REFLECTIONS ON 34 YEARS OF HEALTH& DISEASE STUDIES WITH DESERT TORTOISES

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Acknowledgments

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Distribution of North American Tortoises



When And Where It All Began



Upper Respiratory Tract Disease





TORTOISE HEALTH AND DISEASE STUDIES

Disease Diagnosis

Pathological Evaluations

Pathogen Detection

Health Assessment



Blood Reference Intervals

Epidemiological Studies

Toxicological Studies

Immunological Studies

Test Development and Validation

Challenge Studies



CHRONIC UPPER RESPIRATORY TRACT DISEASE OF FREE-RANGING DESERT TORTOISES (XEROBATES AGASSIZII)

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ABSTRACT: Seventeen desert tortoises, Xerobates agassizii, with upper respiratory tract disease were examined; thirteen were euthanatized for necropsy. Four normal control desert tortoises from a clinically healthy population were similarly evaluated. Hemoglobin and phosphorus values were significantly ($P \le 0.05$) lower and serum sodium, urea, SGOT, and cholesterol values were significantly higher in ill tortoises compared to controls. No significant differences in concentrations of serum or liver vitamins A and E were found between the two groups. While no significant differences were found for concentrations of lead, copper, cadmium, and selenium, the livers of ill tortoises had higher concentrations of mercury and iron. Lesions were found consistently in the upper respiratory tract (URT) of ill tortoises. In all ill tortoises dense infiltrates of lymphocytes and histiocytes obscured the mucosal epithelium and underlying glands. The mucosal epithelium was variably dysplastic, hyperplastic, and occasionally ulcerated. Electron microscopic studies revealed small (350 to 900 nm), pleomorphic organisms resembling Mycoplasma sp., in close association with the surface epithelium of the URT of ill tortoises. Pasteurella testudinis was cultured from the nasal cavity of all ill tortoises and one of four control tortoises. A Mycoplasma sp. was cultured from the nasal passageways of four ill tortoises and was ultrastructurally similar to the pleomorphic organism present on the mucosa in tissue section.

Key words: Upper respiratory tract disease, Mycoplasma sp., Pasteurella testudinis, Desert Tortoise, Xerobates agassizii.

Nasal Cavity Normal Anatomy and Histology







Mycoplasmosis in Desert Tortoises





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Taxonomic Analysis of the Tortoise Mycoplasmas Mycoplasma agassizii and Mycoplasma testudinis by 16S rRNA Gene Sequence Comparison[†]

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The nucleotide sequences of the 16S rRNA genes of two mycoplasmas, *Mycoplasma agassizii* (proposed sp. nov.) and *Mycoplasma testudinis*, isolated from tortoises were determined and used for taxonomic comparisons. Signature nucleotide sequence motifs and overall sequence similarities to other mollicutes positioned these mycoplasmas in the *M. hyorhinis* and *M. pneumoniae* phylogenetic groups, respectively. A third, previously unrecognized tortoise mycoplasma was detected by 16S rRNA gene amplification and sequence analysis and was positioned in the *M. fermentans* phylogenetic group. The 16S rRNA gene of *Acholeplasma laidlawii* was similarly detected in a tortoise isolate, showing that diverse mollicutes can share the same family of reptilian host.

GUIDELINES FOR THE FIELD EVALUATION OF DESERT TORTOISE HEALTH AND DISEASE

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ABSTRACT: Field evaluation of free-ranging wildlife requires the systematic documentation of a variety of environmental conditions and individual parameters of health and disease, particularly in the case of rare or endangered species. In addition, defined criteria are needed for the humane salvage of ill or dving animals. The purpose of this paper is to describe, in detail, the preparation, procedures, and protocols we developed and tested for the field evaluation of wild desert tortoises (Gopherus agassizii). These guidelines describe: preparations for the field, including developing familiarity with tortoise behavior and ecology, and preparation of standardized data sheets; journal notes to document background data on weather conditions, temperature, rainfall, locality, and historic and recent human activities; procedures to prevent the spread of disease and parasites; data sheets for live tortoises to record tortoise identification, location, sex, body measurements and activity; health profile forms for documenting and grading physical abnormalities of tortoise posture and movements, general condition (e.g., lethargy, cachexia), external parasites, and clinical abnormalities associated with shell and upper respiratory diseases; permanent photographic records for the retrospective analysis of progression and regression of upper respiratory and eye diseases, analysis of shell lesions and evaluation of growth and age; and indications and methods for salvaging ill or dying tortoises for necropsy evaluation. These guidelines, tested on 5,000 to 20,000 tortoises over a 10 to 27 yr period, were designed to maximize acquisition of data for demographic, ecological, health and disease research projects; to reduce handling and stress of individual animals; to avoid spread of infectious disease; to promote high quality and consistent data sets; and to reduce the duration and number of field trips. The field methods are adapted for desert tortoise life cycle, behavior, anatomy, physiology, and pertinent disease; however the model is applicable to other species of reptiles. Comprehensive databases of clinical signs of disease and health are crucial to research endeavors and essential to decisions on captive release, epidemiology of disease, translocation of wild tortoises, breeding programs, and euthanasia.

Key words: Chelonian, desert tortoise, diagnosis, disease, field evaluations, *Gopherus agassizii*, health assessments.

Health Evaluations: Modification of Berry & Christopher 2001

FIELD WORKER(S):			Date (ddmmmyyyy)			
Handler(s)			Tortoise ID	Ser	د <u> </u>	Captype
Recorder(s)			MCL (mm)	Gu	lar (mm)	
Observer(s)			PLN (mm)	w	'eight (g)	
STUDY SITE		Time (PST) sper	t handling	start		
UTM (WGS 84)	Easting				end	
N	orthing		On a plot?	yes/no		
County San Bernardino			New growth?	yes/no	SWC	
State California			Photos			
TRANSMITTER:			No	tes		
Transmitter frequency	/?					
Transmitter number?						
Who attached transm	itter?					
LOCATION:		TEMPER	ATURES (°C):			
At cover site? C	over site type?	At 1.5m	lcm	Soil	F	WS 2" start
Tag #	burrow	Activity?	Not at co	ver site?	I	WS 2" end
entering	pallet	resting	in open			
exiting	shrub	basking	other			
on mound	cave	walking	Interaction	ng with an	other torto	oise? yes
inside*	rock shelter	feeding	sex	s	ize	number
*specifically where		-	Type of i	interaction	(s):	_
POSTURE/BEHAV	IOR:					
Behavior appropriate	for the time of day?	yes/no	If no, describe:			
Behavior appropriate for the season?		yes/no	If no, describe:			
Can withdraw tightly	yes/no	If no, describe:				
Alert and responsive?	yes/no	If no, describe:				
Limbs, head hanging	yes/no	If yes, describe	:			
Lethargic?		yes/no	If yes, describe	:		
FORELIMBS		-				
Right	normal/abnormal	If abu	ormal, describe:			
Left	Left normal/abnormal If abno		ormal, describe:			
HINDLIMBS			-			
Right	normal/abnormal	If abnormal, describe:				
Left	normal/abnormal	If abr	ormal, describe:			
OTHER						
Tail	If abr	ormal, describe:				
FORELEGS (adjacen	t to face)					
Dried dirt on forelegs?			yes/no/unk			
Moisture on forelegs?			yes/no/unk			
Dried exudate on scal	es (glossy with drie	d exudate)?	yes/no/unk			

Field workers(s) Date (ddmmmyyyy) Study site name Tortoise ID Sex EVIDENCE OF SHELL/BONE DISEASE Describe Scutes laminae peeling/missing? yes/no/unl Scutes depressed/concave? ves/no/unk Pitting? ves/no/unk Fungal areas? (draw onto diagram and label) ves/no/unk SIGNS OF LESIONS FROM DISEASE: Severity 1 = no signs, 2 = mild, 3 = moderate, 4 = severe Use Table 2 for definitions of mild, moderate and severe for dist tion, severity and chronicity. Bone/scute replacement? yes/no/unk Describe: Severity (Rate 1-4) Location Notes HEAD Distributio Severity Chronicity LIMBS Distributio Severity Chronicity GULAR Distributio Severity Chronicity CARAPACE Distributio Severity Chronicity PLASTRON Distributi Severity Chronicity Legend: Lesions from cutaneous dyskeratosis (specifically), other lesions (enter symbols), describe in

Mod. in 10/2007 from Berry & Christopher (2001). Guidelines for the field evaluation of desert tortoise health and disease. JWD 37:427-450.

7

writing. Note lesions and extent on limbs.



HEALTH ASSESSMENT





EYE EXAMINATION

Examine eyelids, conjunctiva, & periocular area





Blood Collection





Copeia, 1992(1), pp. 237-241 © 1992 by the American Society of Ichthyologists and Herpetologists

FIELD AND CLINICAL TECHNIQUES FOR SAMPLING AND HANDLING BLOOD FOR HEMATOLOGIC AND SELECTED BIO-CHEMICAL DETERMINATIONS IN THE DESERT TORTOISE, XEROBATES AGAS-SIZII.—In general, tortoises are difficult vertebrates to medically evaluate (Jacobson, 1987). As part of health examinations, blood sampling of tortoises for hematologic and plasma biochemical values is routinely performed at the Veterinary Medical Teaching Hospital, University of Florida. Depending upon size and cooperation of the tortoise being evaluated, a variety of sampling sites can be used, each having

Field Blood Collection





Processing Blood





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Effect of venipuncture sites on hematologic and clinical biochemical values in desert tortoises (*Gopherus agassizii*)

Nicole L. Gottdenker, DVM, and Elliott R. Jacobson, DVM, PhD

Summary

Paired blood samples were collected from the postoccipital venous plexus and jugular vein of desert tortoises (Gopherus agassizii) for hematologic and plasma biochemical analyses. Comparison of hematologic values revealed significantly ($P \le 0.05$) lower PCV, RBC count, WBC count, and hemoglobin values for samples obtained from the occipital site. When comparisons were made between plasma biochemical values for the 2 sites, significant ($P \le 0.05$) differences were measured for: glucose, potassium, chloride, uric acid, calcium, phosphorous, total protein, albumin, globulin, alkaline phosphatase, aspartate transaminase, alanine transaminase, and total cholesterol. Significant differences between hematologic and plasma biochemical values from the occipital region samples vs jugular vein samples were attributed to hemodilution of the occipital region samples with extravascular fluid or lymph or both.

ans.^{1,2} The site of blood sample collection also may influence hematologic and blood biochemical values. For instance, sample site influenced blood values in laboratory rats.³⁻⁵

Knowledge of the systematic error of blood sample collection sites in desert tortoises will improve precision of blood data comparison and accuracy of blood value measurement. Other reports described the anatomic location of blood sample collection sites and clinical hematologic and blood biochemical reference values of desert tortoises.^{1,6,7} Although the aforementioned studies used and described a variety of blood sample venipuncture sites and methods for desert tortoises, such as the jugular vein (JV), heart, brachial vein, ventral coccygeal vein, and toenail (clipped short), these reports did not analyze the potential effects of sample collection site on desert tortoise blood value measurement. The objective of the study reported here was to compare effects of sample collection sites on hematologic and plasma biochemical values in desert tortoises.

REFERENCE INTERVALS

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REFERENCE INTERVALS AND PHYSIOLOGIC ALTERATIONS IN HEMATOLOGIC AND BIOCHEMICAL VALUES OF FREE-RANGING DESERT TORTOISES IN THE MOJAVE DESERT

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CLINICAL DISEASE AND LABORATORY ABNORMALITIES IN FREE-RANGING DESERT TORTOISES IN CALIFORNIA (1990–1995)

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ABSTRACT: Desert tortoise (Gopherus agassizii) populations have experienced precipitous declines resulting from the cumulative impact of habitat loss and human and disease-related mortality. Diagnosis of disease in live, free-ranging tortoises is facilitated by evaluation of clinical signs and laboratory test results but may be complicated by seasonal and environmental effects. The goals of this study were: 1) to describe and monitor clinical and laboratory signs of disease in adult, free-ranging desert tortoises at three sites in the Mojave Desert of California (USA) between October 1990 and October 1995; 2) to evaluate associations between clinical signs and hematologic, biochemical, serologic, and microbiologic test results; 3) to characterize disease patterns by site, season, and sex; and 4) to assess the utility of diagnostic tests in predicting morbidity and mortality. Venous blood samples were obtained four times per year from tortoises of both sexes at the Desert Tortoise Research Natural Area (DTNA), Goffs/Fenner Valley, and Ivanpah Valley. Tortoises were given a physical examination, and clinical abnormalities were graded by type and severity. Of 108 tortoises, 68.5% had clinical signs of upper respiratory tract disease consistent with mycoplasmosis at least once during the study period. In addition, 48.1% developed moderate to severe shell lesions consistent with cutaneous dyskeratosis. Ulcerated or plaque-like oral lesions were noted on single occasions in 23% of tortoises at Goffs and 6% of tortoises at Ivanpah. Tortoises with oral lesions were significantly more likely than tortoises without lesions to have positive nasal cultures for Mycoplasma agassizii (P=0.001) and to be dehydrated (P=0.0007). Nine tortoises had marked azotemia (blood urea nitrogen [BUN] >100 mg/ dl) or persistent azotemia (BUN 63–76 mg/dl); four of these died, three of which had necropsy confirmation of urinary tract disease. Laboratory tests had low sensitivity but high specificity in assessing morbidity and mortality; there was marked discrepancy between serologic and culture results for *M. agassizii*. Compared with tortoises at other sites, tortoises at DTNA were more likely to be seropositive for M. agassizii. Tortoises at Goffs were significantly more likely to have moderate to severe shell disease, oral lesions, positive nasal cultures for *M. agassizii*, and increased plasma aspartate aminotransferase activity. The severe disease prevalence in Goffs tortoises likely contributed to the population decline that occurred during and subsequent to this study.

Key words: Chelonian, clinical chemistry, cutaneous dyskeratosis, desert tortoise, hematology, Mojave Desert, Gopherus agassizii, Mycoplasma agassizii, mycoplasmosis, shell disease, upper respiratory tract disease.

DESERT TORTOISE RECOVERY PLAN-1994





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CAUSES OF MORTALITY AND DISEASES IN TORTOISES: A REVIEW

Elliott R. Jacobson, D.V.M., Ph.D.

Abstract: Most of the 40 species of tortoises are experiencing population declines. Of the various causes of mortality in wild populations of tortoises, the interactions of disease and population dynamics are least understood. Although habitat degradation is considered the most significant threat to wild populations of tortoises, disease is being observed more frequently in certain populations. An upper respiratory tract disease has been seen in populations of desert tortoise, *Gopherus agassizi*, in the Mojave Desert, USA, and certain populations of the gopher tortoise, *Gopherus polyphemus*, in Florida, USA. Much more information is available on diseases of captive tortoises than on those of wild tortoises. Of infectious diseases, viral, bacterial, mycotic, and parasitic diseases have all been reported. Noninfectious diseases identified in tortoises include various nutritional diseases, hypothyroidism, and neoplasia. Virtually nothing is known about the effects of pollutants/ toxicants in individual or populations of tortoises.

Key words: Tortoise, mortality, disease.

CUTANEOUS DYSKERATOSIS IN FREE-RANGING DESERT TORTOISES, *GOPHERUS AGASSIZII*, IN THE COLORADO DESERT OF SOUTHERN CALIFORNIA

Elliott R. Jacobson, D.V.M., Ph.D., Thomas J. Wronski, Ph.D., Juergen Schumacher, D.V.M., Carlos Reggiardo, D.V.M., Ph.D., and Kristin H. Berry, Ph.D.

Abstract: High mortality rates and a shell disease originally described as shell necrosis were observed in the population of desert tortoises (Gopherus agassizii) in the Colorado Desert on the Chuckwalla Bench Area of Critical Environmental Concern, Riverside County, California, USA. In a retrospective review of photographic slides of desert tortoises from the Chuckwalla Bench, the disease was evident in 1979 when tortoises on a permanent study site were first photographed. Lesions were present in both sexes and all size classes of tortoises in all years in which tortoises were photographed. In those tortoises where sequential photographs were taken, the most severe lesions were seen in 1988. Although the disease was present on the carapace, plastron, and thickened forelimb scutes, the plastron was more severely affected than other areas of the integument. The affected portions of the shell were gray-white and sometimes orange and had a roughened flaky appearance. The lesion commenced at seams between scutes and spread toward the middle of each scute in an irregular pattern. Shell biopsies of nine affected tortoises were evaluated by light microscopy. No inflammatory infiltrates were present in the lesions, and although bacterial organisms were identified in tissue sections, they were superficially located and were considered to be secondary invaders. Special staining indicated a loss of the normal integrity of the horny material covering affected scutes. For the most part, the epithelial cells that formed a pseudostratified layer under affected portions of each scute remained intact. Although the location and histologic appearance of the lesion were compatible with a dyskeratosis and were suggestive of either a deficiency disease or toxicosis, the exact cause of the disease could not be determined.

Key words: Dyskeratosis, cutaneous, desert tortoise, Gopherus agassizii.

DYSKERATOSIS: Abnormal or imperfect keratinization of the keratinocytes





PATHOLOGY OF DISEASE STUDIES IN DESERT TORTOISES: 1992-1995

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PATHOLOGY OF DISEASES IN WILD DESERT TORTOISES FROM CALIFORNIA

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ABSTRACT: Twenty-four ill or dead desert tortoises (Gopherus agassizii) were received between March 1992 and July 1995 for necropsies from the Mojave and Colorado deserts of California (USA). Diseases observed in these animals included cutaneous dyskeratosis (n = 7); shell necrosis (n = 2); respiratory diseases (n = 7); urolithiasis (n = 3); and trauma (n = 5). In tortoises with cutaneous dyskeratosis the horn layer of shell was disrupted by multiple crevices and fissures and, in the most severe lesions, dermal bone showed osteoclastic resorption, remodeling, and osteopenia. In tortoises with shell necrosis, multiple foci of necrotic cell debris and heterophilic inflammation within the epidermal horn layer were subtended by necrotic dermal bone colonized by bacteria and fungi. Of the seven tortoises with respiratory disease, five were diagnosed with mycoplasmosis. The diagnosis of mycoplasmosis was based on the presence of chronic proliferative rhinitis and positive serologic tests and/or isolation of Mycoplasma sp. Chronic fungal pneumonia was diagnosed in one tortoise with respiratory disease. In the three tortoises with urolithiasis, two were discovered dead, and the live tortoise had renal and articular gout. Traumatic injuries consisted of one tortoise entombed within its burrow, one tortoise burned in a brush fire, two tortoises struck by moving vehicles, and one tortoise attacked by a predator. While the primary cause of illness could be attributed to one or two major disease processes, lesions were often found in multiple organ systems, and a variety of etiologies were responsible for morbidity and mortality.

Key words: Desert tortoise, diseases, Gopherus agassizii, pathology, survey.

24 ill or Dead Tortoises

•Cutaneous dyskeratosis (n = 7)
•Shell necrosis (n=2)
•Respiratory Disease (n=7)
•Urolithiasis (n=3)
•Trauma (n=5)



DESERT TORTOISE CONSERVATION CENTER



Chelonian Conservation and Biology. 1995, 1(4):279-284 1995 by Chelonian Research Foundation

Mycoplasmosis and the Desert Tortoise (Gopherus agassizii) in Las Vegas Valley, Nevada

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ABSTRACT.-*Mycoplasma agassizii* is the cause of an upper respiratory tract disease (URTD) in certain populations of the desert tortoise, *Gopherus agassizii*, in the Mojave Desert of the southwestern United States. This disease and the resulting epizootic influenced the listing, by the federal government, of desert tortoise populations north and west of the Colorado River as threatened. As part of a lawsuit settlement, 875 desert tortoises were removed from specified properties in Las Vegas Valley, Nevada, between June 1990 and December 1991. Clinical signs of URTD were seen in 14.3% of the collected tortoises. Pathologic evaluations of tortoises submitted from the Desert Tortoise Conservation Center, Las Vegas Valley, Nevada, revealed that 8 of 12 tortoises submitted as clinically healthy had lesions consistent with URTD. The presence of lesions indicates that subclinical disease exists in this tortoise population and that determining health status of tortoises requires more sophisticated approaches than clinical appearance alone. The difficulty in accurately assessing health status of wildlife, including chelonians, is an example of one of the problems that conservation biologists will face when trying to evaluate and manage remaining populations of a wide range of threatened or endangered species. Protocols need to be developed as minimal guidelines that can be used in health assessment of those species to be either relocated or intensely managed.

KEY WORDS. – Reptilia; Testudines; Testudinidae; Gopherus agassizii; tortoise; upper respiratory tract disease; pathology; Mycoplasma agassizii; Pasteurella testudinis; Nevada; USA

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Detection of Antibodies to a Pathogenic Mycoplasma in Desert Tortoises (*Gopherus agassizii*) with Upper Respiratory Tract Disease[†]

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Mycoplasma agassizii (proposed species novum) is the etiologic agent of an upper respiratory tract disease in the desert tortoise (Gopherus agassizii), which is threatened in most of its range. An enzyme-linked immunosorbent assay (ELISA) for the detection of *M. agassizii*-specific antibodies in desert tortoises was developed with a monoclonal antibody with specificity for desert tortoise immunoglobulin light chain. Plasma samples from one group of tortoises were tested immediately before and 1 month after challenge either with nasal exudate containing *M. agassizii* or with a purified preparation of *M. agassizii*. Plasma samples from a second group of known healthy and sick tortoises were also tested. In the first group, the ELISA detected seroconversion in individual tortoises following challenge with *M. agassizii*. In the second group, ELISA results were positively correlated with the health status of the tortoises, as determined by clinical and pathologic findings. In addition, the ELISA revealed that tortoise antimycoplasma antibodies were specific for *M. agassizii* when samples were assayed against *M. agassizii*, *M. pulmonis*, *M. testudinis*, and *M. gallisepticum* antigens. The observed direct correlation between the presence of nasal mucosal lesions and *M. agassizii*-specific antibodies proved that the ELISA reliably diagnosed *M. agassizii* infection in desert tortoises and advocates its use for monitoring *M. agassizii*-induced upper respiratory tract disease in free-ranging desert tortoises.

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ELISA Development

Methods

ELISA

1

Plasma banked, most samples run on the same day

p-NPP

Biotinylated anti-tortoise MAb

Mycoplasma



Streptavidin-alkaline phospitatase

Antibodies from tortoise plasma

Plate

Mycoplasmosis



1

CLINICAL AND VACCINE IMMUNOLOGY, Sept. 2007, p. 1190–1195 1556-6811/07/\$08.00+0 doi:10.1128/CVI.00108-07 Copyright © 2007, American Society for Microbiology. All Rights Reserved.

Improved Enzyme-Linked Immunosorbent Assay To Reveal Mycoplasma agassizii Exposure: a Valuable Tool in the Management of Environmentally Sensitive Tortoise Populations[⊽]

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The precarious status of desert (Gopherus agassizii) and gopher (Gopherus polyphemus) tortoises has resulted in research and conservation efforts that include health assessments as a substantial component of management decision-making. Therefore, it is critical that available diagnostic tests for diseases impacting these species undergo rigorous standardization and validation. Since 1992, analysis of exposure of tortoises to Mycoplasma agassizii, an etiological agent of upper respiratory tract disease, has relied on the detection of specific *M. agassizii* antibody by enzyme-linked immunosorbent assay (ELISA). We report here substantive refinements in the diagnostic assay and discuss the implications of its use in wildlife conservation and management. The ELISA has been refined to include more stringent quality control measures and has been converted to a clinically more meaningful titer reporting system, consistent with other diagnostic serologic tests. The ELISA results for 5,954 desert and gopher tortoises were plotted, and a subset of these serum samples (n = 90) was used to determine end-point titers, to establish an optimum serum dilution for analyzing samples, and to construct a standard curve. The relationship between titer and A_{405} was validated using 77 serum samples from known positive (n = 48) and negative (n = 29) control tortoises from prior transmission studies. The Youden index, J, and the optimal cut point, c, were estimated using ELISA results from the 77 control sera. Based on this evaluation, the refinement has substantially improved the performance of the assay (sensitivity of 0.98, specificity of 0.99, and J of 0.98), thus providing a clinically more reliable diagnostic test for this important infection of tortoises.

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Persistence of maternal antibodies against Mycoplasma agassizii in desert tortoise hatchlings.

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Abstract

OBJECTIVE: To investigate Mycoplasma agassizii-specific maternal antibodies in desert tortoise (Gopherus agassizii) hatchlings.

SAMPLE POPULATION: Plasma from 43 captive-reared desert tortoise hatchlings.

PROCEDURE: ELISA for M agassizii-specific antibodies was performed. Four hatchlings from 4 clutches of 3 M agassizii-seropositive females with chronic upper respiratory tract disease (URTD) were tested on the day of hatching (set 1), and 20 hatchlings from 4 clutches of 4 M agassizii-seropositive females with URTD and 19 hatchlings from 4 M agassizii-seronegative healthy females were tested at 4, 8, 12, and 29 months old (set 2). Immunoblot analysis was performed to determine immunoglobulin classes in yolk and plasma of hatchlings. To determine infection status of hatchlings, yolk, egg shell membranes (set 1), and nasal lavage fluid (sets 1 and 2) were examined for M agassizii by use of polymerase chain reaction.

RESULTS: Yolk and hatchling plasma had significantly lower amounts of specific antibodies than did plasma from adult females. The IgG and IgM antibodies were transferred, but M agassizii-specific antibodies were of the IgG class. Hatchlings were not infected with mycoplasmas. Offspring of sick females had significantly higher specific antibody titers than did offspring of healthy females. Titers were still significantly different in 1-year-old hatchlings.

CONCLUSIONS: Desert tortoise females transfer specific IgG and IgM antibodies to their offspring that are still detectable after 1 year.

CLINICAL RELEVANCE: Infection with M agassizii may be misdiagnosed in hatchlings with persistent maternal antibodies. Passively acquired antibodies may have a role in pathogenesis of mycoplasma-induced respiratory tract disease and other diseases.

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RELATIONSHIP BETWEEN CLINICAL SIGNS OF UPPER RESPIRATORY TRACT DISEASE AND ANTIBODIES TO *MYCOPLASMA AGASSIZII* IN DESERT TORTOISES FROM NEVADA

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ABSTRACT: Plasma samples collected in 1990 from free-ranging desert tortoises (Gopherus agassizii) with and without clinical signs of upper respiratory tract disease (URTD) from Las Vegas Valley, Clark County, Nevada (USA), were tested by enzyme-linked immunosorbent assay (ELI-SA) for antibodies to Mycoplasma agassizii, a causative agent of URTD. The relationship between clinical signs and ELISA test results was evaluated. Of the 144 tortoises tested, 45 (31%) had clinical signs while 72 (50%) were seropositive. Presence of clinical signs of URTD was positively related to positive ELISA results (P < 0.0001) regardless of sex or age of the animal. Eighty-four percent of animals with clinical signs tested seropositive. Mucous nasal discharge, the most severe and obvious of the clinical signs, was highly predictive for exposure to M. agassizii based on the ELISA. Ninety-three percent of tortoises with mucous nasal discharge tested seropositive. Serologic testing for M. agassizii antibodies supported clinical signs as useful indicators of URTD, but it also detected potential subclinical infection in 34 (34%) of 99 animals without clinical signs.

Key words: Desert tortoise, Gopherus agassizii, Mycoplasma agassizii, ELISA, Upper Respiratory Tract Disease, URTD, Mycoplasmosis.

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Mycoplasma agassizii Causes Upper Respiratory Tract Disease in the Desert Tortoise[†]

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The desert tortoise is listed by the United States government as a threatened species in part of its range. A major contributing factor in the decline of this animal has been the presence of an upper respiratory tract disease (URTD) which is characterized by a chronic disease which eventually leads to severe occlusion of the nares with viscous exudate and destruction of the respiratory epithelium. Electron microscopy of infected tissues demonstrated the presence of a mycoplasma-like organism attached to the respiratory surfaces. The mycoplasma was isolated and designated as a new species, with the proposed name Mycoplasma agassizii. The current study was designed to fulfill Koch's postulates and determine if M. agassizii was the etiologic agent of URTD. Clinically healthy animals with known antibody status were infused intranasally with pooled exudate (n = 8) from ill donor animals, with M. agassizii alone (n = 9) or in combination with Pasteurella testudinis (n = 8), with P. testudinis alone (n = 9), or with sterile broth (n = 12). The pooled exudate was culture positive for M. agassizii. Tortoises which received exudate or M. agassizii alone or in conjunction with P. testudinis were significantly more likely to develop clinical disease (P < 0.0004) than animals which received P. testudinis alone or the broth controls. Tortoises demonstrated a strong immune response to M. agassizii, and seroconversion was seen in all groups with clinical disease. M. agassizii was isolated from the upper respiratory tracts of clinically ill animals up to 6 months postinfection. On the basis of the results of these transmission studies, we conclude that M. agassizii is an etiologic agent of URTD in the desert tortoise.

TRANSMISSION STUDIES AT THE LIVING DESERT



TRANSMISSION STUDIES AT THE LIVING DESERT





Desert Tortoise Mycoplasmosis



Journal of Wildlife Diseases, 35(4), 1999, pp. 716–727 © Wildlife Disease Association 1999

SEROEPIDEMIOLOGY OF UPPER RESPIRATORY TRACT DISEASE IN THE DESERT TORTOISE IN THE WESTERN MOJAVE DESERT OF CALIFORNIA

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ABSTRACT: Several factors have combined with an upper respiratory tract disease (URTD) to produce declines on some population numbers of desert tortoises (Gopherus agassizii) in the western USA. This study was designed to determine the seroepidemiology of URTD in a population of wild adult tortoises at the Desert Tortoise Research Natural Area (DTNA) study site in Kern County (California, USA). Prior to initiation of the study, there was a dramatic decline in the number of individuals in this population. At each individual time point, samples were obtained from 12 to 20 tortoises with radiotransmitters during winter, spring, summer, and fall from 1992 through 1995. During the course of the study, 35 animals were sampled at one or more times. Only 10 animals were available for consistent monitoring throughout the 4 yr period. Specific antibody (Ab) levels to Mycoplasma agassizii were determined for individual tortoises by an enzyme-linked immunosorbent assay (ELISA) test. Specific Ab levels were not influenced by the gender of the tortoise. Levels of Ab and distribution of ELISA+, ELISA- and suspect animals were not consistently affected by season within a single year or for a season among the study years. Significantly more tortoises presented with clinical signs in 1992 and 1995. The profile of ELISA+ animals with clinical signs shifted from 5% (1992) to 42% (1995). In 1992, 52% of tortoises lacked clinical signs and were ELISA-. In 1995, this category accounted for only 19% of tortoises. Based on the results of this study, we conclude that URTD was present in this population as evidenced by the presence of ELISA+ individual animals, and that the infectious agent is still present as evidenced by seroconversion of previously ELISA- animals during the course of the study. There is evidence to suggest that animals may remain ELISA+ without showing overt disease, a clinical pattern consistent with the chronic nature of most mycoplasmal infections. Further, there are trends suggesting that the clinical expression of disease may be cyclical. Continued monitoring of this population could provide valuable information concerning the spread of URTD in wild tortoise populations.

Key words: Epidemiology, Gopherus agassizii, Mycoplasma agassizii, serology, upper respiratory tract disease.

Chelonian Conservation and Biology, 2002, 4(2):497–507 © 2002 by Chelonian Research Foundation

Application of Diagnostic Tests for Mycoplasmal Infections of Desert and Gopher Tortoises, with Management Recommendations

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Abstract. - Mycoplasmosis is a transmissible upper respiratory tract disease that has affected plans for management and conservation of wild desert and gopher tortoises in the United States. Although impact of mycoplasmosis on populations of desert and gopher tortoises is unknown, increased prevalence of seropositive animals as well as field observations of clinically ill tortoises have occurred in association with declining populations. In order to help in the identification of potentially infected animals, three tests have been developed to diagnose mycoplasmal infections of tortoises: 1) direct mycoplasmal culture; 2) detection of mycoplasmal chromosomal DNA by polymerase chain reaction (PCR); and 3) detection of anti-Mycoplasma antibodies in tortoise plasma by enzyme-linked immunosorbent assay (ELISA). Each test provides different, complementary information that collectively can be used to define tortoise mycoplasmal infection status. The types of samples required, the predictive value, interpretation, and cost vary among tests. These assays have been used for epidemiological surveys and in decision making for relocation, repatriation, or captive management of tortoises to minimize the risk of outbreaks of mycoplasmal respiratory disease and spread of the causative agent of this disease. Certain features of mycoplasmal infections of tortoises and other animals create a diagnostic dilemma. Multiple Mycoplasma species can cause respiratory disease with identical clinical presentations. Further, individual strains of a given species may vary with respect to their virulence potential, and some species may be commensals rather than pathogens. Current diagnostic tests may not differentiate among mycoplasmal species or strains or permit determination of pathogenicity of individual isolates. Thus, the information provided by testing is not a simple "positive" vs. "negative" issue. While these tests provide much needed information on the exposure of tortoise populations to Mycoplasma species, they do not provide a complete picture of the overall health status of individual tortoises or populations. Unfortunately, test results are often used to make life and death decisions concerning disposition of tortoises being displaced by land development without a complete understanding of the limitations of the diagnostic tests or any consideration of other infectious agents that might be present.

KEY WORDS. – Reptilia; Testudines; Testudinidae; Gopherus agassizii; Gopherus polyphemus; tortoise; Mycoplasma; respiratory disease; diagnostics; management

Journal of Wildlife Diseases, 35(4), 1999, pp. 716–727 © Wildlife Disease Association 1999

SEROEPIDEMIOLOGY OF UPPER RESPIRATORY TRACT DISEASE IN THE DESERT TORTOISE IN THE WESTERN MOJAVE DESERT OF CALIFORNIA

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ABSTRACT: Several factors have combined with an upper respiratory tract disease (URTD) to produce declines on some population numbers of desert tortoises (Gopherus agassizii) in the western USA. This study was designed to determine the seroepidemiology of URTD in a population of wild adult tortoises at the Desert Tortoise Research Natural Area (DTNA) study site in Kern County (California, USA). Prior to initiation of the study, there was a dramatic decline in the number of individuals in this population. At each individual time point, samples were obtained from 12 to 20 tortoises with radiotransmitters during winter, spring, summer, and fall from 1992 through 1995. During the course of the study, 35 animals were sampled at one or more times. Only 10 animals were available for consistent monitoring throughout the 4 yr period. Specific antibody (Ab) levels to Mycoplasma agassizii were determined for individual tortoises by an enzyme-linked immunosorbent assay (ELISA) test. Specific Ab levels were not influenced by the gender of the tortoise. Levels of Ab and distribution of ELISA+, ELISA- and suspect animals were not consistently affected by season within a single year or for a season among the study years. Significantly more tortoises presented with clinical signs in 1992 and 1995. The profile of ELISA+ animals with clinical signs shifted from 5% (1992) to 42% (1995). In 1992, 52% of tortoises lacked clinical signs and were ELISA-. In 1995, this category accounted for only 19% of tortoises. Based on the results of this study, we conclude that URTD was present in this population as evidenced by the presence of ELISA+ individual animals, and that the infectious agent is still present as evidenced by seroconversion of previously ELISA- animals during the course of the study. There is evidence to suggest that animals may remain ELISA+ without showing overt disease, a clinical pattern consistent with the chronic nature of most mycoplasmal infections. Further, there are trends suggesting that the clinical expression of disease may be cyclical. Continued monitoring of this population could provide valuable information concerning the spread of URTD in wild tortoise populations.

Key words: Epidemiology, Gopherus agassizii, Mycoplasma agassizii, serology, upper respiratory tract disease.

Serological Studies with *Mycoplasma* and Herpesvirus in and around Ft Irwin







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Seroprevalence of *Mycoplasma agassizii* and tortoise herpesvirus in captive desert tortoises (*Gopherus agassizii*) from the Greater Barstow Area, Mojave Desert, California

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Abstract

Upper respiratory tract disease (URTD) has been implicated as a cause of decline of wild populations of desert tortoises, *Gopherus agassizii*, in the western Mojave Desert. One explanation for outbreaks of disease may be the release or escape of diseased captive tortoises into naïve wild populations. Because *Mycoplasma agassizii* and tortoise herpesvirus have surfaced as important pathogens, 179 captive tortoises were evaluated in the greater community of Barstow, San Bernardino County, California during 2000 and 2001 to determine pathogen exposure. An indirect enzyme-linked immunosorbent assay (ELISA) was performed to detect antibodies against *Mycoplasma agassizii* (n = 179) and tortoise herpesvirus antibodies were detected in 26.6%. A positive association was found between tortoises with anti-mycoplasma antibodies and severity of clinical signs of URTD (p = 0.001) and with age categories, with adults being more likely to be positive (p < 0.001). Neither association was found with herpesvirus exposure. No association was found between gender and pathogen exposure or between being positive for exposure to both



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Short communication

Identification of a novel herpesvirus from a California desert tortoise (Gopherus agassizii)

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Abstract

Herpesviruses are significant pathogens of tortoises, causing upper respiratory tract disease and necrotizing stomatitis, with infections often associated with high mortality rates. Herpesvirus infection in a captive California desert tortoise (*Gopherus agassizii*) was detected by light microscopic observation of intranuclear inclusion bodies in various tissues followed by transmission electron microscopic observation of herpesvirus-like particles, and amplification of herpesvirus nucleic acid sequences using polymerase chain reaction. Using an indirect enzyme linked immunosorbent assay, anti-tortoise herpesvirus antibodies were detected one month after initial onset of clinical signs. This novel herpesvirus is distinct from the previously described tortoise herpesvirus (tortoise herpesvirus-1, THV-1) sharing 83% sequence identity of 60 amino acids of a portion of the DNA polymerase gene and 79% sequence identity across 120 amino acids of a portion of the ribonucleotide reductase gene. Similar to THV-1, this novel herpesvirus, tortoise herpesvirus-2 (THV-2), also clusters with the alphaherpesviruses. © 2005 Elsevier B.V. All rights reserved.

Keywords: Desert tortoise; Gopherus agassizii; Herpesvirus; Reptiles

What Oral Plaques Can Progress To



January 14, 2003





February 11, 2003

NECROPSIES OF SIX DESERT TORTOISES

(Gopherus agassizii) FROM CALIFORNIA

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> Final Report for USGS/Bureau of Land Management Order No. 02WRSAO642

> > 7 July 2002 to 30 September 2006

EVALUATIONS

- Physical Examinations
- Photography digital images and videos
- Hematology and plasma biochemistries
- •Serology Mycoplasma and Herpesvirus
- Necropsy, light microscopy, and electron microscopy
- Organ weights spleen, liver, kidneys
- Urinanalysis
- Aerobic bacterial cultures
- Mycoplasma cultures of nasal cavity and PCR
- Herpesvirus PCR on the brain
- Liver, kidney and shell samples collected for toxicants

Desert Tortoise Necropsy Sample Checklist

Date Collected	Test/Assay	Tissue/Sample	Amount	Tube/Container
	CBC	Whole blood	0.75 ml	1 microtainer lithium heparin tube
	Plasma Biochemicals: reptile vet 20 (including calcium, magnesium, triglycerides, and osmolality) —	Plasma	1.5 ml	5 microtainer lithium heparin tubes
	lonized calcium	Whole blood or plasma	0.5 ml	1 ml syringe coated with Na heparin
	Bile acids	Plasma	0.2 ml	1 microtainer lithium heparin tube
	Iron and iron binding capacity	Serum	0.2 ml	1 microtainer clot tube
	Mycoplasma ELISA	Plasma	0.2 ml	1 lithium heparin microtainer tube
	Mycoplasma culture	Right and left nasal flushes. Swabs of right and left nasal cavity and right and left lung.	4 swabs	B-D Microbial Swabs
	Aerobic bacterial culture	2 swabs of each nasal cavity and two swabs of each right and left lung	8 swabs total	B-D Microbial Swabs
	Urinalysis (including osmolality, Na, K)	Urine	5 ml	Urine cup
	Heavy metals and minerals	Liver, right kidney, scute (LC2,3,4), and dermal bone	1 gram per tissue	2 plastic Cups, 1 whirl pak bag
	Heavy metals and minerals	Whole blood	1.5 ml	2 ml Na heparin tube
	Organochlorines	Liver and right kidney	5 grams per tissue	2 plastic cups
	Organochlorines	Whole blood	2 ml	2 ml Na heparin tube
	Vitamins A, E, and D3	Liver and left kidney	I5 grams per tissue	2 plastic cups
	Vitamins A, E, and D3	Plasma	3 ml (Vitamins A: 1.2 ml, E: 1.2 ml; D3: 0.5 ml	2 4-ml Na heparin tubes
	Arsenic	Scute (RC2,3,4), cranial, and caudal right and left lung frozen and in formalin.	1 gram per tissue	11 plastic cups
	Histology	Full set of tissues	For routine histopathology	38 tissue casettes
	Electron Microscopy	Left and right nasal cavity	1 mm cubes	Plastic cup -Trumps solution
	Herpesvirus PCR	Tongue, submandibular glands	Sagittal section of brain	Sterile eppendorf tube
	Parasitology – E. Greiner	Colon contents	5 grams	Plastic cup
	Bone density -T. Wronski	Right femur	Entire defleshed femur	Plastic cup – 70& ethanol

OXALOSIS IN WILD DESERT TORTOISES, GOPHERUS AGASSIZII

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ABSTRACT: We necropsied a moribund, wild adult male desert tortoise (*Gopherus agassizii*) with clinical signs of respiratory disease and elevated plasma biochemical analytes indicative of renal disease (blood urea nitrogen [415 mg/dl], uric acid [11.8 mg/dl], sodium [>180 mmol/l] and chloride [139 mmol/l]). Moderate numbers of birefringent oxalate crystals, based on infrared and electron microscopy, were present within renal tubules; small numbers were seen in colloid within thyroid follicles. A retrospective analysis of 66 additional cases of wild desert tortoises was conducted to determine whether similar crystals were present in thyroid and kidney. The tortoises, from the Mojave and Sonoran deserts, were necropsied between 1992 and 2003 and included juveniles and adults. Tortoises were classified as healthy (those that died due to trauma and where no disease was identified after necropsy and evaluation by standard laboratory tests used for other tortoises) or not healthy (having one or more diseases or lesions). For all 67 necropsied tortoises, small numbers of crystals of similar appearance were present in thyroid glands from 44 of 54 cases (81%) and in kidneys from three of 65 cases (5%). Presence of oxalates did not differ significantly between healthy and unhealthy tortoises, between age classes, or between desert region, and their presence was considered an incidental finding. Small numbers of oxalate crystals seen within the kidney of two additional tortoises also were considered an incidental finding. Although the source of the calcium oxalate could not be determined, desert tortoises are herbivores, and a plant origin seems most likely. Studies are needed to evaluate the oxalate content of plants consumed by desert tortoises, and particularly those in the area where the tortoise in renal failure was found.

Key words: Calcium oxalate, desert tortoise, Gopherus agassizii, renal failure.

Kidney - Birefringent Crystals

Unpolarized

Polarized





NECROPSIES OF DESERT TORTOISES

(Gopherus agassizii) FROM CALIFORNIA

Elliott R. Jacobson¹ and Kristin H. Berry²

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³United States Geological Survey, Western Ecological Research Center, Box Springs Field Station, Moreno Valley, CA

> Annual Report for Year One (2008-2009) Order No. 07WRCN0020

> 20 September 2009 to 19 September 2010

Serological and Molecular Evidence for Tortoise Herpesvirus 2 infection in Wild Desert Tortoises, *Gopherus agassizii*

Elliott R. Jacobson¹, Kristin H. Berry², James F.X. Wellehan Jr.¹, Francesco Origgi³, April L. Childress¹, Josephine Braun⁴, Mark Schrenzel⁴, Bruce Rideout⁴

Mycoplasma testudineum sp. nov., from a desert tortoise (*Gopherus agassizii*) with upper respiratory tract disease

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Mycoplasma testudineum sp. nov., first cultured from the upper respiratory tract of a clinically ill tortoise (*Gopherus agassizii*) in the Mohave Desert, was distinguished from previously described mollicutes serologically and by 16S rRNA gene sequence comparisons. It lacks a cell wall; ferments glucose, mannose, lactose and sucrose; does not produce 'film and spots'; does not hydrolyse arginine, aesculin or urea; is sensitive to digitonin; and lacks phosphatase activity. The organism causes chronic rhinitis and conjunctivitis of tortoises. The type strain of *M. testudineum* is BH29^T (=ATCC 700618^T=MCCM 03231^T).

NECROPSIES OF TWELVE DESERT TORTOISES (Gopherus agassizii) FROM CALIFORNIA

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> Annual Report for 2008 Order No. 07WRCN0020

20 September 2007 to 19 September 2008

12 Tortoises: Ft. Irwin Translocation Project



12 Tortoises: Ft. Irwin Translocation Project



Evidence for the Pathogenicity of *Mycoplasma testudineum* in Free-ranging Desert Tortoises, *Gopherus agassizii*

Elliott R. Jacobson and Kristin H. Berry

Department of Small Animal Clinical Sciences College of Veterinary Medicine, University of Florida, Gainesville, Florida and United States Geological Survey Western Ecological Research Center, Box Springs Field Station Moreno Valley, California



Normal







Mild



Severe

Bilateral palpebral reduction and concurrent mycoplasmosis in a wild Agassiz's desert tortoise (*Gopherus agassizii*)



A Treatment Protocol for Upper Respiratory Tract Disease of Desert Tortoises, *Gopherus agassizii*

James L. Jarchow, D.V.M.

Abstract: A retrospective study of 10 desert tortoises presented to a veterinary hospital with signs of Upper Respiratory Tract Disease (URTD) was conducted to evaluate a treatment protocol consisting of parenteral administration of enrofloxacin in conjunction with nasal cavity flushes using a combination ofenrofloxacin and dexamethasone. Of the 10 tortoises treated in this manner, 2 had recurrent episodes of clinical URTD while 8 remained asymptomatic on follow-up examinations for 11 to 78 months after treatment.