Epidemiology of Desert Tortoise
Project 567

Investigators: **Richard Tracy, Kenneth Hunter, Fran Sandmeier, Sally DuPre**

Others contributing (UNR): Hamid Mohammandpour, John Gray, Bridgette Hagerty, Rich Inman, Tiffany Sharpe, Stephanie Wakeling, Nichole Maloney, Amy Barber, Tracy Kipke, Simone Brito, Walker Johnson, Matt McMillan, Ryan Cody, Sonja Kokos, Kris Kenny, Scott Sheldon, Seth Cohen, Chris Ruiz, Rich Crawford, John Kraft, Mary Snow, Stanislay Cetkovshy, Damon Dunson, Kate Field, Talia Gebhard, Lauren Johnson, Holly Kaplan, Davi Leite, David Lin, Rachel Mank, Kamille Potter, Elizabeth Ray, Erika Saenger, Kristin Saletel, Aaron Switalki, Matt Davis, Lesley Hanson, Celesea Beebe, Chris Herbst, Suzanne Ankrum, Allison Peters, Deb Hill, Rob Vaghini, Emily Barks, Jenny Ingarra, Sarah Jones, Melissa Scheele, Rachel Zach, Amie Viniciguera

Others contributing (KIVA): Peter Woodman, Mary Ann Hasskamp, William Hasskamp, Bryan Reiley, Kip Kermoian, Patty Kermoian, Rachel Woodard, Brian Hasebe, Chandra Llewellyn, Charlie Jones, Lara McCluskey, Leslie Backus, Sheri Scouten, Laura Pavliscak, Chereka Keaton, Eli Bernstein, Danna Hinderle, Nancy Wiley, Daniel Kent, David Focardi, Cynthia Frman, Kelly Herberson, Las Holbek, Cheraka Keaton, Jessica Liberman, Michael Omama, Jacquelyn Smith, Jennifer Weidensee, Mary Weingarden

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Review

Upper respiratory tract disease (URTD) as a threat to desert tortoise populations: A reevaluation

Franziska C. Sandmeier [a,*], C. Richard Tracy [b], Sally duPré [c], Kenneth Hunter [c]

[a] Ecology, Evolution, and Conservation Biology Program MS 314, University of Nevada Reno, Reno, NV 89557, USA
[b] Department of Biology MS 315, University of Nevada Reno, Reno, NV 89557, USA
[c] Department of Microbiology and Immunology MS 199, University of Nevada School of Medicine, Applied Research Facility, University of Nevada Reno, Reno, NV 89557, USA

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ABSTRACT

The relationships between *Mycoplasma agassizii*, a causative agent of upper respiratory disease (URTD), and desert tortoise (*Gopherus agassizii*), generally illustrate the complexities of disease dynamics in wild vertebrate populations. In this review, we summarize current understanding of URTD in Mojave desert tortoise populations, we illustrate how inadequate knowledge of tortoise immune systems may obfuscate assessment of disease, and we suggest approaches to future management of URTD in desert tortoise populations. We challenge the view that *M. agassizii* causes consistent levels of morbidity and/or mortality across the Mojave desert. Instead, URTD may be described more accurately as a context-dependent disease. In addition, new evidence for relatively high levels of natural antibodies to *M. agassizii* in desert tortoises suggests possible problems in conventional diagnostic tests of disease in tortoises as well as a possible tortoise immune mechanism to protect against *M. agassizii*. Partly because of the problems in diagnostic testing, we recommend abandoning policies to euthanize tortoises that test positive for an immune response to *M. agassizii*. Based on this review, we question management strategies aimed solely at reducing *Mycoplasma* spp. in desert tortoise populations, and advocate a more careful consideration of extrinsic factors as a cause of symptomatic disease.

Is *Mycoplasma agassizii* killing desert tortoises?

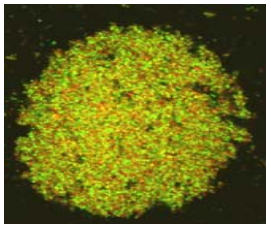
Goals of the Project

- Develop methods for characterizing immune system health of the desert tortoise
- Develop desert tortoise-specific immunological reagents, including a new polyclonal ELISA and Western blot for measuring the antibody response to *M. agassizii*
- Use these new techniques to analyze blood collected from desert tortoises across their Mojave desert range

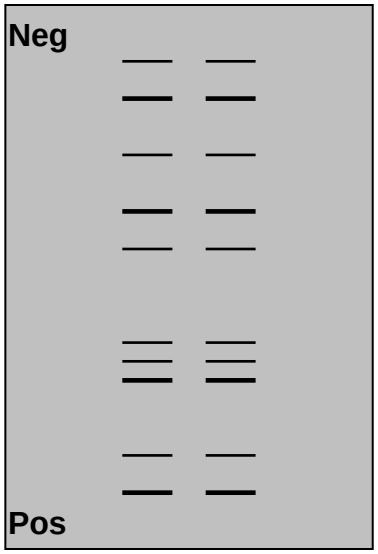
Western Blot Procedure

SDS-PAGE

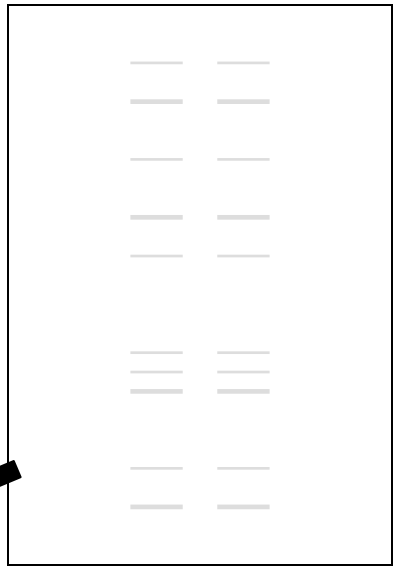
Mycoplasma agassizii

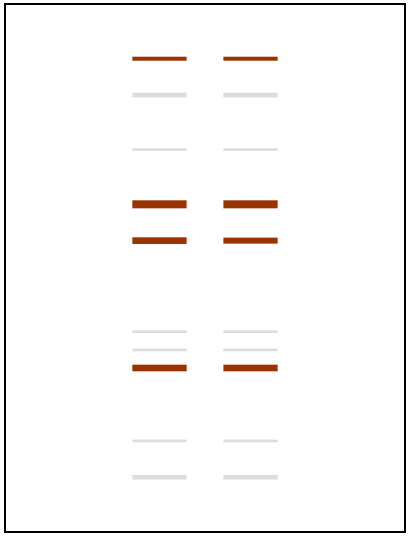


SDS, Δ



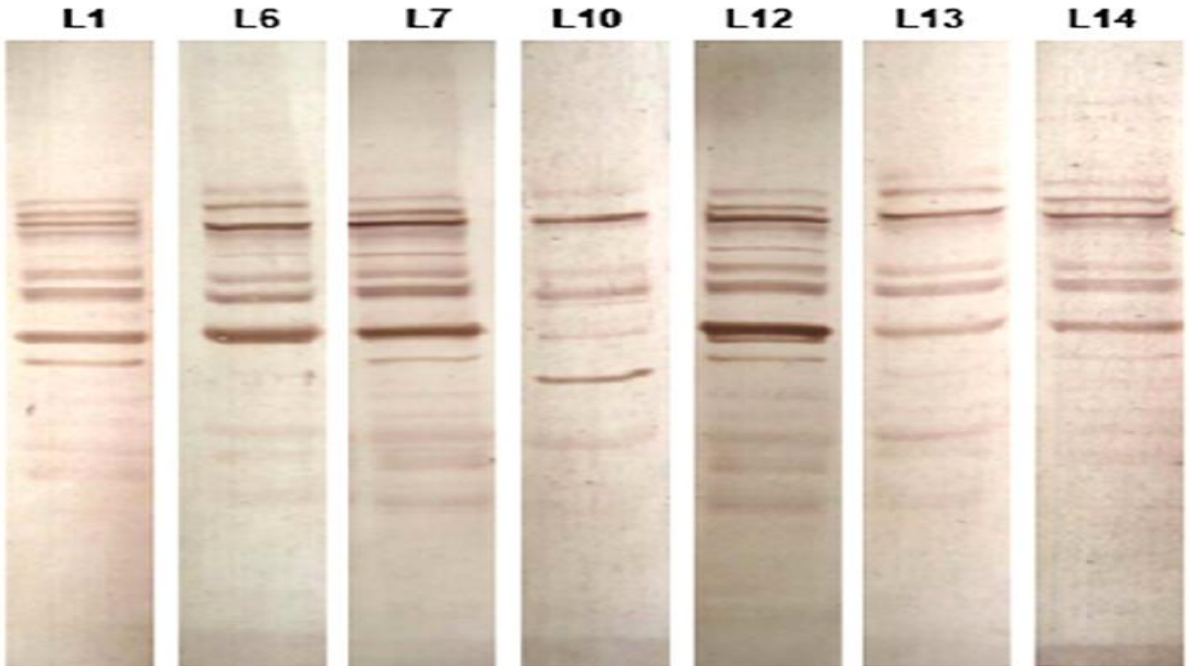
Western Blot



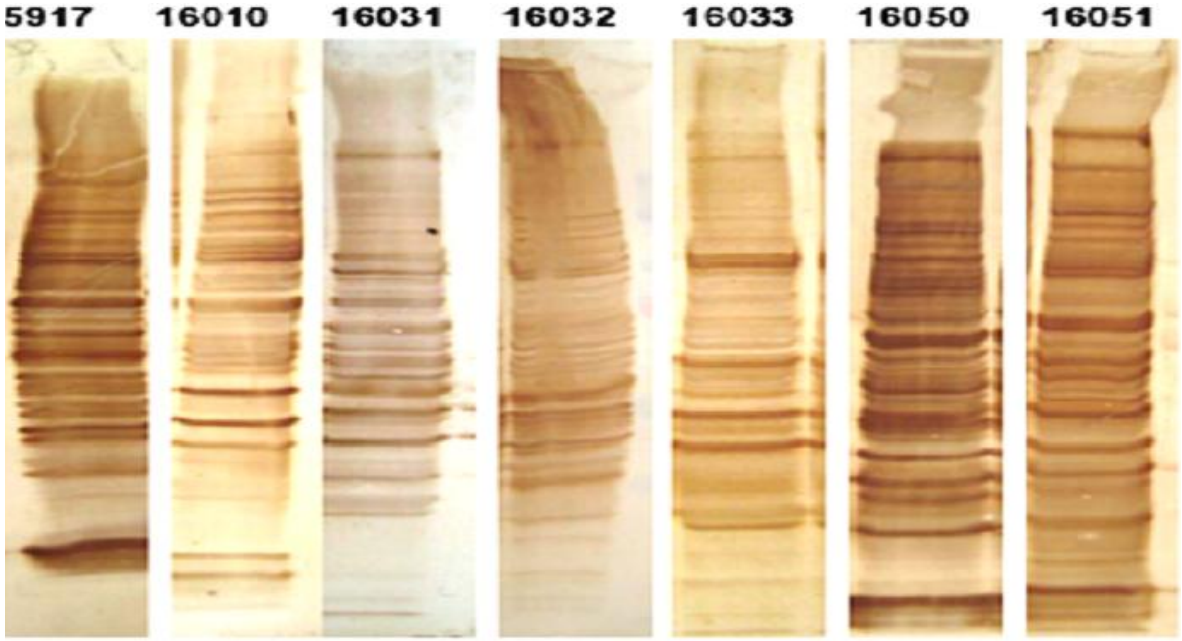


1. **Tortoise Serum**
2. **Rabbit anti-Tortoise Ig**
3. **Goat anti-Rabbit Ig Peroxidase**
4. **TMB Substrate**

Natural Antibody Western Blot Pattern



Acquired Antibody Western Blot Pattern



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Western blot can distinguish natural and acquired antibodies to *Mycoplasma agassizii* in the desert tortoise (*Gopherus agassizii*)

Kenneth W. Hunter Jr. [a,*], Sally A. duPré [a], Tiffanny Sharp [b], Franziska C. Sandmeier [b], C. Richard Tracy [b]

[a] Department of Microbiology and Immunology, University of Nevada School of Medicine, Reno, NV 89557, United States
[b] Department of Biology, University of Nevada, Reno, Reno, NV 89557, United States

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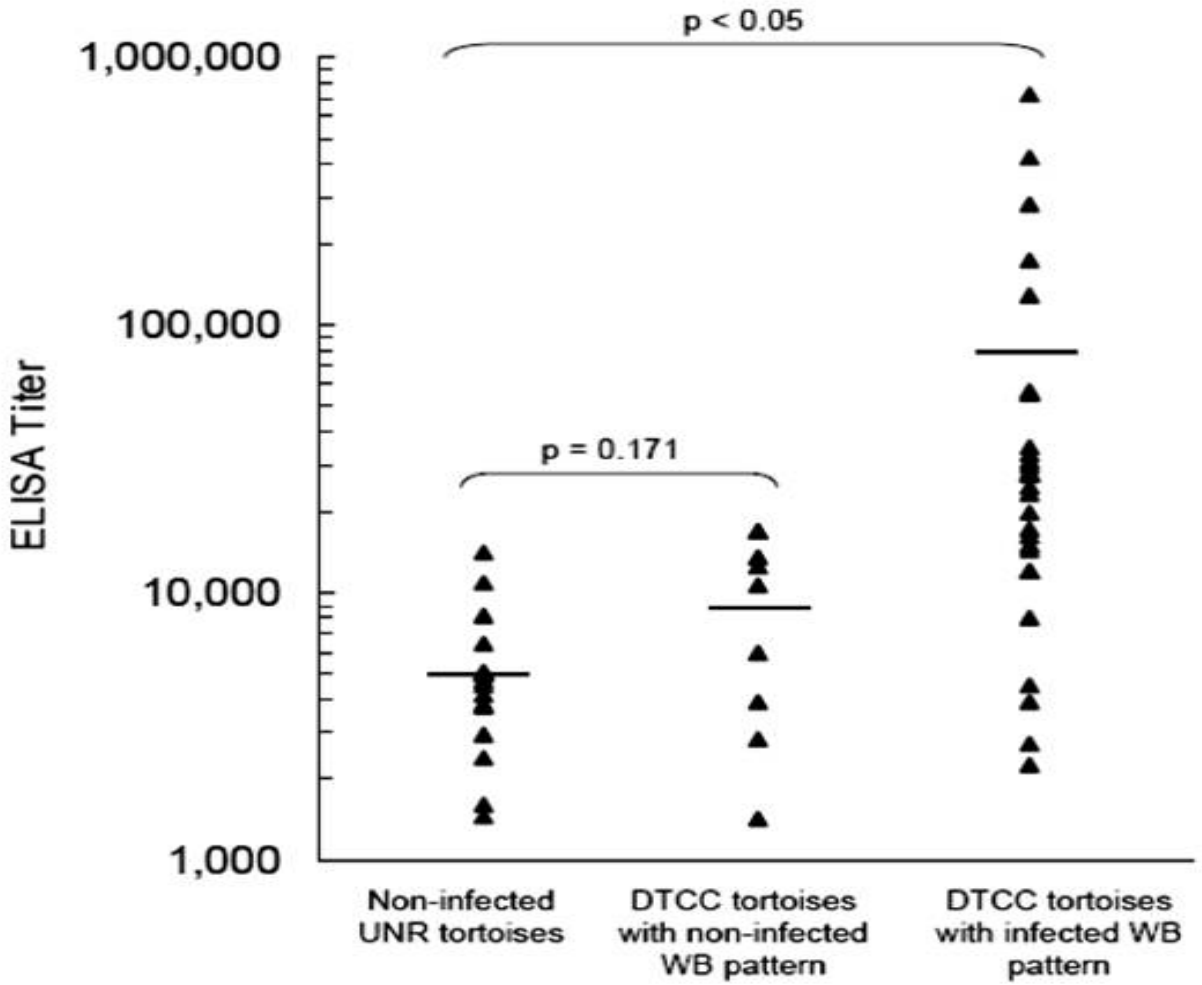
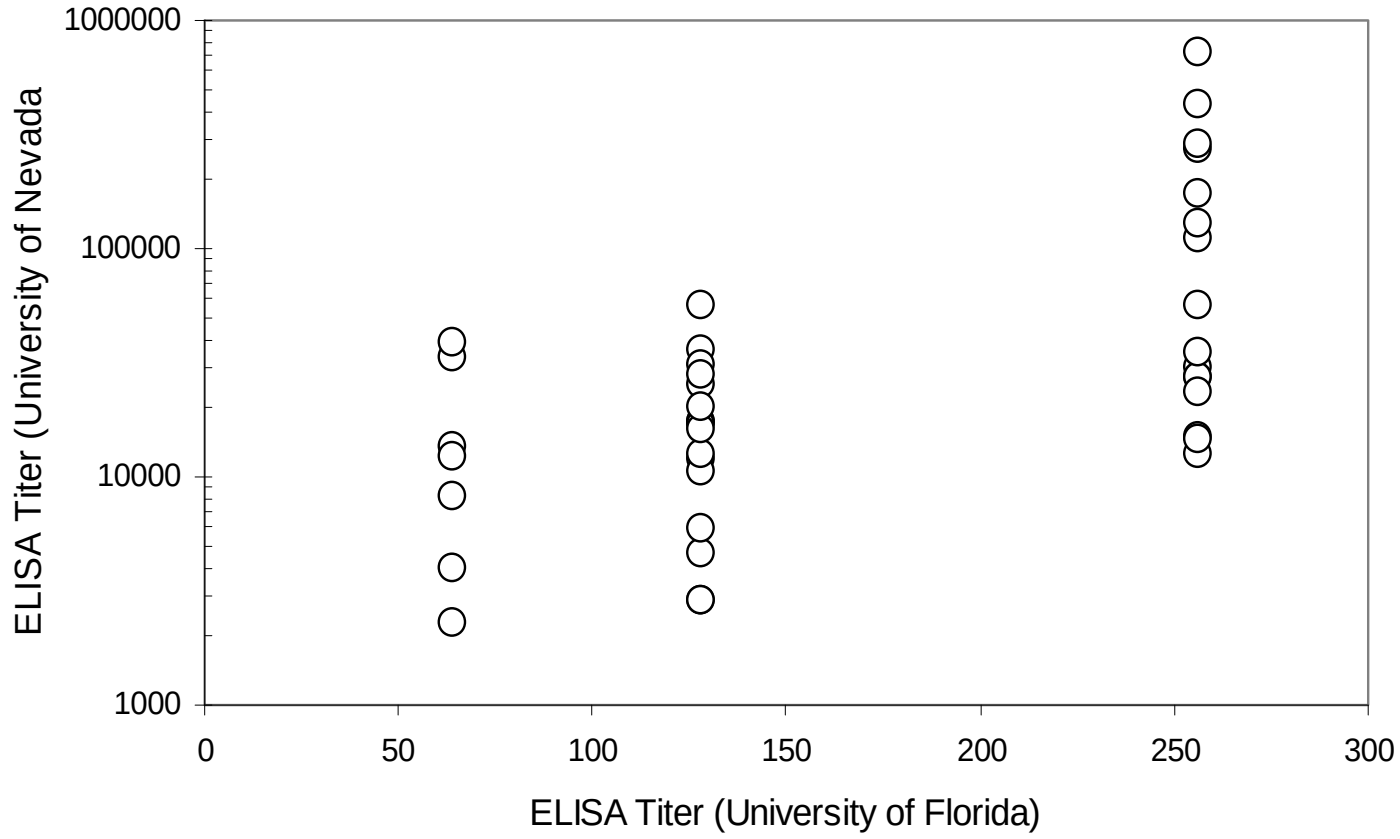
ABSTRACT

Mycoplasma agassizi has been identified as a cause of upper respiratory tract disease (URTD) in the threatened Mojave population of the desert tortoise (*Gopherus agassizii*), and anti-*M. agassizii* antibodies have been found by ELISA in as many as 15% of these animals across their geographic range. Here we report that a cohort of 16 egg-reared desert tortoises never exposed to *M. agassizii* had ELISA antibody titers to this organism that overlapped with titers obtained from some *M. agassizii*-infected tortoises. These natural antibodies were predominantly of the IgM class. Western blots of plasma from these non-infected tortoises produced a characteristic banding pattern against *M. agassizii* antigens. A group of 38 wild-caught desert tortoises was tested by ELISA, and although some of these tortoises had antibody titers significantly higher than the non-infected tortoises, there was considerable overlap at the lower titer levels. However, Western blot analysis revealed distinct banding patterns that could readily distinguish between the non-infected tortoises and tortoises with acquired antibodies, regardless of ELISA antibody titers. We conclude that desert tortoises have natural antibodies to *M. agassizii* that can compromise the determination of infection status by ELISA. However, the Western blot technique can distinguish between natural and acquired antibody patterns and can be used to confirm the diagnosis of *M. agassizii* infections in the desert tortoise.

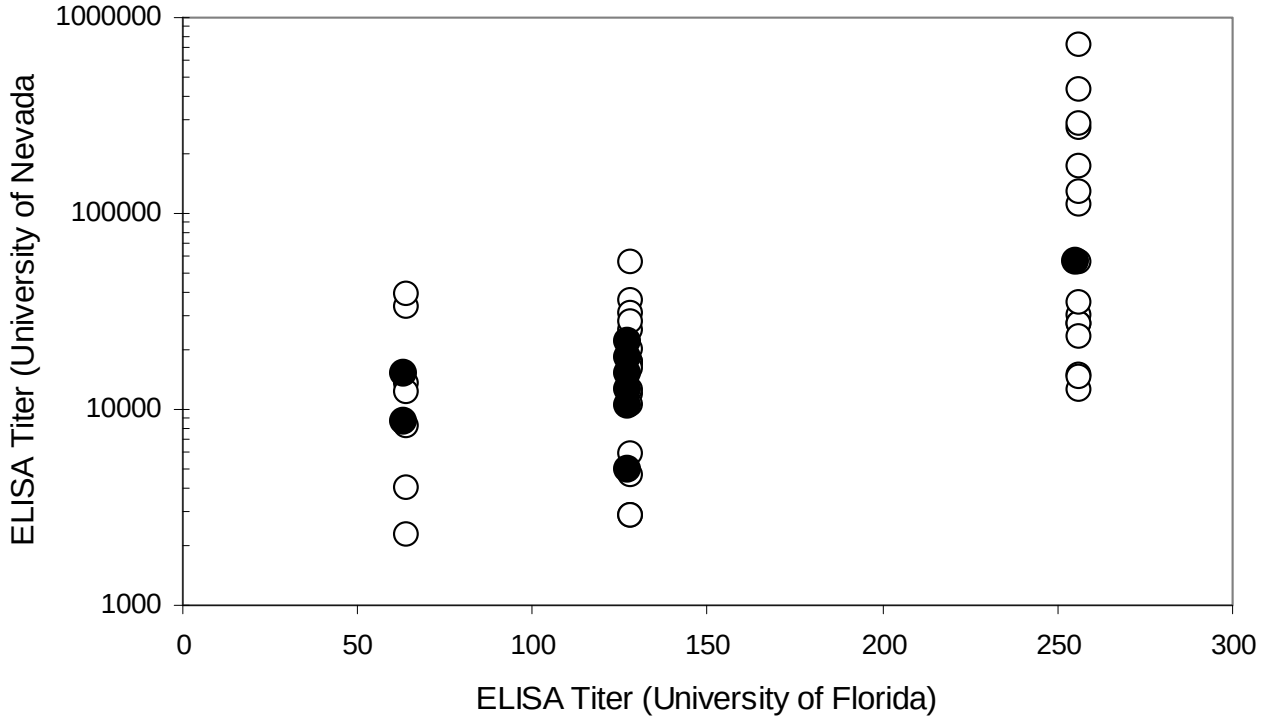
Natural Antibodies to *M. agassizii* Complicate the Interpretation of ELISA Results



Comparison of Monoclonal and Polyclonal ELISA Methods



High Levels of Natural Antibodies to *M. agassizii* Lead to a High False Positive Rate



28% of the tortoises in this study would have been have been incorrectly identified as *M. agassizii* positive and euthanized under the old management strategy

What Are the Implications of Our Findings for Desert Tortoise Management?

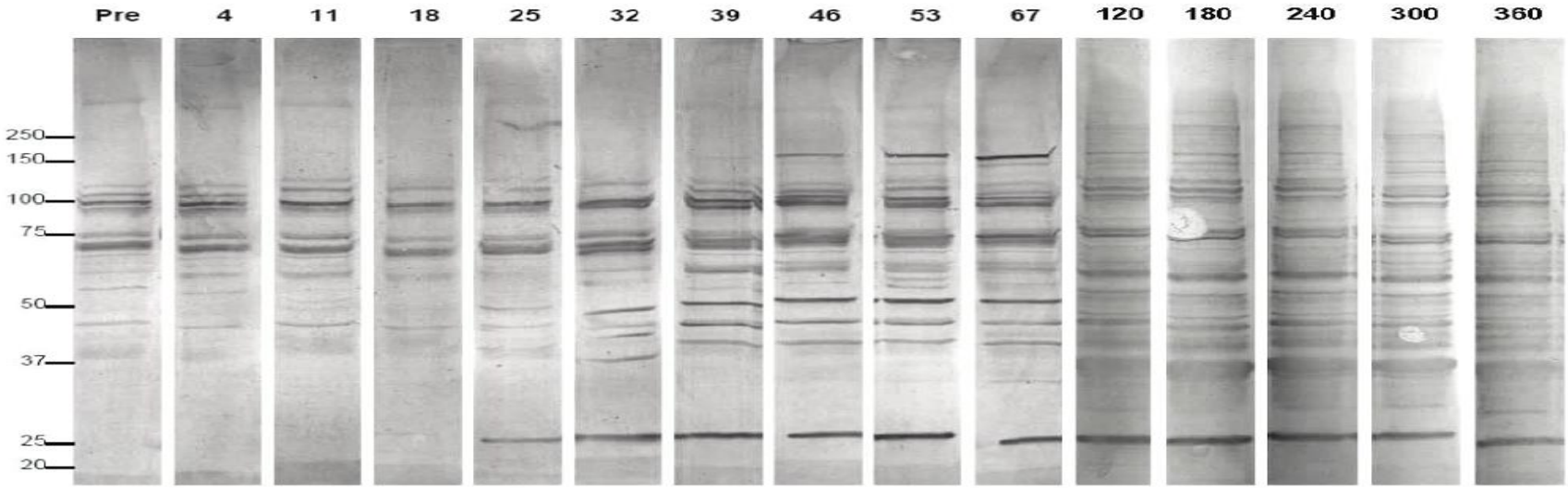
- ELISA cannot be used to unequivocally differentiate tortoises that have been exposed to *M. agassizii* from non-infected tortoises
- Western blot can better identify tortoises that have been exposed to *M. agassizii* and have made an adaptive immune response
- Neither ELISA nor Western blot can determine whether a tortoise is presently infected with *M. agassizii*

Laboratory Infection Study

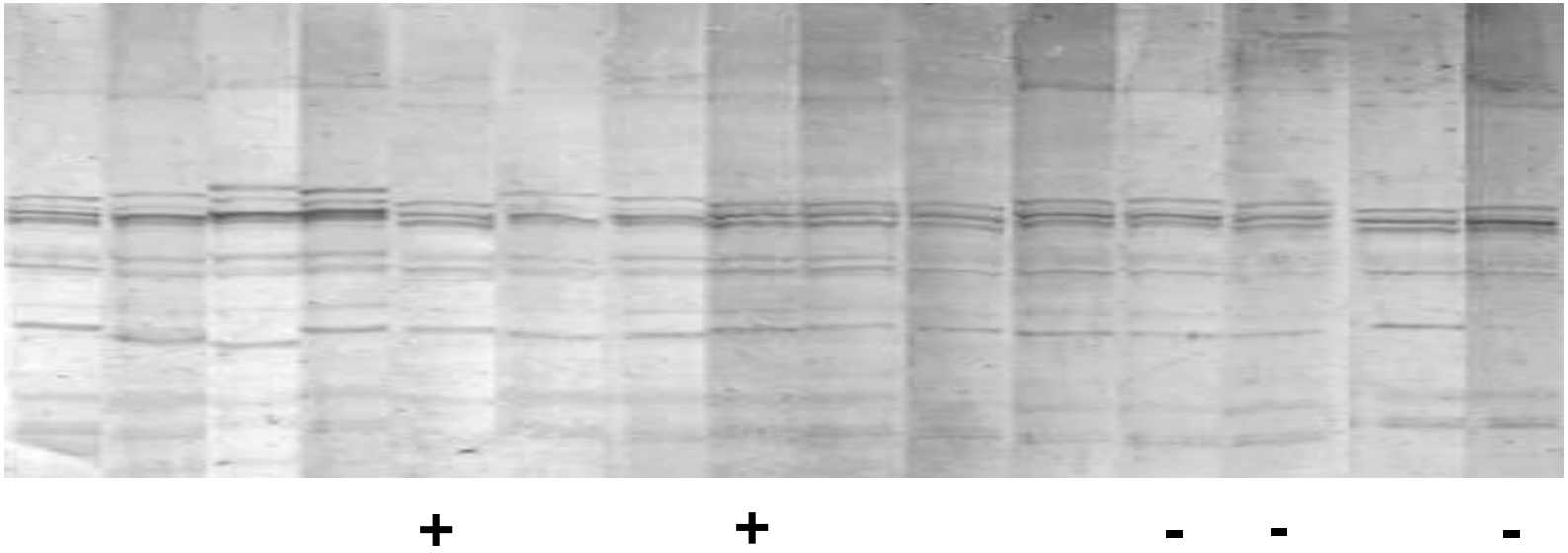
- Six healthy male and female desert tortoises were inoculated intranasally with 3.5 X 10^8 cells of the PS6 strain of *M. agassizii*
- Subcarapacial blood and nasal lavage fluid was collected periodically over the next year
- Plasma was analyzed for antibodies to *M. agassizii* by ELISA and Western blot
- Nasal lavage fluid was analyzed for *M. agassizii* DNA by quantitative polymerase chain reaction (qPCR)

Days After Intranasal Inoculation with *M. agassizii* Cells

Tortoise L26



Tortoise L1



FUTURE WORK

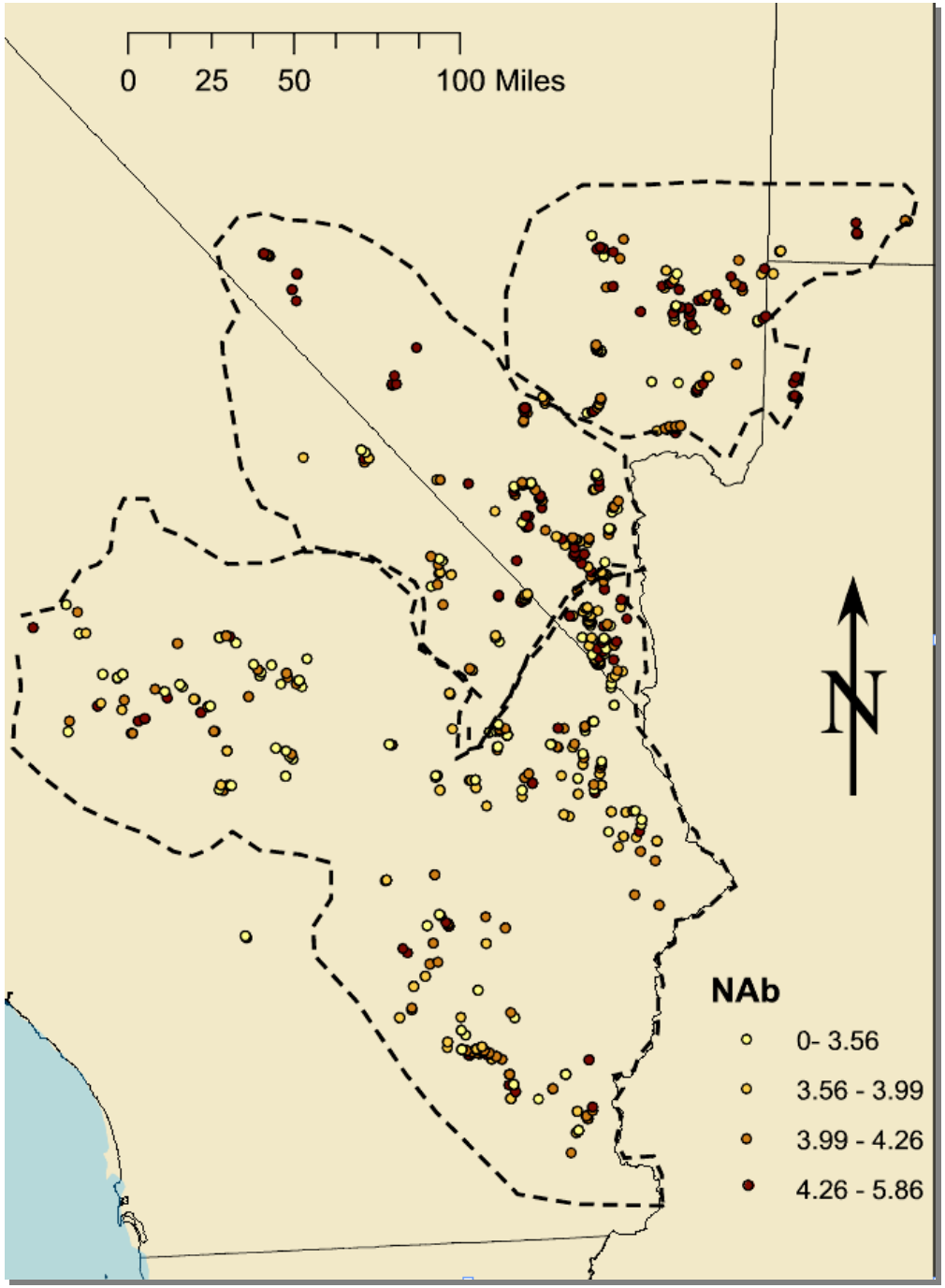
- It is clearly important to know how many desert tortoises in the Mojave desert harbor *M. agassizii* (or other URTD pathogens such as *P. testudinis*) in their upper respiratory tracts (e.g., colonization rate), and how many of these animals have serconverted following infection (e.g., infection rate).
- This information can only be gleaned from microbiological studies that identify the pathogens or their DNA in samples obtained from the upper respiratory tract of wild caught desert tortoises.

Immunity and Disease across Mojave

- NAb (natural antibody) titers of tortoises (ELISA of W. blot-negative tortoises)
- Induced antibody response (W. blot positive/negative)
    - positive = past exposure to *Mycoplasma agassizii* (*Mycoplasma* spp)
- across range/southern NV

Natural Antibody: Quartile Map

- NAb titers are lower in CA genetic cluster
- No difference between NAb: “Las Vegas” and “N. Mojave”



Natural Antibody of Tortoises in LSTS

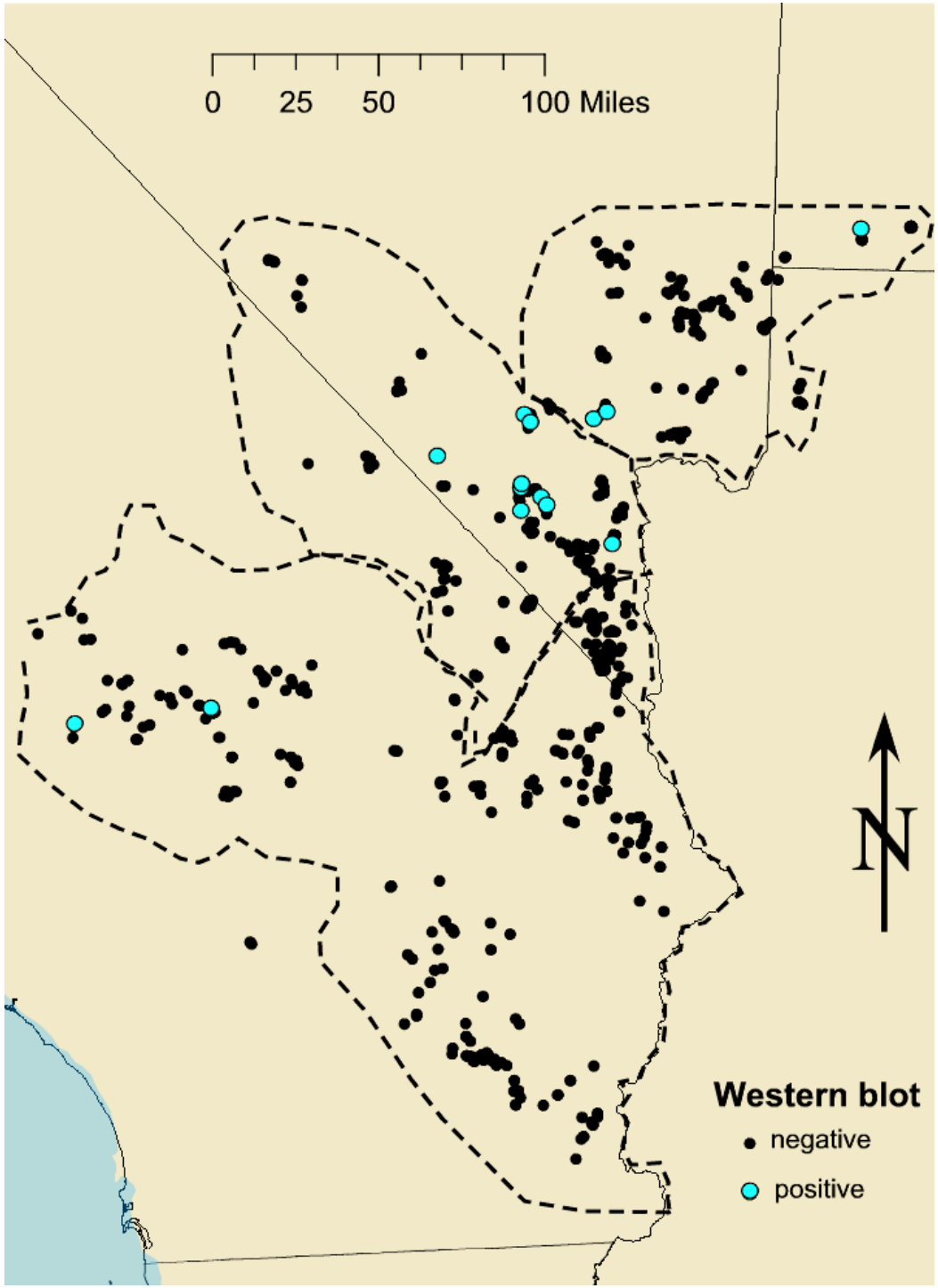
- Nab profile of LSTS population
  - resembles that of the CA cluster (not different $p > 0.5$)
  - Neither resembles that of the Las Vegas nor N. Mojave cluster (different: $p = 0.006$ and $p = 0.001$)

Implications: Range-wide Variation in NAb

- Management policies
  - measure NAb: Las Vegas and N. Mojave subpopulations!
- Possible variation in other immune parameters?
- Possible variation in disease dynamics?
  - endemism in subpopulations of tortoises (different NAb signatures)?

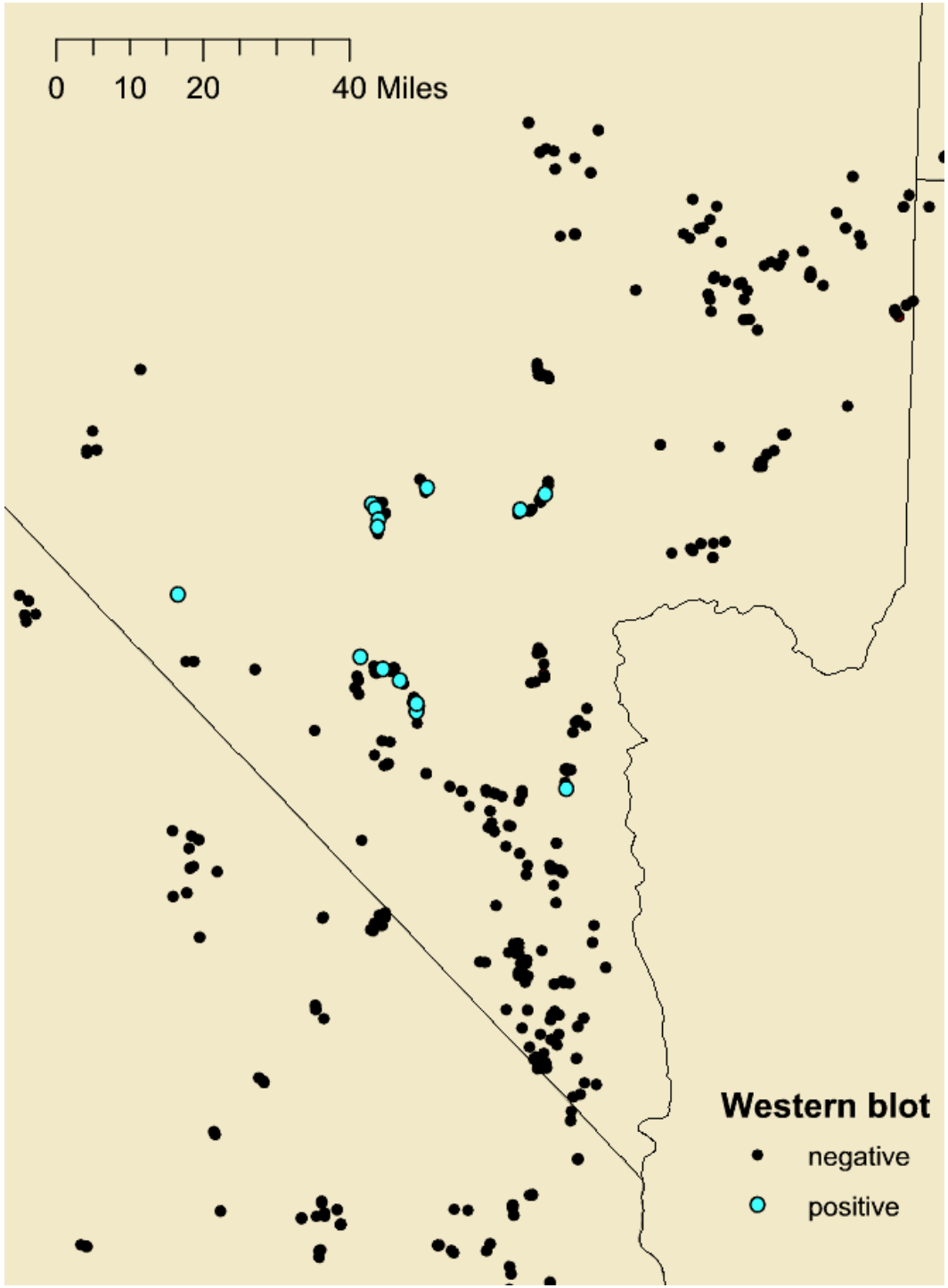
Mycoplasma exposure: Western blot

- More positive W. blots in Las Vegas and N. Mojave than in CA population ($p < 0.001$)



Exposure to *Mycoplasma*: Southern NV

- Western blot-positive tortoises:
  - 5/25 URTD
  - 3/25 exudate
- Western blot-negative tortoises:
  - 9/128 URTD
  - 4/128 exudate

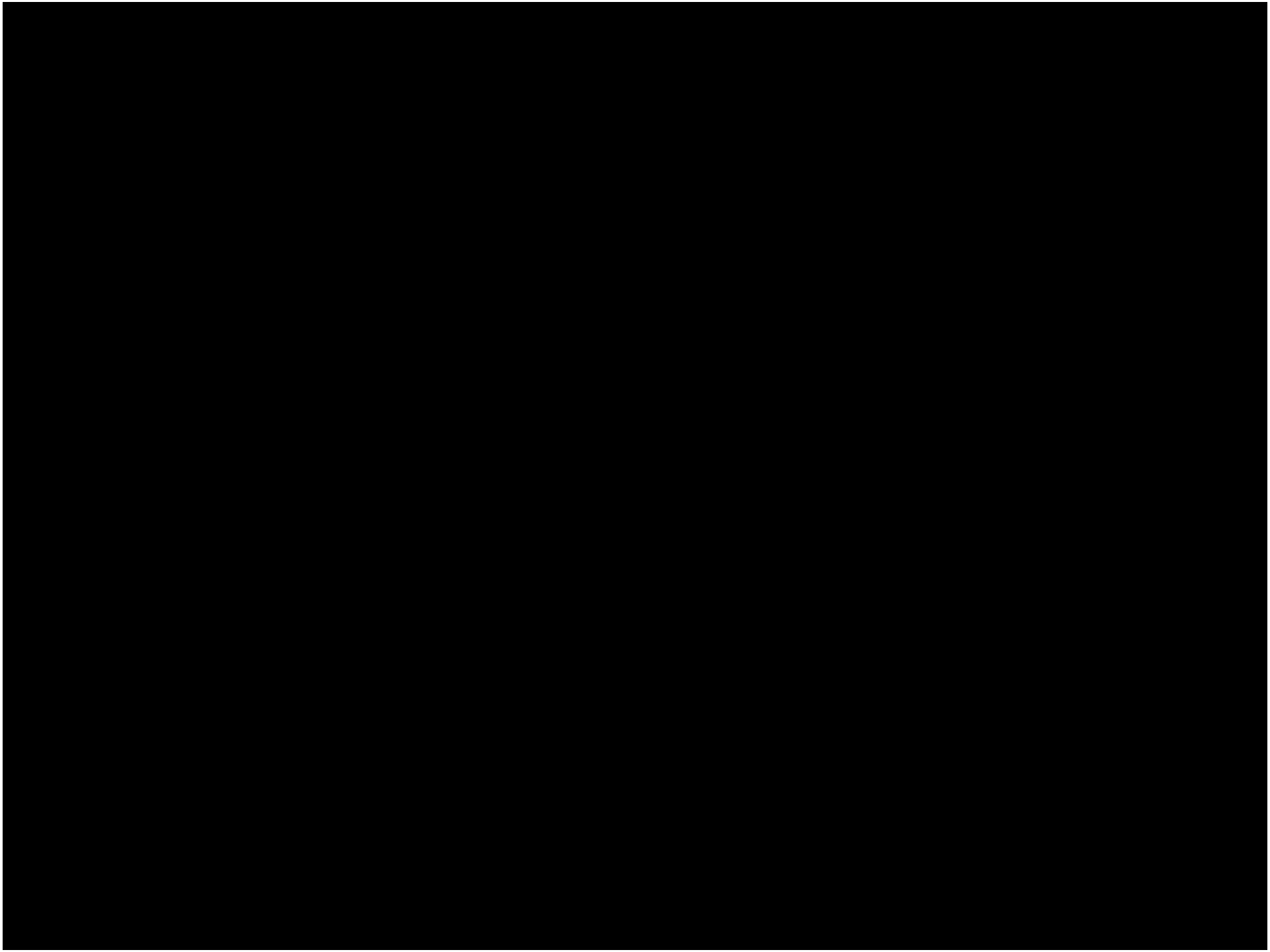


General Patterns

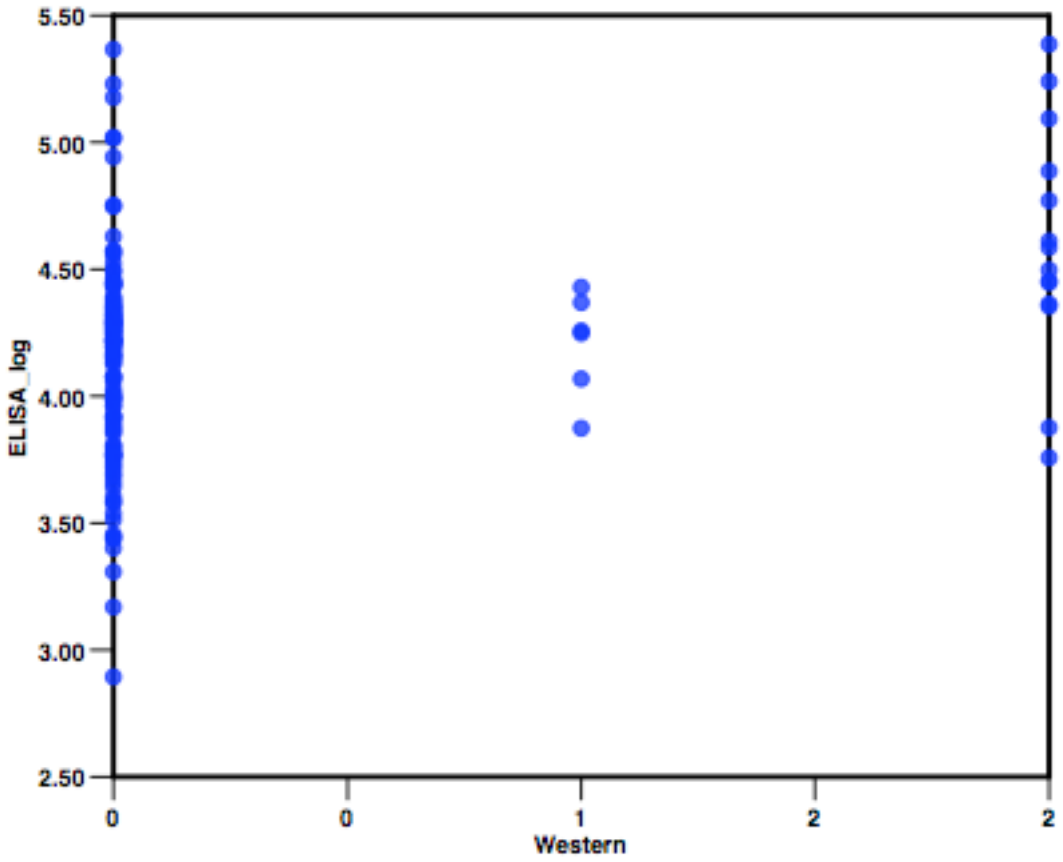
- Positive relationship between URTD and W. blot ($p = 0.025$)
- Symptoms not diagnostic of pos. W. blot
- Both NAb titers and exposure to *M. agassizii* are greater in the Las Vegas and N. Mojave populations

Research Recommendations: Respiratory Disease in Desert Tortoise

- Tortoise immunology
  - efficacy of natural/induced Ab
  - other immune mechanisms
  - interaction with habitat/climate/season
- Microbiology:
  - diversity of strains/spp of *Mycoplasma*/other pathogens
  - evolution of increased/decreased virulence
- Variation across Mojave



ELISA (polyclonal) vs. Western blot
Las Vegas Cluster



Exposure to *M. agassizii*

- Number Western-blot positive by genetic population

2D Contingency Table (Observed)		
Positive W. blot:	0	1
Cluster		
California	291	4
Las_Vegas	137	26
N_Mojave	107	35
$df = 2$	$X^2 = 59.9041$	
$X^2_{0.05} = 5.99146$ $X^2_{0.01} = 9.21034$ $p = < 0.001$		

Hypotheses

•Both NAb titers and exposure to *M. agassizii* are greater in the Las Vegas and N. Mojave populations

IF NAb are protective:

1. variability of NAb creates “reservoirs”

2. increased, variable resistance allows evolution of strains of moderate virulence

Co-occurrence incidental:

3. incidental & both affected by similar mechanisms

4. incidental & both affected by different mechanisms