

**Transmissible Infections and Desert Tortoise Translocation:
A Comprehensive Disease Risk Analysis**

A report to the U.S Fish and Wildlife Service

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PREFACE

The U.S. Fish and Wildlife Service’s Desert Tortoise Recovery Office, the Desert Tortoise Science Advisory Committee’s Disease Workgroup, and the Desert Tortoise Conservation Center/San Diego Zoo Global assembled a workgroup for the task of conducting a disease risk assessment for the Mojave desert tortoise. The workgroup convened 18-19 September 2012 at the Desert Tortoise Conservation Center in Las Vegas, Nevada. This report was prepared by a subset of workgroup participants (Editor: Bruce Rideout; additional input provided by Nadine Lamberski, Kimberleigh Field, Roy Averill-Murray, Jay Johnson, Jerry Simecka, Fran Sandmeier, and Ken Nussear). All participants were given the opportunity to provide input during draft review.

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INTRODUCTION

Many factors may influence the success of wildlife translocations, including presence of threats that may have caused the original decline of the target population; habitat, demographic, and biophysical constraints; genetic mixing and outbreeding depression; social structure; movement and settlement rates; and disease transmission (Berry 1986, Burke 1991, Dodd and Seigel 1991, Reinert 1991, Murphy et al. 2007). This document specifically addresses disease issues associated with the translocation of Mojave desert tortoises (*Gopherus agassizii*). Other considerations about translocation of desert tortoises, including assessing suitability of recipient sites and translocation techniques, are addressed in a separate document (U.S. Fish and Wildlife Service [USFWS] 2012a, currently under revision). Combined, these documents guide management decisions and are essential pieces of what will become a population augmentation strategy for the species.

The use of translocation in both conflict-driven and conservation-driven contexts requires scrutiny, justification, and adherence to practices that reduce risk and increase chances of success. This document presents the results of a qualitative disease risk analysis for Mojave desert tortoise translocations. To evaluate disease risks, we conducted an assessment using the process described in the Guidelines for Wildlife Disease Risk Analysis (OIE and IUCN 2014) and the Manual of Procedures for Wildlife Disease Risk Analysis (Jakob-Hoff et al. 2014a), both of which were in draft at the time of our assessment. Infectious agents assessed were those known at the time of the workshop. Agents recognized after the workshop, but prior to completion of this report, are included in Appendix 1.

When planning for the assessment, we carefully considered the composition of the workgroup that would participate in the in-person workshop. Among the considerations were group size, individuals with key information/skills, and group dynamics (Jakob-Hoff et al. 2014b). The workgroup included an existing group, the Desert Tortoise Science Advisory Committee's Disease Workgroup. Intentionally, several prominent disease and epidemiology experts with experience in desert tortoises were not invited to take part in the workshop, as we wanted people from this specific area of expertise to be able to provide critical review of our process and decisions. Unfortunately, we did not approach these individuals regarding this role prior to conducting the workshop and there was dissatisfaction and disinterest expressed by some individuals in assisting with the assessment as critical reviewers. We strongly suggest contacting all desired participants at the outset, especially when there are known interpersonal, professional, and/or political tensions among key individuals.

Background

Mojave desert tortoise population declines—The Mojave desert tortoise is native to the highly variable environment of the deserts of the southwestern United States. Beginning in the 1970s, data at local levels suggested appreciable declines in many areas (USFWS 1980, Berry 2003, Berry and Medica 1995, Tracy et al. 2004). While portions of the population were given federal protection as early as 1980, the entire Mojave population of desert tortoises (located north and west of the Colorado River in Arizona, Utah, Nevada, and California) was protected across its range through listing as a Threatened species under the

U.S. Endangered Species Act on April 2, 1990 (USFWS 1990). The population that was listed is now recognized as a separate species from those tortoises living south and east of the Colorado River in Arizona and parts of Mexico (Murphy et al. 2011).

In the western portion of its range, where sufficient data were available for examination, the downward trend in populations was determined to be ongoing well after its listing (Tracy et al. 2004). More recently, data from the ongoing range-wide monitoring program confirm that these declines in adult abundance continue in the Western Mojave and are part of a larger pattern of declines involving four of the five recovery units (USFWS 2014). The vast majority of threats to the Mojave desert tortoise and its habitat are associated with humans (USFWS 2011a, Darst et al. 2013). Among others, these threats include collection from the wild, unauthorized breeding as pets, and unauthorized release or escape of captive tortoises to the wild. A large captive population, which likely numbers in the tens of thousands and dates prior to listing under the Endangered Species Act and enactment of State regulations, poses risks associated with transfer of disease to wild populations (USFWS 2011a).

Suspected role of Mycoplasma agassizii infections—Disease was suspected to play a role in desert tortoise population declines (USFWS 1990, Berry 1997). In particular, signs of an upper respiratory tract disease seemed to be increasing, as did loss of marked animals previously noted to have signs of respiratory disease (USFWS 1990). Scientists cautioned that more research was needed to determine what role various agents might play in the observed disease, but bacteria from two genera, *Mycoplasma* and *Pasteurella*, were suspected to be causative (Jacobson and Gaskin 1990, as cited in USFWS 1990). Subsequent research implicated *Mycoplasma agassizii* as the most important causative agent in the observed upper respiratory disease, and Koch's postulates were satisfied (Brown et al. 1994). The population-level effects remain difficult to assess. A second agent, *Mycoplasma testudineum*, has been identified, but additional research is needed to better understand its pathogenicity (Jacobson and Berry 2012). Systemic diseases, such as mycoplasmosis, may also negatively affect keratinization processes of the shell (Homer et al. 2001). As with most diseases, other factors such as stress and inadequate nutrition that compromise the overall health of an individual likely increase susceptibility (Keusch 2003, Sandmeier et al. 2009). Environmental contaminants (such as arsenic and mercury) and drought are two external factors that are thought to affect the desert tortoise's susceptibility to *Mycoplasma* infection and the development of disease (Jacobson et al. 1991, Christopher et al. 2003, Seltzer and Berry 2005). See Sandmeier et al. (2009) and Jacobson et al. (2014) for reviews of information on mycoplasmosis.

Initial risk mitigation for Mycoplasma—Early efforts to mitigate risk of *Mycoplasma* infection were focused on minimizing spread of the pathogen through disinfection of field gear and reducing the possibility of intentionally released or escaped tortoises from captivity (pets) or holding facilities spreading infection to wild tortoises. In California, facilities that included large pens to acclimate former captive tortoises to desert conditions prior to release by state officials (see Murphy et al. 2007) were no longer in operation by this time. However, head-starting facilities, where eggs from wild tortoises were hatched and the young held in group rearing pens, were beginning operation (Morafka et al. 1997).

The California Turtle and Tortoise Club, with its numerous chapters, assisted with management of the pet population through an adoption program, registration of pets with the state wildlife agency, and education to dissuade the release of former pets by the public. Nevada and Utah had holding facilities that accumulated both wild tortoises removed from harm's way and former captives, with the facility in Nevada being of much larger scale than that in Utah. Nevada's Desert Tortoise Conservation Center (DTCC) and associated transfer and holding facility near Las Vegas included a free hotline and pick-up service in an attempt to prevent the public from releasing unwanted tortoises into the wild. In Utah, keeping tortoises as pets within its native range was banned. Both facilities used an enzyme-linked immunosorbent assay (ELISA) that tested for *M. agassizii*-specific antibodies in tortoise blood serum (Schumacher et al. 1993) to identify exposed tortoises. At the DTCC, the USFWS permitted euthanasia of tortoises that showed clinical signs of disease or tested positive for exposure to *M. agassizii* as shown through the ELISA test (USFWS 1996). At the Temporary Care Facility (TCF) in Utah, ELISA-positive tortoises were maintained (i.e., not euthanized) in a separate section of the facility.

Beginning in 1997, tortoises with negative ELISA tests but little assessment of health or of suitability for translocation were allowed to be released from the DTCC to the newly designated Large-Scale Translocation Site (LSTS) (RECON 1996). The LSTS is an approximately 100-km² area enclosed by tortoise-exclosure fencing and rugged topography near Jean, Nevada. The LSTS provided an outlet for tortoises accumulating at the DTCC and allowed for experiments into the efficacy of translocation as a conservation tool where both short-term (Field et al. 2007) and long-term (USFWS in prep.) evaluation could be done. Since 1999 in Utah, ELISA-negative and clinical sign-free tortoises of wild origin have been translocated to Zone 4 of the Red Cliffs Desert Reserve near St. George after temporary holding at the TCF (McLuckie et al. 2012). Zone 4 is an approximately 21-km² area bounded by tortoise-exclosure fencing, the Virgin River, and the northern limits of the species' range (Washington County Commission 1995).

Euthanasia of ELISA-positive tortoises at the DTCC continued for nearly a decade until 2007. At that time, the USFWS formally recognized that the *M. agassizii* ELISA result alone indicated exposure to the organism but gave no indication of a tortoise's current health or disease status, and the policy of euthanizing tortoises at the DTCC based on that single test result was terminated. ELISA-positive tortoises were then held in pens together, to remain in captivity until new recommendations could be developed to direct their disposition. Since 2009, when San Diego Zoo Global joined the partnership for operation of the DTCC, tortoises with clinical signs of upper respiratory tract disease (regardless of ELISA status) were treated with antibiotics (see Wendland et al. 2006 for how to manage upper respiratory disease in chelonians and Lamberski et al. 2014 for examples of antibiotics used). Those that were in poor body condition, exhibited moderate to severe signs of respiratory disease, had recurrent episodes of nasal discharge, or were refractory to treatment could be humanely euthanized.

The practice of translocating only ELISA-negative tortoises was continued as protocols were developed for the translocation of wild desert tortoises from the Fort Irwin Southern

Expansion Area in San Bernardino County, California (Esque et al. 2005). Tortoises there also had to test culture negative via polymerase chain reaction (PCR) (Berry 2006), and any tortoises exhibiting moderate to severe clinical signs were retested. Protocols specified that tortoises that were ELISA and/or culture-positive or suspect for *Mycoplasma* antibodies would be held in quarantine pens until disposition could later be determined (Esque et al. 2005). Only seronegative and culture negative tortoises not showing clinical signs of disease would be translocated to the release sites. Ultimately, ELISA-positive/suspect tortoises and those showing clinical signs of disease were left within the expansion area, rather than moved to pens.

In 2011, following recommendations of wildlife veterinarians and pathologists, the Bureau of Land Management (BLM) in Nevada, Nevada Department of Wildlife (NDOW), and USFWS agreed that tortoises without clinical signs of active upper respiratory tract disease and deemed healthy and suitable for translocation, regardless of ELISA status relative to *Mycoplasma* antibodies, could be released from the DTCC to the large-scale translocation site and other sites agreed upon in the future. The recommendations are being considered in Utah, but have not been implemented for tortoises from the TCF. Wild-to-wild translocations of ELISA-positive tortoises also started to become accepted by some agencies under certain circumstances (see below the section on Conflict-driven Translocations).

Other threats to desert tortoises—While additional pathogens have been isolated from Mojave desert tortoises (see risk assessment below), numerous threats other than transmissible disease affect tortoise population dynamics, and the role of disease relative to other threats needs to be clarified. Shell lesions have been associated with declines in at least two populations (Berry 1997, Christopher 2003) and may be associated with systemic disease of transmissible or nontransmissible origin. In addition to disease, urbanization, human access to the tortoise's habitat, military operations, and illegal use of off highway vehicles appear to have the greatest impacts on desert tortoise populations. Urbanization and human access, through their cumulative and indirect effects, present at least twice the estimated risk to desert tortoise populations as does disease (Darst et al. 2013). This suggests that recovery actions targeting the loss and degradation of habitat are particularly important.

Recovery plan—In 2011, the USFWS released a revised recovery plan for the Mojave desert tortoise (USFWS 2011a). The overall goals are recovery of desert tortoise populations across its range and delisting (i.e., removal from the list of species provided federal protection under the US Endangered Species Act) when threats have been abated to the point that the protections afforded by the Endangered Species Act are no longer necessary. In the plan, six strategic elements are described to guide the recovery program. These elements emphasize a collaborative approach where monitoring is crucial, applied research is essential, and implementation of a working adaptive management process is key to moving forward as information is added to our knowledge base. Recovery criteria related to demography, distribution, and habitat establish targets by which progress toward achievement of recovery objectives can be measured. Specific recovery actions aimed at moving towards the targets set in the criteria are described.

One of the six strategic elements in the recovery plan is to “augment depleted populations through a strategic program.” Population augmentation is viewed as an intermediate strategy aimed at increasing populations, more rapidly than possible through natural processes, in conjunction with elevated threat management, habitat restoration, or directed research on the factors affecting success of augmentations. The plan recommends development of a comprehensive population augmentation strategy to provide specific guidance on translocation and a multitude of factors, including disease. This risk assessment is one of several steps in building the strategy.

Current Situation

Conflict-driven translocations—Recently, the construction of energy production sites, primarily solar, has been destroying desert tortoise habitat. The national priority of increasing renewable energy production allowed for little time to plan prior to the first projects breaking ground in the Mojave Desert. In order to attempt to minimize the impacts that these projects have on already dwindling tortoise populations, projects typically remove tortoises from the habitat to be destroyed and relocate them to nearby areas (i.e., similar to the Fort Irwin expansion).

The USFWS developed in-depth guidance regarding the translocation of tortoises from project sites that took into account the status of knowledge on genetics, release methods, post-translocation dispersal, and disease (USFWS 2010). To complement the translocation guidance, separate health assessment procedures and a disposition decision tree were developed rapidly in cooperation with veterinarians, pathologists, and other scientists and disseminated to biologists working on the solar project-driven translocations (USFWS 2011b). These protocols were adapted from published recommendations (Jacobson et al. 1999, Berry and Christopher 2001) and IUCN guidelines (Woodford 2001). In general, tortoises could be moved between populations exhibiting similar disease prevalence as long as the prevalence was less than 20%. Disease prevalence was calculated to consider exposure as well as active disease and included the proportion of tortoises seropositive for *Mycoplasma agassizii* antibodies, seropositive for *Mycoplasma testudineum* antibodies, and those that had particular clinical signs of disease (USFWS 2011b, USFWS 2012b). The procedures also involved the banking of samples and standardized data collection, such that decisions could be modified after review of available data. While recommended, a centralized database to house these data from projects across the species’ range, and to facilitate review, has yet to be implemented.

Conservation-driven translocations— Previous short-term studies have shown that former captive tortoises transition to life in the wild and survive at rates comparable to resident, wild tortoises (Field et al. 2002, Field et al. 2007, Nussear et al. 2012). This presented an opportunity to use former captive tortoises in efforts to bolster wild populations, without in-depth reconditioning to life in the wild. Although significant behavioral obstacles are not evident, there are two additional critical considerations: genetics and disease. Through reanalysis of existing genetic data, a distance from origin that tortoises can be moved for management purposes while remaining within their genetic unit has been determined (wild-to-wild: 200 km, DTCC-to-wild: 175 km [not evaluated for other holding

facilities/captive situations]) (USFWS 2012c, Averill-Murray and Hagerty 2014). Disease is more complex, as complete captive histories of tortoises are usually unknown. It is possible for privately held desert tortoises to be exposed to other species of tortoises or other reptiles with origins around the world and thus to pathogens that are not found in wild populations of desert tortoises.

The DTCC served as a source of large numbers of desert tortoises, both former privately-held captives and those removed from habitat-destructive projects, which could be used in augmentation efforts contingent on the application of appropriate quarantine and screening protocols. This provided the opportunity to augment populations with tortoises from a variety of age classes, including reproductive adults, without relying on the removal of tortoises from wild population or on resource intensive head-starting programs. Without the potential for these tortoises to be used in wild conservation efforts, their future dispositions became limited to lifetimes in captivity or euthanasia. Under a new partnership with San Diego Zoo Global in 2009, tortoises at the DTCC began to undergo assessments of health that took into account body condition, clinical signs of disease, physical exam findings (e.g., coelomic masses or white mucous membranes), weight history, medical history while at the DTCC, presence of ectoparasites, concurrent illness in pen cohorts, and other factors determined to be important in appropriately assessing the individual's health and determining suitability for translocation (see USFWS 2011b for examples). As described above, the protocols were adapted from published recommendations and built upon by San Diego Zoo Global veterinarians and pathologists in consultation with other veterinarians and scientists with pertinent expertise.

Reevaluation of disease concerns and risk-mitigation strategies—The first large-scale augmentation of a completely free-ranging, depleted population to use tortoises from the DTCC was being considered for spring 2013 (BLM 2013), and translocations to augment other depleted populations were under consideration (and some have been implemented since completion of the risk assessment workshop). Additionally, large-scale projects continue to be proposed within occupied desert tortoise habitat, thereby setting the stage for future translocations to “rescue” large numbers of tortoises from the path of habitat-destructive activities. Due to the increase in translocations from project sites and the desire to augment populations using tortoises that had spent some portion of time in captivity, a reevaluation of current disease concerns and risk mitigation strategies was needed. The workshop (September 2012) described herein was organized to reevaluate disease risks and develop effective risk-mitigation plans. The captive source specifically evaluated was the DTCC. The local and federal management agencies that historically supported the DTCC decided to close the facility at the end of 2014. The large population of desert tortoises in captivity across the range creates potential for continued augmentation of wild populations using former captives, however; an updated risk assessment would need to be done unless those tortoises are sent to single-species quarantine and holding facilities that closely emulate the DTCC in their protocols and policies.

Concepts of Disease

Discussion of the disease risks associated with wildlife translocations can be contentious, in part because of differing viewpoints on the concepts of health and disease. Defining our terms and concepts at the outset can help prevent confusion and conflict.

Disease has traditionally been defined as any impairment in normal structure or function in an individual. This concept of disease is useful when the focus is on managing disease in individuals, but less so when the focus is on ensuring the sustainability of populations. Recognition of disease as a natural population process and a focus on health at the population level has led to a greater emphasis on broad-scale disease risk assessments. In this context, health can be thought of as the ability of a population to perform all of its ecological functions with typical efficiency (Hanisch 2012). Inherent in this is the idea that healthy populations should be able to remain resilient and self-sustaining in the face of naturally occurring disease. It is also important to recognize that diseases do not occur in isolation – there is always a dynamic interplay between the host, the agent, and the environment. This emphasis on wildlife population health in the context of ecosystems enables us to bring all threats into the analysis, so undue attention is not being placed on infectious diseases to the exclusion of other significant threats.

There are no wildlife populations completely free of disease. The purpose of a disease risk analysis is not to help maintain a disease-free state, but rather to maintain healthy (i.e., resilient and self-sustaining) populations by minimizing the risk of a disease scenario to which the target population could not adequately respond. The wildlife disease risk analysis should therefore address the key disease threats in the context of the current or anticipated health of a specific population.

The Disease Risk Analysis Process

Disease risk analysis is a structured process for evaluating the likelihood and consequences of specific disease hazards occurring in a population as a result of a management decision or changing circumstance. The process evaluates disease threats in a specific population context, so a particular disease agent could be determined to be a significant threat to one population but not another (e.g., if one population is determined to be less resilient than another due to other population health impacts it is experiencing). The principles behind a disease risk analysis are adapted from general risk analysis procedures used in a variety of fields, ranging from manufacturing to the military. The process typically involves six steps: problem description, hazard identification, risk assessment, risk management, implementation and review, and risk communication (Jakob-Hoff et al. 2014a).

The value of a formal disease risk analysis for desert tortoises is that the structured process enables all identifiable hazards to be evaluated systematically and objectively by a multidisciplinary group. The ability to evaluate all hazards in context, and to weigh the risk of inaction as well as action, facilitates sound conservation management. The participants in this risk analysis were chosen based on the scientific disciplines deemed important to the process (e.g., veterinary medicine, pathology, epidemiology, disease ecology, population biology, and reintroduction biology) and the agency and stakeholder representation

needed for decision-making (US Fish and Wildlife Service, Nevada Division of Wildlife, and California Department of Fish and Wildlife). See the preface for a list of participants.

THE RISK ANALYSIS

Problem Description

The workgroup defined the current problem as the following:

Urgent conservation actions are being confounded by infectious disease concerns arising from a desire to avoid negative population impacts.

Several assumptions and limitations were acknowledged as the problem description was being developed. These included:

- There are other potentially significant health hazards to wild desert tortoise populations besides mycoplasmosis.
- Our knowledge of the endemic and potentially epidemic disease threats to desert tortoises is very limited.
- Potential hazards to population health include noninfectious as well as infectious diseases.
- Some disease threats are population density dependent, so final population density estimates (i.e., translocated population plus recipient population combined) need to be incorporated into translocation risk assessments.
- There are few screening tests for infectious agents that have been validated for use in desert tortoises. Importantly, an ELISA detects the presence of antibodies for an infectious agent rather than the agent itself.
- Screening or surveillance tests are imperfect and cannot eliminate the risk of a disease introduction.
- Translocations are not the only source of disease risk to desert tortoise populations, so other avenues of disease introduction need to be considered in a translocation risk analysis.
- Solar energy developments will proceed on timelines with limited flexibility, thus not allow time for deep investigations to eliminate uncertainty about potential impacts on desert tortoises.
- Desert tortoises occupying habitat slated for development must be translocated if they are to survive and contribute to recovery of the species.
- Different translocation scenarios entail different disease hazards, so risk mitigation efforts need to be tailored to specific translocation scenarios.
- The number of desert tortoises that need to be translocated is sufficiently large that financial and logistical efficiency need to be a high priority.

The context of translocation, including the source of animals, recipient population, and availability of health history data varies across scenarios. Translocations may be initiated with the primary purpose being to move animals out of harm's way to reduce the number

that would otherwise be directly killed (e.g., solar energy development driven) or to move animals into an area for conservation purposes (e.g., an augmentation of a population). Tortoises may go directly from a wild locality to another wild locality, from the wild to a quarantine and holding facility and then to another wild locality, or from a captive environment to a quarantine and holding facility and then to a locality in the wild. Currently, the most important scenarios are wild-to-wild translocations with a potential layover at an onsite quarantine and holding facility (i.e., tortoises are not brought to a facility where tortoises of other origins are housed) and captive-to-wild translocations wherein the captive history of the tortoises may be unknown. The captive-to-wild scenario specifically assessed was the DTCC, but the assessment could be applicable to other captive scenarios, if they are single-species facilities that closely emulate the DTCC through quarantine, screening, and evaluation procedures. Through implementation of such procedures any deviations in prevalence from those used in our assessment will be illuminated.

Some level of risk will always be present when taking an action intended to be beneficial, when uncertainty is involved. Rather than paralyze conservation action due to an inability to eliminate uncertainty, we must accept a degree of risk, within a defined level of tolerance, in order to move forward with actions intended to benefit the recovery of the desert tortoise. This risk analysis allows us to better understand the levels of risk involved and develop minimization strategies specific to those risks.

Hazard Identification

The workgroup limited the assessment to transmissible infectious agents, as spread of infection that results in detrimental effects on populations is a primary concern in translocation, whereas non-transmissible agents (e.g., uroliths, toxicants, fungi) affect individuals and their own suitability for translocation or survival in the wild. Based on a literature review and personal experience, the workgroup identified the following transmissible infectious agents as being known to cause or be associated with disease in desert tortoises or known to be carried by desert tortoises with potential transmission to other organisms:

- *Mycoplasma agassizii* (Brown et al. 1994, 2001)
- *Mycoplasma testudineum* (Brown et al. 2004, Jacobson and Berry 2012)
- Tortoise herpesvirus-2 (TeHV-2) (Johnson et al. 2005, Jacobson et al. 2012)
- *Chlamydophila* sp. (Johnson et al. 2012)
- *Pasteurella testudinis* (Snipes and Biberstein 1982, Jacobson et al. 1995)
- *Salmonella* spp. (Jacobson 2007)
- *Cryptosporidium* spp. (Braun and Holder, unpublished data)

The workgroup identified the following transmissible infectious agents (or categories of agents) as being plausible pathogens in desert tortoises based on a literature review of pathogens affecting other tortoise species and on the broad host range of the agents (Mader 2005, Jacobson 2007a, and as cited below):

- Paramyxoviruses (Hyndman et al. 2013)
- Adenoviruses (Rivera 2009, Schumacher 2012, Doszpoly et al. 2013)
- Iridoviruses/Ranaviruses (Westhouse et al. 1996, Johnson et al. 2008)
- Pathogenic nematodes (Rideout et al. 1987)
- Pathogenic protozoa (Zwart and Truyens 1975, Jacobson 1993, Jacobson et al. 1994, Griffin et al. 2010)
- Pathogenic ectoparasites (e.g., ticks or mites)
- *Borrelia* sp.
- *Rickettsia* sp.

Intranuclear coccidia (Atkinson and Ayala 1987, Jacobson et al. 1994, Garner et al. 1998, Garner et al. 2006, Innis et al. 2007) were not specifically included in the risk analysis for the following reasons:

- The agents involved have not been documented in desert tortoises or any other native North American chelonians.
- The prevalence is very low in all host species so far identified.
- There is no antemortem screening test. As a result, pre-translocation screening is not possible.
- The agents can be detected postmortem, so routine opportunistic postmortem surveillance is the method of choice for detecting the agents in a population.
- Most chelonian cases reported so far have presented with clinical signs that would result in exclusion of infected animals from release cohorts anyway.
- In the absence of data on the prevalence of these agents in North America, the risk of introduction can only be addressed in general terms and is covered in the section on novel agents, subspecies, or strains.

The workgroup also evaluated the risk of novel pathogen introductions. Pathogens that were recognized after the workgroup's analysis are listed in Appendix 1 to be considered in updates to the analysis.

Hazard Analysis

The workgroup assessed the risks associated with each agent (or category of agent) by assigning a qualitative probability assessment (very low, low, medium, high, very high, or variable) for each of the following steps involved in the introduction and establishment of a pathogen in a population:

- Probability the agent is present in the source population
- Probability the agent is absent from the destination population
- Probability that translocation will be the only source of exposure
- Probability of release and spread
- Probability of establishment
- Probability of negative population consequence

Some of the information that was available to the workgroup through, reports, publications, and personal knowledge is listed in Table 1.

Table 1. Information that was available to the workgroup through reports, publications, and personal knowledge (see Fig. 1 for locations of most of the listed locations). Citations that have dates after the workshop were available as drafts, and/or workshop participants shared knowledge of the data. Cells in grey had no positive detections. The table does not include observations of clinical signs without other diagnostic tests and does not include recovery permit reports prior to 2005.

Site	<i>Mycoplasma agassizii</i>	<i>Mycoplasma testudineum</i>	Citation
Upper Virgin River			
Red Hill (St. George)	1/1 <i>Mycoplasma</i> -like organism present		Jacobson et al. 1991
Red Cliffs (St. George)	22/30 Western blot+ ¹		Sandmeier et al. 2013
Paradise Canyon, Utah	ELISA+ found		Dickinson et al. 1995, 2005
Northeastern Mojave Desert			
Beaver Dam Slope	1/7 Western blot+ ¹		Sandmeier et al. 2013
Coyote Springs	0/11 Western blot+ ¹		Sandmeier et al. 2013
Coyote Springs/Hidden Valley	0/23 ELISA+ 0/22 PCR+ MySpp.	1/23 ELISA+	Drake et al. 2013; Esque 2013
Gold Butte	2/13 Western blot+ ¹		Sandmeier et al. 2013
Halfway Wash	0/12 ELISA+ 1/13 PCR+ MySpp.		Esque 2013
Littlefield, Arizona	ELISA+ found		Dickinson et al. 1995, 2005
Las Vegas Valley, NE	10/19 Western blot+ ¹		Sandmeier et al. 2013
Las Vegas Valley, NW	7/18 Western blot+ ¹		Sandmeier et al. 2013
Mormon Mesa	0/35 Western blot+ ¹		Sandmeier et al. 2013
Muddy Mountains	0/18 Western blot+ ¹		Sandmeier et al. 2013
River Mountains	9/19 ELISA+		USFWS, unpubl. data
Eastern Mojave Desert			
Amargosa Valley	0/11 Western blot+ ¹		Sandmeier et al. 2013
Eldorado Valley	1/46 Western blot+ ¹		Sandmeier et al. 2013
Ivanpah Valley (CA)	0/4 <i>Mycoplasma</i> -like organism present		Jacobson et al. 1991
Ivanpah Valley (CA)	ELISA+ found	ELISA+ found	ISEGS 2010, 2011, 2012
S Ivanpah Valley (CA)	0/13 Western blot+ ¹		Sandmeier et al. 2013
N Ivanpah Valley (NV)	6/32 Western blot+ ¹		Sandmeier et al. 2013

¹ Different antibody test than other studies (Western blot, with polyclonal reagent), which used the test from University of Florida; sampled populations are also typically larger scales than other studies.

² Examined after being held in pens at DTCC. Jacobson et al. state that intermixing of infected and uninfected likely occurred and caused spread.

Table 1. Continued.			
Eastern Mojave Desert, continued			
Site	<i>Mycoplasma agassizii</i>	<i>Mycoplasma testudineum</i>	Citation
Las Vegas Valley ²	12/24 culture+ 17/24 ELISA+		Jacobson et al. 1995
Las Vegas Valley	72/144 ELISA+		Schumacher et al. 1997
Las Vegas Valley, south	15/30 Western blot+ ¹		Sandmeier et al. 2013
Nevada National Security Site	0/7 ELISA+	1/7 ELISA+	Field et al. 2012
Yucca Mtn.	15-23% ELISA+ (n = 49-91)		Lederle et al. 1997
Pahrump Valley	1/8 Western blot+ ¹		Sandmeier et al. 2013
W of Providence Mtns	0/13 Western blot+ ¹		Sandmeier et al. 2013
Shadow Valley	0/15 Western blot+ ¹		Sandmeier et al. 2013
Colorado Desert			
Chemehuevi plot	0/10 ELISA+	0/10 ELISA+	Berry 2011
Chemehuevi	0/45 Western blot+ ¹		Sandmeier et al. 2013
Chuckwalla Bench	0/44 Western blot+ ¹		Sandmeier et al. 2013
Pinto Mountains	0/24 Western blot+ ¹		Sandmeier et al. 2013
Piute Valley	0/21 ELISA+ 0/3 PCR+ MySpp.		Esque 2013
Piute Valley	1/73 Western blot+ ¹		Sandmeier et al. 2013
E of Providence Mtns	0/33 Western blot+ ¹		Sandmeier et al. 2013
Western Mojave Desert			
DTNA	3/8 <i>Mycoplasma</i> -like organism present		Jacobson et al. 1991
DTNA	7-62% ELISA+ (n = 12-35)		Berry 1997, Brown et al. 1999
Daggett	21.7-25.0% ELISA+ (n = 92-104) 0/92 PCR+	6.5-45.2% ELISA+ (n = 92-104) 0/92 PCR+	Berry 2010
Daggett	13.8-14.0% ELISA+ (n = 64-65)	18.5-18.8% ELISA+ (n = 64-65)	Berry 2010
Daggett	10.8-12.9% ELISA+ (n = 65-70)	17.1-21.5% ELISA+ (n = 65-70)	Berry2012
Daggett	11.8-11.9% ELISA+ (n = 67-76)	13.4-14.5% ELISA+ (n = 67-76)	Berry 2012
Daggett	5/66 ELISA+	13/66 ELISA+	Berry 2013
Fremont/Kramer valleys	1/17 Western blot+ ¹		Sandmeier et al. 2013
Ord-Rodman	0/15 Western blot+ ¹		Sandmeier et al. 2013
Ft. Irwin	2/91 ELISA+ 1/91 culture+		Berry et al. 2006
Ft. Irwin 2012	1/3 ELISA+		Esque 2013

Table 1. Continued.

Western Mojave Desert, continued			
Site	<i>Mycoplasma agassizii</i>	<i>Mycoplasma testudineum</i>	Citation
Ft. Irwin 2012	1/33 PCR+		Esque 2013
Ft. Irwin/Superior-Cronese	3 (7?)/699 ELISA+	9/699 ELISA+	Ft. Irwin 2008
Ft. Irwin West	1/55 ELISA+ 0/56 culture+ 1/171 ELISA+	0/54 ELISA+ 0/56 culture+ 0/171 ELISA+	Ft. Irwin 2010
Ft. Irwin West	0/52 ELISA+	0/52 ELISA+	Ft. Irwin 2011
Superior-Cronese	2/11 ELISA+	9/11 ELISA+ 2/11 PCR	Jacobson and Berry 2012
Superior-Cronese	2/31 Western blot ¹		Sandmeier et al. 2013
Superior-Cronese trans.	1/81 ELISA+ 3/65 ELISA+	1/81 ELISA+ 0/65 ELISA+	Ft. Irwin 2009 report
Superior-Cronese trans.	0/12 ELISA+	1/12 ELISA+	Ft. Irwin 2010 report
Sand Hill	1/11 ELISA+ 0/11 culture+	0/11 ELISA+ 0/11 culture+	Berry 2011

Site	Tortoise herpesvirus	Citation
Northeast Mojave Desert		
River Mountains	1/1 PCR+ TeHV2	Jacobson et al. 2012
Coyote Springs 2011	0/21	Esque 2013
Coyote Springs 2012	0/21	Esque 2013
Halfway Wash 2011	0/12	Esque 2013
Hidden Valley	0/24 PCR+ TeHV2	Drake et al. 2013
Eastern Mojave Desert		
Shadow Valley	2/2 ELISA+	Jacobson et al. 2012
Ivanpah Valley 1	1/14 ELISA+	Jacobson et al. 2012
Ivanpah Valley 2	21/25 ELISA+	Jacobson et al. 2012
Colorado Desert		
Fenner	0/2 ELISA+	Jacobson et al. 2012
Upper Ward Valley	0/12 ELISA+	Jacobson et al. 2012
Chemehuevi Valley	2/6 ELISA+	Jacobson et al. 2012
Chocolate Mountains	9/21 ELISA+	Jacobson et al. 2012
Piute Valley 2011	0/22	Esque 2013
Piute Valley 2012	0/21	Esque 2013
Western Mojave Desert		
Fremont Valley	0/1 ELISA+	Jacobson et al. 2012
DTNA	4/18 ELISA+	Jacobson et al. 2012
Fremont-Kramer	6/9 ELISA+	Jacobson et al. 2012

Table 1. Continued.		
Western Mojave Desert, continued		
Site	Tortoise herpesvirus	Citation
Superior-Cronese	1/1 PCR+TeHV2 5/12 ELISA+	Jacobson et al. 2012
Ft. Irwin 2012	0/2	Esque 2013
Ft. Irwin	2/3 ELISA+	Jacobson et al. 2012
Soda Mountains	0/10 ELISA+	Jacobson et al. 2012
Tiefert, Ft. Irwin	2/27 ELISA+	Jacobson et al. 2012
Eastgate, Ft. Irwin	0/9 ELISA+	Jacobson et al. 2012
Lucerne Valley	1/6 ELISA+	Jacobson et al. 2012
Ord-Rodman	10/25 ELISA+	Jacobson et al. 2012
Sand Hill	10/40 ELISA+	Jacobson et al. 2012
Bullion	3/6 ELISA+	Jacobson et al. 2012
Lavic	1/8 ELISA+	Jacobson et al. 2012
Daggett	0/34 ELISA+	Berry 2013

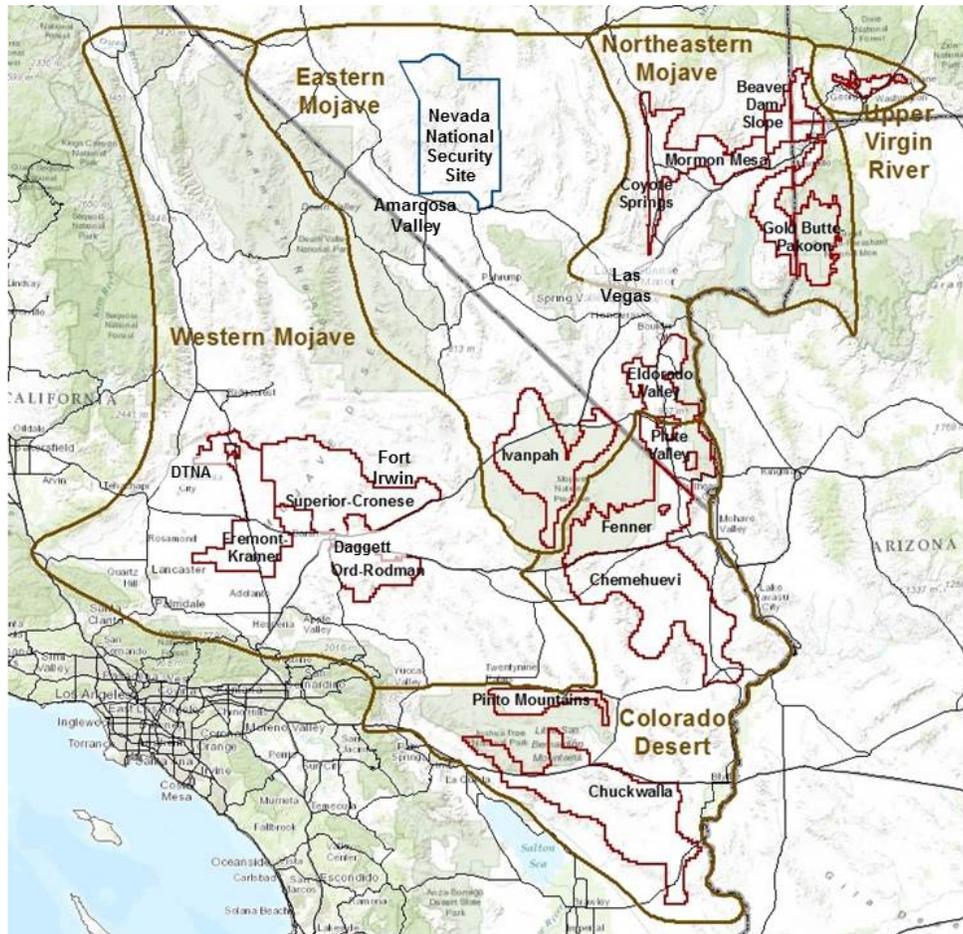


Figure 1. Mojave Desert Tortoise recovery units (outlined and labelled in brown) and other geographic locations listed in Table 1.

The cumulative risk associated with each agent was developed by consensus based on the understanding that the overall probability of a negative outcome is determined by multiplying the probabilities of each step in the sequence leading to that outcome. Although qualitative rather than numerical values were used (so actual multiplication was not possible), the same concept was applied in a commonsense way in this risk assessment (e.g., a low probability multiplied by a high probability would yield a medium probability). The workgroup decided that overall tolerance to risk was low. Table 2 summarizes the analysis for each agent. The following explains the rationale behind each risk assignment.

Mycoplasma agassizii: Although spatial patterns in levels of seroprevalence to *M. agassizii* have been documented, with higher prevalence in close proximity to some urban centers (Schumacher et al. 1997, Jones 2008, Berry et al. 2015), this agent or antibodies to it have been found across the Mojave Desert in all recovery units. It has been found in many wild populations that have been adequately surveyed, so the probability of presence in any source population was considered very high, while the probability that it would be absent in a destination population was considered low. The widespread prevalence of the agent in the wild, as well as uncontrolled movements and releases of desert tortoises by the public, resulted in a low probability that translocations would be the only source of exposure. If the agent were released into a naïve population, the probability of release, spread, and establishment of the agent was considered very high due to its highly contagious nature and ability to persist in a population. The probability of a negative population consequence if introduction occurred to a naïve population was considered high in a high population density scenario, but low in a low population density scenario. In general for mycoplasmas, the presence of antibodies does not necessarily confer immunity (Simecka et al. 1989, Simecka 2005, Szczepanek and Silbart 2014), and immune responses to *M. agassizii* vary in tortoises (see review in Jacobson et al. 2014). The cumulative risk was determined to be medium for a high population density scenario, but low for a low population density scenario.

Mycoplasma testudineum: Although fewer data are available for this agent, the lesions may be less severe than those caused by *M. agassizii*, and the agent may be less pathogenic than *M. agassizii* (Jacobson and Berry 2012), the risk analysis was similar to that for *M. agassizii*. Exceptions are that the probability of a negative population consequence was considered medium for a high population density scenario, and very low for a low population density scenario. The cumulative risk was therefore considered low for a high-density situation and very low for a low-density situation.

Tortoise herpesvirus-2 (TeHV-2): The probability of TeHV-2 being present in a source population was considered high for the following reasons: TeHV-2 has recently been found in wild tortoises from disparate locations (Jacobson et al. 2012); TeHV-2 has only been found in desert tortoises; a herpesvirus was first documented in a desert tortoise 30 years ago (Harper et al. 1982), so it or a similar virus has been present in desert tortoises for at least three decades; and herpesviruses are endemic agents in a very wide range of Testudinidae taxa (probably a majority of taxa) globally (Marschang 2011). In addition, 8 tortoises in the Fenner Valley and 2 tortoises in the Ivanpah Valley, California, had oral lesions consistent with herpesvirus in 1992–93 (Christopher 2003). Thus, there is a strong

possibility the agent is endemic in desert tortoises. Note that ELISAs for TeHV-1 and TeHV-3 are cross-reactive for TeHV-2. While desert tortoises have tested positive for TeHV using the TeHV-3 test, TeHV-3 has not been identified in desert tortoises through PCR identification methods. Therefore, we chose to identify TeHV-2 as the agent being assessed. The probabilities that the agent would be absent from destination populations and that translocations would be the only source of exposure were considered low for the same reasons. If infected tortoises were released into a naïve population, the probabilities of release, spread, and establishment were considered very high because of the highly transmissible nature of herpesviruses. However, the probability of a negative population consequence was considered low because TeHV-2 is known to be present at the DTCC, but has only caused sporadic mortality despite the relatively high density of tortoises at the facility (Josephine Braun, personal communication).

Chlamydophila sp.: The probability of a *Chlamydophila* sp. being present was considered low for a wild population but medium for the DTCC population because the agent has not been found in wild populations (although no specific surveillance has been conducted), but has been found in at least one individual housed at the DTCC and several others originating from there (Johnson et al. 2012). The probability that translocations would be the only source of introduction was considered low because of the frequency of unsanctioned pet tortoise releases and the likelihood that the agent is more prevalent than previously recognized. If an infected tortoise was released into a naïve population, the probability of release and spread was considered low because of the relatively low incidence rate in exposed populations, but the probability of establishment was considered high because the agent tends to persist at low levels in exposed populations. The probability of a negative population consequence was considered very low for a low-density situation, but medium for a high population density situation. The cumulative risk was considered very low.

Pasteurella testudinis: The probability that *Pasteurella* would be present in source populations was considered very high based on previous publications (Jacobson et al. 1995, Snipes et al. 1995, Christopher et al. 2003). The probability of absence in a destination population was considered very low for the same reason. The probability that translocations would be the only source of exposure was also considered low, while the probabilities of release, spread, and establishment were considered very high if a naïve population were exposed. A negative population consequence was considered highly probable in a high-density situation, but low in a low-density one. The cumulative risk was therefore considered medium for a high-density scenario and very low for a low-density scenario.

Salmonella spp.: *Salmonella* species are considered normal flora in all reptiles (Jacobson 2007b), but can be opportunistic pathogens. The risk analysis therefore had to incorporate issues relating to factors that might predispose to opportunistic invasion, which the workgroup recognized was fraught with difficulty due to the large number of highly speculative scenarios that could be considered. Ultimately, the probability of a negative population consequence, and the cumulative risk, were considered very low because of the benign nature of the host-agent relationship.

Cryptosporidium spp.: *Cryptosporidium* species are globally-distributed, microscopic parasites that primarily inhabit the gastrointestinal tract of a wide variety of species, ranging from fish to humans. Infections are typically asymptomatic, but can be fatal in some hosts under certain circumstances. Cryptosporidia have been found in 5/369 (1.4%) tortoises necropsied at the DTCC, but their association with disease remains to be clarified. Although many species appear to have their own host-adapted *Cryptosporidium* sp., host switching has been documented, indicating that these parasites may have relatively broad host-ranges. Tortoises held as pets would have the greatest potential for acquiring cryptosporidia from other hosts, but the broad host range and ubiquitous nature of these parasites could also result in exposure of wild tortoises from other host species (e.g., through water sources contaminated by feces from other reptiles, birds, or mammals). Cryptosporidia have not been documented in wild desert tortoises, but there has been no targeted surveillance in the past. Based on the broad distribution of these parasites in other species, it is possible that cryptosporidiosis is endemic at low levels in desert tortoises. A literature review fails to provide any evidence of negative population-level impacts of cryptosporidiosis in wildlife. In light of these facts, a risk analysis yields a low to medium cumulative risk (see Table 2).

Plausible hazards: The following agents were considered plausible hazards, but have not yet been documented in desert tortoises: Adenoviruses, Iridoviruses (Ranaviruses), Paramyxoviruses, *Borrelia* spp., *Rickettsia/Ehrlichia* spp., and various pathogenic nematodes and protozoa. These agents were evaluated as a group, in part because of lack of data on the prevalence or impacts of such agents on desert tortoises. The probability of the presence of these agents in source populations was considered uniformly low. Postmortem surveillance on over 350 cases from the DTCC has not shown any evidence of these agents. The probability of absence in destination populations was considered correspondingly high. The probability that translocations would be the only avenue of exposure was considered low because the highest risk of exposure would probably come through unregulated release of pet tortoises. The probabilities of release, spread, establishment, and negative population consequences were generally considered low for all of the agents. The cumulative risk was ultimately considered low for all.

Novel agents, subspecies, or strains: The probability of a novel organism being present in a wild source population was considered very low, but medium for the DTCC population due to the unknown exposure histories of privately held tortoises. The probability of absence of novel agents in destination populations was considered very high. The likelihood that translocations from wild source populations would be the only avenue of exposure was considered low because the highest risks would be coming from unregulated releases of pet tortoises. The probability of release, spread, establishment, and negative population consequences would depend on the nature of the agent. As a result, the cumulative risk was determined to be variable and unpredictable. However, it is important that risk mitigation efforts focus on known pathogens that have high population-level impacts rather than undocumented or hypothetical risks (IUCN/SSC 2013), as there are no risk-free scenarios, and inaction as the result of endless what-ifs could result.

Table 2. Risk analysis for each agent. In columns 7 and 8 High D and Low D refers to density.

<u>Agent or hazard</u>	<u>Probability in source population (Wild v. DTCC?)</u>	<u>Probability of absence in destination population</u>	<u>Probability that translocations are only exposure avenue</u>	<u>Probability of release and spread</u>	<u>Probability of establishment</u>	<u>Probability of negative population consequence</u>	<u>Cumulative Risk</u>	<u>Comments</u>
Known Transmissible Hazards								
<i>M. agassizii</i>	VERY HIGH	LOW	LOW	VERY HIGH	VERY HIGH	High D: HIGH Low D: LOW	High D: MEDIUM Low D: LOW	A remote naïve population may be at high cumulative risk in a high density scenario and low risk in low density.
<i>M. testudineum</i>	VERY HIGH	LOW	LOW	VERY HIGH	VERY HIGH	High D: MEDIUM Low D: VERY LOW	High D: LOW Low D: VERY LOW	
TeV-2	HIGH	LOW	LOW	VERY HIGH	VERY HIGH	LOW	VERY LOW	Most taxa have multiple endemic herpesviruses.
<i>Chlamydophila</i>	Wild: VERY LOW DTCC: MEDIUM	VERY HIGH	LOW	LOW	HIGH	High D: MEDIUM Low D: VERY LOW	DTCC source: High D: LOW Low D: VERY LOW Wild source: High D: VERY LOW Low D: VERY LOW	
<i>Salmonella</i>	VERY HIGH	VERY LOW	VERY LOW	VERY HIGH	N/A	VERY LOW	VERY LOW	Very low risk to desert tortoises, but there may be other susceptible species (kit fox).
<i>Pasteurella</i>	VERY HIGH	VERY LOW	LOW	VERY HIGH	VERY HIGH	High D: HIGH Low D: LOW	High D: MEDIUM Low D: VERY LOW	
<i>Cryptosporidium</i>	Wild: VERY LOW DTCC: LOW	Wild: MEDIUM DTCC: LOW	LOW	HIGH	HIGH	High D: LOW Low D: VERY LOW	High D: MEDIUM Low D: LOW	Ubiquitous global distribution; possibly endemic at low levels.
Plausible Transmissible Hazards								
<i>Borrelia</i>	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	
<i>Rickettsia/Ehrlichia</i>	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	

Table 2. Continued.

<u>Agent or hazard</u>	<u>Probability in source population (Wild v. DTCC?)</u>	<u>Probability of absence in destination population</u>	<u>Probability that translocations are only exposure avenue</u>	<u>Probability of release and spread</u>	<u>Probability of establishment</u>	<u>Probability of negative population consequence</u>	<u>Cumulative Risk</u>	<u>Comments</u>
Adenovirus	LOW	HIGH	LOW	LOW	LOW	LOW	LOW	
Iridoviruses/Ranavirus	LOW	HIGH	LOW	MEDIUM	LOW	LOW	LOW	
Paramyxovirus	LOW	HIGH	LOW	MEDIUM	LOW	LOW	LOW	
Novel agents or ssp. and strains	Wild: VERY LOW DTCC: MEDIUM	VERY HIGH	LOW	VARIABLE	VARIABLE	VARIABLE	VARIABLE	

Risk Mitigation

The degree of mitigation should match the threat. In a context of low risk, yet potential conservation benefit, it is important to implement mitigation that allows for action rather than inaction caused by logistics, expenses, or rejection of the risk analysis. In addition, some protocols that may be suggested to mitigate disease risk could actually impose other risks on the species due to increased handling and collection of samples or an inability to swiftly implement conservation actions. For example, some techniques for the collection of biological samples are quite invasive (e.g., nasal lavage, cloacal swab) and may induce detrimental processes in tortoises such as voiding stored water from the bladder. In the wild, not all tortoises will be located subsequent to sampling, thus the ability to mitigate for any negative effects of sampling are limited. Sampling techniques of undetermined value may be appropriate to consider for captive tortoises that have regular follow-up exams when it provides an opportunity to evaluate the techniques.

Our previous strategy was two-fold. First, individual suitability for translocations was evaluated based on attitude and activity, body condition score (Lamberski 2013), clinical signs of disease (primarily nasal discharge and oral lesions), and other notable conditions. Second, ELISA test results (*M. agassizii* and *M. testudineum*) were used in combination with prevalence of clinical signs to translocate “like to like”, wherein we translocated seropositive tortoises in a similar proportion to what was found in the recipient population. Recipient and donor populations could be disqualified if total disease prevalence was determined to be higher than 20%. One option was to continue this approach.

We opted to modify our approach to risk mitigation. The individual-level evaluations, which have been designed to alert us to health issues regardless of cause, remain much the same and still include the collection of biological samples (USFWS 2013, Appendices B and G; see Attachments 1, 2, and 3); however, the population-level strategy will no longer take into account ELISA or other test results in an attempt to match disease prevalence, and no limit of disease prevalence is specified. While a limit for disease prevalence is not specified in our new strategy, this strategy will not apply if there is evidence of an active outbreak in the source or destination populations. Review of health assessment data prior to translocation will help to ensure that potential outbreaks are recognized. Our rationale is that for nearly all agents, the likelihood of presence/absence in the source and destination populations is quite similar, the probability that planned translocations are the only avenue of exposure is low to very low for all agents, and estimated cumulative risk rises to the level of medium in only two cases that are associated with populations at high density (Table 1). In fact, negative population consequences are plausible in high-density populations for reasons other than disease, which includes availability of resources. USFWS does not allow for translocations into high-density populations and as of this report requires that post-translocation densities be limited to similar levels observed elsewhere within the particular region (as measured by the upper standard deviation of the relevant estimated density; USFWS 2012a). Depleted populations are targeted for augmentation with former captives, thus we have already mitigated density-related risks.

All captive tortoises used for population augmentation must continue to enter a period of quarantine prior to final evaluation of their suitability for translocation (Attachment 4). Quarantine periods are consistent with recommendations by the IUCN (IUCN/SCC 2013) and OIE (Woodford 2001). For any captive-to-wild translocations, multiple health assessments should be conducted during the quarantine period prior to release. Only animals that are documented to be free of clinical signs of disease on multiple consecutive evaluations should be considered eligible for release into wild populations (Attachment 2). This strategy will help identify animals with an infectious disease (such as mycoplasmosis) that may manifest intermittent clinical signs.

For wild-to-wild-translocations, bringing animals into captivity for a period of quarantine may be more risky to the animals' health than the overall benefit provided. Rather, we support two in situ health assessments completed 14 - 30 days apart. Additional assessments (outside of 30 days) may be conducted, but a narrow window is necessary to discover animals with intermittent clinical signs. The last assessment should occur immediately prior to the proposed translocation date and may be a physical assessment without another collection of biological samples. The same evaluation of suitability for translocation applies as for captives (Attachment 2). Additional details about how our strategy addresses the specific agents are provided below. Note that comments regarding the DTCC were relevant prior to the closure of that facility and that ultimately three health assessments were completed within 30 days at the DTCC to ensure translocated animals had no signs of disease.

All tortoises to be translocated, as well as a representative sample of resident and control tortoises where applicable, will have blood collected for use in ELISA tests for *M. agassizii* and *M. testudineum*. The results of ELISA tests will give us baseline information for comparison of pathogen seroprevalence over time, but not determine eligibility for translocation. For wild tortoises, a sample will be run immediately and the remainder of the plasma will be banked for future use. For DTCC tortoises, a sample will be run immediately only if no prior ELISAs have been conducted, and all remaining plasma will be banked. DTCC tortoises with clinical signs of upper respiratory tract disease will continue to be treated with antibiotics under veterinary supervision/advisement, and those that are not responsive to treatment may be euthanized.

In addition, all tortoises to be translocated, as well as a representative sample of resident and control tortoises where applicable, will have their oral cavity swabbed and the sample banked for future use. Current uses include the detection of herpesvirus via PCR, and these samples have been shown to be comparable to those collected via nasal lavage when PCR is used to detect *M. agassizii* (Josephine Braun, unpublished; post-workshop: Braun et al. 2014). The health assessment includes examination of the oral cavity, and tortoises with plaques, crusts, or ulcers, regardless of cause, are not recommended for translocation.

One technique that *might* be informative is the collection of a sample via insertion of a swab into the cloaca; however, no studies have been done to determine the value of the technique in detecting pathogens significant to desert tortoises and the technique is likely to increase the risk of a tortoise voiding its bladder. Voiding the bladder has been

documented to increase the likelihood of death (Averill-Murray 2002). Cloacal swabs may be collected from DTCC tortoises in order to evaluate the efficacy of the technique. The collection of biological samples in attempt to screen for pathogens (that would include *Chlamydomphila* sp.) in wild tortoises is not recommended at this time, unless under experimental conditions. *Chlamydomphila* sp. has not been detected in wild tortoises that have been screened, and the efficacy of a technique for collection of informative samples has not been fully evaluated.

Fecal parasite and protozoa screening (via fecal flotation, fecal acid fast stain, and/or the use of a commercial test) is another method of surveillance that could be implemented to detect agents such as *Cryptosporidium*. At this time, we recommend surveillance only in captive tortoises, as *Cryptosporidium* has only been found in tortoises at the DTCC and no other pathogenic agents have been detected using fecal flotation and microscopic evaluation. The risk-matching approach should provide adequate risk mitigation for wild-to-wild translocations.

Postmortem examinations are useful in the surveillance for adenoviruses (Rivera et al. 2009, Schumacher et al. 2012, Doszpoly et al. 2013), iridoviruses (Ranaviruses) (Westhouse et al. 1996), paramyxoviruses (Marschang et al. 2009), *Borrelia* spp., *Rickettsia/Ehrlichia* spp., and similar agents. Opportunistic postmortem examinations of wild tortoises are highly recommended when carcasses are discovered in suitable condition. Postmortem exams are recommended for all DTCC tortoises that die, although we understand that limited resources may only allow for a subset to be examined. Postmortem disease surveillance in the DTCC population resulted in over 350 detailed examinations with no evidence of novel agents (Josephine Braun, personal communication). If any such agents are identified, risk mitigation strategies will have to be developed based on the findings and context of the management actions.

Pasteurella testudinis and *Salmonella* spp. will not be screened for in any of the samples at this time. *P. testudinis* is not considered a primary pathogen (Jacobson et al. 1991, Dickinson et al. 2001), and because it can readily be isolated from healthy desert tortoises, surveillance for this agent would not provide useful information for decision-making. There are numerous serotypes of *Salmonella* with varying levels of pathogenicity. *Salmonella* is a normal part of the intestinal flora of most, if not all, reptiles (Jacobson 2007), and illness from *Salmonella* in reptiles is most commonly associated with bacterial overgrowth during periods of stress or other disease.

Rigorous quarantine periods, repeated health assessments, and necropsies (especially of captive tortoises that had been intended for use in population augmentation) will minimize chances of a novel agent going undiscovered. While possible, novel agents are less likely in wild-to-wild translocations, and no mitigation strategies beyond the repeated health exams will be implemented at this time.

In conjunction with risk-mitigation measures associated with structured, planned translocations, wildlife management agencies should take measures to reduce the risks associated with unauthorized releases to the wild by reducing the segment of the captive

tortoise population that is held by the public. In particular, captive breeding should be discouraged or prohibited unless conducted specifically in conjunction with the recovery program and in compliance with USFWS controlled-propagation policies (USFWS and National Oceanic and Atmospheric Administration 2000).

Implementation and Review

The recommendations in this assessment have begun to be implemented. Implementation occurs through revisions to the translocation guidance (USFWS 2012a), health assessment handbook (USFWS 2013, 2015) and training program, and through permitting of projects.

While we have taken care to conduct a high-quality qualitative risk assessment with responsible risk tolerance and mitigation choices, we must plan for unexpected outcomes. If a translocation appears to result in a disease outbreak, translocation of additional tortoises at that site should cease. Subsequent action will be dependent on the nature of the outbreak. Focused data collection on the health status of tortoises in the population should be undertaken for evaluation and identification of specific corrective actions as recommended by a panel of individuals with expertise in wildlife disease and management.

By requiring monitoring of translocations (USFWS 2012a), information will be collected to test our assumptions and hypotheses and to further inform us about disease dynamics. With new information (e.g., virulence of strains, discovery of novel agents, etc.), we can review the risk assessment and make updates where necessary. New information should be reviewed for inclusion in formal updates to the risk assessment. Should potentially severe disease issues arise prior to formal updates to the analysis, those situations should be addressed immediately and the translocation and monitoring plans updated accordingly.

Risk Communication

We will communicate our assessment of risk via this report. Prior to finalizing the report, we solicited peer review. Several prominent disease and epidemiology experts with experience in desert tortoises were not part of our original panel of experts that conducted the assessment. Intentionally, we wanted people from this specific area of expertise to be able to provide critical review of our process and decisions. We requested review (but did not receive in all cases) from these individuals in addition to others with pertinent expertise. We revised the report based on comments from the reviewers; the comments and responses are on file with the USFWS. We also engaged reviewers in further discussion in some situations.

The final report will be made widely available, such that the contents can be considered by those making decisions relative to translocation of desert tortoises. Specifically, representatives from the BLM and state wildlife agencies will be briefed on the report and given copies for reference. We plan to prepare the contents of this report for submission to a peer-reviewed journal for publication.

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The individuals listed below provided review of this report and/or our risk analysis process. Reviews varied greatly in breadth, depth, and opinion. A summary of comments received and responses is available from the U.S. Fish and Wildlife Service upon request. We thank J. Foley, A. Greenwood, E. Jacobson, J. Mendelson, T. Norton, J. Pramuck, P.K. Robbins, A. Routh, M. Uhart, L. Woolaver, and an anonymous reviewer.

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APPENDIX 1. TRANSMISSIBLE INFECTIOUS AGENTS RECOGNIZED AFTER WORKSHOP

Herpesvirus

The new herpesvirus was reported to be in the Betaherpesvirinae family and was found in a desert tortoise from Fort Irwin, CA. Information provided by Kristin Berry at the USGS/FWS co-lab meeting on 16 July 2013. Wellehan et al. (2014) also reported this novel herpesvirus at the 2014 Desert Tortoise Council Symposium. They indicated that the pathogen was identified via PCR from oral swab samples of a translocated tortoise.

Mycoplasma

A novel mycoplasma was identified from a phallic sample of a translocated tortoise (Fort Irwin, CA?) by Wellehan et al. (2014).

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ATTACHMENT 1

From: U.S. Fish and Wildlife Service. 2013. Health assessment procedures for the desert tortoise (*Gopherus agassizii*): a handbook pertinent to translocation. Desert Tortoise Recovery Office, U.S. Fish and Wildlife Service, Reno, Nevada.

Note: The 2015 revision (post-DTCC closure) has several revisions including removal of the word “normal” from the list of choices in the fields.

www.fws.gov/nevada/desert_tortoise/dtro/dtro_trans.html

Appendix B: Completing the Health Assessments and Data Collection Form

This appendix provides an overview for conducting a physical examination of a desert tortoise and completing the data collection form, but it is not a substitute for appropriate training and hands-on experience. A thorough and accurate physical examination can only be performed if the individual performing the exam has knowledge of normal tortoise physical appearance and behavior. Prior training in assessing desert tortoise health is required. Physical examinations provide valuable information about the health of individual tortoises and insight into population level issues. Because the desert tortoise is a long-lived species, it is extremely important to use standardized techniques for data collection so that information can be compared over time.

This appendix provides a description of how to conduct a basic physical examination of a desert tortoise and follows the order of the provided data collection form. A minimum of two people will be needed in order to conduct a full health assessment that includes the collection of biological samples. One person will serve as the examiner and will also complete the data collection form. This person should be able to stay clean (i.e. not touch the tortoise or contaminated items) until he/she begins to collect the biological samples. The other person (handler) will handle the tortoise such that the examiner can complete the examination and collect samples. If a third person is available, this person (rather than the examiner) may record the data to further reduce contamination risks. It is important to complete certain observational aspects of the physical exam first, so that potential clinical signs are not affected by handling of the animal. For example, head restraint may cause eye bulging or serous discharge so it should be done after initial evaluation. Most of the health assessment can be conducted with minimal, if any, handling of the tortoise. We recommend using the order described below for a systematic health evaluation of every animal. Generally, the tortoise will be evaluated from a distance before direct contact (far to near), beginning at the head and working towards the tail (head to tail), and least invasive procedures will be conducted prior to more invasive procedures (least to most). This approach also helps field staff remember to collect all of the data consistently because they fill out the form as they proceed with the exam. Be sure to carefully describe all anomalies such that comparisons can be made with subsequent examinations. Handling a tortoise for health assessment and sample collection must be completed within 30 minutes or less. This time period does not include rehydrating tortoises that void.

Unless tortoises are being moved into a quarantine holding facility for their health assessment, they should not be moved far (<20 m, if possible) from the location at which they are found in the field. Before the handler touches the tortoise, find a flat shaded area

with ample work space where you will not disturb a tortoise's shelter sites. If shade is not available, it can be created by placing a lightweight cloth over vegetation. Be sure to record the temperature where you plan to work to ensure that it falls within the requirements established by USFWS for handling tortoises. If the temperature is appropriate, lay out equipment in the order that it will be used and place it out of the reach of where you will place the tortoise. Depending on the substrate, the top few centimeters of soil can be cleared away, such that the tortoise is in contact with cooler soil while you work. Prepare all the biological sampling equipment and supplies and set them up in an orderly manner for easy access during the health assessment. An area on one side of the examiner should be designated for clean supplies and equipment and an area on the other side for contaminated items. After taking pertinent initial data (date, start time, project name, site description, tortoise ID, transmitter frequency if applicable, GPS location, temperature, and full names of the biologists), record the time at which you approach the tortoise and are within 2 meters of it. Take care not to startle the tortoise as you approach it. While wearing gloves, the handler should gently lift the tortoise a short distance off the ground by grasping the sides of the shell and slowly carry it to the shaded area while maintaining it close to the ground in its normal spatial orientation.

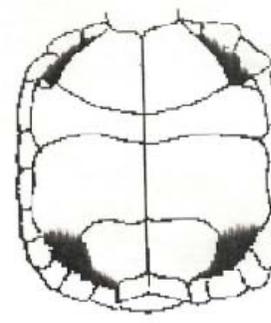
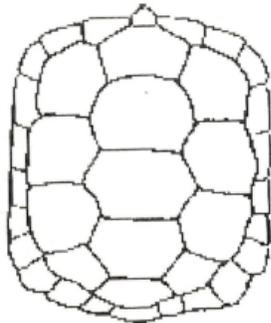
While placing the tortoise on the ground in the work area, be sure that the tortoise does not touch any of the supplies or equipment. The examiner should perform as much of the visual observation portion of the health assessment as possible before the handler holds the tortoise again. Note that when completing the data collection form, right and left refer to the tortoise's right and left sides (not right and left from the examiner's perspective if looking at the tortoise head-on). Descriptions of how to complete each data field on the data collection form follow the presentation of the actual form below.

Desert Tortoise Health Assessment (HA) Data Collection Form – Solar Projects

U.S. Fish and Wildlife Service April 2013

Date (ddmmmyy):		Start time (24h):		Project name:		Site description / current pen #:		Tortoise ID #:		Transmitter frequency:	
GPS datum:		UTM zone:		UTM easting:		UTM northing:		Temp °C:		Full name of biologist(s):	
HA start time:		Attitude/activity: Normal Lethargic/Weak		Respiration: Normal Abnormal sounds Increased effort				Beak: Normal Abnormal Evidence of foraging			
Left naris: Normal Asymmetrical Eroded Occluded			Left naris discharge and severity: None Serous: 1 2 3 Mucous: 1 2 3			Right naris: Normal Asymmetrical Eroded Occluded			Right naris discharge and severity: None Serous: 1 2 3 Mucous: 1 2 3		
Left eye: Normal Sunken Corneal opacity Partially closed Fully closed Serous discharge Mucous discharge Periocular swelling Periocular redness Conjunctival swelling Conjunctival redness					Right eye: Normal Sunken Corneal opacity Partially closed Fully closed Serous discharge Mucous discharge Periocular swelling Periocular redness Conjunctival swelling Conjunctival redness						
Skin lesion location: None Generalized Head Neck L/R forelimb L/R axillary region L/R hindlimb L/R prefemoral region Vent/tail				Condition of skin lesion(s): N/A Active Inactive		Coelomic cavity palpation: No mass L/R mass Not done		Shell characteristics: Compressible N/A Sunken +/- Scutes Peeling/flaking keratin Bone exposed			
Shell abnormality location: Carapace Plastron N/A		Shell abnormalities (describe below): None Localized Multifocal Generalized			Condition of shell abnormalities: N/A Active Inactive		If present, circumstances of skink/shell trauma: N/A Unknown Suspect canid bite Vehicle Other _____				
Sex: M F Unk		Initial weight: _____ (g)		Body condition score: 1 2 3 4 5 6 7 8 9			Photos (take all): ___Front face and body ___Left side face ___Right side face ___Carapace ___Plastron (take when measuring only if abnormal) ___Abnormalities				

Label and describe trauma, anomalies, lesions, missing body parts, and identifying features.



Ticks: 0 1-10 >10			Choana: Not examined Normal Pale Reddened			Tongue and oral mucosa: Not examined Normal Pale Reddened Crust Ulcers Plaques Hypersalivation Impaction			#oral swabs collected: _____								
Location: Soft tissue Seams Scutes Eyes Nares Beaks Collected? N/A Yes No Removed? N/A Yes No			Time of blood draw (24h):			Total sample volume (blood and lymph) collected: _____ (ml)			Total # hep tubes (number each): 0 1 2 3 4			Is the sample contaminated with lymph? Yes No Estimate the degree of lymph contamination: Small (1-10%) Moderate (11-29%) Severe (>30%)			Void during processing: None Urine/urates Feces		
Post void weight: N/A _____ (g)		MCL: _____ (mm)		Width V3: _____ (mm)		Height V3: _____ (mm)		Plastron: _____ (mm)		Hydration method: N/A Soak Nasal-oral Epicelomic Fluid type _____ Vol _____ (ml)			Post fluid weight: N/A _____ (g)				
End handling time (24h):		Disposition: Wild capture location Same pen New pen _____ Other _____			Blood processing time (24h):		Plasma color: Colorless Red Yellow Green		UFL plasma aliquots: 0 1		USFWS plasma aliquots: 0 1 2 3		Total tubes with RBCs saved: 0 1 2 3 4				

Total number of tubes/vials collected: _____

Revised April 4, 2013

Description of data fields on the data collection form

Date (ddmmmyy) – 2 digit day, 3 letter month, and 2 digit year (e.g. 04Mar11).

Start time (24h) – The time when the tortoise is located. This is not the time at which you begin assessing or touching the tortoise, but instead, it's the time at which you visually locate the tortoise. Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Project name – Name and phase of the project.

Site description/current pen # – If the tortoise is newly caught from the wild, provide a description of the capture location, or if the tortoise was already in captivity, provide the quarantine pen number.

Tortoise ID# - Each tortoise will be assigned a unique identification number from a series of numbers assigned by USFWS for that project. The tortoise may have been numbered prior to the health assessment or you may need to number it (refer to numbering protocols provided by the USFWS).

Transmitter frequency – Numbers of the radio transmitter frequency, if applicable. Usually includes 3 digits, a decimal, and 3 more digits (e.g. 164.020)

GPS datum – **Make sure your GPS is set to UTM, WGS 1984.** Datum is the model used to match the location of a feature on the ground to the coordinates of the feature on a map.

UTM zone – In the UTM system, the earth is divided into 60 zones of 6 degrees of longitude wide. Your GPS displays zone as 2 digit number (e.g. 11, 12 that may be followed by N denoting north of the equator).

UTM easting – 6 digit number displayed on your GPS. Easting is measured from the central meridian of the zone, which is given the value of 500,000 m, and increases as you travel east.

UTM northing - 7 digit number displayed on your GPS. Northing is measured relative to the equator, which is assigned a value of 0 in the northern hemisphere, and increases as you travel north.

Temp °C – Temperature in degrees Celsius measured 5 cm above the ground in the shade and protected from wind where you set up your equipment to process the tortoise, or as specified in the most recent USFWS Desert Tortoise Field Manual.

Full name of biologist(s) - First and last names of the biologists handling the tortoise and recording the data. List the Authorized Biologist primarily responsible for conducting the health assessment and biological sampling as the first name, followed by the name of the person assisting by handling the tortoise, then the name of person recording the data, if applicable.

HA start time – Health assessment start time. This is the time at which you approach a tortoise and get within 2 meters of it to begin assessing its condition, even without touching it or moving it.

Attitude and activity – Circle one.

Normal – Tortoise paddles its forelimbs when held, attempts to escape, and repeatedly retracts into its shell when handled; or is shy and tends to remain retracted into its shell when being handled, but has normal strength. During cold temperatures, activity may be minimal, but is considered normal for the temperature or time of year.

Lethargic/weak – Forelimbs may hang limp when tortoise is lifted, tortoise appears weak, is slow to respond to stimuli, and/or does not resist gentle tugging on the limbs.

Respiration – Circle all that apply.

Normal - No sound or a very faint, high-pitched whistle when expelling air out of their nares.

Abnormal sounds - Includes wet, crackling, or gurgling sounds associated with congestion.

Increased effort – Tortoise pumps forelimbs up and down symmetrically when breathing, may indicate lower respiratory disease or compromised lung volume.

Open-mouthed breathing or neck extended while breathing may indicate increased respiratory effort, but this must be distinguished from occasional normal gaping.

Beak – Circle all that apply.

Normal – Usual shape, size, color, and texture. May have pieces of food-related debris or dirt stuck on it.

Abnormal – Unusual shape, size, color, or texture. Describe in area provided.

Evidence of foraging – Presence of food or coloration associated with food, usually green or black, but may be more colorful (e.g. pink) in spring depending on food sources.

Left/right naris – Circle all that apply.

Normal – Usual shape and/or size.

Asymmetrical - One naris is larger and/or wider than the other.

Eroded - Loss of scales and skin around naris opening.

Occluded – Plugged or reduced size of naris opening.

Left/right naris discharge – Circle one.

None – No discharge present.

Serous - Clear, watery discharge present.

Mucous – Cloudy, thick discharge present.

Severity of naris discharge – Circle one.

1. Mild - Moisture present around nares.

2. Moderate - Discharge coming out of the nares, but not running far from the nares themselves.
3. Severe - Discharge coming from nares that is running down the beak.

Left/right eye – Circle all that apply.

- Normal – Usual shape, size, and color.
- Sunken – Eye recessed within the orbit.
- Corneal opacity- Eye is cloudy, hazy, or there is a loss of transparency of the cornea.
- Partially closed – Eye is not fully open.
- Fully closed – Eye is not open at all.
- Serous discharge - Clear, watery discharge present.
- Mucous discharge - Cloudy, thick discharge present.
- Periocular swelling – Area around the eye is swollen.
- Periocular redness – Area around the eye is abnormally pink or red.
- Conjunctival swelling - Membranes around the eye are swollen.
- Conjunctival redness – Membranes around the eye are abnormally pink or red.

Skin lesion location – Circle all that apply, describe in area provided, and draw on diagram.

- None – No skin lesions.
- Generalized – Widespread lesions in many locations all over the body.
- Head
- Neck
- L/R forelimb – Circle left, right, or both.
- L/R axillary region – Circle left, right, or both.
- L/R hindlimb – Circle left, right, or both.
- L/R prefemoral region – Circle left, right, or both.
- Vent/tail

Condition of Skin lesions – Circle one, but for multiple lesions circle all that apply and describe in area provided.

- N/A – Not applicable.
- Active – Very recent or unhealed lesion.
- Inactive - Old or healed lesion.

Coelomic cavity palpation – Circle one.

- No mass – The tortoise was palpated but no masses were detected.
- L/R mass – Circle left, right, or both.
- Not done – The tortoise was not palpated.

Shell characteristics - Circle all that apply and describe in area provided. Note that these characteristics may not always be considered abnormalities, as in the case of sunken scutes on an older tortoise. Therefore, it is possible to circle a characteristic here, but record N/A in the section labeled “Shell abnormality location.”

- Compressible - Capable of being flattened by pressure or pressed into a smaller space.

N/A – There are no unusual shell characteristics.

Sunken – Scutes are sunken lower than the seams of the shell.

+/- Scutes – Circle (+) if there are more scutes than usual and circle (-) if there are fewer scutes than usual on the carapace and/or plastron.

Peeling keratin – Scutes are peeling on the carapace or plastron.

Shell abnormality location - Circle all that apply, describe in area provided, and draw on diagram. Note that it is possible to circle a “Shell characteristic” above, but record N/A in this section since not all characteristics that could be noted there are considered abnormal, as in the case of sunken scutes on an older tortoise. However, if you record something in the section “Shell abnormalities” below, then you must circle a location in this section.

Carapace

Plastron

N/A – There are no shell abnormalities.

Shell abnormalities (describe below) - Circle one, but for multiple abnormalities circle all that apply and describe in area provided.

None – No shell abnormalities.

Localized – Abnormalities are restricted to a particular place or area on the body.

Multifocal–Abnormalities found on two or more distinct places or areas on the body.

Generalized - Widespread lesions in many locations all over the body.

Condition of shell abnormalities – Circle one, but for multiple abnormalities circle all that apply and describe in area provided.

N/A – Not applicable.

Active – Very recent or unhealed abnormality.

Inactive- Old or healed abnormality, or genetic alteration, such as extra or too few scutes.

If present, circumstances of skin/shell trauma – Circle one.

N/A – Not applicable

Unknown – There is no way to determine the cause of the trauma.

Suspected canid bite - Suspected or known predation by coyote, dog, or other canid. Provide details, if known, in the area provided.

Vehicle – Trauma is suspected or known to be caused by a vehicle, including but not limited to a car, truck, military tank, ATV, dirt bike, dune buggy, etc. Provide details in the area provided.

Other _____ - Provide details if the other options do not provide an adequate description of the circumstances of the trauma.

Sex – Circle one.

M – Male

F – Female

Unk – Unknown sex

Initial weight (g)- Weigh the tortoise prior to extensive handling to avoid the risk of the tortoise voiding. Spring scales or electronic balances may be used. When using a spring

scale, weigh the tortoise with the smallest scale appropriate for the individual to get an accurate weight.

Body condition score – Circle one. Scores range from 1 (emaciated) to 9 (morbidly obese). Descriptions of each body score are included in Appendix D.

Photos (take all*) – Photos should be taken with a good quality digital camera. Prior to taking photos of the tortoise, take a photograph of the data collection form clearly showing the Tortoise ID number, date, site, UTM location, biologists, etc. Whenever possible, take photos using a macro lens to show detail, such as those taken to show trauma and signs of disease.

Front face and body- Show nares, forelegs, gular, and anterior shell.

Left side face – Full frame close up.

Right side face – Full frame close up.

Carapace – Full frame close up.

Plastron (*take when measuring, but only if abnormal)-- Take this photo when you are doing the plastron measurement (after sample collection) and only if the plastron is abnormal.

Abnormalities – Full frame close up.

Label and describe trauma, anomalies, lesions, missing body parts, and identifying features – On the line drawings of a tortoise carapace and plastron, draw in any trauma, anomalies, lesions, and identifying features of the tortoise, and circle or point an arrow to the location of missing body parts. Draw in the shape of the gular on the picture of the plastron. Provide a written description in the space provided for details about the tortoise that may require clarification.

Ticks (*Ornithodoros spp*) – Circle one. See Appendix F.2 for details regarding collection.

0 – No ticks were observed.

1-10 – 1 to 10 ticks were observed.

>10 – More than 10 ticks were observed.

Collected? - Circle one.

N/A – No ticks were observed so no ticks were collected.

Yes – Ticks were collected as per the tick collection protocols.

No – Ticks were present but not collected.

Removed? – Circle one.

N/A – No ticks were observed so there were no ticks to remove.

Yes – Ticks were removed as per the tick collection protocols.

No – Ticks were present but not removed.

Location – Circle one. Location where ticks were observed.

Soft tissue – Skin, including limbs, vent, and tail.

Seams – Areas between the scutes on the carapace and plastron.

Scutes – Keratinized plates of the carapace and plastron.

Eyes

Nares

Beak

Choana – Circle one. See Appendix F.3 for details regarding the oral cavity examination.

Not examined – An oral cavity examination was not conducted or it was conducted but the choana was not observed.

Normal – Pale pink or pink.

Pale – Lacking pink coloration, white, or slightly yellow

Reddened – Very dark pink to red.

Tongue and oral mucosa – Circle all that apply. See Appendix F.3 for details regarding the oral cavity examination.

Not examined – The oral cavity examination was not conducted or it was conducted but the tongue and oral mucosa were not observed.

Normal – Pale pink or pink.

Pale – Lacking pink coloration or white

Reddened – Very dark pink to red.

Crust - An outer layer of solid material formed by the drying of a bodily exudate or secretion.

Ulcers - Localized defects or excavations of the surface of a tissue, usually produced by sloughing of necrotic inflammatory tissue.

Plaques - Localized patches or flat areas in the oral cavity. Plaques tend to have a white or yellow appearance.

Hypersalivation - Increased saliva in or around the oral cavity.

Impaction – Lodgment of something, such as food or debris, in the oral cavity

oral swabs collected – Write the number of oral swabs collected. You can collect 0, 1, or 2 oral swabs. See Appendix F.3 for details regarding oral swab sample collection.

Time of blood draw (24h) – This is the time that you finished collecting the blood and removed the needle from the vein. Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Total sample volume (blood and lymph) collected – Total volume in milliliters of each blood collection attempt. Up to three attempts to collect total maximum volumes are allowed. See Appendix F.5 for details regarding blood sample collection.

Total # hep tubes (number each) – Circle one. You can have 0, 1, 2, 3, or 4 tubes containing whole blood and/or lymph samples.

Is the sample contaminated with lymph – Circle yes or no.

Estimate the degree of lymph contamination - Small (1-10%) Moderate (11-29%)
Severe (>30%)

Void during processing – Circle all that apply.

None – The tortoise did not discharge bladder or digestive tract contents.

Urine/urates – Waste material that is secreted by the kidney as a clear fluid or as a semi-solid salt of uric acid that may be white, yellow, or brown

Feces – Bodily waste discharged through the digestive tract; firm excrement

Post void weight – If the tortoise voided during processing, weight the tortoise again at this point before rehydrating. Record the weight in grams. If the tortoise did not void, circle N/A.

Hydration Method – If the tortoise voided urine/urates, circle one, or if multiple rehydration methods were employed, circle all that apply and describe in the area provided. See Appendix F.6 for details regarding rehydration techniques.

N/A – The tortoise did not void urine/urates, so it was not provided with fluids.

Soak – The tortoise was placed in a plastic tote with shallow fresh water for 30 minutes.

Nasal-oral – The tortoise was offered fresh water ad lib with a syringe through the nares and mouth.

Epicoelomic – The tortoise was given an epicoelomic injection of sterile saline.

Fluid type – Fill in blank with fluid(s) used. If multiple rehydration methods were employed, describe in the area provided. See Appendix F.6 for details regarding rehydration techniques. If the tortoise did not void, skip this field.

Water – Fresh drinking water.

Saline – 0.9% sodium chloride.

Vol - Amount of fluids in milliliters injected into the tortoise or offered ad lib. Do not provide a volume if you used the soaking method. If the tortoise did not void, skip this field.

Post-fluid weight – If the tortoise voided, weight in grams after administration of fluids. If the tortoise did not void, circle N/A.

MCL – Straight midline carapace length measured in millimeters by holding the calipers directly over the center line of the tortoise and measuring from the center of the outer edge of the nuchal scute to the most caudal aspect of the carapace. Note that the most caudal end of the carapace may not be the edge of the supracaudal scute.

Width V3 - Width of the carapace measured in millimeters by holding calipers directly over the center line of the tortoise crosswise in the middle of the 3rd vertebral scute. Keep the calipers level with the ground.

Height V3 - Height of the carapace measured in millimeters by holding the tortoise so that one arm of the calipers is held across the center of the 3rd vertebral scute on the carapace and the other arm of the calipers is held in the same position across the plastron so the tortoise is between the arms of the calipers. The arms of the calipers should extend all the way from one side of the tortoise to the other while being held level against the tortoise's carapace and plastron.

Plastron – Length of the plastron measured in millimeters measured by having a handler tip the tortoise to one side (the tortoise’s left or right side) so the examiner can see the plastron. The examiner then uses calipers to measure from the notch between the gular scutes to the notch between the anal scutes. If the calipers do not fit into the notch, then hold them outside the notch on the plastron where the notch comes to a V to get the most accurate measurement. Do not tip the tortoise too far to the left or the right and do not put it on its carapace to take this measurement.

End handling time (24h) – The time at which you complete the processing of the tortoise and return it to its original location (point of capture, pen, etc). Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Disposition - Circle one.

Wild capture location - For tortoises that were caught in the wild and are being placed back at their site of capture immediately following processing (i.e, the tortoise is not being brought into captivity after processing).

Same pen – For tortoises that were already in captivity at the time of processing and are being placed back in the same pen.

New pen – Provide the number of the new pen where the tortoise is being placed after processing, regardless if it was newly captured from the wild, or if it was already in captivity but moving to a new pen.

Other- Describe the new location.

Blood processing time (24h) – The time at which samples were removed from the cooler and placed in the centrifuge. Use the 24 hour clock (e.g. 1:45pm is recorded as 13:45).

Plasma Color – Circle one. This is the plasma color of the sample you will use for the “Hep plasma UFL” cryovial. This should be the best sample you collected for each tortoise, meaning the one with the least lymph contamination. Choose from colorless, red, yellow, and green.

UFL plasma aliquots – Circle one. You can have 0 or 1 aliquots of plasma for UFL. See Appendix F.7 for details regarding sample processing.

USFWS plasma aliquots - Circle one. You can have 0, 1, 2, or 3 aliquots of plasma for USFWS. See Appendix F.7 for details regarding sample processing.

Total tubes with RBCs saved – Circle one. You can have 0, 1, 2, 3, or 4 heparinized microtainers with RBCs in them to save. All of the plasma should have been pipetted out of these microtainers into the UFL or USFWS plasma and plasma/lymph tubes prior to storage, leaving only RBCs in the microtainers. See Appendix F.7 for details regarding sample processing.

Total number of tubes/vials collected – Count the total number of tubes/vials containing samples from the individual.

ATTACHMENT 2

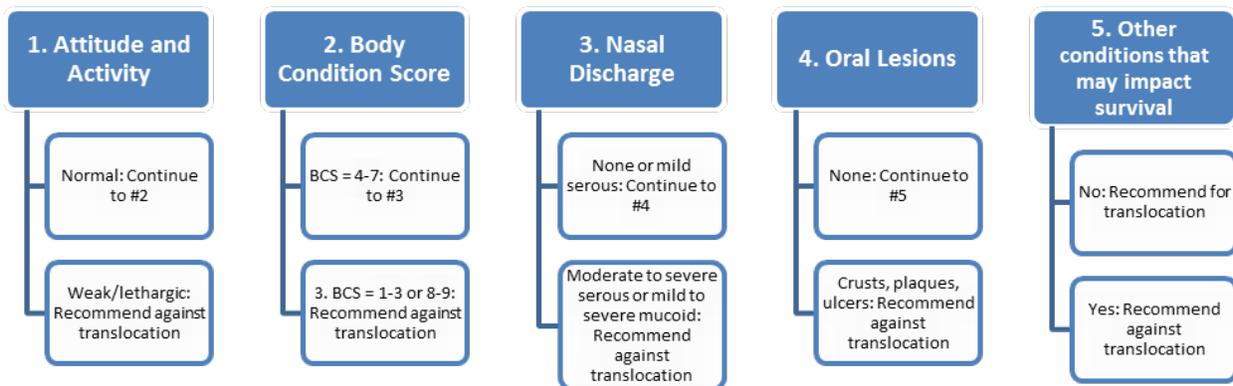
From: U.S. Fish and Wildlife Service. 2013. Health assessment procedures for the desert tortoise (*Gopherus agassizii*): a handbook pertinent to translocation. Desert Tortoise Recovery Office, U.S. Fish and Wildlife Service, Reno, Nevada.

Appendix G. Algorithm for Evaluating if Desert Tortoises are Suitable for Translocation

Goals:

Relocate individuals that have high chances of survival.

Minimize the risk of spreading disease



All recommendations regarding translocation are to be recorded in the proposed disposition plan and submitted to the U.S. Fish and Wildlife Service's Desert Tortoise Recovery Office prior to relocating any tortoises.

ATTACHMENT 3

Health Eligibility Criteria 2013 Translocation from DTCC to Greater Trout Canyon Area

Initial Assessment of Pen Group Eligibility

- Assess **all** individuals occupying pen concurrently.
- The pen group is preliminarily deemed eligible if no tortoises in the pen have signs of disease.
- If one or more tortoises in the pen show mild to moderate signs of disease, the pen is not eligible for release and all tortoises in pen will be treated and observed with re-assessment for eligibility after 3 months.
- If one or more tortoises in the pen has a Body Condition Score ≤ 3 and/or moderate to severe signs of disease, those individuals receive a follow-up health assessment immediately, and the pen is quarantined for 30 days.

Individual Eligibility

- Pre-release comprehensive health assessment, which includes a full physical exam and collection and banking of biological samples (blood, choanal swab, cloacal swab, nasal lavage) conducted
- Normal behavior for season and time of day
- Normal bodily functions
- No active signs of communicable disease
- Serous 1 nasal and/or ocular discharge **does not disqualify** a tortoise from eligibility if there is no scarring or missing scales around the nares and no other health issues
- No oral lesions
- No white oral cavity
- No bladder stones
- No ectoparasites
- No generalized skin conditions
- Body Condition Score 4-7
- History of maintained or increased weight
- 4 legs and normal ambulation
- No gross disfigurements such as severely flattened carapace, unusually domed or peaked carapace, or grossly enlarged carapace
- Midline carapace length ≤ 330 mm

Final approval for release will be given by the DTCC's Conservation Program Specialist or DVM after review of assessments.

ATTACHMENT 4

Desert Tortoise Conservation Center Quarantine Protocol for New Arrivals into the DTCC Collection and Quarantine Protocol Prior to Translocation¹

Revised 9 August 2013

The 2013 DTCC Quarantine protocol for new arrivals has been amended to reflect the following:

1. A decrease in collection size now allows for resources to be available for increased scrutiny of incoming and outgoing animals
2. As a result of a new partnership with Lied Animal Shelter, tortoises coming to DTCC are considered translocation candidates and not unwanted pets
3. DTCC collection animals are treated for disease if clinical signs are observed
4. Incoming animals should be carefully evaluated to prevent the introduction of novel diseases into the DTCC collection
5. Groups of *Mycoplasma* positive and negative animals are needed for disease studies
6. These protocols reflect the recommendations of the IUCN Guidelines for Reintroductions and other Conservation Translocations (<http://data.iucn.org/dbtw-wpd/edocs/2013-009.pdf>) and the O.I.E Quarantine and Health Screening Protocols for Wildlife Prior to Translocation and Release into the Wild (<http://www.iucn-whsq.org/sites/default/files/Quarantine%20and%20Health%20Screening%20Protocol.pdf>).

2013 DTCC Quarantine Protocol for New Arrivals into the DTCC Collection

- All incoming tortoises and cohorts will remain in quarantine for a minimum of 90 days
- All in – all out policy, which means the 3 month period starts when the last animal is added to the pen
- If an animal from a given pen dies or is euthanized, cohorts cannot be moved until necropsy results are reviewed
- During the quarantine period, the following procedures should be performed:
 - Health assessment at the beginning and end of the quarantine period which includes body weight determination and collection of biological samples (blood, oral swabs, feces)
 - *Mycoplasma* Ag/Te ELISA at the beginning and end of the quarantine period. This information will be used for decision-making on destination pens once an individual or cohort is released from quarantine
 - Feces for *Cryptosporidium* spp. PCR
 - Plasma bank at the beginning and end of the quarantine period
 - Visual exam for the presence of clinical signs of disease once weekly (ideal) or 2 times per month (minimum) –
 - Visual exams should be brief and are only meant to capture basic information such as attitude, whether an animal is in or out of a burrow, whether it was observed eating, and whether nasal discharge was observed.
 - If animals are in burrows, they should not be removed just to accomplish a visual exam. Simply note the animal was in a burrow so not observed.

¹ The protocols used at the DTCC were living documents and changed over time. The example here represents the final iteration; however, there was drift from this written protocol over time. This was due to space limitations, reduction of resources, and a compressed timeline due to the closure of the facility in December 2014. The 90-day quarantine period was not always applied to current residents. The collection of individual fecal samples from tortoises at the DTCC for *Cryptosporidium* spp. diagnostic testing proved to be problematic and was not adopted as a routine practice. Testing for *Mycoplasma* spp. at the beginning and end of the quarantine period became cost prohibitive due to the large number of tortoises. However, plasma samples were collected and banked for future studies. DTCC staff included a third health assessment of tortoises during a specified period of time. Only tortoises in good body condition and free from clinical signs of disease on three consecutive evaluations were considered eligible for release.

- Health assessments and visual exam findings must be entered into the DTCC database
- Animals with clinical signs of disease, a decrease in body weight >18%, or a decline in BCS, should be evaluated by a veterinarian
- Additional diagnostics on a case by case basis
- Tortoises may be moved to another enclosure outside of quarantine at the end of that time period as long as they are free of signs of disease.
 - Mycoplasma ELISA neg tortoises and cohorts should be moved from quarantine preferentially into an enclosure with an ELISA neg individual or cohort.
 - Mycoplasma ELISA pos individuals should be moved from quarantine into an ELISA pos pen if space permits. If there is no space in the ELISA pos pens, it should be moved into an enclosure with a mixed cohort.
 - Mycoplasma ELISA pos cohorts should be moved from quarantine into an ELISA pos pen if space permits. If there is no space in the ELISA pos pens, it should be moved into an enclosure with a mixed cohort that has primarily ELISA pos individuals.
 - Mycoplasma ELISA suspect individuals and mixed cohorts should be moved from quarantine into an enclosure with a mixed cohort.
 - Tortoises may also be eligible for translocation after the 90-day quarantine period.

2013 DTCC Pre-release Quarantine Protocol for Tortoises being considered for Reintroduction

- Animals being considered for reintroduction will undergo a pre-release quarantine for a minimum of 90 days
- If an animal from a given pen dies or is euthanized, cohorts cannot be moved until necropsy results are reviewed and the remaining cohorts are deemed healthy
- During the quarantine period, the following procedures should be performed:
 - Health assessment at the beginning and end of the quarantine period which includes body weight determination and collection of biological samples (blood, oral swabs, feces/cloacal swab) if not done previously
 - Visual exam for the presence of clinical signs of disease once weekly (ideal) or 2 times per month (minimum) –
 - Visual exams should be brief and are only meant to capture basic information such as whether an animal is in or out of a burrow, whether it was observed eating, and whether nasal discharge was observed.
 - If animals are in burrows, they should not be removed just to accomplish a visual exam. Simply note the animal was in a burrow so not observed.
 - Health assessments and visual exam findings must be entered into the DTCC database
 - Animals with clinical signs of disease, a decrease in body weight >18%, or a decline in BCS, should be evaluated by a veterinarian
 - Additional diagnostics on a case by case basis