Mycoplasmosis and URTD in Desert Tortoises

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Acknowledgements

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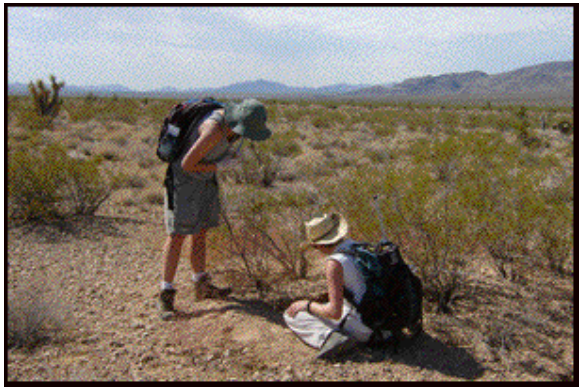
Outline

- Review
  - Sandmeier, FC, CR Tracy, S duPre, K. Hunter. 2009. Upper respiratory tract disease (URTD) as a threat to desert tortoise populations: A reevaluation. Biological Conservation 142:1255-1268.
- Range-wide blood samples (n~660)
  - Natural antibody levels (innate immune system)
  - Western blot to *M. agassizii* (pos/neg)



Review of Literature

- *Gopherus agassizii* (Mojave population)
  - URTD recognized as threat (FWS 1989, 1990, 1994)
- *Mycoplasma agassizii* (PS6)= causative agent
  - URTD & antibody production (Brown et al. 1994)
- *Gopherus* spp: additional causes of URTD
  - *Pasteurella testudinis*, herpes virus, an iridovirus

Hypotheses
(mutually exclusive)

- What is the effect of *Mycoplasma* spp in wild populations?
- 1.*Mycoplasma* spp have the intrinsic ability to cause consistently high rates of morbidity & mortality in desert tortoise populations
  - implicitly accepted in much of literature
- 2. *Mycoplasma* spp do not have the intrinsic ability to cause consistent rates of morbidity and mortality in desert tortoise populations.
  - need to measure factors besides *M. agassizii* to predict/assess affects of URTD

Hypothesis 2

- multiple mechanisms (NOT mutually exclusive):
- a. extrinsic factors influence *M. agassizii* pathogenicity/tortoise immune response
- b. strain variability in pathogenicity of *Mycoplasma* spp
- c. variability in immune resistance in tortoise populations

Importance?

- Hypothesis 1
  - over-simplified
  - implicitly accepted in some of literature, much of management
- Neither hypothesis fully supported by current data
- “Hypothesis 2”
  - multiple, testable hypotheses
  - partial support

Variation among Studies

- Desert tortoises: geographic (temporal?) variability in seroprevalence & prevalence of URTD (Lederle et al. 1997, Schumacher et al. 1997, Brown et al. 1999, Christopher et al. 2003, Dickenson et al. 2005, Berry et al. 2006)
- Gopher tortoises: geographic variability - *M. agassizii* exposure & declines (McCoy et al. 2007)

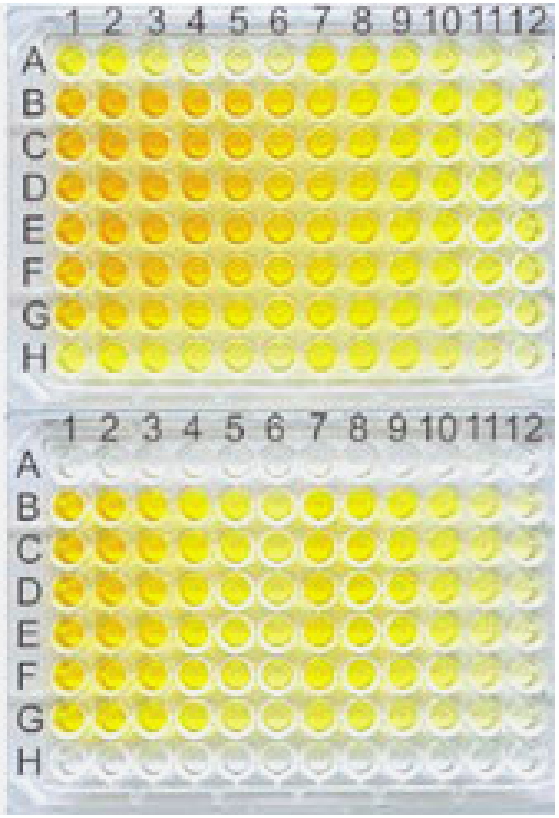
Unknown: Natural Populations

- Progression of *M. agassizii* infection/ antibody response
- Interaction among infection(s), nutritional status of tortoises (vegetation/rainfall), temperature, etc.
- Variation in strains of *Mycoplasma* spp
  - variability in virulence (Brown et al. 2002)
  - possible high rates of antigenic variation
- Variation in genetic resistance to URTD

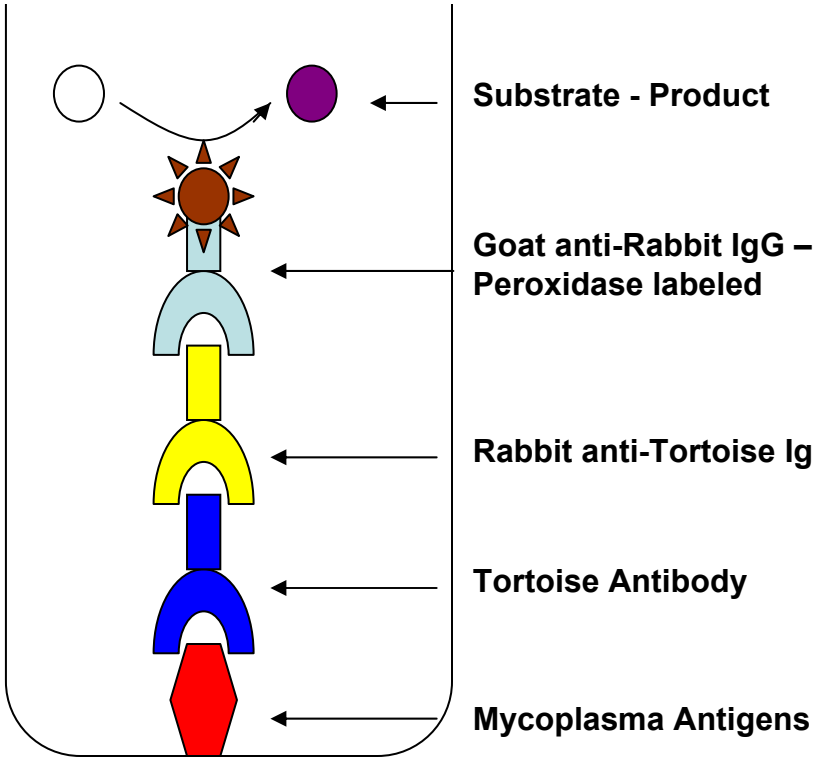
Range-wide Data

- Methods: ELISA & Western blot
- Natural antibodies: evidence & importance
- Range-wide data
  - natural antibody levels
  - exposure to *M. agassizii*

ELISA Method

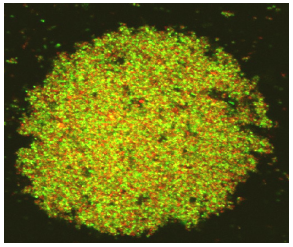


- measures levels of antibody



Western Blot Procedure

Mycoplasma agassizii



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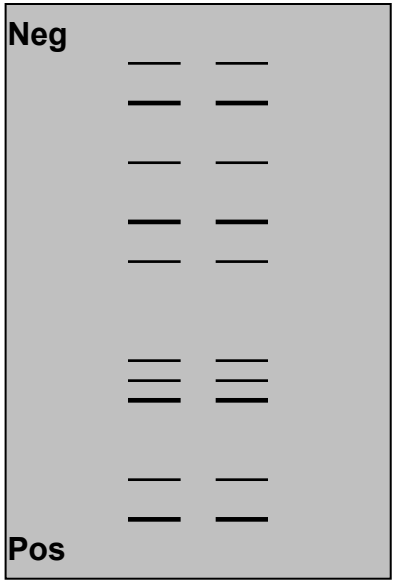
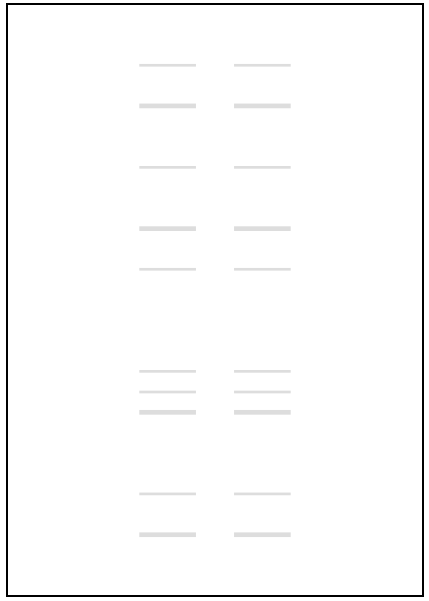
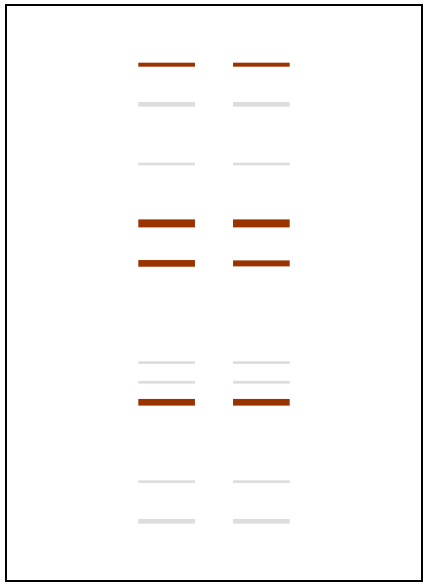


Western Blot

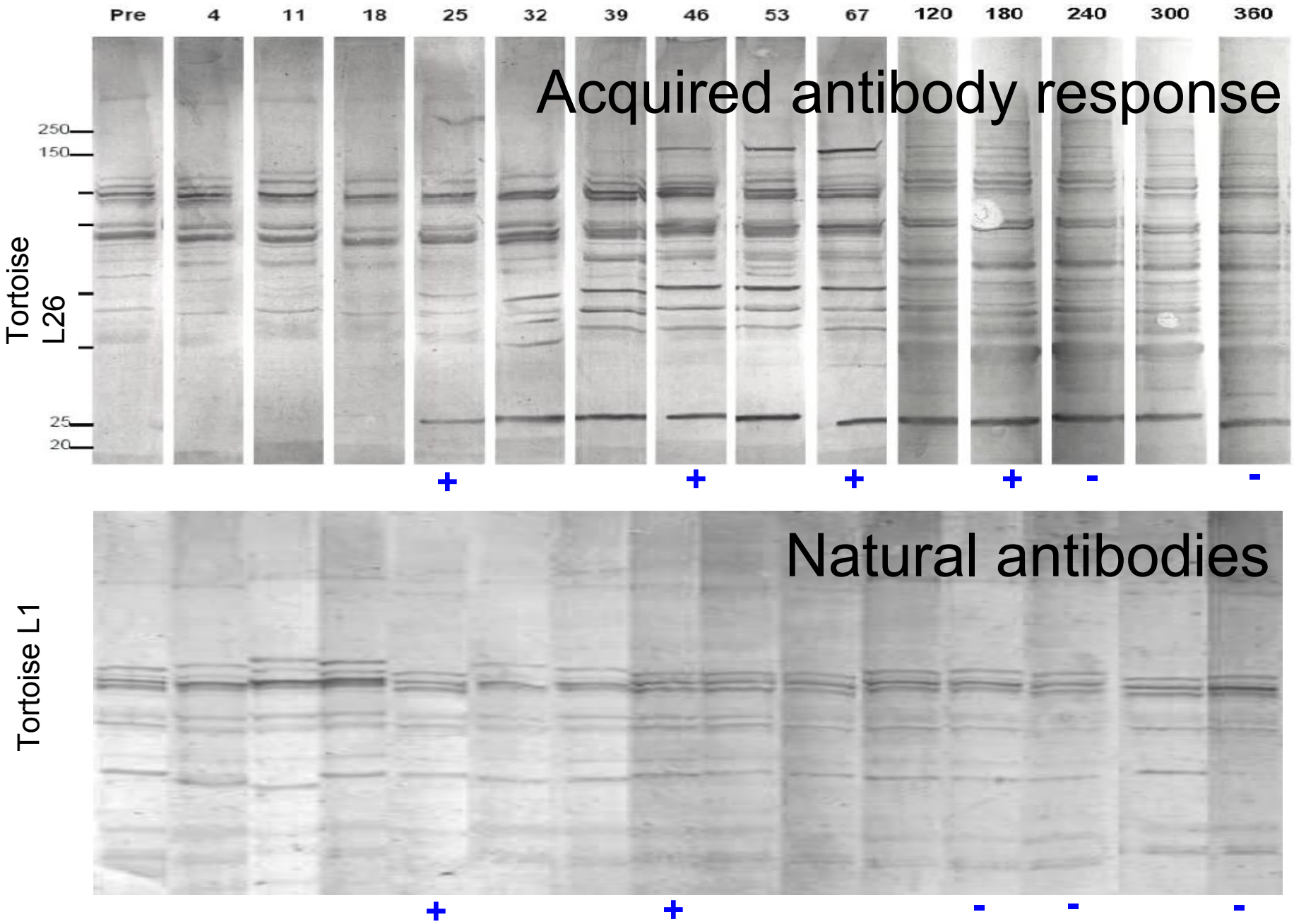




- measures number of different antibodies

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

Days After Intranasal Inoculation with *M. agassizii* Cells



M. agassizii-negative tortoises

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are needed to see this picture.

taken from Schumacher et al., 1993

What does a high ELISA titer mean?

- Acquired Antibodies
- response to exposure to pathogen/antigen
- induced “adaptive” immune response

- Natural Antibodies
- NOT induced by pathogen/antigen
- considered part of “innate” immunity
- “first line of defense”

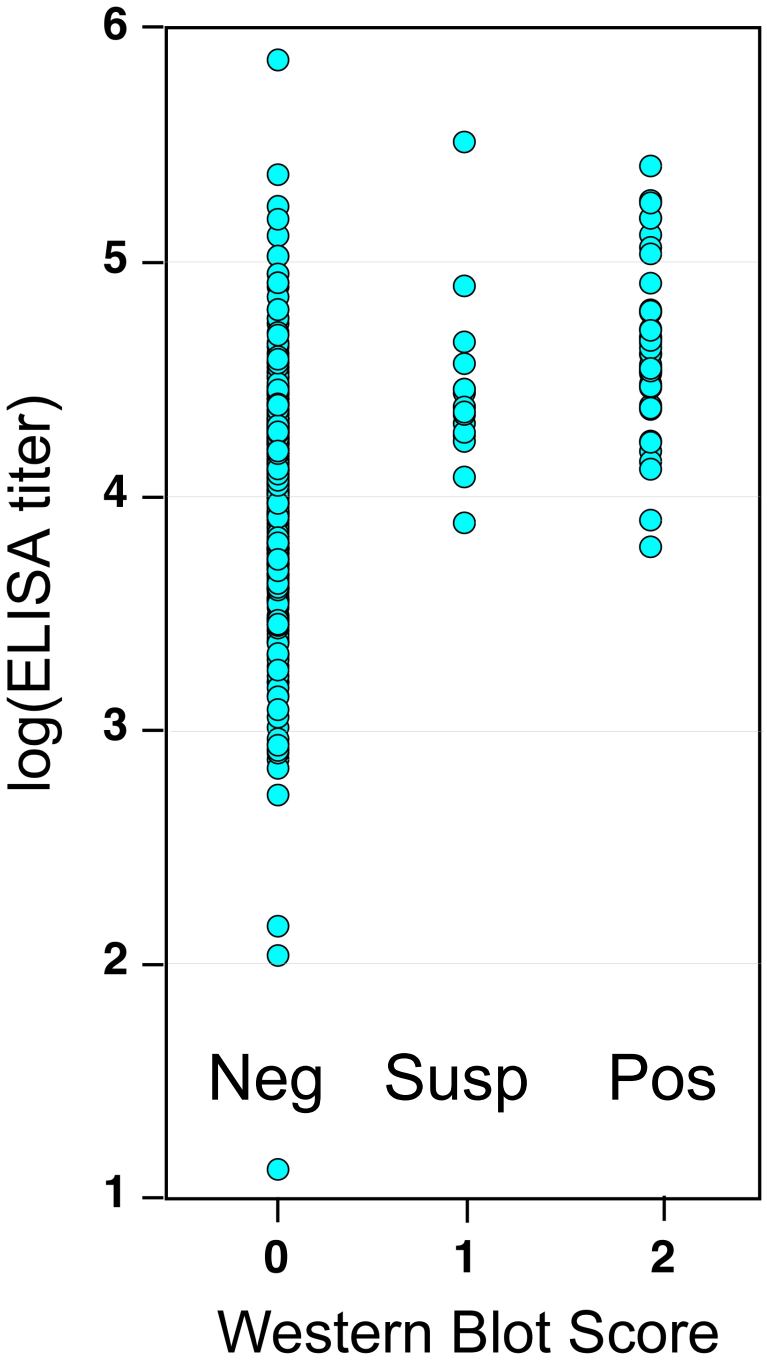
Natural Antibodies

- Desert tortoises: *Mycoplasma agassizii* (Hunter et al. 2008)
- innate immune system
- IgM, polyreactive, low affinity
- High levels of natural antibodies:
  - sandbar, tiger, galapagos sharks (Marchalonis et al. 1993), carp (Kachamakova et al. 2006), chickens (Hangalapura et al. 2006, Parmentier et al. 2004, Van Loon et al. 2004), water python (*Liasis fuscus*) (Madsen et al. 2007)

Natural Populations (n = 660)

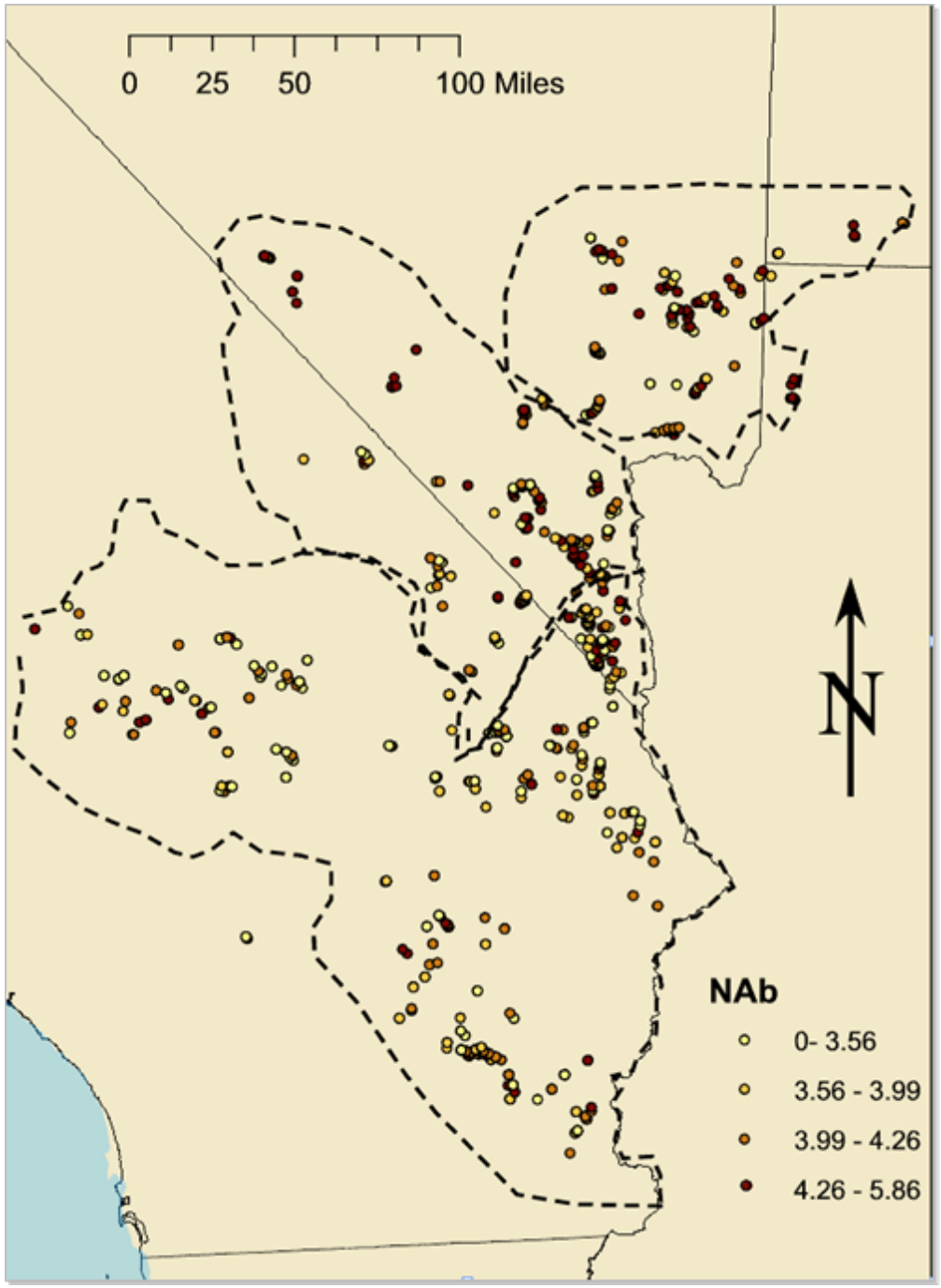
- Natural antibody level of tortoises (ELISA of W. blot-negative tortoises)
- Induced antibody response (W. blot +/-)
  - positive = past exposure to *Mycoplasma agassizii* (*Mycoplasma* spp)

ELISA (polyclonal) vs. Western blot



Natural Antibody: Quartile Map

- Natural antibody levels: lower in CA genetic cluster
- No difference between “Las Vegas” and “N. Mojave” clusters

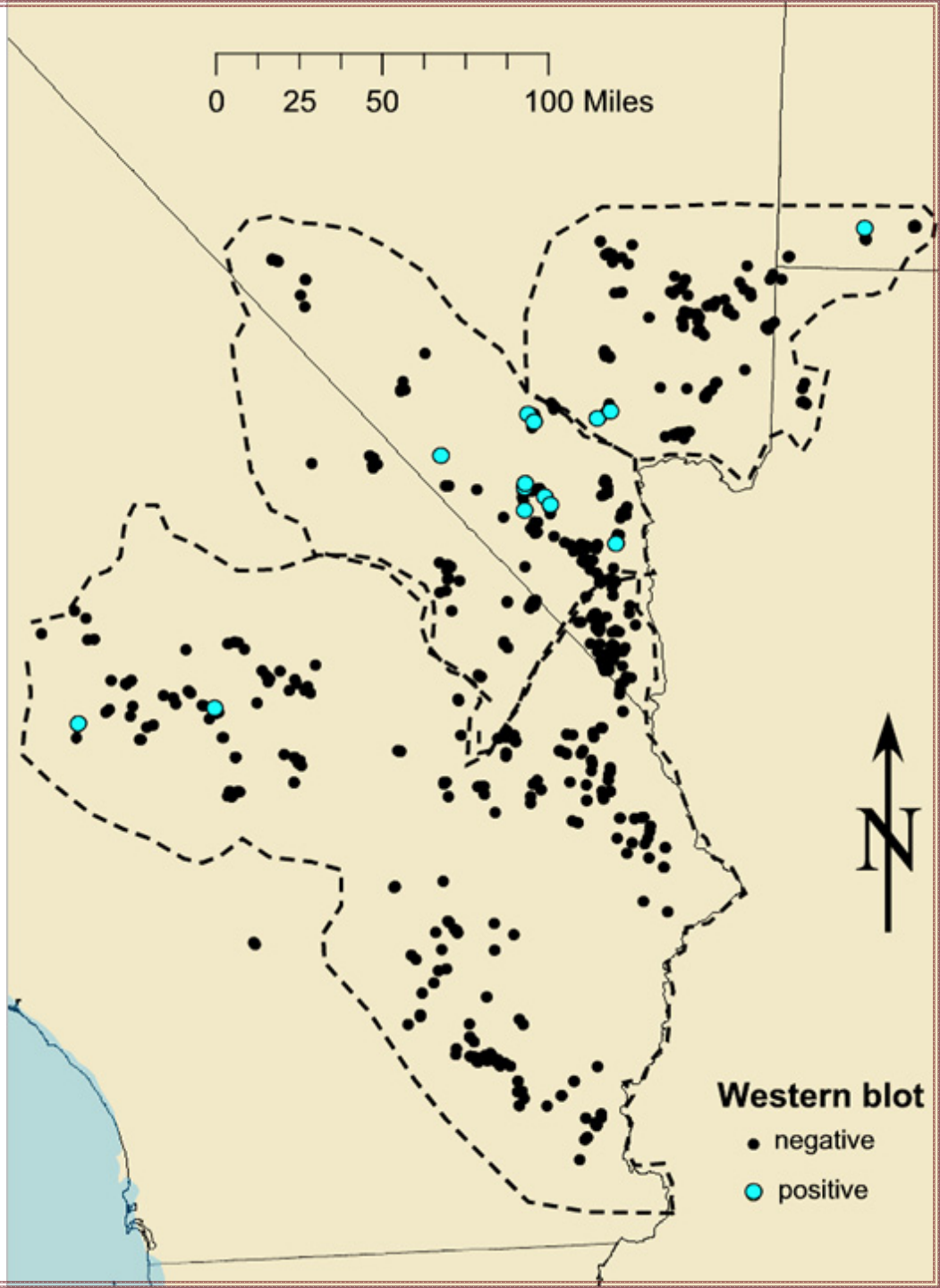


Implications: Range-wide Variation in NAb

- Management policies
  - measure natural antibody levels: Las Vegas and N. Mojave subpopulations
- Possible variation in other immune parameters?
- Possible variation in disease dynamics?
  - endemism in subpopulations of tortoises

Mycoplasma exposure: Western blot

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



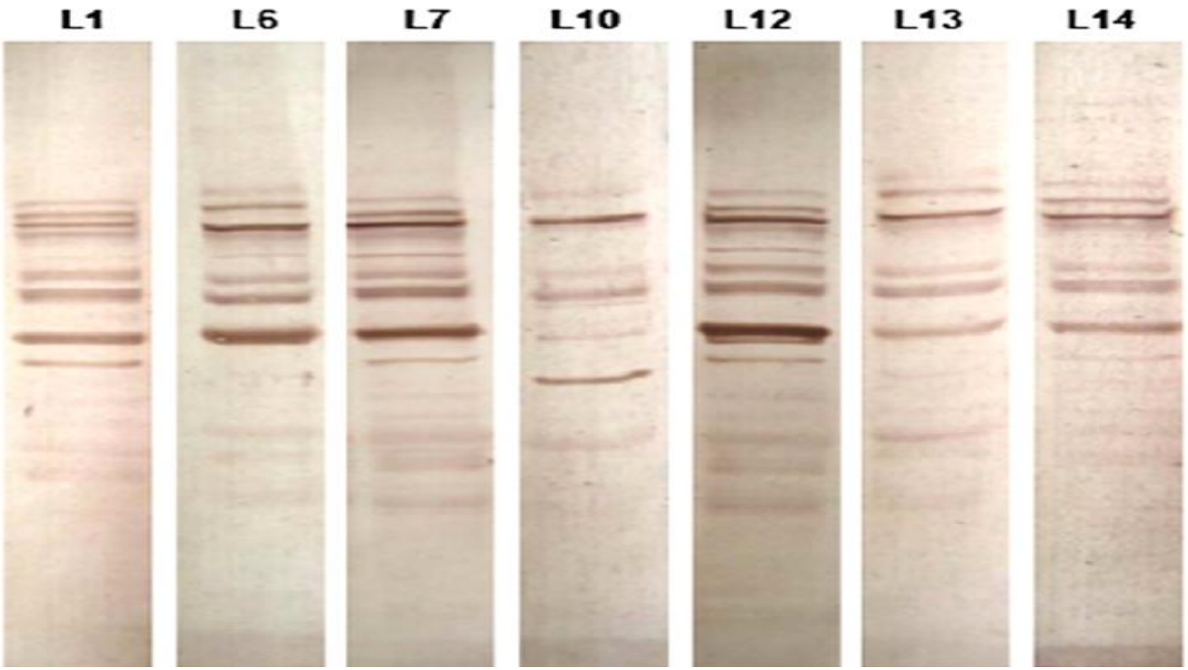
Symptoms of URTD

- W. blot: 63 positive/524negative
- There is a positive relationship between exudate and W. blot ($p < 0.001$)
- Only 24 animals had visible exudate; 12 tested positive by the W. blot

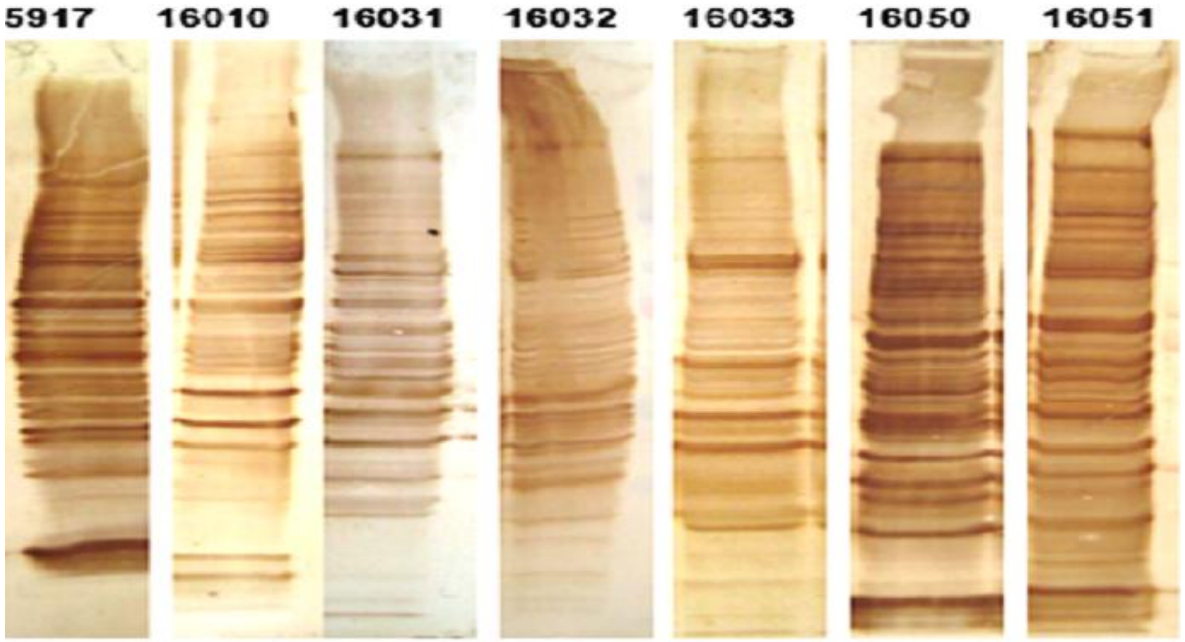
Conclusions & Future Research

- Variation across Mojave
- Hypotheses:
- Tortoise immunology
  - efficacy of natural/acquired antibodies
  - other immune mechanisms
  - interaction with habitat/climate/season
- Microbiology:
  - diversity of strains/spp of *Mycoplasma*/other pathogens
  - evolution of increased/decreased virulence
- Extrinsic factors: temperature, rainfall, vegetation, etc.

Natural Antibody Western Blot Pattern



Acquired Antibody Western Blot Pattern



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Sandmeier et al., 2009

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Jacobson et al., 1995

Jacobson, E.R., M.B. Brown, I.M. Schumacher, B.R. Collins, R.K. Harris, and P.A. Klein. 1995. Mycoplasmosis and the desert tortoise (*Gopherus agassizii*) in Las Vegas Valley, Nevada. Chelonian Conservation and Biology 4:279-284.