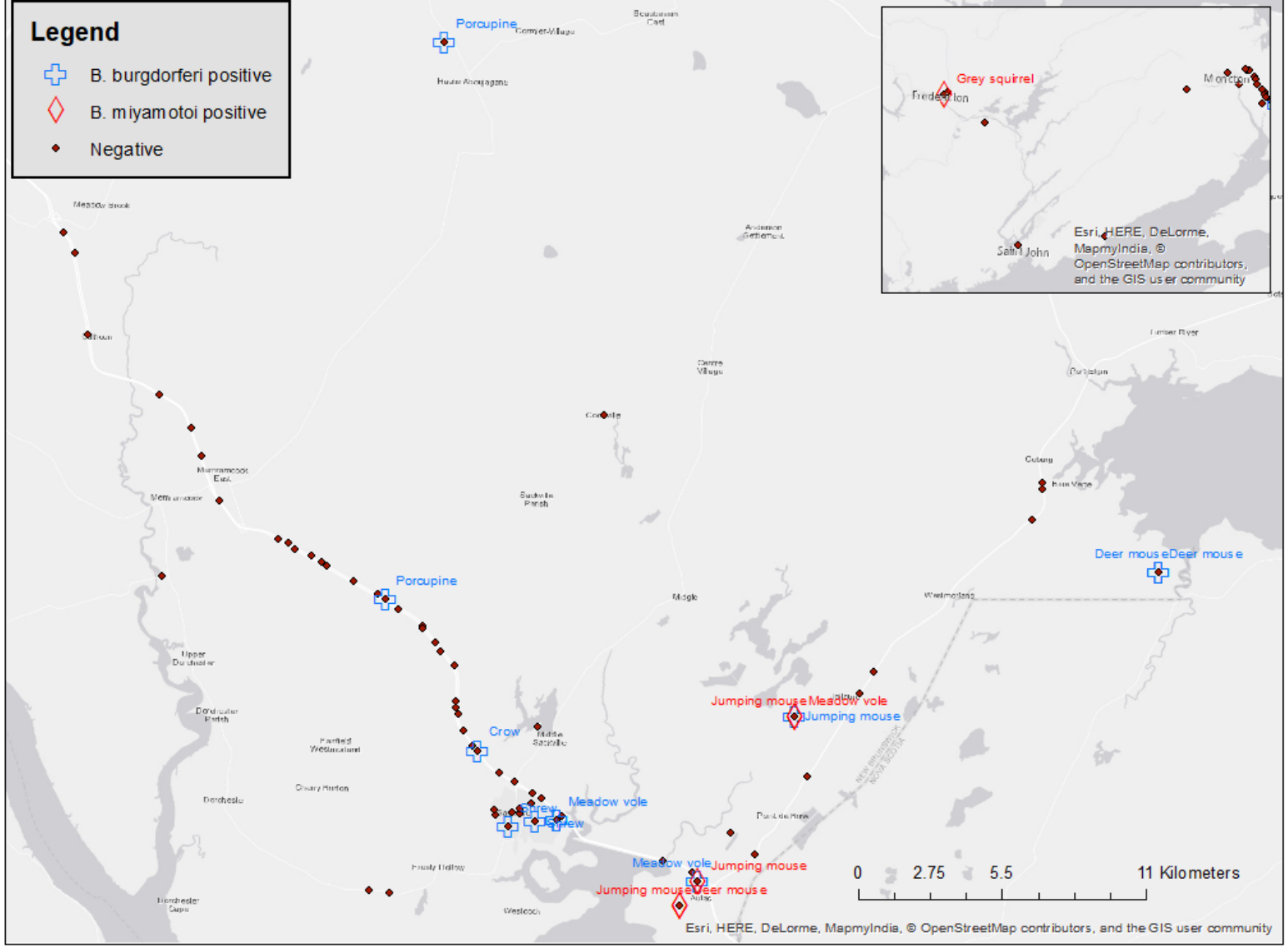


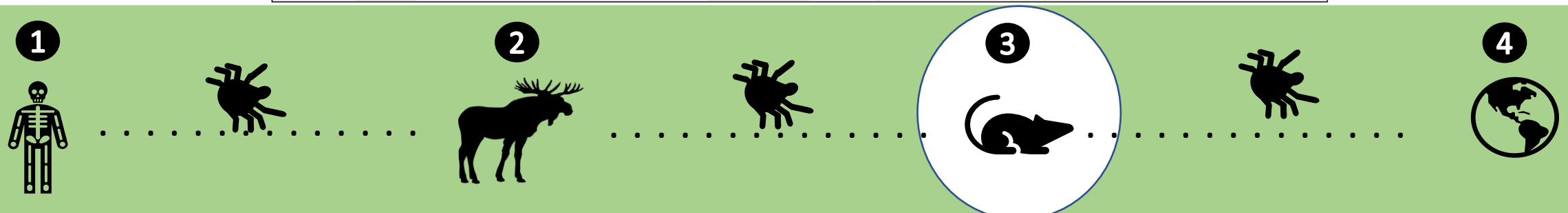
Making Predictions, Spatially Driven?

- Having no significant species means all samples can be used in spatial analysis
- Tested spatial autocorrelation, i.e. are positives more likely to be found together or not
  - Looks at whether “hotspots” occur
  - Done with ArcMap, Getis-Ord test
- Both *Borrelia* tested separately
  - No spatial trends found, random distribution
- Predictions can’t be made
- Species identified give targets for focused sampling





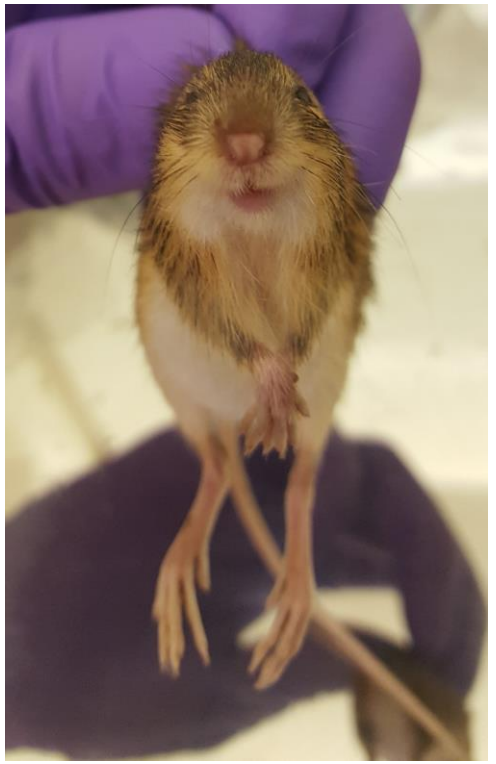

Legend
B. burgdorferi positive
B. miyamotoi positive
Negative
Porcupine
Grey squirrel
Fredericton
Moncton
Saint John
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community
Porcupine
Deer mouse
Deer mouse
Jumping mouse
Meadow vole
Jumping mouse
Crow
Meadow vole
Crow
Crow
Meadow vole
Jumping mouse
Jumping mouse
Deer mouse
0
2.75
5.5
11 Kilometers
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

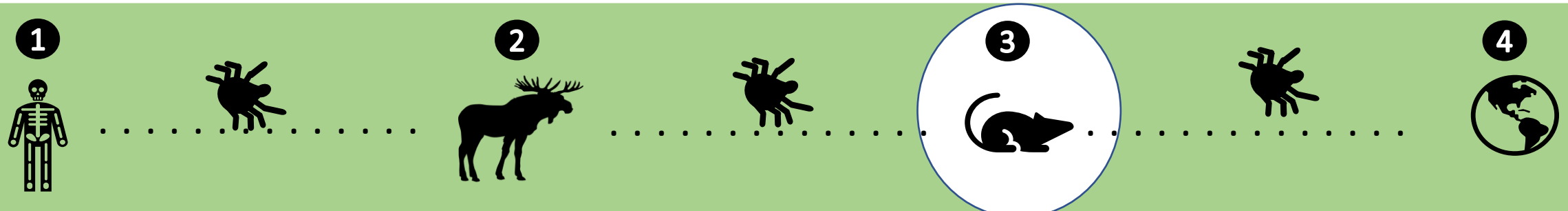

1
2
3
4

Objective 2 - Infection location in Animal

- 11 *B. burgdorferi ss.* Positives
  - 8 fully preserved for further testing
- 5 *B. miyamotoi* Positives
  - Fully preserved
- 1 Co-infection, fully preserved
- 18 species and location matched negatives*

- Tested:
  - Liver
  - Bladder
  - Kidney
  - Muscle
  - Skin
  - Brain
  - Lung
  - Spleen
  - Heart
  - Stomach wall
  - Large intestine
  - Uterine horn*
  - Fetuses*

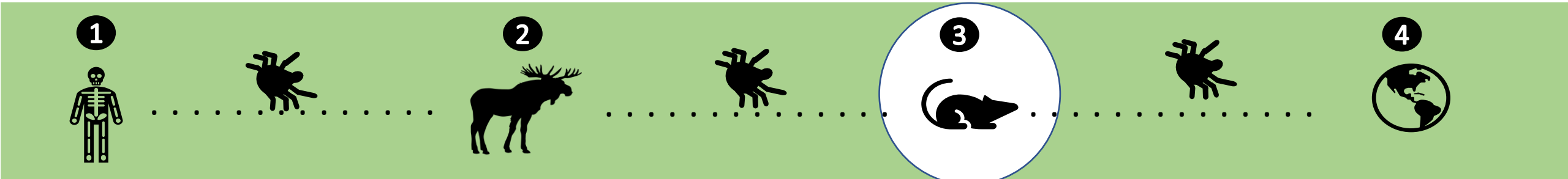




Results – Singly Infected

Sample	Brain	Liver	Kidney	Bladder	Lung
Jumping mouse	-	B	-	-	-
Meadow vole	-	B	-	-	-
Meadow vole	-	B	-	-	-
Meadow vole	-	B	-	-	-
Deer mouse	-	B	-	-	-
Deer mouse	-	B	-	n/a	-
Shrew	-	B	-	-	-
Shrew negative	B	-	-	-	-
Meadow vole	-	-	B	-	-
Meadow vole	M	M	-	M	M
Jumping mouse	-	M	-	-	-
Jumping mouse	-	-	M	-	-
Jumping mouse negative	M	-	-	-	-
Deer mouse	-	M	-	-	-
Grey squirrel	-	M	-	-	-

B = *B. burgdorferi*
M = *B. miyamotoi*

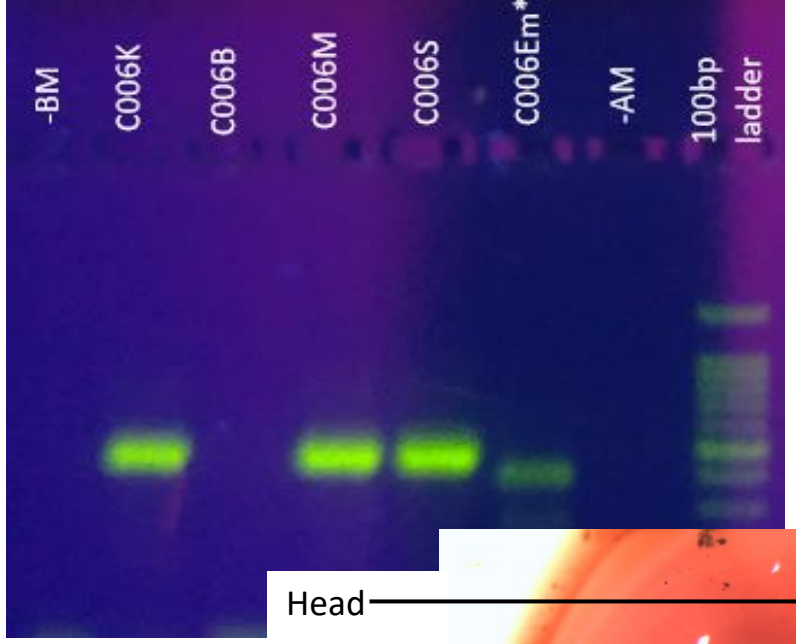


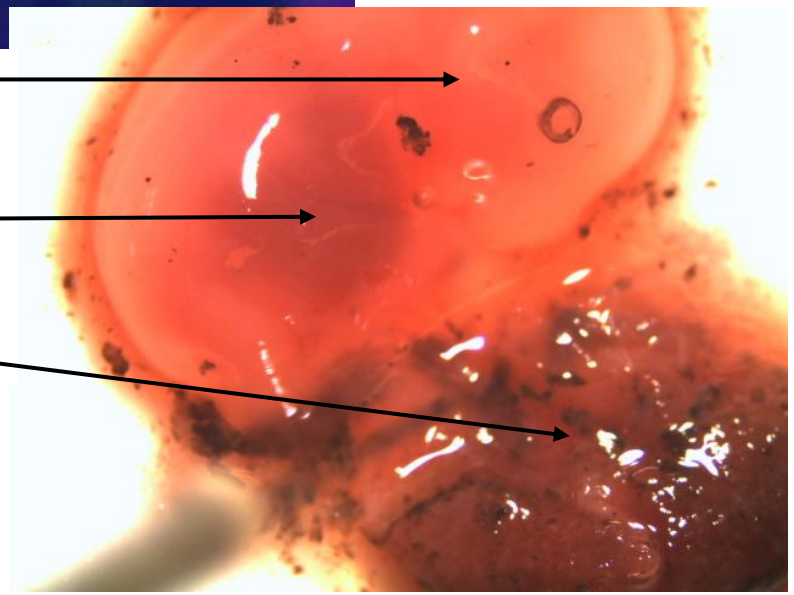
Results – Co-infection

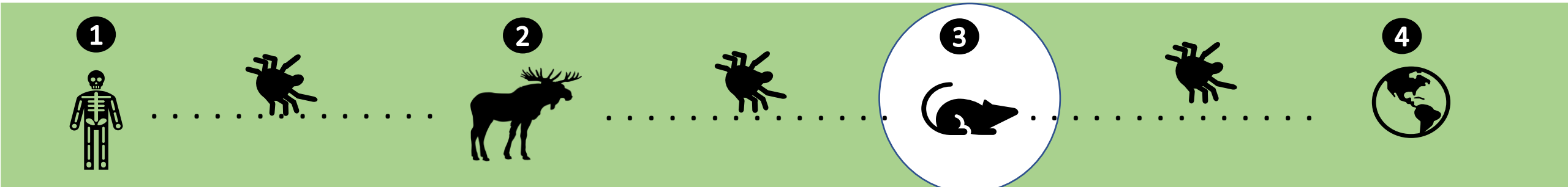
- Was a pregnant female Jumping mouse
  - Two fetuses were recovered by microdissection
  - Their head, gut, and placentae were tested separately

All tested samples, excluding bladder, stomach, and intestine were *B. miyamotoi* positive

Only the liver was *B. burgdorferi ss.* positive

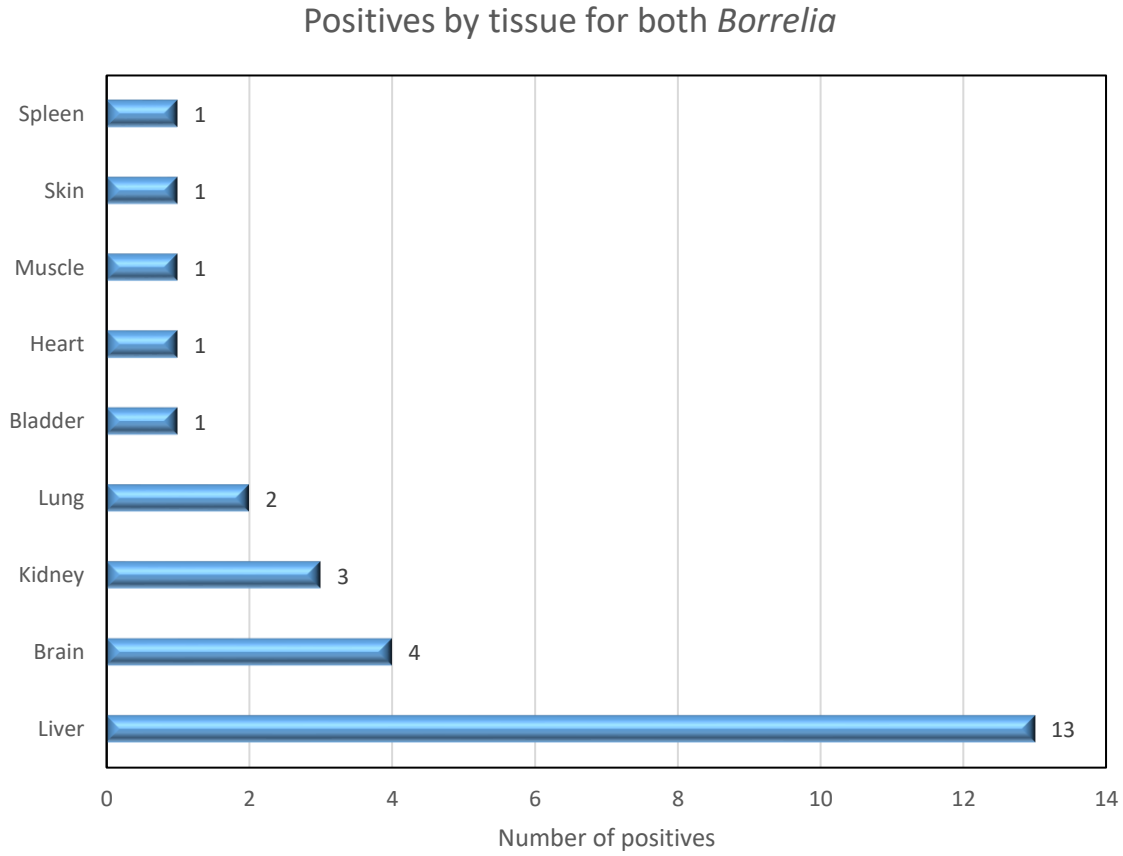


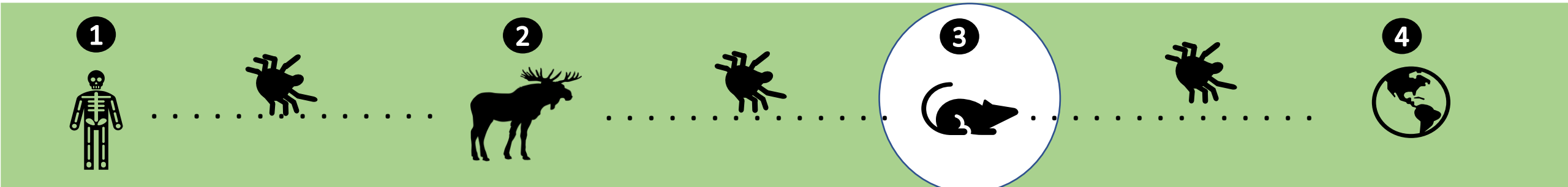




Conclusions - Testing

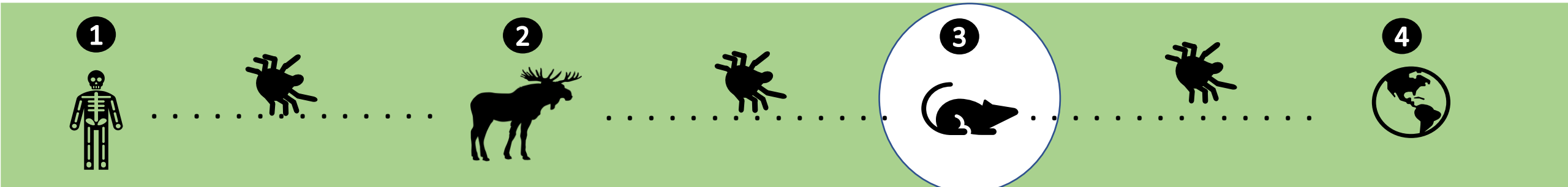
- nPCR detection means the DNA, and bacteria is present
  - Immuno-detection only confirms an infection at some point in time
- Liver best for detecting both *Borrelia*
  - Was a tissue selected for initial screening
- Brain second best tissue
  - Two new positives found due to it
  - Refuge tissue?





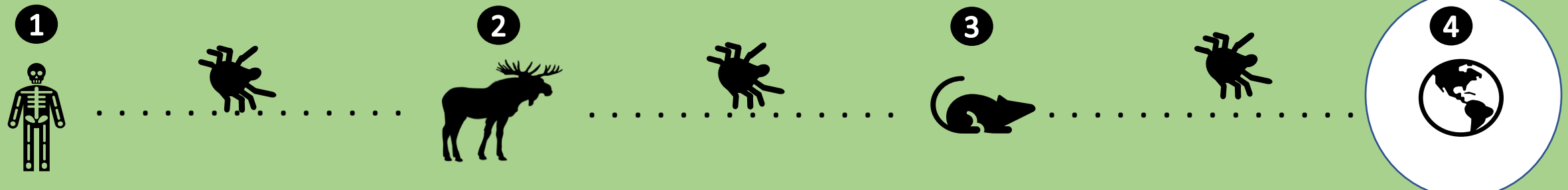
Summary

- Objective 1: Broad scale wildlife surveillance for *Borrelia* in New Brunswick
  - Developed and validated an nPCR protocol to test for *B. burgdorferi* and *B. miyamotoi*
  - Found *Borrelia* in multiple non-migratory, reservoir competent species
    - Jumping mouse, possibly an important species?
  - Wildlife levels reflect tick infection data, and are comparable to other NA endemic areas
- Objective 2: Investigation into the extent of infection within the positive animals
  - Liver the most commonly infected tissue
  - Brain the second most common, two previous negatives found to be positive
    - Possible refuge tissue? Under-representation of positives?
  - Jumping mouse fetuses: *B. miyamotoi* confirmed present
    - Vertical transmission?



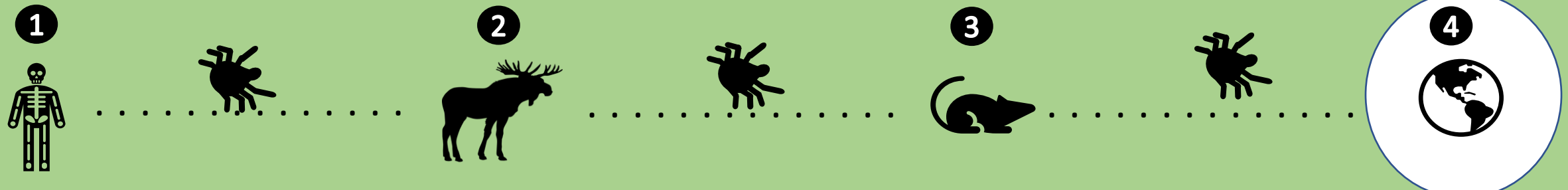
Conclusions

- Reservoir species are abundant
- There are more Borrelia out there than *B. burgdorferi*!
- Different *Borrelia* species show different tissue trophisms in the body
  – *B. miyamotoi* is much more widely dispersed in the body in **wild mice** than *B. burgdorferi*



Significance

- Leptospires are a somewhat basal spirochaete – shed in urine. Adaption to tick-vectoring is a Borrelia-specific innovation.
- Have these Borrelia spirochaetes lost the ability to invade and be shed in urine?
- If *B. burgdorferi* one of the most tick-specialized species, is it a good model for all borrelioses?
- Partnering with the community and patients leads to more powerful research
- The impact of borrelioses on individuals, their families and communities means that a more complete understanding of borrelioses is essential



Thanks to….

- The Lloyd Lab
- Veterinary clinics
- Lyme disease patients and their family
- Supportive physicians
- Citizen scientist tick and specimen donors
- Helpful cats
- Nanuq sequencing service

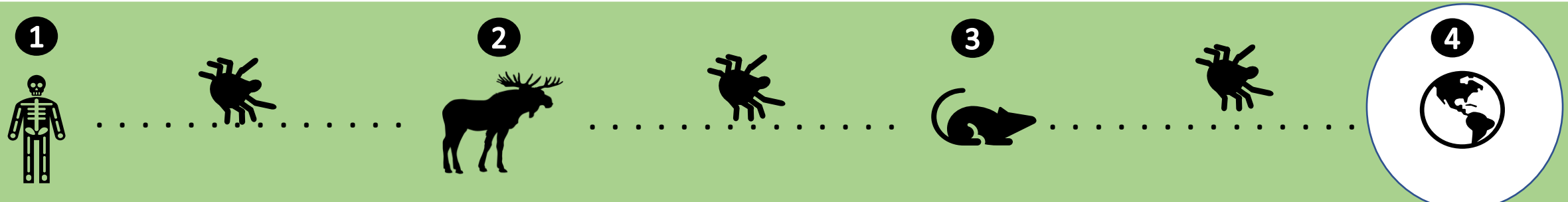
NSERC CRSNG

CanLyme
CANADIAN LYME DISEASE FOUNDATION

Mount Allison UNIVERSITY

LYME RESEARCH NETWORK

NBIF



Questions

Comments

Critiques

Speculation

Discussion