Final Report

Mechanisms of the Host-Pathogen Relationship between the Desert Tortoise (*Gopherus agassizii*) and *Mycoplasma agassizii*

Project Number 2003-UNR-BRRC-248-P

EXECUTIVE SUMMARY

Featured Project:
Mechanisms of the Host-Pathogen Relationship between the Desert Tortoise (*Gopherus agassizii*) and *Mycoplasma agassizii*

Project Type:
Monitoring, research

Species Addressed:
Desert tortoise (*Gopherus agassizii*)

Summary Project Description:
This project's goal is to gain a better understanding about the mechanisms that cause upper respiratory tract disease (URTD) in the desert tortoise. The main aims of the project include:

1. Develop a new ELISA test to support other experiments in this project

2. Quantifying the transmission rate of URTD among URTD positive, negative, and artificially infected tortoises in semi-natural pens in the Mojave desert (DTCC)

3. Quantifying the prevalence of antibodies of *M. agassizii* (i.e. a postive immune response to the *Mycoplasma*) in natural populations of the desert tortoise in Clark County

Project Status/Accomplishments

1. New ELISA test
   We have developed an ELISA test that appears to be approximately 1000 times more sensitive than the ELISA that Clark County uses with their contractor at the University of Florida. Our hope is to develop ELISAs to each of the individual immunoglobulins that will help us identify the course of disease in ELISA positive animals, but that will occur in the next biennium.

2. Transmission rate of URTD
   Blood samples were collected from the experimental tortoises. We have only recently developed our ELISA test, so the actual testing of field data has not occurred as yet. However, that will occur early in the next biennium. ELISA

results will be analyzed to determine transmission rates (i.e. seroconversion) within and among treatment groups. ELISA results will also be use to more fully describe the progression of a tortoise immune response, with respect to season and gender.

3. Prevalence of antibodies of *M. agassizii* in natural populations
   Blood samples were collected (spring/summer 2004, 2005, 2006) in Clark County. Samples will be analyzed via ELISA testing. Data will be turned into GIS coverages as soon as we can convert the samples into ELISA values. This will provide a model of disease prevalence county-wide.

Partners

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Funding

$532,500

Completion Date or Status

Work on this project is ongoing. Completion is expected in 2010

Documents/Information Produced

Reports previously submitted

2003-UNR-BRRC-248-P-1967-03-1
2003-UNR-BRRC-248-P-1967-03-2
2003-UNR-BRRC-248-P-1967-03-3
2003-UNR-BRRC-248-P-1967-03-4
2003-UNR-BRRC-248-P-1967-03-5
2003-UNR-BRRC-248-P-1967-03-6
2003-UNR-BRRC-248-P-1967-03-7
2003-UNR-BRRC-248-P-1967-03-8

Attachments

See appended excel file of all samples taken for the prevalence of *M. agassizii* in natural populations study and a map of those samples.

Project Photos

INTRODUCTION

Description of Project

This project addresses three requirements Clark County must fulfill as part of the Desert Conservation Program. Those are:

1. The DCP proposed desert tortoise translocation into the Larger-scale Translocation Study Site (LSTS Site) to address the problem of disposition of displaced desert tortoises. The U. S. Fish and Wildlife Service (Service) required Clark County to monitor tortoise populations in the LSTS to insure that translocation does not create population threats or result in inhumane treatment of translocated or resident tortoises. This project provides that monitoring effort.
2. The Service required Clark County to experimentally evaluate the impact of tortoise density on tortoise survivorship and health and to monitor density in the LSTS to insure density does not result in decreased survivorship opr health risks to translocated or resident tortoises. This project provides this density evaluation.
3. The Bureau of Land Management and the Service required Clark County to evaluate the historical and current use of the LSTS and to evaluate the future uses and needs for additional areas into which displaced tortoises might be translocated. Clark County contracted to have an Environmental Assessment (EA) prepared for continued desert tortoise translocation. That EA was finalized in January 2005. This project provided technical and scientific assitance to the Desert Tortoise Working Group and the consultant in the evaluation of data and the production of the EA.

Background and Need

The emergency listing of the desert tortoise (*Gopherus agassizii*) was the result of several factors threatening the population of desert tortoises. These reasons included loss of habitat due to development, disruption of habitat by OHV's and the threat of disease. Upper respiratory track disease was considered an imminent threat to the survival of the desert tortoise. Over 10 years later there still is little understanding of how URTD affects populations of desert tortoises (Lederle et al, 1997). There are reports in the literature of massive die offs, as well as the presence of *Mycoplasma agassizii* in desert tortoise populations (Jacobson et al, 1991). Unfortunately, a causal relationship between the two has not been demonstrated. Indeed, natural populations may fluctuate as a result of many factors other than disease, and furthermore, conclusions that all populations have undergone declines have been called into question (Bury and Corn, 1995). We are addressing this gap in knowledge by examining the effects of URTD in small-scale experimental populations.

One of the most frustrating aspects of URTD is the difficulty in identifying individuals that may have the disease, but not the symptoms. The current management plan is to euthanize all animals that show an antibody response, or symptoms of the disease. It has

been shown that exposing desert tortoises to *Mycoplasma agassizii* will elicit an immune response that is detectable with an ELISA test (Brown et al, 1994). The long–term effect on an individual has not been documented. Although there is a significant relationship between ELISA positive status and having the disease, the exact effect of positive animals on a population has never been examined (Jacobson et al 1995, Lederle et al 1993). Previous research has failed to be able to culture *M. agassizii* from ELISA animals on a consistent basis. In Jacobson's 1995 study 50 percent of the individuals did not have an ELISA status that agreed with the culture status (Jacobson et al 1995). In a separate study, *M. agassizii* was cultured from 68 percent of individuals given a nasal flush containing the mycoplasma (Schumacher et al 1994). The reasons for these failures are multiple. First is the difficulty in culturing *M. agassizii*. Therefore the ability to detect the presence of the disease by culture is poor. The relationship between symptomatic animals and the presence of the disease has been questioned due to fears of latent or subclinical phase of the illness (Jacobson 1995). An immune response can help in determining that an individual has been exposed to the disease but it does not differentiate between currently infected individuals, animals exposed to a non–pathogenic mycoplasma, or individuals which have recovered from the disease (Grenfell B.T. pg 78, Schumacher et a, 1997). The removal of recovered individuals from the breeding population could be the least conservative management action. If there is a possibility for individuals to recover from the disease these individuals might have an advantage of resistance or a stronger immune system. Potentially these individuals could ensure the long–term survival of the tortoise.

The immune systems of animals can be very sensitive to a variety of environmental stressors (Lochmiller and Dabbert 1993, Husband 1995), and it has been suggested that immunocompetence plays an important role in the regulation of animal populations (Anderson and May 1982, Lochmiller 1996). For example, declines in amphibian populations have been attributed to environmental factors affecting immunocompetence and increased susceptibility to infectious diseases (Cary et al. 1999), and it has been suggested that environmental factors have played a role in the recent epidemic of fibropapillomatosis in green turtles (Herbst and Klein 1995). Additionally, a change of health state in tortoises infected *M. agassizii,* which is an opportunistic pathogen (Brown pers. comm.), also may serve as a sensitive indicator that desert tortoise individuals are immunologically compromised by environmental stressors. However, studies of the natural history of *M. agassizii* infections in the desert tortoise have been hampered by a lack of reagents to study the tortoise immune response to this microorganism, and we have no tools to study the immune state more directly in tortoise individuals. We need to prepare, characterize, and produce sufficient polyclonal and monoclonal anti-tortoise Ig antibodies to facilitate the development of needed ELISA tests for *M. agassizii.*

Management Actions Addressed

This project is critical for developing tools to do the individual-based monitoring called for in the DTRPAC report.

Goals and Objectives

This project's goal is to gain a better understanding about the mechanisms that cause upper respiratory tract disease (URTD) in the desert tortoise. The main aims of the project include 1) developing a new ELISA test to support other experiments in this project, 2) quantifying the transmission rate of URTD among URTD positive, negative, and artificially infected tortoises in semi-natural pens in the Mojave Desert (DTCC), and 3) quantifying the prevalence of antibodies of *M. agassizii* (i.e. a postive immune response to the *Mycoplasma*) in natural populations of the desert tortoise in Clark County.

METHODS AND MATERIALS

New ELISA test

Rather than using physico-chemical separation techniques that require large volumes of tortoise blood, we have been immunizing tortoises with a test vaccine to isolate pure tortoise Igs in the form of anti-vaccine antibodies by affinity chromatography.

Transmission rate of URTD

Experimental design:

- 23 fenced pens (80m x 90m) at the DTCC
- each pen had 2 treatment groups of six tortoises (sex ration of tortoises 4 females:2 males)
- treatments: Negative (tortoises seronegative for *M. agassizii*, Positive (tortoises seropositive for *M. agassizii*), Infected (tortoises artificially infected with a culture of *M. agassizii* known to produce clinical symptoms of URTD)
- factorial design: 5 pens controls (Negative, Negative) (control), 6 pens (Negative, Positive), 6 pens (Negative, Infected), 6 pens (Infected, Positive); treatments assigned at random to each pen

Data collection:

- brachial blood samples taken monthly from Nov. 2003 – Oct. 2004 (0.1 – 0.5 ml)
- jugular blood samples taken 3-4 times annually in 2004 & 2005 (2.0 – 4.0 ml)

Laboratory analysis:

- serum samples will be analyzed via ELISA testing:
  - positive/negative to *M. agassizii*
  - positive/negative for classes of antibody (IgM, IgY), specific to *M. agassizii*

Data analysis:

- We have only recently developed our ELISA test, so the actual testing of field data has not occurred as yet. However, that will occur early in the next biennium.
- ELISA results will be analyzed to determine transmission rates (i.e. seroconversion) within and among treatment groups

- ELISA results will also be use to supplement the goal of Part 1: to more fully describe the progression of a tortoise immune response, with respect to season and gender.

Prevalence of antibodies of *M. agassizii* in natural populations

Data collection:

- blood samples were collected (spring/summer 2004, 2005, 2006) in Clark County
- 2004, 2005 blood sampling was carried out personally and in collaboration with tortoises monitoring in DWMAs (line distance sampling)
- 2006 blood sampling occurred in selected sites in the Mojave & extensively in Piute Valley DWMA (personal collection, with the help of a few field technicians)

Laboratory analysis:

- samples will be analyzed via ELISA testing
  - positive/negative for antibodies to *M. agassizii*
  - positive/negative for classes of antibodies (IgM or IgY), specific for *M. agassizii*

Data analysis:

- GIS/spatial analysis of distribution of seropositive/seronegative animals
- statistical analysis to determine if significant correlations occur between the frequency of seropositive animals and environmental variables, including annual rainfall, annual temperature, annual rainfall & temperature of preceding years, season, estimated tortoise density, degree of habitat disturbance

RESULTS

We have developed an ELISA test that appears to be approximately 1000 times more sensitive than the ELISA that Clark County uses with their contractor at the University of Florida.

Survey results for prevalence of antibodies to *M. agassizii* in desert tortoises in Clark County (up to 3 years of data, depending on geographic area).

We are beginning to learn more about the seasonal cycles in immune competence as part of the direction we are taking this research.

EVALUATION/DISCUSSION OF RESULTS

This project is simultaneously providing new tools for assessing health of tortoises, it is also providing new knowledge about the nature of the host-pathogen relationship so that we can determine the course of adaptive management in areas in which animals are stressed in ways that could lead to disease. In other words, if we are ever to manage those threats that cause stress and disease, we need to know the biology of that stress and the

biology of infection and how that infection results in morbidity and mortality. Our research is aimed at developing that understanding.

CONCLUSIONS

This project is critical among those that bear directly on threats to persistence of desert tortoise.

RECOMMENDATIONS

We have developed a new ELISA which is much less expensive than that provided by the University of Florida. This ELISA is also much more powerful in its ability to discern an immune response.

It is now clear that the tools we are creating will allow us to monitor stress and disease in tortoise populations. We need to develop the GIS tools to be able to map ELISA status of individuals in Clark County. Changes in these parameters could signal a positive or negative change in threats that would require a change in management.

LITERATURE CITED

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