Michael Dunnoughs

CONFERENCE

HEALTH PROFILES, REFERENCE INTERVALS, AND DISEASES OF DESERT TORTOISES

Soda Springs, California
October 31 - November 3, 1996

Sponsored by the U.S. Geological Survey, with support from the National Training Center at Fort Irwin, California, and the Washington County Habitat Conservation Plan Office, Utah

AGENDA

CONFERENCE ON HEALTH PROFILES, REFERENCE INTERVALS, AND DISEASES OF DESERT TORTOISES

Soda Springs, California
October 31 - November 3,1996

Thursday. October 31, 1996

3:00 - 5:00 p.m.	**Arrivals**
5:00 - 6:30 p.m.	Introductions and Preliminary Discussions about Expectations
6:30 p.m.	Dinner
8:00 p.m.	The purposes and objectives of the conference A brief introduction to the desert tortoise and the reasons for the Federal listing as a threatened species *Kristin H. Berry*

SESSION I. Health profiles and reference ranges of healthy and ill tortoises in the Mojave and Sonoran deserts of the United States

SESSION CHAIR: Dr. Tim Lumsden, University of Guelph, Ontario

8:00 **p.m.** Sex, site and seasonal effects on hematologic and biochemical values of free-ranging Mojave desert tortoises over a 5-year period.
Mary M. Christopher, K. H. Berry, I. Wallis, K. Nagy, B. Henen, and C. Peterson:

9:00 p.m. Reference ranges for blood variables: Issues regarding which data to include, and what to do when all your tortoises are healthy
Patrick E. Lederle, Kurt R Rautenstrauch, Danny L. Rakestraw, and Katherine K. Zander

9:30 p.m. **DISCUSSION**

Friday. November 1, 1996

7:00 a.m. Breakfast

SESSION I, continued.

8:00 a.m. Health studies of desert tortoises in the eastern Mojave Desert of Arizona and Utah and the central Sonoran Desert of Arizona
Vanessa Dickinson, Timothy Duck, Cecil R Schwalbe, James L. Jarchow, and Mark Trueblood

SESSION II. Background Information on Diseases of Tortoises; Assessing Health and Disease

SESSION CHAIR: Dr. Tim Lumsden, University of Guelph, Ontario

9:00 a.m. **Causes of Mortality and Diseases in Tortoises: A Review**
Elliott R Jacobson,

9:20 a.m. **Field methods for evaluating health, diagnosing diseases, and** salving **desert tortoises**
Kristin H. Berry and Mary M. Christopher

9:50 a.m. Break

10:20 a.m. **Drought-induced dehydration and starvation in juvenile and adult desert tortoises from the western and eastern Mojave Desert**
E. Karen Spangenberg Kristin H. Berry, Bruce L. Homer, and Elliott *R Jacobson*

10:50 a.m **Patterns of disease and laboratory abnormalities in three populations of desert tortoises in the western and eastern Mojave Desert (includes data on anomalous health profile material,** shell **lesions, survivorship and mortality)**
Mary M. Christopher, K. H. Beny, I. Wallis, X Nagy, B. Henen, and *C. Peterson.*

11:30 a.m. Lunch

1:00 p.m. **Necropsy of free-ranging desert tortoises - the ultimate health assessment**
Bruce L. Homer, Elliott *R Jacobson, and Kristin H. Berry*

2:00 p.m. DISCUSSION

Questions to be addressed include:

A. How would we expect values for healthy tortoises to vary geographically?

B. What criteria need to be established to determine whether tortoises are "ill" or "healthy," so reference range categories can be established?

C. What are the most reliable and valuable components of the hematological and chemical profiles for determining ill health and pathology?

D. What is the impact of repeatedly drawing blood samples on individual tortoise stress levels and health? It appears that the incidence of disease has increased in each health profile study area following initiation of research. Is this relationship real, or an artifact of the methodology? Is research being conducted in this area?

E. Do positive ELISA values constitute an ill tortoise?

F. What risk factors can we use to determine if a population is at **risk?,** e.g., similar to cholesterol and HDL in humans?

G. What do we know about the immunology and the normal immunological status of tortoises? Does the animal have the usual immunological classes and can cell mediated immunity be demonstrated?

H. What are the management implications of health assessments? How can the information be used to select animals for release to the wild, to be translocated, or to be salvaged from the field?

2:30 p.m. **Break**

SESSION 111. INFECTIOUS DISEASES: The Mycoplasmas and Desert Tortoises

SESSION CHAIR: Dr. Joseph Tully, NIAID

3:00 p.m. An overview of mycoplasmal respiratory infections in animals
Mary B. Brown

3:30 p.m. The establishment of *Mycoplasma agassizii* as the cause of URTD: experimental infection studies. A brief overview of the experimental transmission studies
Mary Brown

3:45 p.m. URTD diagnostic tests and limitations: Culture, PCR, histology, and serology. A comprehensive discussion on current diagnostic methods used, limitations of each, how to evaluate the test results, and what each test does and does not tell us.
Presented by Dan Brown Isabella M. Schumacher, Grace S. McLaughlin, E. R Jacobson, Mary b. Brown, Paul A. Klein, and Daniel R Brown

4:30 p.m. Relationship between clinical signs of upper respiratory tract disease and antibodies to *Mycoplasma agassizii* in desert tortoises from Las Vegas Valley, Nevada
Isabella Schumacher, D. Bradford Hardenbrook, Mary B. brown, Elliott R Jacobson, and Paul A. Klein

5:00 p.m. Serologic survey of desert tortoises, *Gopherus agassizii*, around the National Training Center, Fort Irwin, California, for exposure to *Mycoplasma agassizii*, the causative agent of upper respiratory tract disease
Elliott R Jacobson, Mary B. Brown, Paul A. Klein, Isabella Schumacher, David Morafka, and Rebecca A. Yates

5:30 p.m. Break and Dinner

7:30 p.m. Seroepidemiology of upper respiratory tract disease in the desert tortoise: A four year prospective study at the Desert Tortoise Research Natural Area in the Western Mojave Desert

Seroepidemiology of upper respiratory tract disease in the desert tortoise: A four year prospective study at Ivanpah Valley and Goffs in the eastern Mojave Desert

Mary Brown, Isabella M. Schumacher, Paul A. Klein, Kenneth A. Nagy, and Kristin H. Berry

8:15 p.m. Health and disease monitoring of the desert tortoise population at Yucca Mountain
Patrick E. Lederle, Kurt R Rautenstrauch, Danny L. Rakestraw, Katherine K. Zander, and James L. Boone

9:00 p.m. **DISCUSSION**

Saturday, November 2, 1996

7:00 a.m. Breakfast

8:00 a.m. Upper respiratory tract disease in gopher **tortoises,** *Gopherus* ***polyphemus*****:** Natural disease, experimental studies, and implications for conservation **and** management..
Grace S. McLaughlin, Elliott R Jacobson, Mary B. Brown, Dan R Brown, C. E. McKenna, Isabella M. Schumacher, *and* ***Paul A. Klein***

SESSION IV. Upper Respiratory Tract Disease, Physiology, Reproduction, and Demography

SESSION CHAIR: Dr. Joel Baseman

8:40 a.m. Does mycoplasma **infection** influence water, energy and **food** consumption or reproductive output **of** wild desert **tortoises?**
Ken Nagy, Brian T. Henen, Ian R Wallis, Mary B. Brown, and Charles C. Peterson

9:10 a.m. Upper respiratory **tract** disease in hatchling tortoises
Olav Oftedal, Terry E. Christopher, *and* ***Mary E. Allen.***

9:40 a.m. Short-term effects of upper respiratory tract disease on reproduction in the desert tortoise, ***Gopherus agassizii*****: Hormones,** *egg* production and hatching success
David C. Rostal, Valentine A. Lance, Janice S. Grumbles, and Isabella M. Schumacher

10:10 a.m. Break

10:40 a.m. Have mycoplasmas affected desert tortoise populations?
Dan Brown

11:10 a.m. High mortality rates and population declines in desert tortoises at the Desert Tortoise Research Natural **Area:** 1988-1996
Kristin H. Berry

11:40 a.m. Lunch

12:45 p.m. Summary **of** Upper Respiratory **Tract** Disease: **What** we **know** about URTD, what we **suspect,** and what other questions need **to be** addressed?
Elliott Jacobson

1:00 p.m. Discussion

Discussion Leaders: Drs. Joe Tully and Joel Baseman

A. What are we missing and losing in interpretations **by our** lack of knowledge about the new but unnamed **mycoplasma** (*M. "mysteriosa"*)?

B. What factors affect severity of clinical signs of disease?

C. For individual tortoises, what criteria are required for a definitive diagnosis of mycoplasmosis?

D. What factors lead to death in a mycoplasma-infected animals? How long does mycoplasma take to kill a tortoise? How does mycoplasmosis express itself internally, as well as externally, in the desert tortoise?

E. How does a tortoise fight the consequences of disease?' How do the antibodies for mycoplasma work in the desert tortoise.

F. Is the disease as prevalent now as it was perceived to be in the late 1980s and early 1990s? What environmental factors are different now? What environmental factors predispose individuals and make them more susceptible to the disease?

G. Is the disease an issue across the entire range of the tortoise? Lack of clinical signs and other evidence suggest that the disease is not a significant at Yucca Mountain.

H. Does nutritional status affect:

1. the prevalence of URTD in populations
2. the susceptibility of individuals?
3. the manifestation of signs?

I. Does presence and manifestation of clinical signs of URTD affect

1. shedding of *M. agassizii?*
2. foraging efficiency

J. What do we know about the immunology and the normal immunological status of tortoises? Does the animal have the usual immunological classes and can cell mediated immunity be demonstrated? (Same as F, presented in earlier DISCUSSION)

K. What role have recent droughts played in the health and incidence of disease in tortoise populations?

L. Nutrients of concern

1. Nitrogen (importance of reproduction and perhaps immune response)
2. Potassium (because of the effects on N excretion)
3. Vitamins low in dried plants, e.g., vitamin A and vitamin C
4. Phosphorus? Trace minerals?

M. What is the likelihood that mycoplasma has always been present across the range of the tortoise? Are released captives really the source of a pathogenic strain of the mycoplasma?

N. Present research on disease and health profiles of desert tortoises is basic and descriptive at the present. What are the expected applied results for land managers in the next year, 5 years, or 10 years? A submitted question.

O. How will present and planned disease research facilitate the long-term preservation of healthy tortoise populations in the national parts (or anywhere)? What role do the national parks play in overall research on disease and health research on desert tortoises?

P. Essential research topics and priorities

Sunday a.m., November 3, 1996

SESSION V. MANAGEMENT OF TORTOISES - IMPLICATIONS FOR HEALTH AND DISEASE

SESSION CHAIR: ELLIOTT JACOBSON

800 a.m. **An international overview of turtle and tortoise issues relating to relocation, repatriation, captive breeding, and other related topics.**
John Behler

8:45 a.m. **Health assessment of tortoises: Not an easy task**
Elliott R Jacobson

9:30 a.m. DISCUSSIONS

A. Handling procedures, the use of gloves.

B. Collapsing **of** natural and artificial burrows in pens, enclosures, etc. Disinfecting burrows.

C. Active vs. passive management of disease in wild populations

D. Expectations about recovery **of** populations

10:30 a.m. Elliott Jacobson and John Behler: Development **of** decision tree **for** management of captive tortoises, translocated tortoises, seized animals

11:30 a.m. Future goals and action items

12:00 Noon Lunch and Adjournment

ABSTRACTS

High Mortality Rates and Population Declines in Desert Tortoises at the Desert Tortoise Research Natural Area: 1988-1996

Kristin H. Berry
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In 1988 wild or free-ranging desert tortoises with clinical signs of upper respiratory tract disease (URTD) were observed at the Desert Tortoise Research Natural Area (DTNA) in eastern Kern County, California. The observations were made at two sites: 1) on a long-term research plot in the interior of the Natural Area, and 2) on an adjacent site where graduate student Charles Peterson was conducting research on water balance and energy requirements of adult tortoises. In 1989, Drs. E. R. Jacobson and J. Gaskin visited the site and 12 ill tortoises were salvaged for necropsy. Subsequently the pathogen causing URTD was determined to be a mycoplasma.

Between the 1970s and 1996, several surveys were undertaken to gather data on tortoise population attributes and trends, as well as condition of habitat. One long-term study plot was established in the interior in 1973 and another in the vicinity of the interpretive center in 1979. Short-term studies were established at two other sites for research on: (1) water balance, energy flow, and health profiles (1988-1989) adjacent to the long-term interior plot; and (2) effects of translocation and supplemental irrigation within four enclosures (1989-1991). The most appropriate site for evaluating effects of URTD is the long-term study site in the interior.

The interior study plot was surveyed with 60-day spring surveys in 1979, 1982, 1988, 1992, and 1996, and data on population attributes were collected. Densities of all tortoises and densities of the potentially breeding tortoises ($\geq$ 180 mm mid-carapace length) were calculated using the Stratified Lincoln Index The total population declined from 149 tortoises/km^2 (95% Confidence Interval [CI] = 115-195) to 7 tortoises/km^2 (95% CI = 3-18) between 1979 and 1996. The population of breeding tortoises started with 59 tortoises/km^2 (95% CI = 45-78) in 1979, increased to 92/km^2 (95% CI 71-119) in 1982, and then declined to 5 tortoises/km^2 (95% CI = 2-13) in 1996. Few juveniles remain.

Mortality rates can be calculated in different ways, such as using crude or general death rates or an annualized death rate. Another method is to follow a particular cohort through time. The 1988 cohort of 178 tortoises was registered during the 60-day survey both within and adjacent to the boundaries of the interior plot. These 178 individuals consisted of 156 breeding tortoises and 22 juvenile and immatures. Between 1988 and 1996, 43% of the remains of the small or young adults and 61-65% of the larger adults were found and identified. Only 11% of the 178 tortoises registered in 1988 was observed alive in later years. Among the breeding tortoises, only 5% of the females and 14% of the males were observed alive after 1988. Mortality rates between 1988 and 1996 were high for the breeding tortoises and continue to remain above the 1-2% annualized rate expected for a stable population.

Drought has been suggested as a contributor to the population declines. Precipitation records were compiled for 1972-1995 for two nearby stations (Mojave, Randsburg). The annual precipitation for the rainfall year (October through September) and the winter rainfall (October through March) were compared. Winter rainfall better reflects the forage availability for the desert tortoises in the western Mojave Desert than total annual precipitation. Rainfall records show that winter precipitation was below the norm for four consecutive years between 1972 and 1980 and was below the norm for six years between '80-81 and '94-95. The below winter norms were consecutive in '88-89 and '89-90. In summary, precipitation was below the norm in 10 of 23 years and exceeded the norm in 13 years. The pattern in the 1980's and early 1990's does not appear unusual.

Field Methods for Evaluating Health, Diagnosing Diseases, and Salvaging Desert Tortoises

Kristin H. Berry[1] and Mary M. Christopher[2]
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Most research on populations of wild animals is conducted by wildlife biologists, zoologists, and ecologists without collaboration with veterinary medical specialists. Many research projects, especially those dealing with rare and endangered animals, could benefit from the contributions of veterinarians and other health specialists (Boyce et al., 1992, Kirkwood, 1994) at every phase. The desert tortoise (*Gopherus agassizii*), a wide ranging species of the arid southwestern United States and Mexico, provides an excellent model for how interdisciplinary teams of research scientists developed techniques to evaluate health and diagnose diseases. In this paper we present a model set of standardized field techniques for collecting and analyzing qualitative and quantitative data on clinical and physical signs of health, disease, and trauma for wild desert tortoises. The model is applicable to other chelonians and reptiles. The techniques were designed to maximize acquisition of data for demographic, ecological, health and disease research projects; to reduce handling and stress to tortoises; to avoid spread of infectious disease; to promote high quality and consistent data sets; and to reduce duration and number of field trips.

Preuarations for the Field. Prior to initiating field work, project participants should familiarize themselves with the literature on wild tortoises to optimize time and expedite locating the species. Key documents include ***The Desert Tortoise (Mojave Population) Recovery Plan*** (U. S. Fish and Wildlife Service, 1994), an annotated bibliography by Grover and DeFalco (1995), and several papers in the 1994 issue of Herpetological Monographs No. 8 on physiological ecology, thermoregulation, behavior, and reproductive physiology. Since wild tortoises are easily accessible about 1.7% of each year, knowledge of local and regional differences in daily and seasonal activity periods is essential. Field workers can hone their techniques through practice on captive chelonians.

Procedures to Prevent Spread of Diseases and Parasites. Special precautions must be taken to prevent transmission of upper respiratory tract disease (URTD) caused by ***Mycoplasma agassizii*** (Brown et al., 1994) and other infectious diseases within and between tortoise populations (Jacobson, 1593; 1994). Each tortoise should be handled with a fresh pair of disposable gloves, which is placed in a plastic trash bag after use and disposed of appropriately off-site. Each item of equipment touching the tortoise, including poles used to probe tortoise or other animals burrows, must be disinfected with bleach or ethanol. Precautions must be taken to assure that the tortoise does not touch or rest on the field worker's limbs, clothing, or equipment, without a protective covering. To prevent transmission from site to site, clothes and shoes must be disinfected prior to use on other sites and field vehicles may require thorough external and internal cleaning. Careful adherence to the above procedures can also help to reduce transfer of ticks (e.g., ***Ornithodoros parkeri*** and ***O. turicata***), potential vectors of American tickborne relapsing fever, to humans.

Forms for Recording Field Data. Efficient and effective data collection can be accomplished by following written protocols and recording appropriate data on standardized forms printed on archival paper or incorporated into portable computerized databases. We have used three forms successfully: Journal Notes, Data Sheets for Live Tortoises, and Health Profile Forms.

Journal Notes. Journal Notes should provide background data essential for interpreting whether the activities and behaviors of tortoises are typical of ill or healthy animals, as well as for identifying potential sources of trauma, illness, or disease. Detailed weather conditions and rainfall events are essential. Journal Notes should contain survey times and effort, numbers of live and dead tortoises observed, starting and ending times for field work, time expended in searching for and processing tortoises, and observations of other animals. The Journal Notes are the appropriate place to record precise locations of animals and provide descriptions of habitat condition and evidence of historic and recent human activities (abandoned roads and railroads, mine sites, campsites, evidence of shooting and off-road travel, etc.).

Data Sheet for Live Tortoises. This data sheet is used for recording basic demographic and ecological data for each tortoise observed or captured and contains parameters useful for calculating condition indices and equations related to carapace length and mass. Critical parameters include: date, time and precise location of capture; unique tortoise identification number (notching of shell, numbers glued to scutes, PIT tags; type of capture; sex, body measurements and weight; and activities and behaviors. Other important data include: signs of previous captivity on the tortoise, signs of growth, size and condition of chin glands; and anomalies on the shell. These types of data are useful for determining population attributes and trends; individual and population growth rates; recruitment of young into adult age classes; survivorship by sex and cohort; causes of mortality; and changes in length to weight ratios.

Health Profile Form. The Health Profile Form incorporates standard parameters used to evaluate captive chelonians, as well as new parameters associated with recently described and commonly observed diseases (mycoplasmosis, cutaneous dyskeratosis, shell necrosis) and trauma. The tortoise should first be observed from a distance, and if possible, before it responds with defensive or aggressive postures or movements. Critical factors include postures, particularly position of the head and limbs (e.g., basking, resting, waking); activities and behaviors; and general and specific locations in the environment.

The shell and integument should be examined first for ectoparasites, such as argasid ticks, and their localities on the shell recorded. Then samples of ectoparasites should be taken for identification. The general appearance of the shell and integument should be evaluated after it is cleaned. Dust and dirt are easily removed with a brush, but caked dirt or mud may require washing. Once the shell is clean, factors to evaluate include: clean and glossy vs. dull and caked with dirt; evidence of fungi or discoloration; condition of head and extremities (signs of edema, emaciation, trauma); and ability to retract head, neck and limbs tightly into the shell. Shell lesions should be described in terms of distribution, severity, and chronicity using the diagram on the form or computer database. Most tortoises > 120 mm in mid-carapace length are likely to have some type of lesion on the scutes, underlying dermal bone, and/or extremities from trauma or cutaneous dyskeratosis. Signs of predator attacks should include notes on the potential predator, as well as relative age of the wound, lesion, or scar. Such data, when compiled over several years, can be used to compare survivorship of the different age classes of tortoises to predator attacks and to measure predator pressure on populations. Cutaneous dyskeratosis and other shell diseases should be graded by distribution on the shell, severity, and approximate age of lesion or chronicity for each of three body regions (carapace, plastron, and limbs). Depressions in underlying scutes and bone may be due to nutrition, metabolic bone disease, or a normal part of the aging process.

The beak, nares, eyes, and chin glands provide subtle signs indicative of health or disease. Field workers should look for evidence of recent moisture associated with eyes, nares, and beak; the amount, color, consistency, and turbidity of any exudate or bubbles should be recorded. Dirt on the

beak and *eyes* may be a sign of illness or of recent drinking after a rain storm. Tortoises with a tenacious exudate may have moisture or dried dirt on the medial surface of the forelegs. The color, surface, and condition of the beak may reflect health status as well as recently consumed food items. Chin or mental glands may be abnormally swollen and draining. The surface of the eye, appearance of eyelids, and periocular region should be examined closely for abnormal coloration; presence of dampness, mucus or drainage; and edema--all of which may be signs of URTD or other illnesses. Line drawings were developed of the normal eye and eyes with clinical signs of disease. Embedded dirt and debris are signs of previous moisture, mucus, and drainage.

Permanent Photographic Records. Full-frame images of the head (frontal and lateral views), carapace, plastron, and the posterior costal scutes of each tortoise should be taken with 35-mm slide film at least once during each survey year for identification; to gather data on numbers of growth rings produced and how the growth rings change in appearance over time; to verify how contours of the shell age; to record the condition of the eyes, nares, and beak and to confirm how damaged shell replaces itself over time. Additional photographs can be taken of recent or previously healed injuries to the head, limbs, or shell or unusual abnormalities. If signs of disease are present, then slides can be taken more frequently. The slides have been invaluable in confirming identification for tortoises recaptured after long intervals, after predator attacks when notches have been chewed away, and after death, after the shell is disarticulated or fragmented. Permanent photograph records are invaluable for retrospective analyses of progression and regression of signs of diseases.

Salvage of Tortoises for Necropsy. Necropsies of ill, dying, or recently dead wild tortoises provide a wealth of information about causes of death in populations and should be incorporated into field research protocols. Decisions on criteria for salvage require advance planning and can be placed in three categories: the deliberate and planned salvage of animals with specific disease signs or syndromes for special research projects, obvious cases of injured or dying animals, and the more difficult and subtle cases of individuals that show signs of disease but still appear alert and active. Records from 59 salvaged tortoises were evaluated retrospectively to develop criteria for salvage. Severely debilitated tortoises exhibited lethargy, inactivity, severe emaciation or dehydration, low weight for carapace length, inability to retract limbs into the shell, partial paralysis, severe shell lesions, and severe trauma. Inappropriate behaviors also provided clues to poor condition.

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An Overview of Mycoplasmal Respiratory Infections in Animals

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Mycoplasmas are true bacteria with several unique characteristics. They are the smallest free-living prokaryote, with a genome site of about 750 Kb, about one third that of E. coli. Unlike other bacteria, mycoplasmas lack a cell wall. Clinically, this means that antibiotics which act on the cell wall, such as penicillin, are not effective. Antibiotics which target protein synthesis, transcription and translation are effective, Mycoplasmas *are* recognized as pathogens *of* man. animals, plants, and insects. Pathogenic mycoplasmas have a predilection for serous surfaces. The primary colonization sites are the respiratory tract. joint, *eye*, urogenital tract, and mammary gland. Until recently it was thought that mycoplasmas were rarely invasive; however, it is now known that at least some mycoplasma species can invade and replicate within cells, perhaps providing a potential mechanism for chronicity. Mycoplasmas produce clinically silent, chronic infections. Disease is often exacerbated by environmental factors or by synergistic action of other infectious agents. The primary diseases caused are pneumonia *arthritis*, mastitis and reproductive wastage. Mycoplasmas are in close, intimate contact with host mucosal surfaces. Some even have specialized attachment tips. Mycoplasmas do not survive well outside their host, and are especially susceptible to desiccation, A few species can survive for several weeks in moist environments such as manure or bedding, but probably do not reproduce due to lack of nutrients. Host factors are important in the disease process.

Current methods of diagnosis of mycoplasmal infections involve cultural isolation, immunofluorescence of tissues. or detection of antibody, Cultural isolation requires the use of special media or transport fluid. Culturettes are nor suggested. Some swabs are toxic for mycoplasmas and should not be left in the transport medium. The interval between collection and plating should be kept at a minimum. Once colonies *are* obtained, mycoplasmas are identified presumptively on the basis *of* a few limited biochemical tests and confirmed by serological means such as growth inhibition, immunofluorescence, immunobinding and ELISA, Newer molecular diagnostic tests, such as polymerase chain reaction (PCR) are currently in development, Antibody determination can be used *if* both acute and convalescent sera are available or as a screening tool to determine the negative status of a flock (M. gallisepticum in poultry), or of an individual animal. Serology is most useful to screen exposure to the agent *in* situations where strict maintenance of mycoplasma - free population is needed (poultry, rodents, swine). Histopathology and electron microscopy *of* infected tissues may *also* be used for diagnosis,

Mycoplasmas cause respiratory disease in man, cattle, poultry, laboratory rodents, swine, small ruminants *and* other animal species. The most important respiratory infections in the U.S. are *those* of poultry, swine and rodents. In order for a mycoplasma to cause respiratory disease, it must attach to and colonize *the* respiratory surface. Because the host immune response can potentiate lesion formation, mycoplasmas must induce a immune response by the host to cause disease. Respiratory mycoplasmoses *are* often clinically silent and chronic in nature. Exacerbation by environmental factors or stress as well as synergistic action with other infectious agents increase the severity of the disease and the appearance of clinical signs,

Most respiratory mycoplasmal infections are characterized by upper tract lesions, epithelial and lymphoid hyperplasia, sequential destruction of the respiratory epithelium, and the loss of ciliated cells and normal epithelium. Influxes of inflammatory cells in focal areas are common.

The common respiratory diseases are summarized in the following table.

Economic Importance	Host	Etiologic Agent
Major	Rats, Mice	M. pulmonis
	Swine	M. hyopneumoniae
		M. hyorhinis
	Poultry	M. gallisepticum
		M. synoviae
		M. meleagridis (turkeys)
	Cattle, Buffalo (non U.S.)	small colony M. mycoides
Minor	Cattle	U. diversum
		M. bovis
		M. dispar
		M. bovigenitalium
	Poultry	M. iowae
	Goats, Sheep	Large colony M. mycoides "cluster"
		M. capricolum
		M. ovipneumoniae

Ruminants:

In ruminants, CBPP is a major economic problem worldwide but is not found in the U.S. Disease is transmitted via aerosols from affected animals, respiratory expulsion of infected material from pulmonary sequestra of "carrier" animals, urinary shedders, and transplacental infection. Subclinically affected and recovered animals are clinically inapparent reservoirs of infection.

There is increasing evidence that infections with mycoplasmas indigenous to the U.S. cause respiratory disease in cattle. The main problem is that these mycoplasma spp. have been recovered from the respiratory tract of normal cattle, Disease is normally associated with young (3-6 mo) dairy calves. Calf pneumonia is often multiple etiology, including a complex of viral, bacterial, and mycoplasmal agents. M. bovis, M. dispar, M. bovigenitalium and U. diversum can cause calf pneumonia. M. dispar has been associated with pneumonia in naturally and experimentally infected calves, although in many cases the pneumonia would have gone unrecognized if the calves had not been killed and examined at necropsy. M. bovis causes moderate to extensive pulmonary consolidation in experimentally challenged calves. Pyrexia, dyspnea, depression, and lameness were associated clinical signs. Ureaplasma diversum has been associated with pneumonia, particularly in "weak calf' syndrome. There is evidence that ureaplasma may be acquired in utero.

With the exception of M. dispar, "cuffing" pneumonia is seen. Cuffing pneumonia is characterized by hyperplasia of peribronchial lymphoid tissue. M. dispar causes interstitial pneumonia and alveolitis. Although usually self-limiting, severe disease occurs when other infectious agents are present Arthritic complications may be seen with M. bovis. The practice of feeding infected milk to calves has resulted in transmission and isolation of mycoplasmas from the calf lung. Diagnosis is usually made by culture. Disease is often self limiting. Vaccines have been used for M. dispar in Europe.

Rodents:

M. pulmonis infection is is a major disease problem in laboratory rodents. Almost all conventional rodent colonies have MRM. It is clinically silent until the terminal stages of the disease. Primary lesions are rhinitis, otitis media, laryngotracheitis. and bronchopneumonia. In the terminal stages. animals exhibit rales, eye rubbing, head tilt, roughened coat, nasal discharge.

and weight loss. Transmission occurs by aerosol, but can be transmitted in utero. Control is based on identification of negative breeders, Cesarian derivation, strict barrier isolation, and monitoring of sentinel animals, preferably retired breeders. The current screening test used is the ELISA. However, then is a period of 1-3 months between the decline of maternal antibody and production of antibody by the rat which causes diagnostic confusion. No vaccine is available, although there has been work with temperature-sensitive mutants which provide partial protection. The disease is potentiated by high ammonia levels and viruses, especially Sendai. Host factors are important and there are differences in strain susceptibility, In mice, C3H/Hen and CO-1 mice are susceptible; C5BL/6 are resistant. F344 rats are resistant; LEW rats are susceptible.

Swine:

Three recognized species of mycoplasmas are commonly pathogenic for swine. M. hyorhinis causes respiratory disease, polyserositis, and polyarthritis in 3-10 week old swine. causes polyarthritis in swine after 10 weeks of age. M. hyopneumoniae causes enzootic pneumonia. This disease is world-wide, and 30-70% of market age hogs have classic lesions. Although there is a high morbidity, mortality is essentially 0. The disease is chronic and characterized by a dry, nonproductive cough. Finishing pigs are most affected, and up to 20% decrease in feed/gain ratio is seen. Characteristic lesions are lobular, glistening, and purple. Airways may have a sticky exudate. Spread is by droplet infection. Current control is by tiamulin or tylosin antibiotic therapy - but this does not cure disease and carriers are established. A vaccine is currently used with moderate success. Early vaccines caused arthritis,

Poultry:

M. gallisepticum, M. meleagridis, M. synoviae, and M. iowae cam cause respiratory disease in poultry. M. iowae is of minor importance. Uncomplicated disease has low mortality. If other agents (E. coli. Infectious Bronchitis virus, Newcastle disease virus) are present, mortality may :reach 30%. Clinical signs are rales, cough, nasal discharge, and air sacculitis. Major economic losses are due to downgrading of carcass, decreased feed conversion, decreased egg production, and increased medication costs. Turkeys are more susceptible rhan chickens. Transmission is via direct contact, aerosol, and eggs.

Diagnosis is by culture or more commonly serology (rapid plate test, ELISA), Egg yolks can be used in lieu of serum in ELISA. Treatments may not be cost-effective, Tylosin and tiamulin are used. Control is based on identification of clean breeder flocks, serological monitoring, and removal of reactor flocks, Vaccines are limited in effectiveness; F strain is still pathogenic for turkeys but reduces egg loss. New vaccines using tcmpcrature-sensitive mutants are currently in the evaluation stage.

A recent outbreak of MG infection had been seen in house finches and now appears to be spreading to other songbirds and finches. The disease is charactrized by severe ocular lesions, leading to blindness. It is believed that backyard feeding stations could contribute to the spread of disease.

Reptiles:

In addition to upper respiratory tract disease in tortoises, mycoplasma species have been associated with rspiratory tract disease and arthritis in intensively managed crocodiles and alligators. The disease has high morbidity but low mortality in crocodiles. In alligators, a higher death rate was seen and there appeared to be a peptic phase as the organism could be isolated in high number from the CNS.

Seroepidemiology of Upper Respiratory Tract Disease in the Desert Tortoise: A Four Year Prospective Study at the Desert Tortoise Research Natural Area in the Western Mojave Desert‡

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Dramatic declines in some populations of the desert tortoise (Gopherus agassizii) over the past 20 years led the U.S. Fish and Wildlife Service to list the species as threatened in 1990 under the Endangered Species Act of 1973, as amended (U.S. Fish and Wildlife Service, 1994). Several factors, primarily induced by human activities, have combined with an upper respiratory tract disease (URTD) to produce severe negative impacts on some desert tortoise populations (U.S. Fish and Wildlife Service, 1994; Berry, 1996). In 1988, desert tortoises at the Desert Tortoise Research Natural Area (DTNA), Kern County, California, were seen (Knowles, 1989) with clinical signs of illness similar to those observed in captive desert tortoises (Fowler, 1977; Rosskopf et al, 1981). The observations of clinical disease occurred at the time of dramatic population declines (Berry, 1996). The purpose of this study was to evaluate Ab levels to M. agassizii in a population of desert tortoises in the western Mojave Desert at the DTNA which underwent catastrophic decline (Berry, 1996), to address how Ab levels changed with season and year, to determine if differences existed in the immune response (Ab levels) by sex, and to determine if Ab levels correlated with other general measures of health or body condition.

The samples obtained for testing were part of a larger on-going study of to assess overall health and to determine reference intervals for numerous hematologic and biochemical variables. This study plot has been surveyed regularly since 1979 for population density changes. Blood samples were obtained from adult tortoises by venupuncture of the jugular vein of wild desert tortoises fitted with radio-transmitters at the Desert Tortoise Natural Area (DTNA) in the western Mojave Desert, Kern County From the winter of 1992 through the fall of 1995, four samples were obtained yearly: in late winter (late February or early March), just prior to emergence from hibernation; in spring (May) during the time of peak activity; in summer (July/August) during the time of peak stress as a result of increased temperature and decreased rainfall; and in fall (October) during the time of decreased activity and initiation of hibernation. Exact numbers of tortoises sampled varied with the season and year. In 1992, samples were obtained from 12-14 adult tortoises; in 1993, from 14-16 adult tortoises; in 1994, from 13-15 adult tortoises; and in 1995, from 15-21 adult tortoises. Replacement tortoises were added to the study population as needed; therefore a total of 36 individuals were sampled during the study period. At the time of collection, a field assessment of overall health was recorded for each tortoise, including weight and carapace length at the midline (MCL), and packed cell volume (PCV). Samples of plasma were frozen above liquid nitrogen in the field, and were sent frozen on dry ice to the University of Florida for determination of Ab to M. agassizii. Samples were stored at -20°C in a manual defrost freezer until assayed, usually within two weeks of receipt.

The ELISA procedure was performed as previously described (Schumacher et al, 1993) using M. agassizii strain PS6 asd antigen. A biotinylated monoclonal Ab against desert tortoise IgY was used as the conjugate, and alkaline-phosphatase labeled Streptavidin was used as the substrate. Absorbence of each well was determined at 405 nm. Plasma of a desert tortoise which was culture negative for M. agassizii and free of lesions indicative of URTD was used as the negative control. Plasma from a desert tortoise which was experiementally infected with M. agassizii and

had lesions indicative of URTD was the positive control. The same reference sera were used for assays in 1992 ans 1993; depletion of stocks necessitated that new reference reagents be used in 1994 and 1995. Samples were categorized as positive if the ratio of sample absorbence to negative control absorbance was ≥ 3.0; samples were categorized as negative if the ratio of sample absorbance to negative control absorbance was ≤ 2.0 (Schumacher et al, 1993). Samples with a ratio value between 2 and 3 were deemed suspect.

The effects of sex and season on Ab levels were analyzed by analysis of variance (ANOVA).. The distribution of positive, negative and suspect animals was analyzed by Chi square analysis. Changes in Ab levels of individual animals over time were evaluated by paired T *test,* with values compared only between the *same* season and only between 1992 and 1993 or between 1994 and 1995.

The serological response of individual tonoises during each sampling period, as well as the individuals included within each sample, are shown in Table 1. Several patterns *are* apparent in the sample animals. Animals in Group I remained in the population consistently and were frequently sampled throughout the entire four year study period (92-95). These 10 animals provided a stable base and accounted for about 50% of the population sampled Groups II and V were: *composed* of tonoises which were present primarily during only one year of the *study* period. Group III and IV were composed of tortoises which were present primarily during two years of the study period.

During the course of the study. most animals retained their serological status. Animals which tested positive remained positive with a few exceptions of values which dropped into the suspect, and *on* rare occasions, the negative range.

The overall frequency of positive, negative, and suspect animals in the populations at each sample time is summarized in Figure 1. Distribution among the same season of different years was different only for winter of 1993 and 1994. There were statistically significant differences in the distribution of animals with positive reactions as compared with the distribution in winters of 1992 and 1995, P = 0.04. There were no differences between the observed and expected frequencies for the seasonal distribution within a single year in 1992 (P = 0.96), 1994 (P = 0.75) and 1995 (P= 0.87). However, in 1993 increases (P=0.02) were observed in the number of positive animals in fall and in the number of suspect animals in the summer. No other differences were significant From Table 1 , it *is* clear that these changes can be explained by the changes in rhe individual animals comprising the population sample at those rimes, No differences were observed between the observed and expected frequencies for the different study years within a specific season for spring (P=0.43), summer (P=0.29), or fall (P=0.66). However, differences were observed in winter (P=0.04). Within a given *year,* differences were Seen in 1993 (P=0.02) where increases were seen in the number of positive and suspect animals in fall and summer, respectively. No other differences were significant.

In 1992 and 1993, there werc no statistically significant seasonal effects (P = 0.08). There did appear to be a tendency for higher antibody levels to be observed in 1992 (P =0.06). Differences observed in the 1994 and 1995 samples were similar to those seen in 1992-1993 in that there was no apparent effect of season (P=0.43) or year (P = 0.14). It should be noted that what might appear to be differences may be explained by the failure to obtain samples from all animals at all time points as well as the introduction *of* new animals into the study. Thus if only a limited number of animals are available, the results of analysis of variance could be skewed to some extent. As can be seen in Table 1 , the individual animals contributing to the population sample differed markedly.

The most dramatic differences in antibody levels were seen in the fall. In both 1993 and 1995, a decline was observed (P= 0.009 and 0.004, respectively). No other significant differences were noted for the 1992 vs. 1993 sample years. However, in 1994 Vs 1995, significant decreases were seen in 1995 in samples obtained in winter (P = 0.05), spring (P = 0.04). and summer (P= 0.02).

The relationship between Ab levels and MCL and weight as well as PCV for each season of each year were tested by simple regression analysis. None of these relationships were significant (data not shown)-

Clinical signs compatible with URTD were recognized in 1988 (*Berry*, 1996). The clinical signs were especially pronounced during the 1989-1990 seasons preceding the serological sampling times. Clinically ill animals from this population were extensively evaluated in 1989 (Jacobson et al, 1991) and had lesions consistent with URID. Population density surveys in the DTNA study plot are summarized in Figure 2, During the 17 years of populadon monitoring, significant decreases occured in the population densities ($P < 0.001$). The population declines have been described in detail elsewhere (Berry, 1996). The most dramatic declines in population occured concurrent with, and subsequent to, the observation of clinical signs of URTD in the papulation (*Beny*, 1996).

Seraepidemiology is a powerful tool for analysis of population health. Samples taken at a single point in time can provide a "snapshot" of the past exposure of a population to infectious agents. To understand *the* dynamics involved in the interaction between the host and infectious agent, it is necessary to follow populations prospectively over time. The present survey is an excellent example of the value of continuously monitoring a population to obtain the status of a free-ranging wild population with respect to disease and overall health.

Based on these observations and the change in Ab status over time, we hypothesized that the pattern observed in DTNA was suggestive of a population which had been exposed to an infectious agent responded immunologically by production of specific Ab, and may now be in the chronic and/or convalescent stage of infection. We do not know if these tortoises have (a) recovered from infection and cleared the mycoplasma, (b) remain infected at low levels which preclude transmission, or (c) remain infected and can transmit the disease. The observation that Ab levels either remained stable or decreased over time could be a result of decreased antigenic stimulation (i.e., the mycoplasma has been cleared by the tortoise or is no longer present in high numbers) or immunosuppression. Of particular interest were seronegative animals in the DTNA population. Although these tortoises were likely to come in *direct* contact with Ab-positive animals, their own Ab levels remained relatively low. This suggests that the Ab-positive animals might have decreased ability to transmit the disease, which is consistent with the hypothesis of a convalescent population. The observation of milder clinical signs also supports this hypothesis.

This study showed that a key factor which must be considered in the continued monitoring of a population is that the introduction or removal of individuals from a population can influence interpretation of data, especially when the overall numbers of animals monitored is low. For example, half of *the* population studied in 1995 was different from the population members seen in 1992. Although antibody levels can give an idea of the magnitude of response by individual animals, the population profile as a qualitative assessment of seropositive animals may be more helpful.

Assessment of health status is particularly difficult in free-ranging animals. Semepidemiology represents a powerful tool which can be used to monitor the spread of disease in populations. Continued monitoring of populations could provide valuable information with respect to the spread of URTD in wild tortoise populations as well as the predictive value of serological profiles in this disease. Changes in the percentage of seropositive animals within a population or changes in Ab levels could precede the appearance of clinical diseasc and provide an early warning of potential disease outbreaks in populations. Because URTD is clinically silent in the majority of animals, this early warning is especially important. Similarly, seroconversion of newly introduced animals in a population which has seemingly recovered from disease could indicate that the infectious agent *is* still present. Knowledge of the prevalence of infection in populations will allow better management decisions concerning possible geographical areas to be targeted for habitat preservation or populations which are at risk to acquire or to spread URID.

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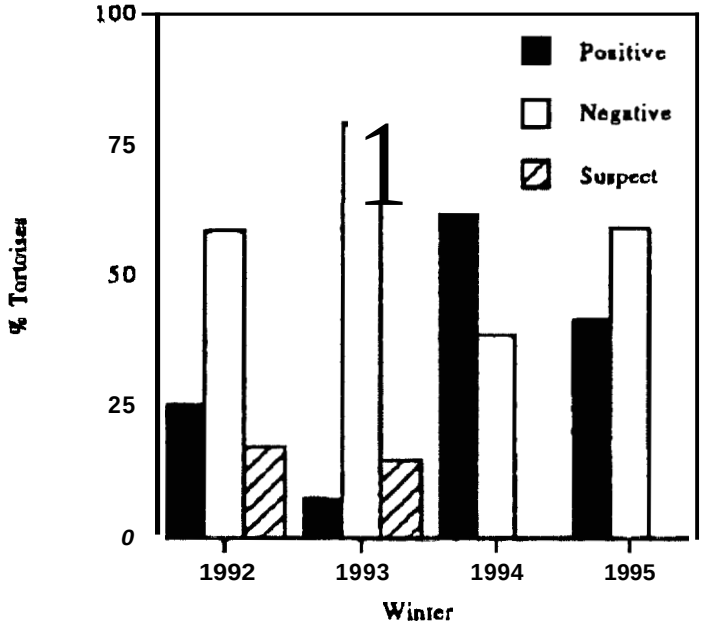
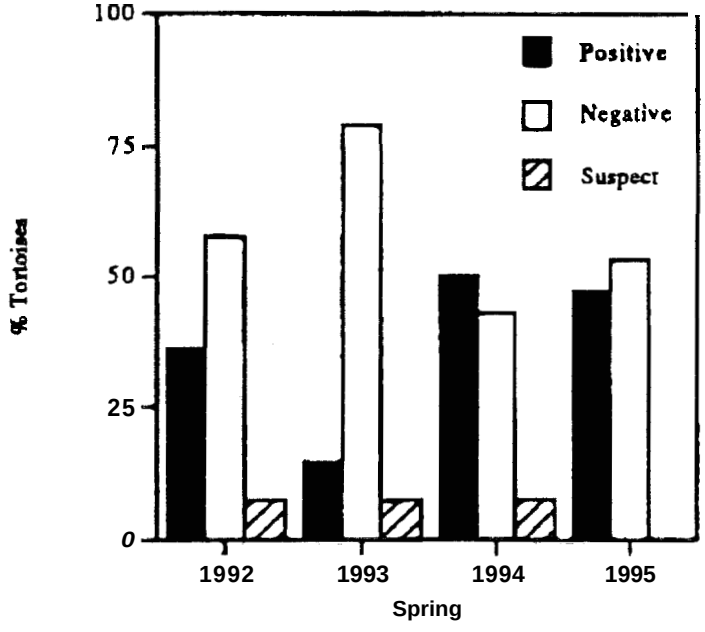
Table 1. Serological response of individual desert tortoises to *Mycoplasma agassizii* over a four year period.

Individual Tortoise	1992 W	1992 Sp	1992 Su	1992 F	1993 W	1993 Sp	1993 Su	1993 F	1994 W	1994 Sp	1994 Su	1994 F	1995 W	1995 Sp	1995 Su	1995 F
Group I:																
D01M	N	N	N	N	N	N	N	N		N	N	N	N	N	N	N
D05M	P	S	P	P	S	P	S	P	P	P	P	P	P	P	P	P
D11F	N	N	N	N	N	N	S	N	N	N	N	N	N	N	N	N
D13F	N	P	S	S	N	N	S	S	P	P	S	N	N	N	S	S
D15F	S		P	P	S	N	S	N	P	P	P	S	P	P	P	P
D25M	S	P	P	P	N	S	S	P	P	P	P	P	P	P	P	P
D26F	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
D27M	N	N			N							N	N	N	N	N
D28M	N	N				N	N	N	N	N	N	N	N	N	N	N
D29F		N	N	N	N	N	N	N							N	N
Group II:																
D09M	P	P														
D10M	N				N											
D22M	N	N														
D30F			N													
Group III:																
D31F		N	N	N	N	N	N	N								
D32M		P	P	P	N	N	N	P	P							
D33M		N	N	S	N	N		P	N							
D34F			N	N	N	N	N	P								
D35F			N	N	N	N	N	N	N							
Group IV:																
D36M							P		P	P	P	P	P	P		
D37M							P	P	P	P	P	P	P	N	P	P
D38M								N	N	N	N	N	N	N		
D39F										N	N	N	N	N		
D40M										S	N	N	N			
D41M												N	N	N		
Group V:																
D42F													P	P	P	P
D43M														N	N	P
D44F														P	P	P
D45M															N	N
D46F															N	N
D47F															N	N
D48M																N
D49F																P
D50M																P
D51M																N

Results are expressed as positive (P), negative (N) or suspect (S). If no result is noted, then the animal was not sampled at that time point. Group I is composed of tonoises which were present throughout the entire four year study period (92-95). Groups II and V are composed of tortoises which were present primarily during only one year of the study period (1992 and 1994, respectively). Group III and IV *are* composed of tortoises which were present primarily during two years of the study period (1992-93 and 1994-95, respectively).


100
75
50
25
0
% Tortoises
Positive
Negative
Suspect
1992
1993
1994
1995
Winter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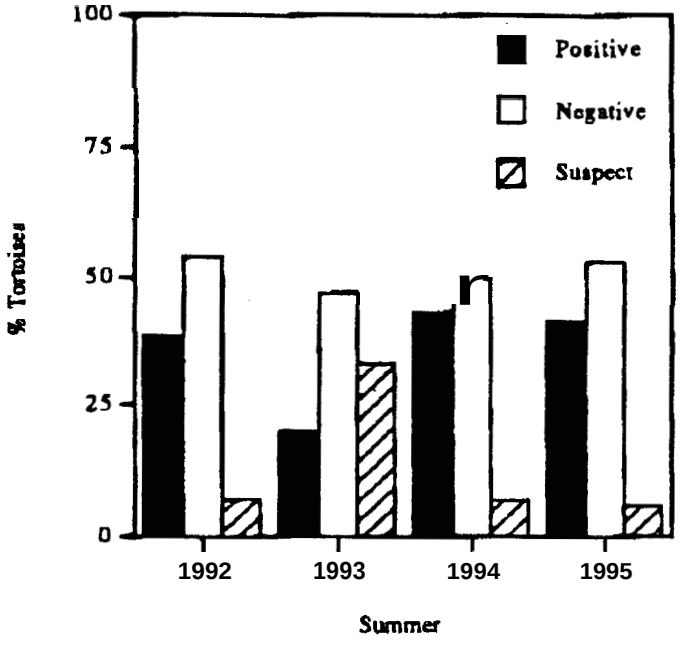

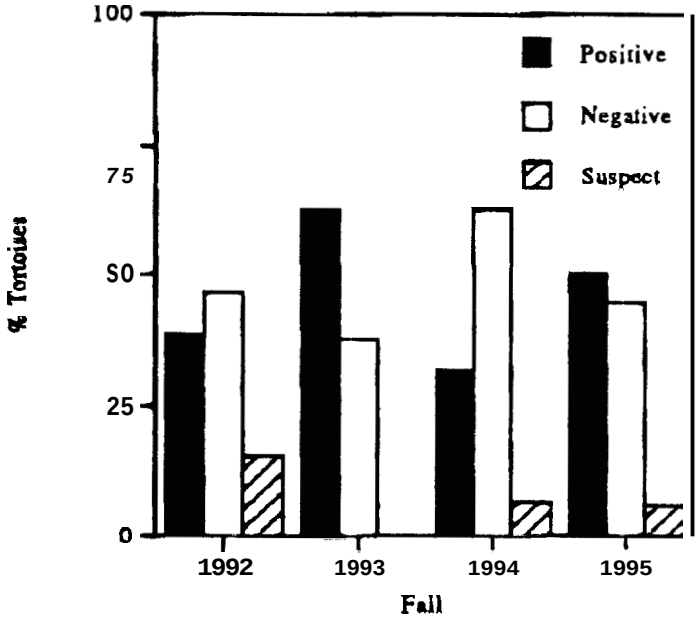

Figure 1. Distribution *of* tortoises with positive, negative or suspect ELISA values in desert tortoises from the Desert Tortoise Research Natural Area (DTNA) in Kern County, California In winter of 1993 and 1994, there were statistically significanr differences in the distribution of animals with positive reactions as compared with the distribution in winters of 1.992and 1995, P = 0.04. Within a given year, differences were seen in 1993 (P= 0.02)where increases were seen in

the number of positive and suspect animals in fall and summer, respectively. No other differences were significant.

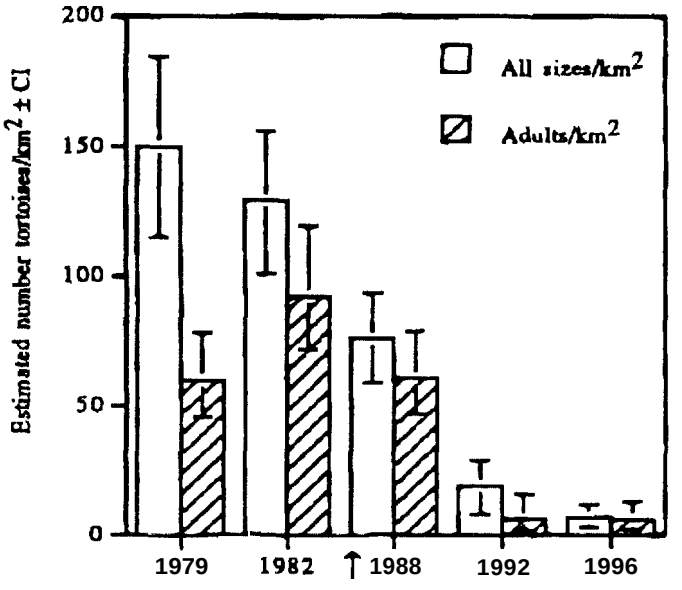

Figure 2. Density estimates for the *years* 1979, 1982. 1988, 1992, and 1996 at the desert tortoise study plot in the DTNA. Results are expressed as the mean number *of* tortoises ± *the* confidence interval for true population density. The arrow indicates the relative time at which clinical signs of URTD were first observed in tortoises at the DTNA.

Christopher MM, Berry KH, Wallis I, Nagy K, Henen B and Peterson C: Sex, site and seasonal effects on hematologic and biochemical values of free-ranging Mojave desert tortoises over a 5-year period.

The Health Profile Study was a 5-year program (1990-1995) instituted by the Bureau of Land Management in response to developing disease problems (including upper respiratory tract disease and shell diseasc) in free-ranging Mojave desert tortoises. The objectives of the study were to define rcfcrence ranges for hematologic and biochemical variables; to characterize physiological alterations in laboratory values on the basis of sex, geographic site and season; to identify ill tortoises on *the* basis of laboratory and other abnormalities; and to identify useful laboratory tests for diagnosis of disease and impending mortality. Venous blood samples were obtained by jugular venipuncture from approximately 20 adult tortoises of both sexes at four seasons each year: February/March (post-hibernation); May (resource-rich); July/August (summer), and October (pre-hibernation).). Tortoises that werc missing or died were replaced by new tortoises through 'the course of the study, with the number of each sex maintained at about equal numbers. A Total of 110 tortoises were sampled, 40 at *the* Desert Tortoise Rcscarch Natural Area (western Mojave); 37 tortoises at Goffs (eastern Mojave) and 33 at Ivanpah Valley (northeastern Mojave). Each tortoise was sampled an average of 10 times during the 5 *year* period for a total of 1071 samples. Whole blood, blood smears and heparinized plasma samples were processed and analyzed using standard methodology for complete blood counts and chemistry panels at a veterinary diagnostic laboratory.

All data were checked for accuracy. A change in chemistry analyzers in 1993 appeared to affect results for some analytes, so that iron and creatininc values obtained prior to July 1993 (October 1993 for Goffs) were not utilized. Of the 110 tonoises, *64* were seronegative for M.agassizii, 29 were seropositive at some point during the study pcriod, 13 were not tested for M. agassizii, and 4 of 16 tonoises confirmed dead in the course of the 5 year period were necropsied. Only datu from seronegative tortoises were analyzed for the first 2 objectives of the study. Percentile plots of all data from seronegative tortoises were evaluated for the presence of outliers. Calculation of reference ranges was complicated by the repeated determinations and low number of tortoises for any given site, sex, season and year. Use *of* the range *of* mean values, differentiation of wet vs. dry years, or use of mid-95th percentiles of repeated values are alternative techniques far developing rcfcrence intervals dictated by significant sex, site and seasonal differences. The latter method may best reflect the spectrum of values based on intra-tortoise, inter-tonoise and year-to-year variation.

Because of year-to-year variation in rainfall and forage, mean values for each season were considered more representative for evaluation of seasonal, sitc and sex differences in tortoise populations. and facilitated use of repeated measures analysis of variance. Body weight, mid-carapace length, erythrogram paramctcrs and AST activity were significantly greater at all seasons for males (n=27) vs. females (n=26). Females had significantly greater values for cholesterol, triglyceride, calcium, phosphorus. and magnesium concentration. Tortoises at DTNA had significantly greater plasma iron and lower total protein and globulin concentration; tortoises at Ivanpah had significantly higher basophil counts. Seasonal differences were marked for body weight, erythrogram parameters, total WBC, lymphocytes, uric acid, cholesterol, triglycerides, calcium, phosphorus, mngnesium. proteins, alkaline phosphatase and AST (lower in March, higher in May). BUN and total CO, were significantly higher in March and lower in May. Osmolality and electrolytes increased in summer and fall, particularly at Goffs. There was an inverse relationship between BUN and uric acid, suggesting modifications in nitrogen metabolism in dehydration vs. hydration.

~41,000 data points ex 110 tortoises over 5 years

Health studies of desert tortoises in the eastern Mojave Desert of Arizona and Utah and the central Sonoran Desert of Arizona

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Abstract: Desert tortoises (*Gopherus agassizii*) are long-lived reptiles found in the deserts of the southwestern United States. Concerns in 1989 over declines in the Mojave desert tortoise population prompted 2 5-year health studies. The first 5-year study began in 1989 in the eastern Mojave Desert and consisted of 3 free-ranging populations: City Creek, Washington County, Utah; Paradise Valley, Washington County, Utah; and Littlefield, Mohave County, Arizona. The second 5-year study began in 1990 in the central Sonoran Desert and consisted of 2 free-ranging populations in Arizona: Little Shipp Wash, Yavapai County, and the Harcuvar Mountains, La Paz County. We captured and radio-tagged 92 tortoises in the Mojave Desert from 1989-93, and 36 in the Sonoran Desert from 1990-94, then attempted to recapture them 3 times a year. We weighed, measured, and evaluated each tortoise for clinical signs of upper respiratory tract disease (URTD). We chemically immobilized each tortoise and collected blood, nasal aspirate, choana and cloacal swabs, and fecal matter to perform hecnatology, blood chemistry, microbiological, and parasitology assessments.

In the Mojave Desert, tortoise blood chemistry parameter values differed ($P < 0.001$)

between sites and sexes, and among seasons and years. Females had higher ($P < 0.05$) levels of cholesterol, triglycerides, calcium, phosphorus, and vitamin E than did males. Seasonal and annual differences related to rainfall patterns, forage availability, and presence: of disease. Ten tortoises had positive titers for *Mycoplasma agassizii* and 11 had clinical signs of upper respiratory tract disease. Two of 17 cloacal bacteria we isolated were pathogenic. Ninety-one percent of the tortoises had nonpathogenic pinworm ova in their feces, the only intestinal parasite we found.

In the Sonoran Desert, tortoise blood chemistry parameter values differed ($P < 0.001$) between sites and sexes, and among seasons and years. Females had higher ($P < 0.05$) levels of cholesterol, triglycerides, calcium, phosphorus, and vitamin E than males. Seasonal and annual differences in hematology and blood chemistry related to rainfall patterns, forage availability, and presence of disease. We found 1 species of pathogenic bacteria in tortoise cloacae (*Pseudomonas* spp.), and 1 pathogenic bacteria in the nasal cavity (*Pasteurella testudinis*). Three tortoise were exposed to *Mycoplasma agassizii*, the causative! agent of URTD (2 Harcuvar Mtns., 1 Little Shipp). We found 1 tortoise with a suspect *titer* for *M. agassizii* in 1993, and 1 tortoise with a positive titer in 1994 using an enzyme-linked immunosorbent assay (ELISA). Results from a polymerase chain amplification (PCR) test initiated in 1994 confirmed *the* presence of *M. agassizii* in the tortoise. with a positive ELISA result, but also gave a positive result to a tortoise with a negative ELISA titer. Ninety-one percent of the tortoises sampled had nonpathogenic pinworm (*Trachygonetria* spp.) ova in their feces, the only intestinal parasite found in the study.

NECROPSY OF FREE-RANGING DESERT TORTOISES - THE ULTIMATE HEALTH ASSESSMENT

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The purpose of the post-mortem examination (necropsy) is to determine the **definitive** cause of illness or death by gross and microscopic examination of tissues and by conducting the appropriate hematologic, biochemical, serologic, microbiologic and toxicologic examinations. Necropsy is indicated when a population of animals exhibits increased mortality, reduced procreation, or a newly emerging disease; or when individuals within the population exhibit chronic weight loss, chronic progressive disease, or die and are still fresh when recovered. Necropsy examination identifies infectious diseases, moderate toxicities, moderate nutritional deficiencies, some other metabolic diseases, traumatic diseases, parasitic diseases, reproductive anomalies, and tumors.

SIGNALMENT, OBJECTIVE, AND " H O D S

Twenty-five ill or dead desert tortoises, ***Gopherus agassizii,*** were received from March, 1992 through July, 1995 €or necropsies from the Mojave and Colorado deserts of California. The major objective was to determine the causes of illness or death of the tortoises. Complete necropsy examinations included live tortoise health assessment; identification of gross and microscopic lesions; analysis of liver, kidney and plasma for metals (including copper, iron, selenium, cadmium, lead, arsenic, mercury, zinc, thallium, phosphorus, chromium, magnesium, vanadium, sodium, cobalt, manganese, molybdenum, calcium, nickel, tin, and barium) and organic compounds; complete hematologic and biochemical profiles and *Mycoplasma* serology on blood collected prior to necropsy; and bacterial and *Mycoplasma* isolation from the nasal cavity, nasopharynx and colon. An additional 29 tortoises were included in the survey of metal and minerals, including 24 tortoises from Las Vegas Valley, Clark County, Nevada (19 of which had lesions of mycoplasmosis) and five juvenile tortoises with shell osteopenia consistent with malnutrition. These tortoises were divided into groups according to five recovery unit populations: Western Mojave, Eastern Mojave, Northeastern Mojave, Northern Colorado, and Eastern Colorado.

SUMMARY OF RESULTS

Major disease categories and specific lesions

Major disease categories included cutaneous dyskeratosis (n=7); shell necrosis (n=2); respiratory diseases (n=7) including mycoplasmosis (n=5); urolithiasis (n=3); trauma (n=5); and nonspecific liver disease (n=1). Lesions associated with these diseases are listed in Table 1. In tortoises with cutaneous dyskeratosis the epidermal horny layer was disrupted by multiple crevices and fissures and, in the most severe lesions, dermal bone showed osteoclastic resorption, remodeling, and osteopenia. Lesions of cutaneous dyskeratosis were also found in one tortoise with shell necrosis and one tortoise with urolithiasis. In tortoises with shell necrosis, multiple foci of necrotic debris and heterophilic inflammation within the epidermal horny layer were subtended by necrotic dermal bone colonized by bacteria and fungi. The diagnosis of mycoplasmosis was based on characteristic pathologic findings, especially chronic proliferative rhinitis, and positive serologic tests and/or isolation of *Mycoplasma* sp. from the nasal cavity. In two other tortoises with respiratory disease, chronic fungal pneumonia was present in one, while inflammation of uncertain etiology was present in the nasal cavity, eyelids, and chin and salivary glands of the other. Two tortoises with uroliths were discovered dead. Renal and articular gout were present in the live tortoise. One tortoise had been entombed within its burrow and developed a cutaneous fungal infection. Another was entrapped in a brush fire. Both tortoises had multicentric visceral inflammation. Two tortoises struck by moving vehicles sustained multiple shell fractures and coelomic hemorrhage, and one developed acute pneumonia. One tortoise was attacked by a predator and developed a terminal bacterial pneumonia.

Tortoises with diseases represented in all major categories had some degree of liver degeneration. Livers varied from small and dark brown to swollen with rounded edges, pale tan coloration and friable consistency. A few pale livers floated in formalin. However, many livers with histopathologic changes were unremarkable on *gross* examination. Liver lesions included hepatocellular vacuolar change (hydropic degeneration and lipidosis), hemosiderosis, anisokaryosis, atrophy and increased deposition and aggregation of melanin (melanosis). Liver lesions were most severe in tortoises with chronic respiratory diseases and urolithiasis.

Chronically ill tortoises often had evidence of weight loss. In some tortoises, limbs were thin due to muscle atrophy. The coelomic cavity usually contained little or no adipose tissue. Liver masses in *six* tortoises were approximately 25 to 60% smaller than that of other comparably sized tortoises, only ranging from *0.85* to 2.36% of the body weight compared to 0.85-6.0% for all tortoises in the study. Hepatocytes in atrophic livers were shrunken and the cytoplasm was homogenous. Pancreatic acinar

cells in 5 of 6 tortoises with liver atrophy were shrunken and devoid of zymogen granules, consistent with atrophy. Hypertrophy and intracytoplasmic mucus accumulation of urinary bladder epithelium were often associated with evidence of dehydration (sunken eyes, dry tacky subcutaneous and coelomic tissues, and weight loss).

Summary of metal analyses

For each metal assayed, a mean concentration, range, and standard deviation were determined. The concentration of a metal was considered high if it was greater than 2 standard deviations above the mean for that metal. The results of metal analyses are listed in Table 2 Ranges, means and standard deviations of hepatic and renal metals were reported in the abstracts of the 1996 annual Desert Tortoise Council meeting. One or more toxic metals, including mercury, cadmium, and lead were found in tortoises from all recovery unit locations but the Eastern Colorado Desert. This may reflect the relatively small number of metals assayed for that population. Tortoises with infectious diseases or urolithiasis appeared to have the highest incidence of metal accumulation. Elevated nickel was only found in tortoises with shell disease. Cadmium was elevated in two tortoises with urolithiasis. Metal assays were not conducted on liver and kidney of the third tortoise due to advanced autolysis. Nine of 26 tortoises with respiratory disease had elevated concentrations of one to three of the following metals: cadmium, mercury, selenium, vanadium, cobalt, molybdenum, copper and iron. Three of four juvenile tortoises from the Western Mojave Desert recovery unit had elevated concentrations of one or two of the following metals: lead, copper, zinc, manganese and sodium. Of two tortoises killed by vehicular trauma, one had elevated renal iron, probably associated with internal hemorrhage. Five healthy tortoises from the Las Vegas Valley did not have elevated concentrations of metals.

Summary of biochemical and hematologic profiles

Sensitivity and specificity of several plasma enzymes and other components were evaluated relative to pathologic changes in liver or urolith formation. For 12 tortoises with hepatocellular hydropic degeneration or lipidosis, sensitivity vs. specificity of alkaline phosphatase (ALP) = 42% vs. 66%; bilirubin = 25% vs. 66%; aspartate aminotransferase (AST) = 25% vs. 66%; lbile acids = 25% vs. 40%; alanine aminotransferase (ALT) = 8% vs. 75%; and the sensitivity of cholesterol = 0%. For 6 tortoises with hepatocellular atrophy, sensitivity vs. specificity of bile acids = 100% vs. 83%; bilirubin = 50% vs. 77%; cholesterol = 50% vs. 92%; ALT = 33% vs. 92%; AST = 33% vs. 77%; and ALP = 17% vs. 69%. For 3 tortoises with hepatocellular necrosis, sensitivity vs. specificity of ALT = 66% vs.

93%; cholesterol = 66% vs. 93%; AST = 66% vs. 81%; bile acids = 100% vs. 71%; bilirubin = 66% vs. 75%; and the sensitivity of ALP = 0%. For 3 tortoises with urolithiasis, sensitivity vs. specificity of blood urea nitrogen = 100% vs. 78%; and uric acid = 66% vs. 94%. Leukocyte and erythrocyte counts and differentials were evaluated relative to tortoises with systemic inflammation. For 7 tortoises with systemic inflammation, sensitivity vs. specificity of abnormal total leukocyte counts (WBC) = 86% vs. 67%; monocytosis = 86% vs. 33%; anemia (reduced packed cell volume) = 86% vs. 54%; and a combination of abnormal WBC and monocytosis = 71% vs. 92%.

Summary of Serologic Examination

Results of *Mycoplasma* serology and culture were evaluated relative to the presence of proliferative rhinitis. For 5 tortoises with mycoplasmosis, the sensitivity vs. specificity of serology = 80% vs. 87%; culture = 40% vs. 100%; and a combination of serology plus culture = 100% vs. 100%.

Summary of Microbial Investigations

Potentially pathogenic bacteria were isolated from the nasopharynx and colon of 15 tortoises. All but *Pasteurella testudinis* were isolated from only one of the two sites. Potential pathogens included *Xanthomonas maltophilia* (n=2) and *Klebsiella oxytoca* (n=1) isolated from the two tortoises with shell necrosis; *Pseudomonas* sp. (n=2) from one tortoise with shell necrosis and one with cutaneous dyskeratosis; *Pasteurella testudinis* (n=9) from tortoises with a variety of lesions; and *Citrobacter* sp.(n=6) mostly from the colon of tortoises with a variety of lesions. *Mycoplasma* was isolated from the choanae and the nasal cavity of two tortoises. The species has not yet been identified by culture or polymerase chain reaction analysis; it was not *M. agassizii*.

CONCLUSIONS

1. Necropsy is beneficial to determine causes of disease and death, and to interpret health profiles.
2. Environmental toxins are widespread in tortoises in the Mojave and Colorado deserts and play a role in disease of free-ranging desert tortoises.
3. Hematologic and biochemical profiles are valuable tools to identify ill tortoises, especially those with liver lesions, urolithiasis, and systemic disease.
4. The unnamed species of *Mycoplasma* is associated with disease similar to that of *M. agassizii*.
5. Although there is no single definitive test for mycoplasmosis, a combination of serology and culture appears to be useful in identifying free-ranging tortoises with the disease.

Table 1. Lesions found in 24 desert tortolses collected or salvaged between March 1992 and July 1995 for necropsies from the Mojave and Colorado Deserts of California. Tortoises are grouped according to major disease categories. The tortoise with nonspecific liver disease was cot included.

ORGAN	CUTANEOUS DYSKERATOSIS	SHELL NECROSIS	RESPIRATORY DISEASE	UROLITHIASIS	TRAUMA
BLADDER	Mu		I,Mu	Mu,I,Uro	Mu
BONES/ JOINTS				Gout	
BONE MARROW	Hyper	Hyper	Hyper		Hypo,Hyper
ESOPHAGUS/ PHARYNX	I	I	I		I,S
EYELIDS	I		I,Ed		
HEART		I	I		I
INTESTINE	I,N	I	I,N,U,C	I,S	I,N
KIDNEY			An	N,Gout	D
LIVER	D,L,M,An,A	D,M	A,M,H,D,An	A,H,M,An	D,H,L
LUNG	I		I	I	I
MUSCLE	I, Endo, D	D, Endo	I,D,Endo,A	A,D	D,N
NASAL CAVITY	I		I		I
OVARIES	I	I,Ec			I
PANCREAS	F,I		A	A	
SHELL	KD,R,F,I,O,C	N,I,C	Θ		I,Fr
SKIN	KD,Deb,I,C	Deb,I,C	Deb,I,C		I,U,C
SPLEEN	Ly Dep,I	I,H	I,Ly Dep	H,Ly Dep	I,H Ly Dep
STOMACH				I,S	
TONGUE	I,S				I,S,U

Table 1. continued

A = Atrophy

An = Anisokaryosis

C = Colonization by bacteria and fungi

D = Degeneration

Deb = Plant material and foreign debris in stratum corneum

Ec = Ectopic egg

Ed = Edema

Endo = Endoparasitism (Sarcocystis sp.)

F = Fibrosis

Fr = Fracture

H = Hemosiderosis

Hyper = Hyperplasia **of** heterophils

Hypo = Hypoplasia **of** heterophils

I = Inflammation

KD = Keratin Dysplasia

L = Lipidosis

Ly Dep = Lymphoid Depletion

M = melanosis

Mu = Mucosal epithelial mucus accumulation

N = Necrosis

O = Osteopenia

R = Reactive bone and bone resorption

S = Cactus spine penetration

U = Ulcer

Uro = Urolith

Table 2. Recovery unit location, major diseases and/or lesions, and metals and minerals found in elevated concentrations in liver and kidney of 48 desert tortoises collected or salvaged between April 1991 and July 1995 for necropsies. Five tortoises from the northeastern Mojave Desert were healthy, did not have elevated concentrations of metals and were not included in the table. The number of affected tortoises is indicated in parentheses.

LOCATION	MAJOR DISEASES	METALS AND MINERALS
Northeastern Mojave Desert, California and Nevada	Shell osteopenia and malnutrition (1)	No elevated metals*
	Urolithiasis (1)	Liver: Mo*; Mg; Ba Kidney: Cd; Mg; Ba; Ca
	Mycoplasmosis (19)	Liver: Hg (1); Cd (1); Se (1); Cu (1); Fe(1) Kidney: Hg (3)
Western Mojave Desert, California	Fungal pneumonia (1)	Liver: Hg; Cd; Se
	Fungal dermatitis and multicentric inflammation (1)	Kidney: Hg
	Mycoplasmosis (4)	Liver: Hg (1); V (1); Fe (2) Kidney: V(l); Co (1); Mo (1); Cu (1)
	Blunt trauma (1)	No elevated metals
	Shell necrosis (1)	Kidney: Ni (1)
	Osteopenia and malnutrition (4)	Liver: Pb (1); Cu (2); Na (1) Kidney: Pb (1); Zn (1); Mn (1)
	Urolithiasis and gout (1)	Liver: Cd, Zn Kidney: Cr, Na
	Nonspecific inflammation of nasal cavity, eyelids and salivary glands (1)	No elevated metals

Table 2. Continued

LOCATION	MAJOR DISEASES	METALS AND MINERALS
Western Mojave Desert, California	Cutaneous dyskeratosis (1)	No elevated metals
Eastern Mojave Desert, California	Acute bacterial pneumonia secondary to predation (1)	No elevated metals**
	Burn injury (1)	Kidney: Kg
Northern Colorado Desert, California	Cutaneous dyskeratosis (1)	Liver: Cr, Ni
	Shell necrosis (1)	Liver: Pb
	Liver disease (1)	No elevated metals**
	Blunt trauma (1)	Kidney: Fe
Eastern Colorado Desert, California	Cutaneous dyskeratosis (5)	No elevated metals [a]
	Mycoplasmosis (1)	No elevated metals

Only Cu, Fe, Cd, Pb, As, and Zn assayed.

* Only Cr, Fe, Se, Cd, Pb, As, and Hg assayed.

†Mo - molybdenum; Mg - magnesium; Ba - barium; Cd - cadmium; Ca - calcium; Hg - mercury; Se - selenium; Cu - copper; Fe - iron; V - vanadium; Co - cobalt; Ni - nickel; Pb - lead; Na - sodium; Zn - zinc; Mn - manganese; Cr - chromium

HEALTH ASSESSMENT OF TORTOISES: NOT AN EASY TASK

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The family Testudinidae consists of approximately 40 species of tortoises, ranging in adult size from 100 gram padloppers (*Homopus signatus*) to 400 kg Aldabra tortoises (*Geochelone giganteus*). As a group, tortoises are difficult animals to evaluate clinically. Once within their shell they become a "bony box". Clinicians experienced in evaluating tortoises have devised methods for coaxing them from the confines of their shell. For medium to small-sized tortoises, one method is to push in or gently touch the hindlimbs, which often results in head extension. At times, sedation or anesthesia is required. Drugs such as ketamine, telazol, and succinylcholine have been used to allow a detailed physical examination, particularly with large and giant tortoises. Even in those tortoises which move about freely despite manipulations by the clinician, judging their health status is no simple matter, The difficulties are compounded in the field where the investigator may only be able to judge the animal at a distance, or when collected, there is limited equipment and time to do a thorough evaluation. The work being done will dictate how much information can be collected. Thus, health assessment in the field vs. health assessment in a clinical practice or university teaching hospital may require a somewhat different approach.

Coming up with a simple approach to a rather complex issue is what we are all looking for when devising schemes for categorizing tortoises, and other animals, as healthy or ill. When animals have dramatic, overt clinical signs such as exudate being expelled from the nares or palpebrae swollen and closed, the categorization Is rather simple to do. However, in many diseases. signs may be subclinical as is often the cases with mycoplasmosis in tortoises, In such situations a much more sophisticated approach is needed to determine the health status of an animal. When

working with species of tortoises where there is a limited data base on such biomedical specimens such as blood and urine which are routinely used in evaluating health of humans and domestic animals, the task seems insurmountable. Extrapolation becomes the rule, using values for other species to make comparisons.

As a clinician who has worked with tortoises for many years, the approach has been modified over the last 10 years as new applied technologies have become available and more information on diseases and causes of mortality have been identified. Still, the approach is not that different then what is used in assessing other reptiles, birds, and mammals. For captive tortoises, and other animals, medical records are essential for documenting health problems and maintaining all of the findings and reports for future reference. Medical record forms and folders vary from institution to institution and have not been standardized on a national or international level, Access to the information also varies between institutions with an emphasis on computerization of all medical records and data so that retrieval will be rapid and the information easily accessible. Similarly, record keeping is essential in field situations where notebooks and field sheets are necessary for recording information. Again the computerization of this information should be a goal sat by anyone involved in this work.

For captive tortoises, history becomes essential when interpreting findings and understanding the basis for many medical problems. For tortoises in the field similar information is important for assessing the overall health of an individual and viability of a population. For instance, diet of captive tortoises, or should I say, lack of a proper diet often leads to various health problems. Thus collecting information on the diet of the captive tortoise is important when trying to assess the health status of the animal, Similarly, quality of vegetation in the field needs to be assessed when determining the status of individual wild tortoises or populations. For captive tortoises diet is often limited by what *is* commercially available and the knowledge of the owner on the subject. For wild tortoises, diet is affected by environmental conditions such as drought or habitat degradation through overgrazing and destruction of plant communities by use of off-road vehicles or introduction of exotic plants. Developing a good data base on environmental conditions for both

captive and wild animals is necessary when trying to assess health of these animals.

A thorough physical examination is the staring point for assessing tortoise health. Weight (W) and carapace length in the midline (MCL) measurements are simple to take (except for giant tortoises) and may provide valuable information, This data should be collected anytime a tortoise is handled for health assessment. In 1980, Jackson presented data suggesting that body weight in relation to carapace length in captive spur-thighed tortoises (*Testudo graeca*) and Hermann's tortoises (*T. hemanii*) could be used to assess their clinical condition. In trying to understand causes of infertility and attempting to evaluate body condition of captive Aldabra tortoises (*Geochelone gigantea*), Spratt in 1990 compared weight vs. carapace length relationships between captive and wild tortoises. When the logarithm of carapace length (cm) was regressed on the logarithm of body weight (kg), no significant difference was found between the slopes or intercepts of the regression for wild and captive tortoises. To determine if the relationship between W and MCL could be used to discriminate between healthy desert tortoises (*Gopherus agassizii*) and desert tortoises with signs of upper respiratory tract disease (URTD), the logarithm of MCL was regressed on the logarithm of W for both groups. While a significant difference was found between the regression lines for the two groups, with tortoises with clinical signs weighing about 7% less than clinically healthy tortoises, still several affected tortoises weighed more for their length when compared with healthy tortoises. These findings suggest that while these measurements are extremely important, and can be used in health assessment, they should not be used alone in categorizing a tortoise as healthy or ill.

Following the determination of W and MCL, a full clinical examination should be performed. The adnexal tissues of the orbit should be examined for changes in size, shape, and color. An eye examination should be performed for minimally determining clarity of the cornea and lens. When possible, the oral cavity should be visualized for presence of lesions or foreign bodies. The patency of the nares needs to be noted. The profile of the head should be viewed both frontally and laterally for conformational abnormalities. The quality of the skin and scutes should be inspected for irregularities and lesions. In captive tortoises, pyramiding of scutes,

especially on the carapace is commonly seen and is thought to have a dietary basis. The seams between adjacent scutes should be inspected for shell growth. With some smaller species of tortoises it may be possible to palpate shelled eggs through the soft tissue axillary region of the hindlimbs. In a veterinary hospital radiography can be used to evaluate a variety of internal structures such as lungs, gastrointestinal system, reproductive tracts, and bladder. Ultrasound imaging, particularly of the reproductive tract, can be done both in a clinic and in the field. Finally, biological samples such as blood, urine, biopsy specimens, and exudates should be collected for a variety of clinical evaluations. A wide variety of blood collection sites are reported for tortoises, each having advantages and disadvantages. Blood values have been reported for several species of tortoises including the desert tortoise, gopher tortoise (*Gopherus polyphemus*), Mediterranean tortoises (*T. graeca* and *T. hermanii*), and radiated tortoise. However values between captive and wild tortoises will more than likely vary with season, age, and sex differences making interpretation a complex affair.

Continued data collection for free-ranging tortoises is needed to assess health of tortoises , both in the filed and in captivity. Correlative studies need to be performed since data only becomes meaningful in the context of the whale animal. As more and more data is accumulated, and new methods end technologies become available, such as ELISA testing *of* blood for exposure to potential pathogens, health assessment will become more of a science than an art. With this comes the need for disserniinating this information so that minimal standardized guidelines can be used for the multiple species being evaluated and studied around the world.

SEROLOGIC SURVEY OF DESERT TORTOISES, *GOPHERUS AGASSIZII*, IN AND AROUND THE NATIONAL TRAINING CENTER, FORT IRWIN, CALIFORNIA, FOR EXPOSURE TO *MYCOPLASMA AGASSIZII*, THE CAUSATIVE AGENT OF UPPER RESPIRATORY TRACT DISEASE

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The bacteria, ***Mycoplasma agassizii*,** is the causative agent of **a** chronic upper respiratory tract disease (URTD) of **desert tortoises,** both In captivity and in the wild. Epizootics of **URTD have** been seen **at** multiple **sites** in the Mojave Desert of the southwest United States. **An** enzyme linked Irnrnunosorbent **assay (ELISA)** has been developed specifically for use in **desert tortoises** to determine exposure **to** *M. agassizii*. Data is slowly accumulating **on** prevalence **of** exposure ***to*** this organism **in** certain wild populations. To add **to** the data base, commencing In June **1993, samples** were collected from desert tortoises In and around **the** National Training Center, Fort Irwin, California. Tortoises were **sampled** from the following locations: **1)** captive tortoises in **Barstow, CA.; 2) captive** tortoises in **Ft.** Irwin **Park end at** Ft. Irwin Veterinary Clinic: 3)FISS site (southeastern Fort Irwin boundary); **4)** Tiefort Mts.; and **4)** North Alvord Mts. Clinical signs of **URTD have** been **seen** in captive tortoises In **Barstow, and** tortoises in **Ft.** Irwin **Park** and Ft. Irwin Veterinary **Clinic; 14** of **25** tortoises sampled **from these** locations were seropositive tor exposure ***to M. agassizii***. However, **only** four **of 61** tortoises sampled from **the FISS** site were seropositive, and none of **11 tortoises** sampled **from** the Tieforts were seropositive. From the North Alvords, **35** tortoises **were sampled,** of which ***6*** were seropositive; **one *of*** the seropositive **tortoises also had a** nasal discharge. **To** date, **of the** 108 wild tortoises sampled, **10** were found **to be** seropositive, **6** of which were from **the** North **Alvords.** Nasal **samples** from tortoises In **these** locations are currently being collected **and** evaluated **by a** recently developed polymerase chain reaction technique **to** determine presence **of *M. agassizii.***

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CAUSES OF MORTALITY AND DISEASES IN TORTOISES: A REVIEW

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Of the 40 extant species of tortoises, most, if not all, are experiencing population declines. Although collection of tortoises for the pet trade and use by local human populations as food items in many areas of the world have contributed, habitat degradation and destruction of the environment probably accounts for the most significant worldwide declines. While a number of diseases have surfaced as significant problems for several species of captive and wild tortoises, few thorough retrospective or prospective studies have been conducted on causes of mortality in either of these groups. In the wild, by the time a dead tortoise is found, generally all that remains are the hard parts. Virtually nothing is known about causes of mortality in neonates, a life stage that is only beginning to be studied in detail. A considerable body of information is available on population declines of the desert tortoise, *Gopherus agassizii*, in the western Mojave desert, USA. The following is a list of those problems indicative of downward trends in these populations: 1) declines in densities, 2) declines in numbers registered during a 6--day survey, 3) declines in proportions of juveniles and small immature tortoises, 4) reduction in recruitment of young individuals into the adult population, 5) abnormally high mortality rates for the breeding population, 6) abnormally high mortality rates for juveniles, 7) human-induced sources of mortality, and 8) deterioration of habitat. Of diseases, two have surfaced as significant health problems in wild desert tortoises. An upper respiratory tract disease (URTD), first recognized in the western Mojave Desert in 1988 is now known to be caused by a mycoplasma, *Mycoplasma agassizii*. Transmission studies have confirmed its etiologic role and since the recognition of URTD in 1988, desert tortoises with URTD have been seen at multiple locations in the Mojave Desert. This disease also has been seen in gopher tortoises, *Gopherus agassizii*, in Florida, USA. The second

significant disease in desert tortoises is a cutaneous dyskeratosis which has been associated with population declines on the Chuckwalla Bench Area of Critical Concern, Riverside County, California. Recently, illness and deaths have been seen in wild Galapagos tortoises, *Geochelone elephantopus,* in the Galapagos and wild geometric tortoises, *Psammobates geometricus*, in South Africa. The causes of these losses are being investigated.

When we look at captive tortoises, much more information is available on specific health problems. The European tortoises, *Testudo hermanii* and *T. graeca*, have been seen with herpesvirus stomatitis, pharyngitis, and pneumonia in England, Germany, Switzerland, and France. Herpesvirus pharyngitis also has been reported for captive desert tortoises. A large shipment of Argentine tortoises, G. *chilensis,* died with herpesvirus pharyngitis soon after importation into the United States. It is clear that herpesviruses are significant pathogens in several species of tortoises. Iridoviral hepatic necrosis was described in a Hermann's tortoise, *T. hermanii* , and iridoviral tracheitis and pneumonia for a gopher tortoise.

Compared to viral infections, relatively few bacteria have been incriminated as causes of illness and death in captive tortoises. URTD was known to occur in captive desert and gopher tortoises long before being described for wild tortoises. It wasn't until money became available to study the disease in wild populations that the causative agent was clearly established, *Salmonella* is recognized as a normal inhabitant of the gastrointestinal tract of many species of reptiles, including tortoises. However, disease in tortoises resulting from such infections is uncommon. A filamentous organism resembling *Dermatophilus* has been seen in skin lesions of captive desert tortoises and padloppers, *Homopus: signatus.* There are several reports of fungal pneumonias in captive giant tortoises (G. *elephantopus* and G. *gigantea*), with maintenance at suboptimal environmental temperatures being a predisposing factor.

Of endoparasites affecting captive tortoises, the most significant include the protozoa *Entamoeba invadens* and *Hexamita parva,* and the nematodes *Angusticaecum* and *Proatractis*. Ectoparasites include fly larvae of the dipteran *Cistudinomyia cistudinis*, the mite *Eutrombicula alfreddugesi,* and ticks in the genera *Amblyomma, Hyalomma, and Ornithodoros.*

The most common non-infectious disease in captive tortoises is metabolic bone disease, developing as a result of: 1) prolonged deficiencies in calcium; 2) deficiencies in phosphorus; 3) improper ratios of calcium to phosphorus in the diet; and 4) vitamin D deficiency. There are relatively few reports of neoplasia in captive tortoises.

Reference ranges for blood variables: Issues regarding which data to include, and what to do when all your tortoises are healthy. Patrick E. Lederle, Kurt R. Rautenstrauch, Danny L. Rakestraw, and Katherine K. Zander, Science Applications International Corporation, Las Vegas, NV.

As a corollary to our evaluation of the effects of increased human activities on the dynamics of antibody levels associated with *Mycoplasma agassizii*, we also analyzed blood samples (standard cell count and chemistry) for most of the tortoises we were monitoring. We are in the process of developing reference ranges for blood cell count and chemistry variables for desert tortoises from the Yucca Mountain population.

We are following the advice of Lumsden and Mullen (Can. J. Comp. Med. 1978, 42:293-301), yet there are a number of issues yet to be resolved before reference ranges can be finalized. A total of 105 tortoises were sampled 1-3 times during September of 1993, 1994 and 1995. In terms of understanding disease dynamics it is useful to repeatedly measure the same individual through time, yet the problem of lack of independence among time periods must be addressed. Whether or not to include all data points, or only one sample from each animal will be discussed. In addition, many blood variables are non-normally distributed and in those cases, the common practice of reporting the mean ± two standard deviations is clearly inappropriate.

Another common practice is to establish reference ranges for both ill and healthy tortoises. Presumably, if values differ between ill and healthy tortoises they can be used as a diagnotic tool. Although nearly 20% of the samples obtained at Yucca Mountain tested positive for antibodies specific to *Mycoplasma agassizii*, clinical signs of upper respiratory tract disease were essentially absent from the population. Since prior exposure does not indicate the disease is present, we concluded that we were dealing with a healthy population of tortoises and did not classify any as ill for the purposes of developing reference ranges.

A preliminary overview of variables measured and tentative reference ranges for healthy Yucca Mountain tortoises will be presented along with several interesting outliers.

Incidence of positive ELISA tests at Yucca Mountain for antibodies associated with the causative agent of upper respiratory tract disease. Patrick E. Lederle, Kurt R. Rautenstrauch, Danny L. Rakestraw, Katherine K. Zander, and James L. Boone, Science Applications International Corporation, Las Vegas, NV.

We studied a population of desert tortoises at Yucca Mountain to evaluate the effects of increased human activities on the dynamics of antibody levels associated with the causative agent of upper respiratory tract disease. We compared a control group of tortoises to two treatment groups that were characterized by the intensity and duration of human activities. Blood samples were collected from radiomarked tortoises four times from September 1993 through September 1995. and clinical signs were evaluated each time tortoises were handled. Plasma samples were analyzed for presence of antibodies associated with the causative agent of upper respiratory tract disease using enzyme-linked immunoassay tests (ELISA:).

Effects of gender were minimal, both in likelihood to test positive, and for evaluation of antibody levels, so data were pooled. Because samples were not independent between periods (i.e., some tortoises were sampled during all periods, some only once, etc.), analyses of the likelihood to test positive were conducted separately for each sampling period. Likelihood to test positive was independent of treatment groups in all sampling periods (all $\chi^2 < 6.53$, df = 4, all $P > 0.16$), and sampling periods were found to be homogeneous ($\chi^2 = 8.644$, df = 12, $P = 0.73$). Overall, 18.7% of all samples were positive (range = 14.6-23.3% per sampling period). Repeated measures analysis of variance was used to evaluate log-transformed antibody levels for 31 individuals that were measured during all four sampling periods. No effects due to treatment group or treatment by sampling period interaction were found. There was, however, a significant effect due to sampling period ($F_{3,84} = 20.08$, $P < 0.01$), which was attributable to overall antibody level ratios increasing gradually over time in the repeatedly sampled tortoises (mean untransformed ELISA values increased from 1.9 to 3.4). The increase in antibody levels over time was of equal magnitude across treatment groups, indicating that the disturbances at Yucca Mountain did not influence the levels of antibodies encountered in the population. Although samples were not independent, no such increase in ELISA values over time was found when data from all tortoises sampled were examined.

Of 1,294 examinations conducted in the field under non-laboratory conditions, clinical signs were observed only six times (0.5%). Clinical signs of the disease also were observed 6 of 283 times (2.1%) during physical examinations performed in the laboratory prior to drawing blood.

in all six cases observed in the laboratory, the signs were scored as 1 which is the lowest category (0, no signs; 3, most severe). It is possible that clinical signs observed in tortoises transported to the laboratory may have been the result of handling stress.

There was no difference in reproductive output during 1994 and 1995 between females that tested positive or negative for antibodies during September of those years ($F_{1,51} = 0.023$, $P = 0.879$). During 1994, however, there were marginally significant differences between in reproductive output between females that tested positive ($n = 5$) or negative ($n = 19$) for antibodies during June of that year ($F_{1,21} = 2.960$, $P = 0.100$). However, samples sizes were too small to make this test conclusive.

Survival of adults ($n = 104$, all greater than 180 mm) in the Yucca Mountain population over four years exceeded 99% per year, and represents one of the highest survival rates recorded in any population of desert tortoises. High survival rates over this time period indicate that disease was not a significant factor in the Yucca Mountain population.

Discriminant function analysis on 16 blood chemistry and cell-count variables failed to distinguish between those tortoises testing positive or negative (negative plus suspects were combined for this analysis) (Wilke's $\lambda = 0.916$, $F_{16,193} = 1.035$, $P = 0.424$). Similar results were found for analyses broken down by sampling period. Univariate F-tests confirmed that blood values differed little between positive vs. negative tortoises; only one of the variables showed a significant difference between groups. Further, principal components analysis showed a complete overlap between positive and negative groups in multivariate space described by the first two principle components. In contrast, when male and female tortoises were compared, discriminant function analysis easily distinguished between the two groups (Wilke's $\lambda = 0.601$, $F_{16,193} = 1.035$, $P < 0.001$), and univariate F-tests showed that 10 of 16 variables were significantly different between males and females.

We concluded that the Yucca Mountain Site Characterization Project did not result in any detectable increases in the presence of antibodies associated with the causative agent of upper respiratory tract disease. In addition, lack of clinical signs and the low incidence of positive ELISA tests, coupled with very high survival rates of adults and minimal impacts on fecundity, indicate that upper respiratory tract disease was not affecting the tortoise population at Yucca Mountain during this study. It has been argued that upper respiratory tract disease is manifested during times of stress caused by factors such as drought or overcrowding. Although antibodies to *Mycoplasma agassizi* were detected at Yucca Mountain, manifestation of the disease may not

have occurred because this study was conducted during a period of above-average rainfall and tortoises appeared to be in good physiological condition. Alternatively, there may be pathogenic and non-pathogenic strains of the mycoplasma bacteria, both of which are detected by the ELISA test. Lack of clinical signs suggests that the pathogenic strain of the causative agent may not have been present in the Yucca Mountain population.

Little is known about the transmission and dynamics of upper respiratory tract disease in natural populations. Released pet tortoises are thought to have played a significant role in disease transmission in other locales and may be the source of a highly pathogenic strain of the mycoplasma. Compared to other groups of desert tortoises studied in southern Nevada or California, the Yucca Mountain population is relatively isolated because the study area has largely been protected from uncontrolled human activities since the early 1950s. The likelihood of released tortoises being present at Yucca Mountain is extremely small.

Upper Respiratory Tract Disease in Gopher Tortoises, *Gopherus polyphemus*: Natural Disease, Experimental Studies, and Implications for Conservation and Management

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Upper respiratory tract disease (URTD) could become a major factor affecting the maintenance of viable populations of gopher tortoises, *Gopherus polyphemus*, in the state of Florida and throughout the species' range. In order to provide regulatory agencies with data on which to base management decisions, we investigated the pathological and immune responses of tortoises to, and transmission of, the causative agent of URTD, *Mycoplasma agassizii*. Natural and experimental infections with *M. agassizii* cause mild to severe damage to mucosal and olfactory nasal epithelia, with increasing damage evident in longer-term infections. Possible systemic effects are indicated by increased blood urea nitrogen and decreased albumin levels in chronically ill animals, and increased presence of melanomacrophages and hemosiderin in the liver. Although mild clinical signs, especially ocular discharge and palpebral edema, are usually evident by 2 wk postinfection (PI), an immune response detectable by an enzyme-linked immunosorbent assay (ELISA) is not evident for 6 - 8 wk PI. Subclinical infections occur in some tortoises, although mycoplasma may be recovered from their nasal flushes, and seroconversion occurs. Some strains of *M. agassizii* are highly infectious, with doses of 10 or fewer colony forming units causing disease. There is no dose related effect, with infections caused by 10 or 1000 organisms eliciting the same clinical and immune responses as doses of 10^8 organisms. Infection of a limited number of tortoises with one isolate produced no significant

clinical disease, nor did it elict a detectable immune response. Initial exposure to *M. agassizii* does not provide a protective immunity. Subsequent exposure results in a more rapid and more severe clinical response than initial exposure. Plasma antibody levels begin rising more quickly on repeated exposure than on initial exposure. Horizontal transmission probably occurs via direct contact, but may occur on food plants or items, or via water. Transmission is more likely to occur from a tortoise that is clinically ill and has positive culture or polymerase chain reaction test results. Some long term captives with high ELISA results never showed clinical signs of disease, nor did their partners become clinically ill or seroconvert. There does not appear to be significant transmission when a tortoise enters a burrow previously occupied by an ill tortoise. There is no demonstrable vertical transmission, although there is transfer of maternal antibodies. The level of antibodies in egg yolk or hatchling plasma is approximately 20% of that in maternal plasma. Adult tortoises that test positive for exposure to *M. agassizii* by ELISA are not good candidates for relocation, repatriation, or restocking efforts. However, they can be used in captive breeding efforts and their offspring released into the wild. Exceptions to this caveat may be those animals that have been carefully monitored for at least 2 full years, with no evidence of active disease or infection.

Funded by The Walt Disney ~~Disney Development~~ Co.

0.5 - 3 cc blood taken
gopher tortoise sera - lower than desert tortoise
No inhibition of mycoplasma in medium

Abstract for Desert Tortoise Health and Disease Research Conference at Zzyzx, CA October 31, 1996

DOES MYCOPLASMA INFECTION INFLUENCE WATER, ENERGY AND FOOD CONSUMPTION OR REPRODUCTIVE OUTPUT OF WILD DESERT TORTOISES?

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Continuous doubly labeled water measurements of field metabolic rates and water influx rates (which reflect rates of food consumption) were made during winter of 1991-1992, and through spring, summer, and autumn of 1992 in free-ranging adult desert tortoises in the Mojave Desert of California. The three study sites were: the Desert Tortoise Natural Area (western Mojave), the Fenner Valley near Goffs (eastern Mojave), and in the Ivanpah Valley (northeastern Mojave). Plant productivity at all three sites was relatively high during this study, due to above-average rainfall associated with the El Niño (ENSO) conditions then. Blood plasma samples taken at the end of each seasonal measurement period were tested for antibodies to *Mycoplasma agassizii* using the ELISA method. Rates of energy metabolism and water influx for tortoises testing ELISA-positive were compared with rates for tortoises testing negative within each season at each site, using Student's t-test or the Mann-Whitney u-test if variances were heterogeneous.

The data set permitted statistical comparisons to be made for 17 of the 24 possible season-site cases. Only one case showed a significant difference ($P = 0.027$): rates of water influx for Ivanpah tortoises testing positive were 25% lower than those of Ivanpah tortoises testing negative in spring 1992. Although small sample sizes in some of these comparisons reduced the chances of detecting significant differences that may have been present, the occurrence of only one instance of a significant difference out of 17 cases may have been a random event. Because of small sample sizes in some of these two-sample comparisons, and because of uncertainties about the threshold for determining positive and negative results for ELISA tests that year, we reanalyzed the results by regressing field metabolic and water influx rates on the absorbance values determined in the ELISA analyses. This approach sidesteps the decision of positive or negative ELISA result, and it asks the question: were rates of energy or water metabolism progressively higher or lower or unchanged in tortoises having progressively higher antibody levels? Least-squares linear regression analyses were done on the results from four seasons at three sites (12 cases) using the absorbance value from the sample taken at the beginning of the season or at the end of that season (12 cases). Only one of the 24 analyses displayed a statistically

significant correlation (and it had a small sample size), indicating that the presence of antibodies to ***M. agassizii*** was not clearly detrimental to field metabolic rate or water intake rate. We conclude that long-term, integrated field measurements of energy and water metabolism are not useful predictors of *M. agassizii* antibody status in wild tortoises during ENSO years, and that the levels of antibody observed in this study **(up** to 0.8 absorbance units) were not associated with changes in the tortoises' daily cost: of living or water consumption, nor with their levels of activity or their feeding rates (which are closely reflected by metabolic and water intake rates) during "good" conditions in the field.

Breeding female tortoises living at the Desert Tortoise Natural *Area* and near Goffs were studied from March through June of 1993, which was another "good" (ENSO) year. Egg numbers and sizes, and the number of clutches produced per year were measured by periodic x-ray radiography using a portable x-ray machine. Simultaneous sampling of blood plasma allowed antibody levels to be determined by the ELISA method. Reproductive output of these tortoises was relatively high (about seven eggs per year), and there was no significant influence of antibody titer on reproductive output in females in either population.

URTD in Hatchling Tortoises

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Introduction

Little is known about the incidence and severity of Upper Respiratory Tract Disease (URTD) among juvenile desert tortoises in the Mojave desert. If URTD is transmitted to hatchling and juvenile tortoises which then fail to survive, the potential impact on recruitment to the population may be severe, but may not become evident for many years. We are reminded of the situation on the Galapagos island of Pinzon in which introduced rats had apparently destroyed all hatchlings of the endemic: Galapagos tortoise for decades, but this was not recognized for a long time because the long-lived adults remained abundant.

Evidence about the appearance and transmission of URTD among small juveniles is therefore important. We report herein on the widespread incidence of URTD among a set of 137 captive hatchlings at the Desert Tortoise Conservation Center (DTCC) near Las Vegas, Nevada.

The Origin and **Care of** Hatchling Tortoises

In the spring of **1994** we became aware that about **150** hatchlings (from the **1993** cohort) were being housed in raven-proof outdoor pens at the DTCC and requested that these be made available for nutritional research studies. As of mid-May our staff took over the care of these animals, as we intended to use them in nutritional studies in the fall once necessary research permits had been issued.

These hatchlings had been placed in the **11** outdoor raven-proof pens in late summer and fall **1993.** Two sets **of** pens were identified: 1. two pens housing ***27*** animals that had been found in adult tortoise pens maintained by BLM (**"BLM** hatchlings"), and 2. eight pens housing **110** animals that were being maintained by Clark County contractors ("County hatchlings"). The BLM hatchlings were all captive hatched at DTCC, and as they had been transferred directly to the raven-proof pens, their sole prior exposure had been to adult tortoises in their pens **of** origin. The County hatchlings included both animals that hatched in DTCC pens and animals that had been recovered from development sites or in urban areas in the Las Vegas Valley. Thus the County group may include backyard hatchlings that escaped from private residences. Prior to placement in the raven-proof pens the County hatchlings had been maintained in large group pens in which tortoises of different origin were exposed to each other.

The winter had been dry and the spring was both dry and hot. Some food and water had been provided in April and May by BLM and

County staff, but no records of feeding or drinking were maintained. Based on body condition, we suspected that many of the hatchlings had not been eating and that some were dehydrated. The animals were moved on May 15-18 to indoor plastic bins in a climate-controlled building (ca. 78-82° F) where they could be closely monitored. The tortoises from each outdoor pen were subdivided into two to five bins that were labeled according to the pen of origin. Each bin contained 2-6 animals. At an initial cursory examination the hatchlings were weighed and measured; 3 animals deemed ill based on closed eyes, lethargy and soft plastron were isolated in a separate bin.

We were surprised that a large proportion of the hatchlings were somewhat lethargic or reluctant to eat. An intensive effort was undertaken to encourage drinking and eating. Animals were watered twice per week (including a 15 minute soak period once per week) and were fed ad libitum on a nutritionally-complete pelleted diet that had been ground and sometimes moistened, and that was mixed with finely chopped produce including broccoli, globe mallow leaves and flowers, carrots and purple cabbage.

Morbidity and Mortality

About 24% of the animals refused to eat and died within 3 months; most did not show overt signs of URTD, but their shells became very soft due to resorption. Death rates were similar among BLM and County hatchlings. This initial inappetence and mortality may have been related to a difficult overwintering and spring that these animals had endured, although an infectious problem cannot be ruled out.

On August 24, all hatchlings were examined in detail under a dissecting microscope. Apparently healthy animals had open nares with no discharge, eye openings that were circular or deeper than wide ("old man" eye), rapid movement of nictating membranes and no swelling of eye lids. There was no evidence of discharge around the mouth. They were vigorous when handled, did not open their mouths to breath, and did not undertake a head-rubbing "ritual" (rubbing of eyes and head, extension and retraction of head, blowing of bubbles) when soaked. By these criteria only 16 of 85 surviving County hatchlings were healthy, but all 19 of the surviving BLM hatchlings were healthy. Five of the healthy County hatchlings were the sole occupants of one bin, and two the sole occupants of another bin. Both of these bins derived from one of the raven-proof pens.

Many of the signs of illness noted in the 69 sick County hatchlings involved the eyes. These included: slow movement of the nictating membrane, inflammation and swelling of the nictating membrane, swelling around the eyes leading to a reduction in size and change in the shape of the eye opening (from "old man" to round to seed-shaped to slit to closed), and excess fluid. Other prominent signs included occlusion of or discharge from the nares, eye- and head-rubbing, mouth-breathing, and after animals had been soaked in water, copious discharge from the mouth.

Animals with mild signs, including nasal occlusion, slight swelling around the eyes, slow movement of the nictating membrane, and eye-rubbing usually retained good appetite and vigor. However, animals with severe eye signs (e.g., trouble opening eyes, nictating membrane swollen and only partly retracted, slit shape of eye opening) and mouth discharge ate poorly and became! rapihly debilitated.

Necropsies on six animals with severe signs documented ***Mycoplasma aqassizii*** as a primary pathogen in upper and lower respiratory tract infections of these animals although ***Pasteurella testudinus*** was also isolated from most of them **(C.** Schiller, **APL** Veterinary Laboratories). The respiratory inflammation was more extensive than previously described for older tortoises with URTD.

The prevalence of URTD signs in the County hatchlings was a good predictor of subsequent mortality. Despite a high plane of nutrition and regular care, 51 of the **85** County hatchlings had died by the end **of** July 1996; by contrast, none of the **BLM** hatchlings died in this period. Seven County hatchlings from two apparently disease-free bins have remained healthy [although one **of** these tested positive for ***Mycoplasma agassizii*** antibody by the **ELISA** method in 19961. **Of** the remaining **27** County animals surviving in July 1996, **25** tested positive, one tested suspect and one tested negative for ***Mycoplasma agassizii*** antibody. **All** surviving BLM animals tested negative.

Disease **Transmission**

The very different pattern of disease in the two groups of hatchlings despite their identical treatment by us indicates that the difference stems from prior exposure to other animals. Although we do not know if the adults in the pens from which the **BLM** hatchlings were retrieved will test negative for ***Mycoplasma agassizii*** antibodies, the hatchlings escaped infection, and did not become infected in our bins even though infected County hatchlings were housed in adjacent bins. We suspect that the county hatchlings were exposed to the disease when they were housed in large groups prior to being placed in the raven-proof pens. These large groups included animals removed from urban areas and development sites. It appears that the disease is readily transmitted among hatchlings. Of the **11** raven-proof pens from which we obtained County hatchlings, only one was apparently disease-free: the seven surviving tortoises from this pen remain healthy to this date (although a positive **ELISA** test for one animal should be confirmed). All tortoises from the remaining 10 pens have died, developed signs of URTD and/or have tested positive for ***Mycoplasma agassizii*** antibody.

It is remarkable that none of the **BLM** hatchlings developed URTD signs or a positive **ELISA** test despite the fact that the bins in which they were housed were interspersed with the bins in which the County hatchlings were housed, and BLM hatchlings were handled immediately after County hatchlings were handled. We did

not use rubber gloves or wash our hands between bins, but avoided touching the faces of tortoises. Tortoises from different bins were never placed together, and all bins, food dishes, water dishes and soaking pans were thoroughly washed with detergent and soaked in a bleach solution between uses. Severely ill or debilitated tortoises were placed in isolation bins and were handled last when tortoises were being fed, watered and cleaned.

The fact that all BLM hatchlings and two bins of County hatchlings remained URTD-free suggests that the disease was not transmitted from bin to bin, and that the difference in infection rate among the bins was a consequence of prior exposure. It appears that handling per se did not lead to disease 'transmission. Our findings are consistent with the hypothesis that transmission of ***Mycoplasma agassizii*** requires direct animal contact or direct transfer of secretions between animals.

CONCLUSIONS

1. Hatchling tortoises appear to be highly susceptible to **URTD** once exposed to the infectious agent. Hatchlings housed together readily share the disease.

2. Eye signs observed under a dissecting microscope are useful indicators of URTD in hatchlings. Hatchlings with mild eye signs may continue to eat and grow, despite occluded nares. This is not true of hatchlings with severe eye signs.

3. Respiratory inflammation may be especially severe in young tortoises.

4. Even on a high plane of nutrition and under favorable environmental conditions, **most** infected hatchlings die. Thus it is unlikely that infected hatchlings in the wild survive to maturity.

5. The difference in infection rate between tortoises obtained from **BLM** and County pens was probably due to prior exposure.

EFFECTS OF ACUTE UPPER RESPIRATORY TRACT DISEASE ON REPRODUCTION IN THE DESERT TORTOISE, *Gopherus agassizii* HORMONES, EGG PRODUCTION AND HATCHING SUCCESS

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INTRODUCTION

Recent dramatic declines in wild populations of the desert tortoise, ***Gopherus*** *agassizii*, have led to its federal listing as a threatened species. These population declines have been largely attributed to habitat loss and degradation. However, the impact of upper respiratory tract disease (URTD) on population declines **is unknown.**

The acute effects of upper respiratory tract disease on reproduction in the desert tortoise ***(Gopherus*** *agassizii*) were monitored from **1991** to **1993** at the Desert Tortoise Conservation Center. The disease broke out in several pens that had been set up to study reproduction (Rostal et al., **1994).** This appeared to result fiom the introduction of a undiagnosed diseased female or male into several of these pens (Jacobson et al., **1995).** During the course of the study, the disease was allowed to run its course since an effective treatment was unavailable.

MATERIALS AND METHODS

SUBJECTS

Fifty tortoises were maintained in ten reproductive groups composed of **2** males and **3** females each. Male mean straight carapace length was **261.25 ± 3.93** mm (SE; n = **20).** Female mean straight carapace length was **241.07 ± 2.32** mm (SE; n = **30).** The tortoises were housed in **15** m **X 30** m pens. Each pen had five artificial burrows, two sod plots, and two watering stations each. The pens were also supplemented with alfalfa hay. The tortoises were exposed to ambient weather conditions (i.e., air temperature and rainfall).

BLOOD COLLECTION AND RADIOIMMUNOASSAY

Blood samples **(3** to **5** cc) were collected from **20** males and **30** females fiom August **1991** to July **1993** (as described in Rostal et al., **1994).** Blood samples were centrifuged and the plasma was removed and frozen for later analysis. Plasma testosterone, progesterone and corticosterone were measured in duplicate aliquots of plasma extracted with **10** vols **of** ethyl acetate: n-hexane **3:2** vol/vol and incubated overnight with highly specific antibodies from ICN diagnostics and tritiated steroids from Amersham-Searle (see Rostal et al., **1994** for details). Plasma estradiol was measured using a modified iodine-125 kit fiom Diagnostic Products Corp. The samples were extracted with ethyl acetate:hexane, but incubated with only ½ the volume of antibody as specified by the kit, and the bound from free steroid was separated using dextran-charcoal instead of the second antibody. These modifications increased the sensitivity **of** the assay to **1** pg/tube.

ULTRASOUND EXAMINATIONS

The reproductive status of the females was monitored at two month intervals using ultrasonography (as described in Rostal et al., 1994). Ovarian follicular growth and the presence of shelled oviductal eggs was monitored using an Aloka 500V portable real-time ultrasound scanner with a 7.5 convex linear transducer. During the nesting season, females were scanned every two weeks to determine nesting.

CLASSIFICATION OF HEALTH

The status of an animal's health was based on both the presence of antibodies to *Mycoplasma agassizii* as determined by ELISA technique (Schumacher et al., 1993) and clinical signs of URTD. Clinical signs of URTD ranged from mild serous exudate from the nares to mucopuruient exudate from the nares with encrusting dirt on face and forelegs. Animals with severe signs were also emaciated and lethargic.

RESULTS

Plasma testosterone levels were significantly lower in diseased males (n = 8) than healthy males (n = 10) during the second fall breeding season (July 1992 to October 1992). Female plasma testosterone levels were not significantly different between healthy females (n = 12) and diseased females (n = 12). Among the diseased females, however, there was a significant difference in plasma testosterone levels between reproductively active (nesters; n = 6) and reproductively inactive (non-nesters; n = 6) females during the second reproductive season (July 1992 to June 1993). Plasma estradiol levels were significantly lower in diseased females (n = 12) than healthy females (n = 12) during the second reproductive season (July 1992 to June 1993). Among the diseased females, there also was a significant ciifference in plasma estradiol levels between reproductively active (nesters; n = 6) and reproductively inactive (non-nesters; n = 6) females during the second reproductive season (July 1992 to June 1993). Plasma corticosterone levels, however, were not significantly different between diseased and healthy males or females.

Vitellogenesis and follicular growth was retarded in diseased females during the second reproductive season. Maximum follicle size measured in September 1992 was significantly smaller in diseased females (n = 9) than healthy females (n = 10). Hatchling size and growth rates were similar for hatchlings produced from both healthy females (n = 15 hatchlings) and diseased females (n = 15 hatchlings) that reproduced.

DISCUSSION

Endocrine function, ovarian development, egg production and hatching success were monitored during the study. Upper respiratory tract disease was observed to influence circulating hormone levels in diseased females. Testosterone levels were significantly lower in diseased males during the second reproductive season (July 1992 to October 1992). Estradiol levels were significantly lower in diseased females during the second reproductive season. Estradiol is critical for normal ovarian and follicular development. When estradiol levels were compared between nesting and non-nesting diseased females the effect was even more pronounced with circulating levels being 1/4 their normal level during October 1992. The month of October is critical for final follicular maturation prior to hibernation. Upper respiratory tract disease did reduce egg production in females actively showing signs of the disease. Only 50% of the diseased females produced eggs in 1993 while 100% of non-

diseased females produced eggs. Clutch size was not significantly different between reproducing diseased females and non-diseased females (mean clutch size was 5.60 ± 0.40 eggs for diseased females versus 5.64 ± 0.58 eggs for non-diseased females). *Also,* those diseased females that did reproduce showed equally high hatching success as non-diseased females and there was no difference in hatchling size or growth rates. Reproduction declined from 91.7% to approximately 54.5% in diseased females by the second year while healthy females remained high (91.7% in 1992; 100% in 1993).

FUTURE RESEARCH NEEDS:

1. Determine long term effects of URTD on egg production in the desert tortoise.
2. Monitor seasonal reproductive hormone levels and determine if a long term effect resulting in lower circulating hormone levels can be attributed to exposure to URTD.
3. Determine viability of eggs produced by tortoises following long term exposure to URTD.

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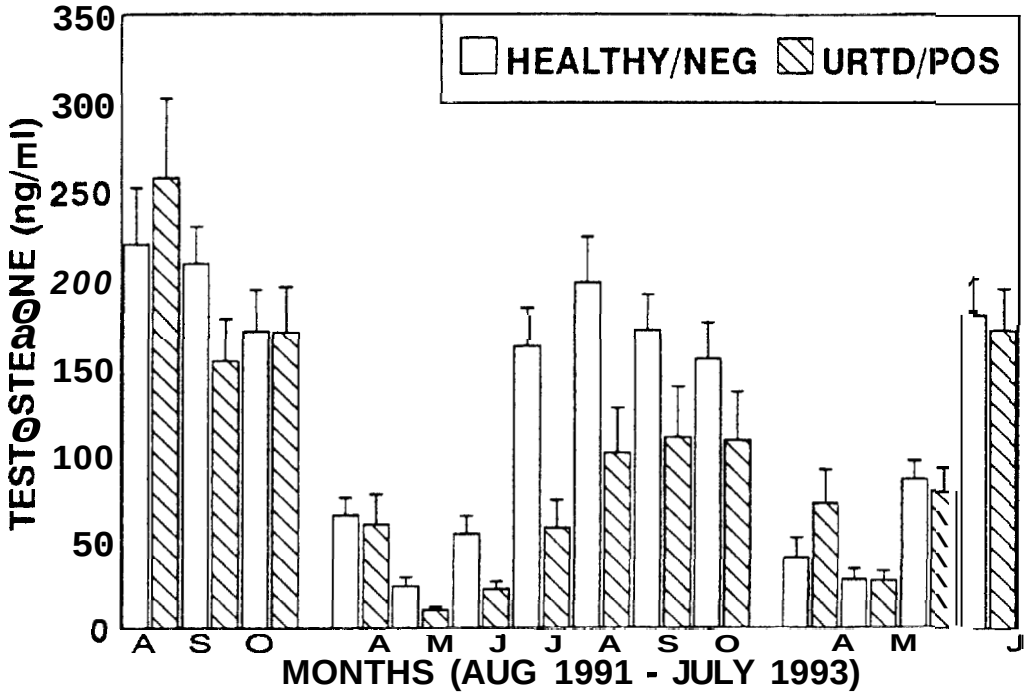
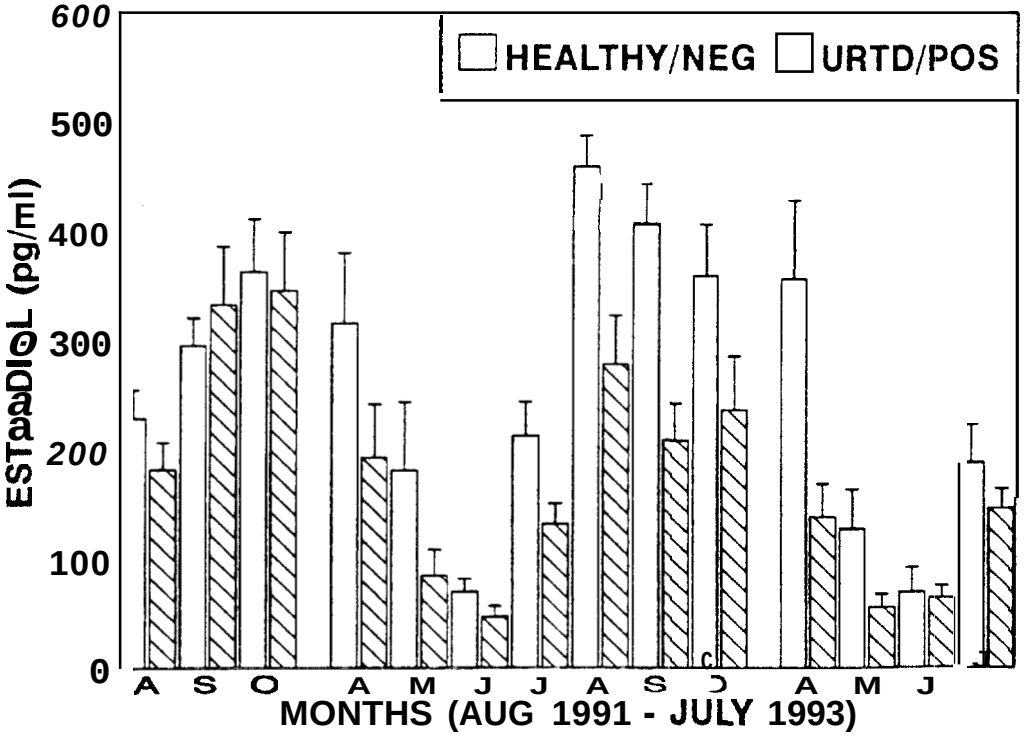
MALE DESERT TORTOISES

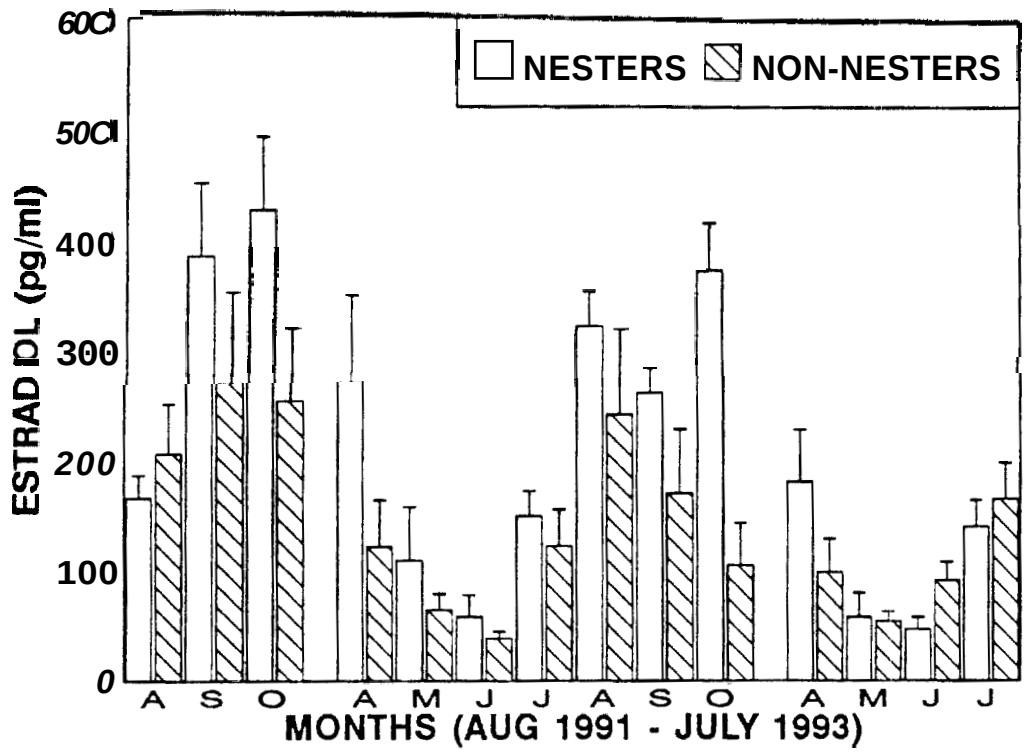
HEALTHY VS URTD



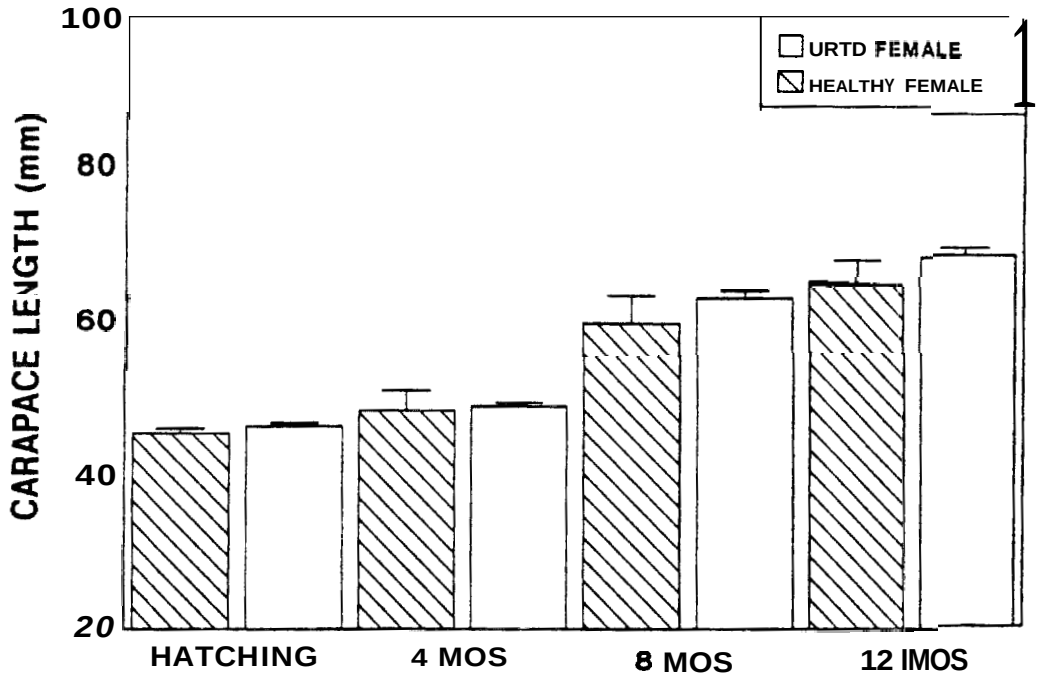
FEMALE DESERT TORTOISES



URTD/POS FEMALES



DESERT TORTOISE HATCHLINGS



Hatchlings from healthy and URTD females as determined by ELISA for Mycoplasma agassizii.

Draft prepared for the Conference on Health Profiles, Health Reference Ranges, and Diseases in Desert Tortoises, CSU Desert Studies Center at Soda Springs CA, October 31 - November 3, 1996.

Diagnostics of Mycoplasma Infections of Tortoises: Applications for Management and Conservation

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Abstract

Three tests have been used to diagnose mycoplasmal upper respiratory infections of captive and free-ranging tortoises: direct mycoplasmal culture, detection of mycoplasmal chromosomal DNA by polymerase chain reaction (PCR), and detection of anti-mycoplasma antibodies in tortoise plasma by enzyme-linked immunoassay (ELISA). These diagnostic tests each measure different things, and therefore differ in the types of samples required, sensitivity, specificity, cost, and interpretation. The results of the tests are complementary for defining tortoise mycoplasma infection status. They can be used for epidemiological surveys and in decision making for management of captive tortoises or repatriation programs.

RELATIONSHIP BETWEEN CLINICAL SIGNS OF UPPER RESPIRATORY TIRACT DISEASE AND ANTIBODIES TO MYCOPLASMA AGASSIZII IN DESERT TORTOISES FROM LAS VEGAS VALLEY, NEVADA

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ABSTRACT: Plasma samples 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 (ELISA) for antibodies to Mycoplasma agassizii, a causative agent of URTD. The relationship between clinical signs and ELISA test results was examined. 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 ($P < 0.0001$, Chi-square = 33.1) 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.

INTRODUCTION

The Mojave population of the desert tortoise (Gopherus agassizii), occurring north and west of the Colorado River in Utah, California, Arizona, and Nevada **(USA)** is listed as threatened by the U.S. federal government (Department of Interior, 1990). Mycoplasma agassizii is an etiologic agent of a contagious upper respiratory tract disease (URTD) in the desert tortoise (Brown et al., 1994). Upper respiratory tract disease was first observed in desert tortoises two decades ago and may have contributed to the decline of this species (Jacobson et al., 1991). In its early stage URTD is characterized by palpebral edema and serous nasal and ocular discharge while intermittent episodes of mucous nasal and ocular discharge are seen in its chronic stage. The contagious nature of URTD complicates tortoise management decisions. Due to land development, relocation of tortoises is often employed as a conservation effort. Thus, a reliable diagnosis of mycoplasma infection is essential in curbing the spread of URTD. Chronically infected tortoises with URTD have intermittent clinical signs, thus making a diagnosis of this disease by clinical signs alone unreliable. Enzyme-linked immunosorbent assays (ELISAs) measure antibodies that are produced in response to mycoplasma exposure and thus have the potential to detect subclinically infected tortoises. Diagnosing URTD by clinical signs alone would miss silent carriers, possibly resulting

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in the spread of URTD into a naive tortoise population. While serological testing cannot distinguish between an active infection and exposure to a pathogen it is still the method of choice for screening large numbers of samples. In this study we examined the relationship between clinical signs of **URTD** and exposure to M. aeassizii as measured by ELISA (Schumacher et al., 1993) in a free-ranging desert tortoise population.

MATERIALS AND METHODS

In 1990, blood samples were collected by jugular venipuncture (Jacobson et al., 1992) from wild, free-ranging desert tortoises (Gopherus agassizii) in the Las Vegas Valley, Clark County, Nevada (USA) (35°57'N, 115°15'W). Blood was originally collected for complete blood counts and plasma biochemistry to establish baseline health profiles for 277 tortoises prior to relocation into a nearby conservation research facility (Hardenbrook, unpubl.). Surplus plasma samples from 144 tortoises were initially frozen in liquid nitrogen and then stored at -80 C until June 1993, when they were shipped to the University of Florida, Gainesville, Florida (USA) and tested by ELISA for antibodies to Mycoplasma agassizii using an enzyme-linked immunosorbent assay (ELISA) (Schumacher et al., 1993). The samples represented 61 adult males (43%), 55 adult females (38%), and 28 (19%) immature tortoises. Adults were defined as tortoises with a median carapace length (MCL) ≥ 204 mm whose sex could be determined using secondary sexual characteristics (Berry, 1984).

For **ELISA** a plasma sample was considered to be positive if the absorbance at 405 nm of either one or both of its dilutions (two-fold dilution and 10-fold dilution) was greater than twice the absorbance of the negative control plasma at the same dilution. Samples with values equal to or lower than twice the negative control were considered negative.

At the time of blood collection, a physical examination was conducted. The following clinical signs were recorded: moisture around the nares, nasal discharge (ranging from serous leakage to a mucous discharge from the nares), occluded nares, labored breathing, wheezing, and palpebral edema. Based on clinical signs two groups were established. The first group consisted of tortoises with either only one or any combination of the above described clinical signs. The second group was **a** subgroup of the first group and consisted of tortoises with mucous nasal discharge only or in combination with any of the other clinical signs.

Data were analyzed using Chi-square analysis and Fisher's Exact P-value (StatView, Abacus Concepts, Inc., Berkeley, California, **USA).** Positive and negative predictive values with

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95% confidence intervals were calculated to identify the relationship between clinical signs and ELISA results (Smith, 1995). Kappa statistic (Rosner, 1995) was used to investigate the concordance of assessment of URTD status by clinical signs with assessment by ELISA. A kappa ≥ 0 and < 0.4 indicated marginal concordance, kappa ≥ 0.4 and 0.75 indicated good concordance, and kappa > 0.75 indicated excellent concordance. A $P > 0.05$ indicated statistical significance.

RESULTS

Of the 144 tortoises tested for M. agassizii using an ELISA, 72 (50%) were seropositive and 72 (50%) were seronegative. Of 61 male tortoises, 38 (62%) were seropositive and 23 (38%) were seronegative. Of the 55 female tortoises, 27 (49%) were seropositive, and 28 (51%) were seronegative. Seven (25%) of the 28 immature tortoises were seropositive and 21 (75%) were seronegative.

Ninety-nine (69%) of the 144 tortoises did not exhibit any clinical signs of URTD (Table 1). Sixty-five (66%) of these 99 animals without clinical signs tested seronegative; yet, 34 (34%) tested seropositive indicating exposure to M. agassizii. Thirty-eight (84%) of 45 animals with clinical signs tested seropositive. Seven tortoises showed clinical signs but tested seronegative (9.7% of all seronegatives). Five of those seven tortoises (Table 1) were reported with either moisture around the nares, labored breathing, or making wheezing sounds and two had mucous nasal discharge (Table 2). Clinical signs were significantly ($P < 0.0001$, Chi-square = 33.1) related to a seropositive ELISA result, regardless of sex and age (males $n = 22$, $P < 0.0001$, Chi-square = 16.1; females $n = 17$, $P = 0.044$, Chi-square = 4.55; immatures $n = 6$, $P = 0.0012$, Chi-square = 13.9). The probability for the examined population that a tortoise with clinical signs of URTD also tested seropositive (positive predictive value of clinical signs or sensitivity of the ELISA test) was 84% with a confidence interval of 70 to 93%. Positive predictive values of clinical signs and the corresponding confidence intervals for adult male, adult female, and immature tortoises were 96% (77-100%), 71% (44-90%), and 83% (36-100%), respectively. The negative predictive value for clinical signs or the specificity of the ELISA test for the total population was 66% with a confidence interval of 55-74%. Negative predictive values of clinical signs and the corresponding confidence intervals for adult male, adult female, and immature tortoises were as follows: 56% (40-72%), 61 (43-76%), and 91% (71-99%), respectively. Kappa for concordance of clinical signs with ELISA results for the entire population was 0.43 ($P = 0.0001$). For adult

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male, adult female, and immature tortoises kappa was 0.48 ($P = 0.0001$), 0.28 ($P = 0.017$), and 0.7 ($P = 0.0001$), respectively.

Twenty-six of **28** (93%) tortoises whose clinical signs included mucous nasal discharge tested positive by ELISA for exposure to M. agassizii ($P =< 0.0001$, Chi-square = 25.5) (Table **2).** The remaining two tortoises with mucous nasal discharge tested negative (2.89'0of the 72 seronegative tortoises). The relationship between mucous nasal discharge and a positive test result was significant for both male tortoises ($P < 0.0001$, Chi-square = 14.3) and female tortoises ($P = 0.025$, Chi-square = **5.52).** In immature tortoises no relationship between mucous nasal discharge and a positive test result ($P = 0.15$, Chi-square = 3.11) was detected. The probability for a tortoise with mucous nasal discharge also being seropositive in the ELISA test was 93% with a confidence interval of 77-99% for the total population. In males it was 100% with a lower confidence limit of 84%, in females 88% (47-100%), and in immatures 67% (9.4-99%). Kappa for concordance of mucous nasal discharge with ELISA results for the entire population was 0.33 ($P = 0.0001$). For adult male, adult female, and immature tortoises kappa was 0.38 ($P = 0.0001$), 0.23 ($P = 0.0094$), and 0.29 ($P = 0.039$), respectively.

DISCUSSION

For the tortoises examined from Las Vegas Valley, Nevada there was a positive relationship between clinical signs of URTD and exposure to M. agassizii as measured by ELISA. **All** clinical signs recorded at the time of blood collection were compatible with URTD (Jacobson et al., 1991). Clinical signs reported in this study were good indicators for M. agassizii infection in adult tortoises, as the majority of adult tortoises that showed clinical signs of URTD were also M. agassizii-positive by ELISA. The small number of seropositive immature tortoises in this study caused a wide confidence interval which made it impossible to predict the serological status of immature tortoises by clinical signs. Mucous nasal discharge, alone or in combination with other clinical signs was the most reliable indicator of a M. aeassizii infection in adult tortoises. All male and most female tortoises with nasal discharge were accurately predicted to test positive for mycoplasma infection by visual inspection for mucous nasal discharge. Immatures were impossible to predict. Again, this was due to the small number of seropositive immature tortoises.

Visual inspection of tortoises for clinical signs of URTD proved to be very specific in detecting mycoplasma infection in the adult tortoises of the examined population. However, absence of clinical signs in a tortoise **does** not mean that it has not been exposed to M. agassizii.

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The sensitivity of visual inspection for clinical signs was poor. One third of the tortoises in this study appeared clinically healthy at the time of blood collection but tested seropositive. We have seen captive tortoises with confirmed (by ELISA and culture) M. agassizii infections to be clinically normal for more than one year before suddenly exhibiting clinical signs of URTD. This can be attributed to the chronic nature of URTD, which causes intermittent appearance of clinical signs throughout the course of the disease. The serological status of these animals typically remains positive for antibodies to M. agassizii during the subclinical periods. Jacobson et al. (1995) reported on subclinical mycoplasmosis in a tortoise population in Las Vegas Valley. The environmental and behavioral stressors which may cause a latent M. agassizii infection to become clinically manifest have not been investigated yet. Tortoises that have no clinical signs of URTD and still test seropositive for M. agassizii, may be silent carriers and act as reservoirs for the pathogen. In some tortoises antibodies to M. agassizii may develop before any clinical signs of disease are seen, as demonstrated in a recent transmission study in which desert tortoises were inoculated with M. agassizii (Brown et al., 1994). Some tortoises may be able to clear the mycoplasma infection while remaining seropositive, others may have been exposed to the pathogen without becoming infected. Unfortunately, the tortoises sampled for the present study were subsequently used by other investigators for a variety of studies. Consequentially, logistical and funding constraints precluded a systematic follow-up in terms of the development of clinical signs of URTD in these tortoises. When a newly developed polymerase chain reaction (PCR) (Templeton, 1992) was used to screen presumed isolates of M. agassizii, 10 of 35 isolates did not have the same 16S rRNA sequence and represented a new species (Brown et al., 1995). However, when the new mycoplasma was used as the ELISA antigen in lieu of M. agassizii, the ELISA values obtained were virtually identical (Schumacher, unpubl.). Based on preliminary infection studies, the unknown mycoplasma, like M. agassizii, can cause clinical signs of URTD in gopher tortoises (Gopherus polyphemus). To date the pathogenicity of the new mycoplasma has not been tested in the desert tortoise. There may be other agents that do not cause clinical disease, but share antigenic determinants with M. agassizii, thus causing tortoises exposed to those agents to react positive in the serologic test.

Of the tortoises with signs of URTD, seven were seronegative for exposure to M. agassizii. There are several explanations which may account for this. Other pathogens, like Pasteurella testudinis, have been found in tortoises (Snipes and Bieberstein, 1982) and were suggested to cause clinical signs similar to those observed in the seven M. agassizii-negative animals. However, in a recent study Pasteurella testudinis alone did not cause clinical signs

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(Brown et al., 1994). Also, some clinical signs reported may not have been indicative of illness but may have been caused by stimuli other than mycoplasmosis. Wet nares in tortoises can be caused by eating or drinking or in response to dust or other allergens. In some tortoises the appearance of clinical signs may precede the production of detectable levels of M. agassizii antibodies (Schumacher, unpubl.). Interpretation of ELISA results used in population management as a step to curb the spread of a contagious disease should err on the side of false positives rather than false negatives. However, the low cut-off in the ELISA used to define seropositive animals in order to avoid false negative results may still have been too high. Finally, the observed clinical signs in all or some of the seven tortoises may have been caused by one or several as of yet undiagnosed pathogens.

Although there was significant concordance between visual inspection for URTD and detection by ELISA of antibodies against M. agassizii, kappa was only between 0.2 and 0.4, indicating only fair or good concordance between the tests. This was a direct result of the way in which kappa is calculated. Kappa is derived by taking into account, at the same time, the number of seropositive animals with clinical signs and the number of seronegative animals without clinical signs. Since the sensitivity of the ELISA (84%) was much better than the sensitivity of the visual inspection (53%), the overall concordance of the two tests was low. This reinforces,the importance of serological testing of tortoises that have to be relocated in order to not miss animals that do not have clinical signs but that are infected with M. agassizii and able to spread the pathogen. Even the most careful observer may miss clinical signs, and there are cases were clinical signs are absent, either because infected chronically ill animals were visually assessed in between episodes of overt disease, or because some tortoises were silent carriers that may, although infected, never show clinical signs.

The ELISA can be used to determine whether individual tortoises have been exposed to M. agassizii as well as the prevalence for M. agassizii- exposure within a population. But based upon a single blood sample, ELISA tests cannot be used to diagnose an active infection because the presence of M. agassizii organisms in an animal cannot be determined by serologic tests. Rising titers between paired samples taken approximately 2 mo apart would be evidence for a recent infection. Information on M. agassizii presence can be obtained by culture (Tully, 1977) or by PCR (Brown et al., 1995). These tests have the potential to detect the microorganism or its genetic material in tissues or in nasal flushes. However, the sensitivity and specificity of the ELISA merits its use as the most reliable indicator of exposure to M. agassizii in free-ranging tortoise popuiations. Also, blood samples for ELISA testing are more conveniently obtained under field

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conditions and less costly than sterile nasal flush samples for culture.

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TABLE 1, The number of desert tortoises with and without clinical signs of URTD compared to the number of desert tortoises with and without antibodies to M. agassizii as measured by **ELISA.**

ELISA result	Clinical signs a / b / c	No clinical signs a / b / c
Seropositive	21 / 12 / 5	17 / 15 / 2
Seronegative	1 1 5 1 1	22 / 23 / 20

a Number of male tortoises.

b Number of female tortoises.

c Number of immature tortoises.

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TABLE **2.** The number of desert tortoises with and without mucous nasal discharge compared to the number of desert tortoises with and without antibodies to M. agassizii as measured by **ELISA.**

ELISA result	Mucous nasal discharge a / b / c	No mucous nasal discharge a / b / c
Seropositive	171712	21 / 20 / 5
Seronegative	01111	23 / 27 / 20

[a] Number of male tortoises.

[b] Number of female tortoises.

[c] Number of immature tortoises.

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Drought- induced dehydration and starvation in juvenile and adult desert tortoises from the western and eastern Moiave Desert

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Eleven desert tortoises *(Gopherus agassizii)* were collected or salvaged between April 1990 and March 1995 from three sites in the Mojave Desert. Two were found alert, four found weak or ill, and five found recently dead. Salvaged tortoises included five adults, one immature, and 5 juveniles. Tortoises salvaged from the field displayed one or more following clinical signs: lethargy and inactivity; limp limbs or head; eyes partially closed, swollen or sunken; respiratory signs; thin or dehydrated appearance; or low in weight for size. The objective was to determine if dead or dying tortoises appearing to be dehydrated and starving, were suffering from inadequate water and nutrient uptake or had succumbed to other causes.

Average annual precipitation (October to September) and winter precipitation (October io March) were calculated from long-term precipitation records compiled by the National Climate Data Center at stations near study sites at Goffs, Ivanpah Valley, and Ft. Irwin Study Site (FISS), San Bernardino County, California. The eastern and central Mojave Desert received annual and winter precipitation below long-term norms during the 1988-89, 1989-90, and 1993-94 rainfall years.

Expected weights for study animals were calculated from data bases of tortoises at long-term study sites. Tortoises were selected for analysis using two criteria: 1) they were from the same study site; 2) they had survived one or more droughts during the last twenty years; and 3) they were within $\pm$ 10 mm carapace length at the midline of a study tortoise. Weights of selected tortoises were regressed against the carapace lenghts to determine expected weights of study animals. Necropsies examinations including gross and microscopic examinations, laboratory analysis, hematologic and plasma biochemical profiles, urinalysis, serology, parasitic examination, and metal and organic compounds, were conducted and results were compared to reference ranges from health profiles of free-ranging desert tortoises in California.

All adult tortoises for which blood and urine samples were collected (n = **4)** had hemosiderosis of the liver, the deposition or storage of iron in heptocytes and macrophages throughout the liver. Three out of four adult tortoises had low weight for size, modestly high bilirubin, and urolithiasis. Two adults had elevated urea nitrogen (BUN), elevated plasma chloride, low plasma calcium, and elevated aspartate aminotransferase (AST) . One out of four adults had a low red blood cell count, low packed cell volume, elevated sodium, and potassium in the urine. In addition, the adult salvaged from **FISS** had slightly high creatinine, high cholesterol, elevated uric acid, high osmolality, elevated bile acids, and periarticular and renal gout. Two out of five adults had no auxiliary fat adjacent to the proximal end of the humeri and the thymus was barely visible or could not be located. Serology **(ELISA)** tests were negative for 4 adults and suspect for one.

Hematologic and plasma biochemical results for the immature tortoise revealed low glucose, elevated sodium, chloride, BUN, cholesterol, and AST, and potassium in the urine. One juvenile was **anemic** with low plasma proteins **and** calcium. All juveniles; exhibited moderate to severe osteopenia. In addition, FISS juveniles exhibited skeletal muscle atrophy; hemosiderosis of the liver; no coelomic adipose tissue; and elevated hepatic lead, copper, zinc as well as, elevated renal lead and zinc.

Elevated BUN, sodium and chloride were indications of dehydration and inadequate water balance. Urolithiasis and gout are often an end stage of dehydration and/or kidney disease. Elevated bilirubin was indicative of liver atrophy or hemolysis. Hemosiderosis in the liver often occurs secondary to chronic disease and could indicate hemolysis **or** excess exogenous iron.

Study tortoises showed signs of anorexia (lack of appetite), cachexia (skeletal muscle atrophy **and** weakness), emaciation (thin because of starvation or illness), dehydration (inadequate fluid intake), and starvation (protein/calorie undernutrition) . Behavior in the field was characterized by being above ground at inappropriate times of the year, failing to return to burrows at night or during hot times of the day, remaining in a resting position in one place day after day, failing to eat when forage was readily available, general weakness **and** diminished response. Drought is likely to magnify the effects of other diseases. Toxins in tortoises **may** increase vulnerability to drought and illness.

BIOSKETCHES

10/23/96

BIOSKETCH

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1969-1971 NIH Postdoctoral Fellow, Department of Microbiology and Molecular Genetics, Harvard Medical School. Studied hormonal regulation of animal cells in culture in the laboratory of H. Amos.

1971-1976 Assistant Professor, Department of Bacteriology and Immunology, The School of Medicine, The University of North Carolina at Chapel Hill

1976-1980 Associate Professor, Department of Bacteriology and Immunology, The School of Medicine, The University of North Carolina at Chapel Hill

1981- Professor and Chair, Department of Microbiology, The University of Texas Health Science Center at San Antonio

1986- Professor, Department of Medicine, The University of Texas Health Science Center at San Antonio

HONORS AND AWARDS:

NIH Research Career Development Award
Member, NIAID Microbiology and Infectious Diseases Advisory Committee
Editorial Board, *Infection and Immunity*
Member, Committee on Medical Microbiology and Immunology of the Public and, Scientific Affairs Board
Member, Texas Research and Technology Foundation, Scientific Advisory Board
Chairman, American Society for Microbiology President's Fellowship Committee
Director, Microbiology for Public School Science Teachers in South Texas, Carnegie Corporation

ASM Foundation for Microbiology Lecturer
Member, NIAID Bacteriology and Mycology 2 Study Section
President, Association of Medical School Microbiology and Immunology Chairs
Member, Board of Directors, International Organization for Mycoplasmology
Member, National Caucus of Basic Biomedical Science Chairs
Panel Member, Molecular Immunology and Vaccine Development, NIH Strategic Plan, Bethesda, Maryland
Stuart Mudd Memorial Lecturer, University of Pennsylvania and American Society for Microbiology, Philadelphia, Pennsylvania
Member, **NM** Intramural Program Review, Rocky Mountain Laboratories, Hamilton, Montana
Senior Editor, Federation of European Microbiological Societies (FEMS) Immunology and Medical Microbiology.

PROFESSIONAL SOCIETIES:

American Society for Microbiology
Sigma Xi
American Association for the Advancement of Science
Association of Medical School Microbiology and Immunology Chairs
International Organization for Mycoplasmology
National Caucus of Basic Biomedical Science Chairs
American Association of Medical Colleges GREAT (Graduate Research, Education and Training) Group

REFEREED PUBLICATIONS ***(since 1993):***

Su, C.J., S.F. Dallo, and J.B. Baseman. 1993. Possible origin of sequence divergence in the P1 cytadhesin gene of *Mycoplasma pneumoniae.* Infect. Immun. 61:816-822.

Mernaugh, G.R., S.F. Dallo, S.C. Holt, and J.B. Baseman. 1993. Properties of adhering and non-adhering populations of *Mycoplasma genifalium.* Clin. Infect. Dis. 17:S69-78.

Marais, A., J.B. Bove, S.F. Dallo, J.B. Baseman, and J. Renaudin. 1993. Expression in *Spiroplasma citri* of an epitope carried on the G fragment of the cytadhesin P1 gene from *Mycoplasma pneumoniae.* J. Bact. 175(9):2783-2787.

Staggs, T., M.K. Greer, J.B. Baseman, S. Holt, and V.V. Tryon. 1994. Identification of lactoferrin-binding proteins from *Treponema pallidum* subspecies *pallidum* and *Treponema denticola.* Molecular Microbiol. 12(4):613-619.

Tully, J.G., D.L. Rose, J.B. Baseman, **S.F.** Dallo, A.L. Lazzell, and C.P. Davis. 1995. Mixed *Mycoplasma pneumoniae* and *Mycoplasma genitalium* in a synovial fluid isolate. J. Clin. Microbiol. 33(7):1851-1855.

Reddy, **S.P.,** W.G. Rasmussen, and J.B. Baseman, 1995. Molecular cloning and characterization of an adherence-related operon of *Mycoplasma genifalium.* J. Bacteriol. 177(20):5943-5951.

Baseman, J.B., M. Lange, N.L. Criscimagna, J.A. Girón, and C.A. Thomas. 1995. Interplay between mycoplasmas and host target cells. Microb. Pathog. 19: 105-116.

Girón, J.A., M. Lange, and J.B. Baseman. 1996. Adherence, fibronectin binding, and induction of cytoskeleton reorganization in cultured human cells by *Mycoplasma penetrans*. Infect. Immun. **64**(1): 197-208.

Reddy, S.P., W.G. Rasmussen, and J.B. Baseman. 1996. Correlations between *Mycoplasma pneumoniae* sensitivity to cyclosporine-A and cyclophilin-mediated regulation of mycoplasma cytadherence. Microb. Pathog. 20: 155-169.

Dallo, S.F., A.L. Lazzell, A. Chavoya, S.P. Reddy, and J.B. Baseman. 1996. Biofunctional domains of the *Mycoplasma pneumoniae* P30 adhesin. Infect. Immun. 64:2595-2601.

Reddy, S.P., W.G. Rasmussen, and J.B. Baseman. 1996. Isolation and characterization of transposon Tn*4001*-generated, cytadherence-deficient transformants of *Mycoplasma pneumoniae* and *Mycoplasma genitalium.* FEMS, Immunology and Medical Microbiology. (in press)

BOOK CHAPTERS/REVIEWS *(since 1993):*

Baseman, J.B., and **V.V.** Tryon. 1993. Microbial adhesion and arthritides: Other Pathogens, In Musculoskeletal Infection, J.L. Esterhai, Jr., A.G. Gristina and R. Poss (eds.) American Academy of Orthopaedic Surgeons, Dallas, Texas, p. 89-115.

Tryon, V.V., and J.B. Baseman. 1993. Pathogenic determinants and mechanisms. In Mycoplasmas: Molecular Biology and Pathogenesis, J. Maniloff, R.N. McElhaney, L.R. Finch, and J.B. Baseman (eds.). American Society for Microbiology, p. 457-471.

Baseman, J.B. 1993. The cytadhesins of *Mycoplasma pneumoniae* and *Mycoplasma genitalium.* In Subcellular Biochemistry: Mycoplasma Cell Membrane, **S.** Rottem and I. Kahane (eds.), Plenum Publishing Co., New **York,** p. 243-259.

Baseman, J.B., S.P. Reddy, and S.F. Dallo. 1996. Interplay between mycoplasma surface proteins, airway cells and the protean manifestations of mycoplasma-mediated human infections. Am. J. Resp. Crit. Care Med. (in press)

RESEARCH INTERESTS:

Our laboratory examines the molecular pathogenesis of microbial disease emphasizing the virulence potential of specific procaryotes and the host response to parasitic attack. Currently, we are studying *Mycoplasma* species using a variety of experimental approaches including recombinant DNA, hybridoma and synthetic peptide technologies.

A major thrust of the laboratory is to characterize molecules possessed by pathogenic mycoplasmas which mediate adherence and are important in the development of disease.

Studies are also underway to characterize host cell receptors which serve as targets for mycoplasma cytadherence, and to define subsequent events which lead to mycoplasma invasion of the intracellular spaces of host target cells. Another area of interest focuses on recent evidence suggesting a role of *Mycoplasma* in AIDS-associated disease and autoimmune-related pathologies.

JOHN L. BEHLER
Curator, Department of Herpetology

John L. Behler has served as Curator of the Herpetology Department since 1976 at the Wildlife Consemtion Society's Bronx Zoo. Behler started his career with the Society in the Reptile Department as a New York Council on the Arts curatorial trainee in 1970.

Behler has assumed leadership positions in the development of captive breeding programs for endangered and threatened crocodilians, iortoises, and freshwater turtles. Through his efforts, first-rime births of batagur turtles and false gharials were achieved at the Bronx Zoo. He received the American Zoo and Aquarium Association (AZA) Edward H. Bean Award in 1980 for most significant reptile birth for his work with Chinese alligators. Behler's interests also focus on the ecology and behavior of reptilians and reptilian diseases.

Among his many conservation affiliations, Behler chairs the World Conservation Union's Tortoise and Freshwater Turtle Specialist Group and is the Species Coordinator and Studbook Keeper for the Chinese Alligator Species Survival Plan. He was a founding member of the AZA's Crocodilian Advisory Group and also serves on their Wildlife Conservation and Management Committee. Behler works closely with the New York State Department of Environmental Conservation's Endangered Species Unit and serves on his community's Conservation Board.

Behler received a Bachelor's degree in Zoology/Botany from the University of Miami and a Master's degree in Biological Sciences from East Stroudsburg University. He is a doctoral candidate at the University of Kent at Canterbury, United Kingdom.

A native of Pennsylvania, he now resides in Amawalk, New York with his wife. He has authored more than 40 popular and scientific articles and three guide books highlighting reptiles and amphibians.

BIOSKETCH FOR KRISTIN H. BERRY

U.S. Department of the Interior, U.S. Geological Survey, Biological Resources Division, Riverside Field Station
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ACADEMIC EDUCATION

1964	Stanford University. B.A in Biology
1968	University of California at Los Angeles. M.A. in Biology (Animal Behavior)
1972	University of California at Berkeley. Ph.D. in Zoology (Population Biology)

POSITIONS

1974-1993 USDI, Bureau of Land Management, Riverside, California: Staff Leader for Wildlife, California Desert Plan Program (1974-1980); Coordinator for Research, Studies, and Monitoring **(1980-1983);**Desert Tortoise Research/Monitoring Program **(1983-1990);** Staff Supervisor and Research Scientist, Desert Biology **(1990-1993)**

1993-1996 USDI, National Biological Service, Riverside, California. Research Scientist, Desert Tortoise Research Project.

1996- USDI, Geological Survey, Biological Resources Division. Research Scientist, Desert Tortoise Research Scientist

Recent Appointments and Memberships on National Scientific Committees or Teams

1989-94 Member, Desert Tortoise Recovery Team, appointed by the U. S. Fish and Wildlife Service. The Team produced: ***Desert Tortoise (Mojave Population) Recovery Plan* (1994)** and ***Proposed Desert Wildlife Management Areas for Recovery of the Mojave Population of the Desert Tortoise* (1994).**

Selected Papers Published in Peer-Reviewed Journals, Books or Proceedings

Berry, K. H. 1967. Wildflowers. Pp. 87-106 in Amer. Assoc. Univ. Women, China Lake Branch (eds.), ***Indian Wells Valley Handbook.*** 4th edition.

Berry, K. H. 1974. The ecology and social behavior of the chuckwalla, ***Sauromalus obesus obesus*** Baird. Univ. California Publ. Zool. 101:1-60.

Berry, K. H. 1978. Livestock grazing and the desert tortoise. Trans. North American Wildlife and Natural Resources Conference. 43:505-519.

Berry, K. H. 1980. A review of the effects of off-road vehicles on birds and other vertebrates. Pp. 451-467 *in* R. DeGraff and N. Tilghman (eds.), ***Management of Western Forests and Grasslands for Nongame Birds: Workshop Proceedings.*** USDA Forest Service Gen. Tech. Rept. INT-86.

Berry, K. H. (ed.) 1984. The Status of the Desert Tortoise (*Gopherus agussizii)* in the United States. Desert Tortoise Council Rept. to U.S. Fish and Wildlife Service, Sacramento, on Order No. 11310-0083-81. *858* pp.

Berry, K. H. 1986. Desert tortoise *(Gopherus agassizii)* research in California, 1976-1985. Herpetologica 42:62-67.

Berry, K. H. 1986. Desert tortoise *(Gopherus agassizii)* relocation: implications of social behavior and movements. Herpetologica 42:113-125.

Berry, K. H. 1986. Incidence of gunshot deaths in desert tortoises in California. Wildl. Soc. Bull. 14:127-132.

Berry, K. H. 1986. Introduction: development, testing, and application of wildlife-habitat models. Pp. 3-4 *in* J. Verner, M. L. Morrison, and C. J. Ralph (eds.), *Wildlife 2000: Modeling Habitat Relationships of Terrestrial Vertebrates.* Univ. Wisconsin Press.

Berry, K H. 1989. *Gopherus agassizii*. Pp. 5-7 *in* I. R. Swingland and M. W. Klemens *(eds.), The Conservation Biology of Tortoises*. IUCN/SSC Tortoise and Freshwater Turtle Group. Gland, Switzerland.

Berry, K H., and W. I. Boarman. 1995. Common ravens in the southwestern United States, 1968-92. Pages 73-75 *in* E. L. LaRoe, G. S. Farris, and C. E. Puckett *(eds.),* Our Living Resources: A report to the nation on the distribution, abundance, and health of U. S. plants, animals, and ecosystems. USDI, National Biological Service, Washington, D.C. 530 p.

Berry, K. H., and P. Medica. 1995. Desert tortoises in the Mojave and Colorado deserts. Pages 135-137 *in* E. L. LaRoe, G. S. Farris, and C. E. Puckett *(eds.),* Our Living Resources: A report to the nation on the distribution, abundance, and health of U. S. plants, animals, and ecosystems. USDI, National Biological Service, Washington, D.C. 530 p.

Berry, K H., T. Shields, A. P. Woodman, T, Campbell, J. Roberson, K. Bohuski, and A. Karl. 1986. Changes in desert tortoise populations at the Desert Tortoise Research Natural *Area* between 1979 and 1985. Proc. Desert Tortoise Council Symp. 1986:100-123.

Berry, K. H., and F. B. Turner. 1984. Notes on the behavior and habitat preferences of juvenile desert tortoises *(Gopherus agassizii)* in California. Proc. Desert Tortoise Council Symp. 1984:111-130.

Berry, K. H., and F. B. Turner. 1986. Spring activities and habits of juvenile desert tortoises, *Gopherus agassizii,* in California. Copeia 1986 (4):1010-1012.

Jacobson, E. R., M. Weinstein, K. H. Berry, B. Hardenbrook, C. Tomlinson, D. Freitas. 1993. Problems with using weight versus carapace length relationships to assess tortoise health. Veterinary Record 132:222-223

Jacobson, E. R., T. J. Wronski, J. Schumacher, C. Reggiardo, and K. H. Berry. 1994. Cutaneous dyskeratosis in free-ranging desert tortoises, *Gopherus agassizii,* in the Colorado Desert of Southern California. J. Zoo and Wildlife Medicine 25(1):68-81.

Book Chapters and Proceedings Papers in Press

Berry, K. H., T. A. Rado, and P. D. Mack In Press. The California Desert Conservation Area Database for Vegetation, Wildlife, Soils and Hydrology with Examples of Research needs for land management. *In* J. L. Latting *(ed.), The California Desert: An Introduction to Natural Resources and Man's Impact.*

The following four papers are in: J. Van Abbema *(ed.),* Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles-An International Conference. July 1993, Purchase, New York. Wildlife Conservation Society Turtle Recovery Program and the New York Turtle and Tortoise Society, New York. (to be published in 1996)

Berry, K H. 1996. The Desert Tortoise Recovery Plan: An ambitious effort to conserve biodiversity in the Mojave and Colorado deserts of the United States.

---, 1996. Demographic consequences of disease in two desert tortoise populations in California, USA

Christopher, M. M., K. A Nagy, I. Wallis, and K. H. Berry. 1996. Laboratory health profiles of desert tortoises in the Mojave Desert: A model for health status evaluation of chelonian populations.

Morafka, D.J., K. H. Berry, and E. K. Spangenberg. 1996. Predator-proof enclosures for enhancing hatching success and survivorship of juvenile tortoises.

Professional and Conservation Affiliations

1976-	Co-founder and Member of Board of Directors, The Desert Tortoise Council, Inc.
1974-	Member of Board of Trustees, Desert Tortoise Preserve Committee, Inc.
1975-81	Member, Board of Directors, Southern California Academy of Sciences.
1977-81	Board of Directors, Southern California Chapter of The Nature Conservancy
1988-	Deputy Vice-chairman. International Union for Conservation of Nature, Species Survival Commission, Tortoise and Freshwater Turtle Specialist Group

DANIEL R. BROWN

Departments of Pathology and Pathobiology
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352/392-4700 x3971
FAX 352/392-1619

Born October 8, 1956, Clinton, Iowa U.S.A. Social Security #311-64-2304. Family status: married.

Education

Ph.D. (1984 - 1987) Genetics, University of Arizona, Tucson AZ.
M.S. (1978 - 1980) Quantitative Genetics, Louisiana State University, Baton Rouge LA.
B.S. (1974 - 1978) Agricultural Sciences, Purdue University, West Lafayette IN.

Research

OPS Professional Biological Scientist (1993 - current) Create and conduct nucleic acid (PCR-RFLP) and immunological (immunoblot, ELISA) molecular diagnostics of mycoplasma and other bacterial and viral pathogens for clinical identification, phylogenetic classification, and applications in ecology and conservation biology. Managed over $250,000 in research grant funds in three years.

Assistant Research Scientist (1992 - 1993) Department of Animal Sciences, University of Arizona. Conducted PCR-RFLP, single-stranded DNA conformation polymorphism, and DNA sequence analyses of variation in genes affecting hereditary and infectious diseases of livestock (malignant hyperthermia, spongiform encephalopathy, leukocyte adhesion deficiency), **and** variation in genes affecting quantitative traits of economic importance (somatotropin, calpain) in animal agriculture.

Postdoctoral Associate (1987 - 1991) Departments of Immunology and Medical Microbiology, University of Florida, and Animal Science, Iowa State University. Conducted basic research on poliovirus RNA replication and mitochondrial genetics. Utilized molecular biology techniques including PCR and RT-PCR, DNA cloning, DNA / RNA sequencing, *in vitro* transcription, transfection of cultured HeLa cells, nucleic acid purification and fractionation, and Southern / Northern blot hybridization analyses. Developed a PCR-based diagnostic for viral meningitis. Cultured bacterial and bacteriophage vectors of recombinant DNA, and purified recombinant protein expressed in bacteria. Utilized radioisotopes (^{3}H, ^{14}C, ^{32}P, ^{35}S, ^{125}I) in immunoassays and nucleic acid analyses.

Research Associate (1980 - 1984) Animal Science Department, Louisiana State University. Supervised the operations of an analytical chemistry laboratory and assistants. Conducted quantitative chemical analyses and bioassays with microorganisms and laboratory animals. Member of Association of Official Analytical Chemists. Taught a graduate laboratory course in vitamin and mineral analysis.

Teaching

Adjunct Instructor (1991, and 1993 - current) Santa Fe Community College, Gainesville FL.
BSC 2005 General Biology (4 cr) with wet laboratory, teach classes of 20 to *50* students.
MCB 2010 Introduction to Microbiology (4 cr) with wet laboratory, teach classes of 25 to ***55*** students.

Lecturer (1992 - 1993) University of Arizona.
ANS 213, Animal Genetics (3 cr), taught class of 65 students.
ANS 399, Independent Study (3 cr), supervised lab projects of 3 undergraduate students.
ANS 696, Graduate Seminar (1 cr), served as coordinator and supervised **4** graduate students.

Publications

Co-authored 24 refereed scientific publications on molecular microbiology, genetic marker analysis, mitochondrial genetics and metabolism, other animal science, and many abstracts. Reviewed textbook: Microbiology, Tortora et al., 6th ed.

Professional and Honorary Membership

American Association for the Advancement of Science
American Society for Microbiology
International Organization for Mycoplasmology

Phi Kappa Phi
Gamma Sigma Delta
Sigma Xi
Alpha Zeta .Agriculture Honorary

Abbreviated Curriculum Vitae

Mary B. Brown, M.S., Ph.D.
Associate Professor

Department of Infectious Diseases
IFAS Box 110926
University of Florida

Education:

B.S.	1971	Biology/chemistry	University of South Carolina
M.S.	1974	Microbiology	University of Florida
Ph.D	1985	Biology	University of Alabama at Birmingham

Professional Experience:

1992- present — Associate Rofessor, Department of Pathobiology, University of Florida, Gainesville, FL

1985 - 1992 — Assistant Professor, Department of Infectious Diseases., University of Florida, Gainesville, FL

Professional Societies:

American Society for Microbiology (ASM)
International Organization for Mycoplasmology (IOM)

Honors:

Fellow, Moms Animal Foundation
1990-92 Membership Secretary, International Organization for Mycoplasmology
1990-96 Board of Directors, International Organization for Mycoplasmology
Member, 1992-96 working teams on bovine and on wildlife and zoological mycoplasmas, International Research Program on Comparative Mycoplasmology (IRPCM)
1992 Division G Councillor, ASM
1992-1996 Treasurer, International Organization for Mycoplasmology
1996-, Chair, Animal Mycoplasmas (Companion, Laboratory and Zoo/wildlife) Team, International Research Program on Comparative Mycoplasmology (IRPCM)

Publications (selected)

Davidson, M.K., Lindsey, J.R., Brown, M.B., Schoeb, T.R., and Cassell, G.H. 1981. Comparison of methods for detection of *Mycoplasma* pulmonis in experimentally and naturally infected rats. J. Clin. Microbiol. 114: 646-655.

Cassell, G.H., Lindsey. J.R., Davis, J.K., Davidson, M.K., Brown, M.B., and Mayo, J.G. 1981, Detection of natural *Mycoplasma pulmonis* infection in rats and mice by an enzyme-linked immunoassay (ELXSA). Lab. Ani. Sci. 131: 676-682.

Brown, M.B., Cassell, G.H.. Taylor-Robinson, D., and Shepard, M.C. 1983. Detection of antibodies to *Ureaplasma urealyticum* by an enzyme-linked immunosorbent assay. J. Clin. Microbiol. 117:288-295.

Brown, M.B., Cassell, G.H., McCormack, W.M., and Davis, J.K. 1987. Measurement of antibody to *Mycoplasma hominis* by enzyme-linked immunoassay and detection of class specific antibody responses in women with postpartum fever, Am. J. Obstet. Gynecol. 156: 710-708.

Brown. M.B., J.K. Shearer, and F. Elvingcr. 1990. Mycoplasmal mastitis in a dairy herd. J. Am. Vet. Med. Assoc. 196:1097-1101.

Brown, M.B. and M.A. Ambrose. *1990.* Caseinolytic activity of *Mycoplasma mycoides* subsp. *mycoides* GM12 for alpha, beta, and kappa casein. Zbl. Bakt. Hyg. S20:658-660.

Brown, M.B., and A.E. Scasserra. 1990, Antibiotic resistance in streptococcal species isolated from the bovine mammary gland. Am J. Vet Res. 51:2015-2018.

Butcher, G.D. and M.B.Brown. 1990. Reduction of clinical signs in budgerigars experimentally infected with *Mycoplasma gallisepticum*. J. Assoc. Avian Vet. 4:227-230.

Jacobsen, E.R., J.M. Gaskin, M.B. Brown, R.K.Harris, C.H. Gardiner, J.L. LaPointe, H.P. Adams, and C. Reggiardo. 1991. Chronic respiratory tract disease of free-ranging desert

tortoises, *Xerobates agassizii*. J. Wild. **Dis. 27:296-316.**
Brown, M.B., M. Stoll, J. Maxwell, and **D.F.** Senior. **1991. Survival of** feline mycoplasmas **in** urine. **J. Clin.** Microbiol. **29:1078-1080.**
Brown, M.B. and L. Reyes. **1991,** Immunoglobulin class- **and** subclass-specific responses to *Mycoplasma pulmonis* in **serum** and secretions **of** naturally-infected Sprague Dawley female **rats. Infect. Immun. 59:2181-2185.**
Brown, M.B. and G.D. Butcher. **1991.** *Mycoplasma gallisepticum* **as a model to assess efficacy of** inhalant **therapy** in budgerigars **(*Melopsittacus undulatus*). Avian Dis.** 35:834-839.
Brown, M.B., M.L. Stoll, **A.E.** Scasserra, and **G.D.**Butcher. **1991,** Comparison **of** egg **yolk and** seum samples for detection **of** antibodies **to** ***Mycoplasmu gallisepticum*. J.** Clin. **Microbiol. 29:2901-2903.**
Steiner, **D.A. .and M.B.** Brown. **1993.** Impact **of** cxperimcntal genital **mycoplasmosis on** pregnancy outcome in Sprague Dawley rats. Infect. Immun. **61:633-669.**
Schumacher, I.M., M.B. Brown. **E.R.** Jacobson, **B.R.** Collins, **and P.A. Klein.** 1993. Detection of **antibodies** to a pathogenic mycoplasma in desert **tortoises *(Gopherusagassizii)*** with upper **respiratory *tract* disease** (URTD), **J.** Clin. Microbiol, **31:1454-1460.**
Stciner, D.A., E.W. Uhl, and **M.B.** Brown. 1993. In **utero** transmission **of *Mycoplasma pulmonis*. Infect.** Immun. **61:2985-2990.**
Brown, M.B., I.M. Schumacher, **P.A.** Klein, **K.** Harris, T. Correll, and **E.R. ,Jacobson.** 1994. ***Mycoplasma agassizii*** **causes** upper respiratory tract **disease** in **the** desert tortoise. Infect. **Immun. 62:4580-4586.**
Brown, **D.E., B.C.** Crenshaw, G.S. McLaughlin, I.M. Schumacher, C.E. McKenna, **P.A. Klein, E.R.** Jacobson, **and** M.B. Brown. 1995. Taxonomic **analysis** of the tortoise **mycoplasmas *Mycoplasma agassizii*** and ***Mycoplasma testudinis*** **by** 16S rRNA **gene** sequence **comparison.** Int. J. **Syst.** Bacteriol. **45** : 348-350.
Brown, D.R, G.S. MacLaughlin, **and M.B. Brown.** 1995. Taxonomy of **the** feline mycoplasmas ***Mycoplasma felifaucium*, *Mycoplasma feliminutum*, *Mycoplasma felis*, *Mycoplasma garae*, *Mycoplasma leocaptivus*, *Mycoplasma fleophayngis*** **and** ***Mycoplasma simbae*** **by 16S** rRNA gene sequence comparison. Int. J. **Syst.** Bacteriol. 45:560-564.
Jacobson, E.R., **M.B. Brown, I.M.** Schumacher, B.R. Collins, **R.K. Harris,** and **P.A. Klein. 1995**. Mycoplasmosis **and the desert tonoise *(Gopherus agassizii)* in Las** Vegas Valley, **Nevada. Chelonian** Conservation **Biol.** 1:281-286.
Ewing, **M.L.,** S.K. Kleven, and **M.B. Brown. *1996.*** Comparison **of** Enzyme-linked immunosorbent assay and hemagglutination inhibition **for** detection **of** antibody to ***Mycoplasma gallisepticum*** **in commercial** broiler, **fair and** exhibition, and experimentally-infected **birds.In Press. Avian Dis. 40:13-22.**
Brown, M.B. **and** D.A. Steiner. 1996, Experimental genital mycoplasmosis: time **of** infection **influences pregnancy** outcome. **Infect, Immun.** 64:2315-2321.
Ewing, M.L., **L.H.** Lauerman, **S.K. Kleven, and** M.B. **Brown.** Evaluation **of** diagnostic procedures to detect **Mycoplasma synoviae** infection in commercial multiplier-breeder **farms and commercial hatcheries** in **Florida Accepted. Avian** Diseases.

Book Chapters (selected).

Brown, M.B., **J.M.** Bradbury, and J.K. Davis. 1996. ELISA in small **animal hosts,** rodents, birds. In: Molecular **and** Diagnostic Procedures in **Mycoplasrnology.** Vol. II. **J.G. Tully and S,** Razin, eds., Academic Press., pp. 93-104..
Senior, **D.P.,** and **M.B. Brown. 1996, The** role **of** Mycoplasma **species and Ureaplasma** species **in feline lower urinary tract disease. Vet. Clinics N. Amer.: Small** Anim. Pract. 26:305-308.

CURRICULUM **VITAE**

Mary Monica Christopher

BIRTH: March **25, 1956, Evergreen Park,** Illinois

BUSINESS ADDRESS: **Department of** Pathology, **Microbiology & Immunology**
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PROFESSIONAL EDUCATION:

1980	**D.V.M. Iowa State University**
1988	**Ph.D., University of Minnesota**

SPECIALTY BOARDS:

1990	**Diplomate, American College of Veterinary Pathologists** (Clinical Pathology)

PROFESSIONAL EXPERIENCE:

1980-1983	**Associate Veterinarian** **Equine/Small Animal Medical Center, Lakeville, MN**
1983-1988	**Veterinary Medical Associate, Clinical Pathology** **Dept. of Veterinary Pathobiology, College of Veterinary Medicine** **University of Minnesota, St. Paul, MN**
1988-1989	**Postdoctoral Research Associate** **Dept. of Medicine, College of Medicine** **University of Minnesota, Minneapolis, MN**
1989-1994	**Assistant Professor, Clinical Pathology** **Depts. of Physiological Sciences, College of Veterinary Medicine** **University of Florida, Gainesville, FL**
1994-present	**Associate Professor, Clinical Pathology** **Dept. of Pathology, Microbiology & Immunology** **School of Veterinary Medicine** **University of California, Davis, CA**

PROFESSIONAL MEMBERSHIPS:

American Veterinary Medical Association
American Society for Veterinary Clinical Pathology
International Society of Animal Clinical Biochemistry
American Association for Clinical Chemistry
The Oxygen Society/International Society for Free Radical Research
Association for Women Veterinarians

HONORS AND SCHOLARSHIPS:

1977-	Phi Zeta, Veterinary Honor Society
1996	Sigma Xi, Scientific Research Society
1996	Favorite Teacher Award, Class of *1998,* UCD
1995	Favorite Teacher Award, Class of *1997,* UCD
1993	Teacher of the Year, College of Vet Med, UF
1993	Teacher of the Year, Class of *1995,* UF
1993	Daniels Young Clinical Investigator Award
1989	National Phi Zeta Research Award
1988	Finalist, Oelshlegel Research Award in Red Cell Metabolism and Function
1987	Phi Kappa Phi, University of Minnesota
1986	Gamma Sigma, Honor Society of Agriculture
1985	C.L.Davis Foundation, Veterinary Pathology Award
1980	Graduation with Distinction, Iowa State University
1978	Frank Waish Memorial Scholarship
1977	Highest 2% of Veterinary Medical Class
1973	Admitted to Iowa State with Recognition and Award

REFEREED PIJBLICATIONS:

1. **1984** **Fernandez F, Davies AP,** Teachout D, Christopher M, and Perman V: Vitamin K-induced Heinz body formation in dogs, J Amer Anim Hosp Assoc 20:711-*720.*

2. 1985 Krake A, Arendt T, Raffe M, Christopher M, Stowe CM, and Perman V: Cetacaine™-induced methemoglobinemia in domestic cats, J Amer Anim Hosp Assoc 21:527-534.

3. **1985** Weiss DJ and Christopher MM: Idiopathic aplastic anemia in a dog. Vet Clin Pathol 14:23-25.

4. **1986** Christopher MM, Metz AL, Klausner J, Polzin D, and Hayden DW: Acute myelomonocytic leukemia with neurological manifestations in the dog. Vet Pathol 23:140-147.

5, 1986 Christopher MM and Wallace LJ: Synovial fluid eosinophilia: A, case report in a dog and review of the literature. Vet Clin Pathol 15:25-31.

6. 1986 Clem MF, O'Brien TD, Christopher MM, Feeney DA, and Schultheiss P: Pulmonary chondrosarcoma in a horse. Comp Cont Educ 8:964-967.

7, 1988 Christopher MM, Perman VP, and Hardy RM: A reassessment of cytologic values in canine cerebrospinal fluid using cytocentrifugation. J Amer Vet Mcd Assoc 192:1726-1729.

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9. 1989 Christopher MM, Perman VP, and Eaton JW: Contribution of propylene glycol-induced Heinz body formation to anemia in cats. J Amer Vel Mcd Assoc 194:1045-1056.

10. 1989 Christophcr MM: Relation of endogenous Hcinz bodies to disease and anemia in cats: 120 cases (1978-1987). J Amer Vet Med Assoc 194:1089-1095.

11. 1989 Christopher MM, Perman VP, White JG, and Eaton JW: Propylene glycol-induced Heinz bodies and D-lactic acidosis in cats. Prog Clin Biol Res 319:69-92.

12. 1990 Christopher MM, Eckfeldt JH, and Eaton JW: Propylene glycol ingestion causes D-lactic acidosis. Lab Investigation 62:114-118.

13. 1990 Ney PA, Christopher MM, and Hebbel RP: Synergistic effects of oxidation and deformation on erythrocyte monovalent cation leak. Blood 75:1192-1198,

14. 1990 Weiss DJ, McClay CB, Christopher MM, Murphy M, and Perman V: Effects of propylene glycol-containing diets on acetaminophen-induced methemoglobinemia in cats. J Amer Vet Med Assoc 196:1816-1819.

15. 1990 Christopher MM, White JG, and Eaton JW: Erythrocyte pathology and mechanisms of Heinz body-mediated hemolysis in cats. Vet Pathol 27:299-310.

16. 1992 Nleman RA and Christopher MM: Identification of intracytoplasmic inclusion bodies in mononuclear cells from the cerebrospinal fluid of a dog with canine distemper. Vet Pathol 29:84-85.

17. 1992 Christophcr MM, Periera J, and Brigmon R: Adaptation of an automated assay for determination of β-hydroxybutyrate in dogs using a random access analyzer. Vet Clin Pathol 21:3-8.

18. 1992 **Thompson** JP, **Christophcr** MM, Ellison GW, and **Homer** RL: **Paraneoplastic** leukocytosis associated with **rectal** adenomntous **polyp in a dog.** J Amer Vet **Med Assoc** 201:737-738.

19. 1992 **Christopher MM: Bonc** marrow **contamination** of **canine cerebrospinal** fluid, Vet **Clin** Pathol 21:95-98.

20. 1992 **Mohammed SS,** Christopher **MM, Mehta P, Kedar A, Gross S. and** Derendorf H: Increased erythrocyte **and** protein **binding of** codcinc **in** patients with sickle **cell** disease. J Pharmaceut Sci 82:1112-1117.

21. 1994 **Christophcr** MM, **Brigmon R, and** Jacobson E: Seasonal alterations **in** plasma **b-**hydroxybutyrate **and related** biochemical **parameters in the** desert tortoise ***(Gopherus agassizii).*** Comp Biochem **Physiol 108A:303-310.**

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24. 1995 Christopher **MM, Broussard,** JD, and Peterson, ME: Increased serum D-lactate **associated with** diabetic ketoacidosis. Metabolism; Clinical and Experimental 44:287-290.

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26. 1995 **Christopher MM** and Drew **DL: Gender** portrayal in **veterinary** medical **advertising:** Implications for **occupational** segregation. **J Women** Minorities Sci Engineering 2:49-63.

27. 1996 Christopher MM, **Belknap EB,** Mcyer DJ, **Lackey MN, and Vap LM: Comparison** of methodology for **sodium** and potassium determination **in** llama **urine. Am** J Vet Res 57:25-30.

28. ***1996*** **Fallin** CW **and** Christopher MM: ***In vitro*** cffects **of** ketones **and hyperglycemia on** feline **erythrocyte oxidation and** D- **and L-lactate** production.. Am J Vet **Res 57:463-467.**

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32. Burkhard MJ, Lappin M, Christophcr MM, and Meyer DJ: Efficacy of a flavanoid on acetaminophen toxicity in cats, Res Vet Sci (Submitted)

33. Lcskosky L and Christopher MM: Effect of faculty gender on veterinary student teaching evaluations. J Vet Med Educ (Submitted)

BOOK CHAPTERS:

1. 1987 Christopher MM: Pleural effusions. Vet Clin North Amer 17:255-270.

2. 1992 Christopher MM and Harvey JW: Specialized hematology tests. Seminars Vet Med Surg 7:301-310.

3. 1995 Christopher MM: Hematologic complications in diabetes mellitus. Vet Clin North Amer 25:625-637.

4. 1995 Christopher MM, Broussard JD, Harvey JW, Warner CB, and Peterson ME:L Feline Heinz bodies as *in vivo* markers of oxidative injury: association with ketoacidosis in diabetic cats. IN: The Oxygen Paradox. Davies KJA and Ursini F, eds. CLEUP University Press, Padova, Italy, pp. 137-147.

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6. Christopher MM: Heinz body anemia. JN: Tilley LP and Smith FWK, cds. Williams & Wilkins, Malvern PA. The 5 Minute Veterinary Consult (text and CD ROM, In press.

GOVERNMENT REPORTS:

1. 1992 Christopher MM, Nagy KA, Peterson CC, Henen BT, Wilson MA, Longmate J, and Jacobson E: Laboratory health profiles of free-ranging desert tortoises in California: Use of clinicopathologic data in the evaluation of physiologic and pathologic alterations (May 1989-March 1990). Report to the USDI/Bureau of Land Management, Contract No. B950-C1-0600, 125 pages, 8 tables, 49 figures.

2. 1993 Christopher MM, Wallis I, Nagy KA, Henen BT, Peterson CC, Meienbergcr C, Girard I, and Klaassen JK: Laboratory health profiles of free-ranging desert tortoises in California: Interpretation of physiologic and pathologic alterations (October 1990-October 1991). Report to the the USDI/Bureau of Land Management, Contract No, B950-C 1-0600, 136 pages, 12 tables, 35 figures.

3. 1993 Christopher MM, Wallis 1, Nagy KA, Henen BT, Peterson CC, Wilson MA, Meienberger C, and Girard I: Laboratory health profiles of free-ranging desert tortoises in California: Interpretation of physiologic and pathologic alterations (March 1992-October 1992). Report to the the USDI/Bureau of Land Management, Contract No. B950-C1-0600, 133 pages, 13 tables, 18 figures.

4. 1994 Homer BL, Berry KH, Christopher MM, Brown MB and Jacobson ER: Necropsies of desert tortoises from the Mojavc and Colorado deserts of California and the Sonoran Desert of Arizona. Report to the the USDI/Bureau of Land Management, Contract No. B950-C1-0062, 85 pates, 11 tables.

5. 1995 Christopher MM, Berry K, Nagy KA, Henen BT, Peterson CC, Wallis I, Wilson MA, Girard I: Laboratory health profiles of free-ranging desert tortoises in California: Interpretation of physiologic and pathologic alterations (March 1993-October 1993). Report to the USDI/U. S. Fish and Wildlife Service, National Biological Service, Contract No. 14-48-0006-95-003, 91 pages, 10 tables, 18 figures. Accepted for publication.

ABSTRACTS:

1. 1984 Christopher MM, Perman VP, and Hardy R: A reassessment of cytologic values of canine cerebrospinal fluid using cytocentrifugation. Proceedings: Am Coll Vet Pathol, 35th Annual Meeting, p. 77.

2. 1987 Christopher M M , Eaton JW, and Perman VP; Propylene glycol-induced Heinz bodies in cats. Am Federation Clin Res 35;422A.

3. 1988 Christopher MM: Endogenous Heinz bodies in cats: Relation to disease and anemia. Vet Clin Pathol 18:11.

4, 1988 Christopher MM, Pennan VP, and Eaton JW: Propylene glycol-induced Heinz bodies and D-lactic acidosis in cats. Proceedings: Am Coll Vet Pathol, 39th Annual Meeting, p. 20,

5, 1990 Weiss DJ, McClay CB, Christopher MM, Murphy M, and Pennan V: Role of additive oxidant stress in hematologic alterations induced by propylene glycol-containing cat foods. Vet Clin Pathol 19:7.

6. 1991 Christopher MM, Pereira J, Brigmon R, and Schaer M: Automated determination of β-hydroxybutyrate for the assessment of ketoacidosis in animals, Proceedings: 9th Annual Vet Medical Forum, Am Coll Vet Internal Med, p. 90.

7. 1992 Christopher MM, Nagy K, Longinate J, Jucobsen E, Peterson CC, Henen BT, and Wilson MA: Clinical biochemical profiles of free-ranging desert tortoises: Effects of life cycle, habitat, and disease. Proceedings: Vth Congress, Int'l Soc Animal Clin Biochem, p. 401.

8. 1993 Christopher MM, Nagy K. Wallis I, Klaassen J, and Berry KH: Laboratory health profiles of free-ranging desert tortoises in California: Evaluation of physiologic and pathologic alterations, Proceedings: 18th Annual Desert Tortoise Symposium, p. 8.

9. 1993 Christopher MM, Nagy KA, Wallis I, Klaassen JK, and Berry K: Laboratory health profiles of desert tortoises in the Mojave Desert: A model for health status evaluation of chelonian populations. Proceedings: Int'l Conf Conservation, Restoration and Management of Tortoises and Turtles, p. 25.

10. 1993 Christopher MM, Broussard J, and Peterson ME: Evidence for *in vivo* oxidative damage associated with ketoacidosis: Heinz body formation in diabetic cats. Free Rad Bioi Med 15:480.

11. 1993 Christopher MM, Broussard J, and Peterson ME: Heinz bodies are associated with kctoacidosis in cats with diabetes mellitus. Vet Pathol 30:429

12. 1993 Christopher MM, Nagy K, Wallis 1, Klaassen J, and Berry K: Clinicopathologic studies of desert tortoises over a two year pcriod: lnsights into biochemical physiology and response to disease. Vet Pathol 30:432.

13. 1994 Meeks JC, Christopher MM, Chrisman CL Hopkins AL, and Homer BH: The maturation of canine cerebrospinal fluid. J Vet Int Med 8:177.

14. 1994 Christopher MM, Warner CB, and Peterson ME: Increased serum D-lactate in cats with ketoacidotic diabetes. Proceedings; VIth Congress, Int'l Soc Animal Clin Biochem, Lumsden J (ed), p. 81.

15. 1994 Gatof M and Christopher M: Economic feasibility of clinical chemistry analyzers in small animal practice. Proceedings, Vlth Congress, Int'l Soc Anim Clin Biochem, Lumsden J (ed), p. 139.

16. 1995 Christopher MM and Lee SE: Red cell morphologic alterations in cats with hepatic disease. Vet Digest, pp. 23-25.

17. 1995 Christopher MM, Berry KH, Nagy KA, Henen, BT, Peterson CC, Wallis I, Wilson B, and Girard I: Progression of abnormal laboratory data in tortoises in the Mojave Desert from 1990-1993. Proceedings, 20th Annual Desert Tortoise Council Symposium, p. 16.

18. 1995 Lucroy MD, Kraegel SA, Christopher MM and Madewell BR: Examination of anemia associated with canine lymphoma. Proceedings, Vet Cancer Soc Annual Meeting,

19. 1996 Werner LL, Christopher MM and Snipes J: Spurious leukocytosis and abnormal histograms associated with Heinz bodies in cats. Vet Pathol

PROCEEDINGS AND OTHER PUBLICATIONS:

1. 1985 Christopher MM, Weiss DJ, and Perman V: Heinz body formation and anemia in cats, Minnesota Vet 25:26-31.

2. 1991 Christopher MM: Invited comments: Effects of hemolysis on serum chemistries. Adv Small Anim Med Surg 3:5-6.

3. 1993 Christopher MM: Social portraits of a profession. Proceedings: Celebrate Diversity: Enhancing the Learning Environment in Veterinary Medical Education, University of Wisconsin, Madison, pp. 24-30.

4. 1996 Christopher MM: Hyperlipidemia and other clinicopathologic abnormalities. Proceedings: Int'l Symposium on Canine Hypothyroidism, University of California, Davis, pp. 40-42.

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BIOSKETCH FOR BRUCE L. HOMER

EDUCATION: B.S., Veterinary Medicine, University of Illinois, Urbana, IL, 1975.
D.V.M., University of Illinois, Urbana, IL, 1977.
Ph.D., Veterinary Pathology, College of Veterinary Medicine, Texas A & M university, College Station, TX, 1986.

PROFESSIONAL EXPERIENCE:

Small Animal Practice, Miami, Florida. 1977-1979.
Instructor, Biscayne Paramedical Institute, Miami, Florida. 1979-1980.
Laboratory Animal Pathology Resident, Papanicolaou Cancer Research Institute, Miami, Florida. 1978-1980.
Veterinary Clinical Associate and Eli Lilly Fellow, Department of Veterinary Pathology, Texas A & M University, College Station, Texas. 1980-1985.
Assistant Professor, Department of Pathology, Division of Comparative Pathology, University of Miami, School of Medicine, Miami, Florida. 1985-1987.
Visiting Instructor, Department of Comparative and Experimental Pathology, College of Veterinary Medicine, University of Florida, Gainesville, Florida. 1987-1988.
Assistant Professor, Department of Comparative and Experimental Pathology, College of Veterinary Medicine, University of Florida, Gainesville, Florida. 1988-1993.
Associate Professor with tenure, Department of Comparative and Experimental Pathology, College of Veterinary Medicine, University of Florida, Gainesville, Florida. 1993-present.

SPECIALTY BOARD CERTIFICATION:

The American College of Veterinary Pathologists

PROFESSIONAL LICENSURE:

Florida, Illinois, Missouri

REFEREED PUBLICATIONS RELATED TO WORK WITH TURTLES:

1. Homer, B.L., Jacobson, E.R., Schumacher, J., Scherba, G.: Chlamydiosis in mariculture - reared green sea turtles (Chelonia mydas). Vet Pathol 31:1-7, 1994.

2. Westhouse, R.A., Jacobson, E.R., Harris, R.K., Winter, K.R., Homer. B.L.: Respiratory Iridovirus infection in a gopher tortoise (Gopherus polyphemus). In Press. J Wildl Dis.

3. Garner, M.M., Herrington, R., Howerth, E.W., Homer. B.L., Nettles, V.F., Isaza, R., Shotts, E.B., Jacobson, E.R.: Shell disease in river cooters (Pseudomys concinna) and yellow-bellied turtles (Trachemys scripta) in a Georgia lake. Accepted J Wildl Dis.

4. Garner, M.M., Homer., B.L., Jacobson, E.R., Raskin, R., Berry, K.H., Hall, B., Weis, W.A.: Evaluation of bone marrow from desert tortoises ***(Gopherus agassizii)***. Accepted. Am J Vet Res.

NON-REFEREED PUBLICATIONS RELATING TO DESERT TORTOISE WORK:

1. Homer, B.L., Berry, K.H., Christopher, M.M., Brown, M.B., Jacobson, E.R.: Necropsies of desert tortoises from the Mojave and Colorado deserts of California and the Sonoran Desert of Arizona. Final report to United States Department of the Interior, Bureau of Land Management, Riverside California. Contract No. B950-C1-0062. 85 pp. 1994.

2. Homer, B.L., Berry, K.H., Jacobson, E.R.: Necropsies of eighteen desert tortoises from the Mojave and Colorado deserts of California: 1994-1995. Final report to United States Department of the Interior, National Biological Service, Riverside California. Work Order Number 131; Unit Cooperative Agreement No. 14-16-009-1544 120 pp. 1996.

ABSTRACTS RELATED TO DESERT TORTOISE WORK:

1. Garner, M., Homer, E., Raskin, R., Jacobson, E.: Evaluation of bone marrow from desert tortoises (Gopherus agassizii). Desert Tortoise Council Eighteenth Annual Symposium, Palm Springs, CA, 1993; p.10.

2. Homer, B.L., Jacobson, E.R., Christopher, M.M., Brown, M.B.: Physiological and morphological effects of burn-injury in a desert tortoise, Gopherus agassizii. Desert Tortoise Council Nineteenth Annual Symposium, Tucson, AZ, 1994; pp. 12-13.

3. Homer. B.L., Jacobson, E.R., Christopher, M.N., Brown, M.B.: An update on shell disease (cutaneous dyskeratosis) in desert tortoises, Gopherus agassizii. Desert Tortoise Council Nineteenth Annual Symposium, Tucson, AZ, 1994; pp. 13-14.

4. Homer, B.L., Berry, K.H., Christopher, M.M., Brown, M.B., Greiner, E.C., Jacobson, E.R.: Necropsies of fourteen desert tortoises from the Mojave and Colorado Deserts of California and Sonoran Desert of Arizona. Desert Tortoise Council Twentieth Annual Symposium, Las Vegas, NV, 1995; pp. 23

5. Jacobson, E.R., Homer, B.L.: Health problems of wild populations of desert tortoises, Gopherus agassizii, in the southwestern United States. Proceedings of the American Association of Zoo Veterinarians annual meeting. East Lancing, MI, 1995, pp.68-69

6. Homer, B.L., Berry, K.H., Ross, F., Reggiardo, C., Jacobson, E.R.: Potentially toxic metals and minerals in liver and kidney of desert tortoises in California. Desert Tortoise Council Twenty-first Annual Symposium, Las Vegas, NV, 1996; pp. 19-20

REFEREED PUBLICATIONS RELATED TO WORK WITH METAL TOXICITY

1. Homer, B.L., Pierce, KR.: Morphometric cytochemistry of diminution of catalase-containing peroxisome in copper-loaded liver. Histochemical Journal 21:63-71, 1989.

2. Homer, B.L., Pierce, K.R., Womack, J.E., Sowa, B., Bridges, C.B.: Inhibition of copper-associated erythrocyte ghost membrane lipid peroxidation by hepatic cytosolic low moiecular weight proteins. Toxicologic Pathology 1991.

3. Heaton-Jones, T., Homer, B.L., Heaton-Jones, D., Sundlof, S.: Tissue distribution of mercury in alligators (*Alligator mississippiensis*) from the Everglades and elsewhere in Florida. In Press. Journal of Zoo and Wildlife Medicine

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CURRICULUM **VITAE**

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EDUCATION AND DEGREES

B.S. 1967. Biology, Brooklyn College of the City University of New York.

M.S. 1969. Zoology (Physiological Ecology), New Mexico State University, Las Cruces, N.M.

D.V.M. 1975. University of Missouri, Columbia, MO.

Ph.D. 1975. Zoology (Endocrinology), University of Missouri, Columbia, MO.

Resident Training Program. 1979. Laboratory Animal and Wildlife Medicine, University of Florida, Gainesville, Fl.

PROFESSIONAL EXPERIENCES

Assistant Professor, Department of Veterinary Science, University of Maryland, College Park, Md. 1975-1977.
Assistant Professor of Wildlife and Zoological Medicine, College of Veterinary Medicine, University of Florida, Gainesville, Fl. 1979-1984.

Associate Professor of Wildlife and Zoological Medicine, College of Veterinary Medicine, University of Florida, Gainesville, Fl. 1984-1989.

Professor of Wildlife and Zoological Medicine, College of Veterinary Medicine, University of Florida, Gainesville, Fl. 1990 - present.

RESEARCH INTERESTS

Infectious and noninfectious diseases of reptiles.
Antibiotic pharmacokinetics in reptiles.
Health assessment of reptiles.
Reptile immunology.

TRAINING EXPERIENCE

Master's Degree Students: 1 completed; 2 current
Ph.D. Degree Students: 1 completed
Post-Doctoral Fellows: 1 completed
Residents: 18 completed/ 3 current

COURSES AND SEMINARS TAUGHT (Last 3 years)

VEM 5361 - Wildlife and Zoological Medicine
VEM 5821 - Wildlife and Zoological Medicine Clerkship

SELECTED PUBLICATIONS (From a list of 2 books; 141 refereed publications; 35 non-refereed publications; 27 book chapters)

1. Jacobson, E.R., Brown, M.P., Chung, M., Vliet, K., Swift, R. 1988. Serum Concentration and Disposition Kinerics of Gentamicin and Amikacin in Juvenile American Alligators. J. Zoo. Animal Med. 19:188-194.
2. Jncobson. E.R., Mansell, J.L., Sundberg, J. P., Hajjar, L., Rcichmann. M.E., Ehrhart, L.M., Walsh, M., Murru, F. 1989. Cutaneous Fibropapillomas of Gracn Turtles. J. Comp. Path. 101:39-52.
3. Jacobson, E.R., and S.R. Telford. 1989. Chlamydia and Poxvirus Infection of Monocytes in a Flap-Necked Chameleon. J. Wildlife Dis. 26:572-577.
4. Jacohnnn. E.R., Cnskin, J.M., Brown, M., et al. 1991. Chronic Upper Respiratory Tract Disease of Free-Ranging Desert Tortoises, Xerobates agassizii. J. Wildlf. Din. 27:296-316.
5 Jacobson, E.R. .Schumnchcr, J. and Green, M.E. 1992. Techniques for Sampling nnd Handling Blood for Hematologic and Plasma Biochemical Determinations in the Desert Tortoise, Xerobates agassizii. Copeia. (1): 237-241.
6. Jacobson, E.R.. Schumacher, J. and Green, M.E. 1392. Techniques for Sampling and Handling Blood for Hematologic nnd Plasma Biochemical Determinations in the Desert Tortoise, Xerobates agassizii. Copeia. (1):237-241.
7. Jacobson, E.R., Cnskin, J.M., Wells, S., Bowler, K. and Schumacher, J. 1992. Epizootic of Ophidian Paramyxovirus in a Zoological Collection: Pathological. Microbiological, and Serological Findings. J. Zoo Wildl. Med. 23:318- 327.
8. Jacobson. E.R., Weinstein, M., Berry, K., et al.1993. Problems with Using Weight Vs. Length Relationships to Assess Tortoise Henlth. Vet. Rec. 132:222-223.
9. Jacobson, E.R. 1993. Implications of Infectious Disease for Captive Propagation and Reintroduction Programs of Threatened/Endangered Reptiles. J. Zoo Wildl. Med. 24:245-255.
10 Jncnbson. E.R. 1994. Causes of Mortality and Diseases of Tortoises: A review, J. Zoo Wildl. Med. 25:2- 17.
11. Jacobson, E.R., Wronski. T. Schumacher, J.. Reggiardo, C. and Berry. K.H 1994. Cutaneous Dyskeratosis in Free Ranging Desert Tortoise, Gopherus agassizii, in the Colorado Desert of Southern California. J. Zoo Wildl. Med. 25:68-81.
12. Jacobson, E.R., Schumacher, J., Telford, S.R., Grcincr, E.C., Buergelt, C.D.and Gardiner, C.H.1994. Intranuclear Coccidiosis in a Radiated Tortoise (Geochelone radiata). J. Zoo Wildl. Med. 25:95-102.
13. Jacobson, E.R., Brown, M.B., Schumacher, I.M., Collins. B.K., Harris, R.K.and. Klein., P.A. 1995. Subclinical Mycoplasmosis nnd the Desert Tortoise, Gopherus agassizii, in Las Vegas Vnlley, Nevada. Chelon. Conscrv. Biol. 1:279-284.
14. Jacobson, E.R., Kopit, W., O'Brien, B. 1996. Co-infection of n Bearded Dragon, Pogona vitticeps, with Adeno- and Dependo-like Viruses. Vet Path. 33:343-346.
15. Jacobson, E.R. 1996. Metabolic Scaling of Anlihiorics in Reptiles: Basis and Limitations. Zoo Biol. .15: 329-339.

Curriculum Vitae

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EDUCATIONAL BACKGROUND

1966	Long Island University, New York, NY; B.S. cum laude, full tuition scholarship
1968	College of William and Mary, VA; M.A. Biology, recipient of NSF traineeship award
1974	University of Hong Kong, Hong Kong; Ph.D. Zoology

PROFESSIONAL BACKGROUND

09/68 - 12/73	Senior Demonstrator; Department of Zoology, University of Hong Kong, Hong Kong
01/74 - 09/77	Research Associate; Department of Biology, Boston University, Boston, MA
09/77 - 05/78	Lecturer; Department of Biology, Boston University, Boston, MA
08/78 - 05/82	Assistant Professor; Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA
08/82 - 08/87	Research Assistant Professor; Department of Medicine, Tulane University School of Medicine, New Orleans, LA
09/87 -	Endocrinologist, Head of Endocrinology; Research Department, San Diego Zoo, San Diego, CA

Associate editor, Journal of Experimental Zoology

RESEARCH INTERESTS Hormonal control of seasonal reproduction, sex determination and differentiation, hormonal control of pregnancy, physiology and endocrinology of reptiles, evolution of pancreatic hormones.

Publications Sevent-nine peer-reviewed papers and 21 reviews and book chapters.

PROFESSIONAL SOCIETY MEMBERSHIPS American Society of Zoologists, The Endocrine Society, Society for the Study of Reproduction, AAAS, AAZPA.

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EDUCATION

Ph.D. Zoology, 1995, Michigan State University, East Lansing, MI

M.S. Biology, 1982, University of Minnesota—Duluth, Duluth, MN

B.S. Zoology, 1979, Michigan State University, East Lansing, MI

EXPERIENCE

ANIMAL ECOLOGIST
Science Applications International Corporation, Environmental Sciences Department, Las Vegas, NV, October 1995 to present.

ANIMAL ECOLOGIST
EG&G Energy Measurements, Environmental Sciences Division, Las Vegas, NV, April-September 1995.

RESEARCH COORDINATOR
Department of Zoology, Michigan State University, East Lansing, MI, and Terrestrial Vertebrates Group, Crystal Falls, MI, April 1983-September 1994..

CONSULTANT
American Scientific International, USEPA, Duluth, MN, May-September 1988

RESEARCH ASSISTANT
Wildlife Research Group, Minnesota Department of Natural Resources, Grand Rapids, MN, June 1981-May 1982, November 1982-March 1983.

WILDLIFE TECHNICIAN
Section of Wildlife, Minnesota Department of Natural Resources, St. Paul, MN, June-October 1982.

AFFILIATIONS

American Ornithologists' Union
Association of Field Ornithologists
Sigma Xi, Associate Member
Wilson Ornithological Society
American Society of Mammalogists
Cooper Ornithological Society
The Wildlife Society, Certified Wildlife Biologist

Biosketch

John H. Lumsden, DVM, Dip Clin Path, Msc, Diplomate, ACVP

Graduate of the Ontario Veterinary College, University of Toronto in 1960. Private practitioner, partner in multiple person mixed practice until 1969. Returned to Ontario Veterinary College, now part of University of Guelph, to complete a one year diploma course in clinical pathology followed by an M Sc. Employed as assistant professor in the Department of Pathology in 1971, associate professor in 1975 and full professor in 1980. Became a Diplomate, American College of Veterinary Pathology, Clinical Pathology, in 1974.

Active teaching role at undergraduate and graduate levels. Broad research interests with primary support in applied aspects. Supervisor of 22 graduates students at the Diploma, MSc and DVSc level. Author, or co-author, of 85 refereed publications.

Initial Department of Pathology responsibilities were divided between teaching, research and laboratory management. Review of laboratory instrumentation, quality control and method development including diagnostic cytology were primary interests. Obtained a travel scholarship in 1972 to visit several research and teaching institutions in Europe. Primary objectives were to discuss methodology and quality control with Drs. Dacie and Lewis at the Royal Hammersmit Hospital in London and diagnostic cytology with Profs Soderstrom at Lund, anti Zajicek at the Karolinska Insitute in Stockholm, Sweden, respectively. Research leave for 10 months in 1978-79 provided opportunity to visit institutions in the UK and Europe with active neutrophil or endocrinology research programs, or teaching programs in diagnostic cytology. Located at Uppsala, Sweden for 6 months. In 1986 invited as a Commonwealth Visiting Professor, for 6 months at James Cook University, Townesville, Australia (1986). Consulted 5 months with the Ministry of Agriculture and Fisheries, New Zealand, primarily providing input into continuing education programs at the regional level in clinical pathology.

Served on committees for the American College of veterinary Pathology, American Society of Veterinary Clinical Pathology. Member of Editorial Board for Veterinary Clinical Pathology and Canadian Journal of Veterinary Research, Associate Editor 2 years for Veterinary Pathology. Invited to consult, present lectures and workshops in Mexico, Colombia, Venezuela, China, Australia and New Zealand. Chair of Local Organising Committee for the VIth Congress of the International Society of Animal Clinical Biochemistry, Guelph, 1994, President, 1994-966, and currently Vice-president, ISACB.

KEN NAGY did his Ph.D. thesis research on a desert lizard, the chuckwalla, at a study site in the Mojave Desert not far fiom here. He graduated in 1971, and has been on the Biology faculty at UCLA ever since. He teaches courses on Physiological Ecology of Desert Animals and on Herpetology, and has done research on desert animals in Africa, Asia and Australia, as well as here in the Southwestern USA.

Biographical Summary - Olav **T.** Oftedal

Olav **T.** Oftedal has been research nutritionist at the National Zoological Park, Smithsonian Institution, Washington DC since 1980. He received his **A.B.** in biology from Harvard University (1971) and his Ph.D. in nutritional sciences from Cornell University (1981). Major areas of research include lactation performance in mammals and nutrition of reptiles. The latter includes studies of the desert tortoise in Nevada (1991-present), the green iguana in Panama and Costa Rica (1987-1991), and the land iguana in the Galapagos Islands (1989-present). Dr. Oftedal served on the National Research Council's Committee on Animal Nutrition, which oversees the development of nutrient requirement standards for animals, as well as on its Subcommittee on Laboratory Animal Nutrition, which prepared a comprehensive review of the nutrient requirements of laboratory rodents published in 1995. He was a founder of the Comparative Nutrition Society and serves as its first president. He has supervised the research of **12 M.S.** and Ph.D. students and 7 post-doctoral fellows.

Dr. Oftedal has authored or coauthored about one hundred scientific papers, including 70 peer-reviewed papers in scientific journals and books. Substantive reviews include papers on the systematic status of a microteiid lizard genus (1974), equity effects of nutrition and health care in less-developed countries (1977), mammalian milk composition and yield (1984), lactation in pinnipeds (1987), nutrition of foraging primates (1991), nutrition of carnivorous reptiles (1994), gross and fatty acid composition of mammalian milks (1995) and the nutrition of mammals in zoos (1996).

David C. Rostal, Ph.D.

Dr. David Rostal received his Ph.D. in Zoology from Texas A&M University in May 1991. His research focused on the reproductive biology of the Kemp's ridley sea turtle, *Lepidochelys kempi*. From Texas A&M, he went on as a post-doctoral research fellow at the Center for Reproduction of Endangered Species, San Diego Zoo where he studied the reproductive biology of the desert tortoise, *Gophenrs agassizii* at the Desert Tortoise Conservation Center in Las Vegas Nevada. He is presently an Assistant Professor of Biology at Georgia Southern University, Statesboro, Georgia. He specializes in the reproductive physiology and behavior of reptiles and amphibians as it applies to the conservation and management of endangered species. This work has included the leatherback (*Dermochelys conacea),* black *(Chelonia agassizi*), and olive (*Lepidochelys olivacea*) sea turtles; the Galapagos (*Geochelone elephantopus*) and gopher (*Gopherus polyphemus*) tortoises; the eastern indigo snake (*Drymarchon corais cooperi*), the flatwoods salamander *(Ambystoma cingulatum*), the striped newt (*Notophthalmus peristriatum*) and the gopher frog (*Rana capito capito*).

Some of his publications are listed below:

Rostal, D.C., Robeck, T.R., Owens, D.W., and Kraemer, D.C. (1990) Ultrasound imaging of ovaries and eggs in Kemp's ridley sea turtles (*Lepidochelys kempi*). Journal **of *Zoo*** and 'Wildlife Medicine 21: 27-35.

Robeck, T.R., Rostal, D.C., Burchfield, P.M., Owens, D.W., and Kraemer, D.C. (1990) Ultrasound imaging of reproductive structures and eggs in Galapagos tortoises, *Geochelone elephantopus* **spp.** Zoo Biology 9: 349-359.

Rostal, D.C., Lance, V.A., Grumbles, J.S., and Alberts, A.C. (1994) Seasonal reproductive cycle of the desert tortoise (*Gopherus agassizii*) in the eastern Mojave desert. Herpetological Monographs 8:72-82.

Rostal, D.C., Grumbles, J.S., Lance, V.A., and Spotila, J.R. (1994) Non-lethal sexing techniques for hatchling and immature desert tortoises *(Gophenrs agassizii*). Herpetological Monographs 8:83-87.

Spoala, J.R., Zimmerman, L.C., Binckley, C.A., Grumbles, J.S., Rostal, D.C., List, Jr., A., Beyer, E.C., Phillips, K.M., and Kemp, S.J. (1994) Effects of incubation on sex determination, hatching success, and growth of hatchling desert tortoises, *Gopherus agassizii.* Herpetological Monographs 8:103-116.

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Niblick, H.A., Rostal, D., and Classen, T. (1994) Role of male-male interactions and female choice in the mating system of the desert tortoise. Herpetological Monographs 8:124-132.

Rostal, D.C., Paladino, F.V., Patterson, R.M., and Spotila, J.R. (1996) Reproductive physiology of the leatherback sea turtle (*Dermochelys coriacea*) at Las Baulas de Guanacaste National park, Costa Rica. Chelonian Conservation and Biology: in press (October 1996).

Plotkin, P.T., Rostal, D.C., Byles, R.A., and Owens, D.W. (1996) Reproductive and developmental synchrony in female *Lepidochelys olivacea.* Journal **of** Herpetology: in press (March 1997).

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  Became interested in reptiles (in particular snakes and tortoises) through **my husband, Dr. Juergen Schumacher, who was Dr. Jacobson's grad. student at the time.**

- **1991** to **date...still at the University of Florida, Research Scientist in The Biotechnology Program's Immunological Analysis Laboratory.**

  Ongoing research that I am involved in:

- **Upper Respiratory** Tract **Disease** (URTD) **in** Tortoises

- **Mycoplasmosis Outbreak in Alligators**

- **Green Turtle Fibropapillomatosis (GTFP) and Herpesvirus**

- **Vitellogenin Levels of Green Turtles as an Indicator of Environmental Contaminant Effects**

- **Effect of Methyl-Mercury on the** Immune **System** in **the** Common **Egret**

October 22, 1996

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Biosketch:

Karen Spangenberg graduated with a B. A. in Biology in 1970 from Lawrence University, Appleton, Wisconsin. After graduating, she worked in the Microbiology Division of Stanford Research Institute, Menlo Park, California. From 1982 to 1989, she was on the Board of Directors of the Independent Documentary Group, a non-profit corporation which produced documentary films on environmental and social issues. She was Associate Producer on "Dark Circle", a feature length documentary about nuclear power and nuclear weapons which received an Honorable Mention from the Academy of Motion Pictures and the Blue Ribbon Award from the American Film Festival. From 1980 to 1993, Ms. Spangenberg worked as a Sound Editor in the motion picture industry. Her credits include "The Right Stuff', "Amadeus", "The Incredible Lightness of Being", "Mosquito Coast", "Indian Jones and the Last Crusade", and "The Prince of Tides". She was Supervising Dialog Editor on "The Dead Poet's Society", "Fried Green Tomatoes", "Unforgiven", and "A Perfect World". She volunteered with the Bureau of Land Management's Desert Tortoise Research Group in 1993, and studied the foraging patterns of adult female and immature desert tortoises in the central Mojave. In 1994, she entered California State University, Dorninguez Hills, in Carson, California, where she studied with Dr. David Morafka doing field research studying time-activity budgets ofjuvenile desert tortoises. She received her masters degree in Spring 1996. She is currently Project Leader at Ft. Irwin Study Site (FISS), a desert tortoise hatchery/nursery located at the United States Army's National Training Center near Barstow, California and on the Board of Trustees of the Desert Tortoise Preserve Committee, Inc. which manages the Desert Tortoise Research Natural Area in the western Mojave in association with the BLM.

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Major: Zoology; Minor: Biochemistry

M.S. Degree, 1951, Brigham Young University, Provo, UT
Major: Bacteriology; Minor: Biochemistry
Thesis: The occurrence of salt tolerant fungi in the Great Salt Lake area and some metabolic studies on one of the organisms.

Ph.D. Degree 1955, University of Cincinnati College of Medicine, OH
Major: Microbiology;
Minors: Biochemistry, Pharmacology, Preventive Medicine
Thesis: Preparation, characterization, and immunogenicity of a soluble protective antigen from Shisella types.

Brief Chronology of Employment:

1955-57 - Assistant Professor of Microbiology, University of Cincinnati College of Medicine, and Attending Microbiologist, Cincinnati General Hospital
1957-61 - Microbiologist, Department of Microbiology, Division of Special Operations, Walter Reed Army Institute of Research, Washington, D.C.
1961-62 - Chief, Department of Microbiology, Walter Reed Army Institute of Research, Washington, D.C.
1962-73 - Chief, Mycoplasma Section, Laboratory of Microbiology, National Inst. of Allergy & Infectious Diseases
1973- Chief, Mycoplasma Section,,Laboratory of Molecular Microbiology, National Inst. Allergy & Infectious Diseases

Military Service: United States Navy, Fleet Marine Force
April 26, 1943 to April 26, 1946

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China Medical Board-Louisiana State University Fellow to Central America (April-May **1956**)
Chairman **(1974-83)**, Subcommittee on Taxonomy **of** Mycoplasmas, American Society for Microbiology
Chairman **(1971-72)** and Vice Chairman **(1970-71)**, Mycoplasmology Division, American Society for Microbiology
Chairman, Organizing Committee, International Organization for Mycoplasmology **(1974-75)**
Chairman **(1976-78)**, International Organization for Mycoplasmology

Honorary Doctoral Degree, University of Bordeaux 11, Bordeaux, France, November **1980**
Divisional Lecturer, American Society for Microbiology, **1982**

J. Roger Porter Award, U.S. Federation **of** Culture Collections and the American Society for Microbiology, March **1982**
Emmy Klieneberger-Nobel Award, International Organization for Mycoplasmology, September **1982**

Chairman's Award, 10th International Congress, Internation Organization for Mycoplasmology, Bordeaux, France, July **1994**
Associate Editor, International Journal of Systematic Bacteriology **(1982-91)**

Member: FAO/WHO Program on Comparative Mycoplasmology **(1970-80)** and Chairman of the Board **(1972-77)**
Member, International Committee on Systematic Bacteriology, Subcommittee **on** the Taxonomy **of** Mollicutes **(1968-);** Secretary **(1992-94)**

Board for International Research Program on Comparative Mycoplasmology, International Organization for Mycoplasmology **(1980-)**
Advisory Board, 9th edition, Bergey's Manual **of** Systematic Bacteriology **(1981-83)**
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Member, Board of Trustees, Bergey's Manual Trust **(1991-).**

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Editor/co-editor: Five volume series **of** The Mycoplasmas, Academic Press **(1979-89);** Methods in Mycoplasmology, **2 Vols.**, Academic Press, **(1983);** Molecular and Diagnostic Procedures in Mycoplasmology, **2** Vols., Academic Press **(1995-96).**

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Tully, J.G., and Whitcomb, R.F.: The genus Spiroplasma. In Balows, **A.,** Truper, **H.G.,** Dworkin, M., Harder, **W.,** and **K.H.** Schleifer. (Eds.): The Prokarvotes, Vol. *2*, 2nd Edition, Springer-Verlag, New York, 1991, pp. 1960-1980.

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Tully, J.G. Current status of the mollicute flora **of** humans. Clin. Infect. Dis. 17 (suppl.1):S2-S9, 1993.

Tully, J.G.: Mollicute-host interrelationships: current concepts and diagnostic implications, p. 1-21. In Molecular and Diagnostic Procedures in Mycoplasmology, Vol. II (J.G. Tully and **S.** Razin, eds.), 1996. Academic Press, San Diego.

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Bonilla, H.F., **C.E.** Chenoweth, J.G. Tully, L.K. Blythe, **J.** Robertson, V.M. Ognenovski, and C.A. Kaufman. Mycoplasma felis arthritis in a patient with hypogammaglobulinemia. Clin. Infect. Dis. **XX,** in press, 1996.

Taylor-Robinson, **D.,** and Tully, J.G.: Mycoplasmas, Ureaplasmas, Spiroplasmas, and Related Organisms. **In** Topley and Wilson, Principles and Practice **of** Microbiology, vol. 2, Chapter 33, pgs. xxx-xxx. Arnold Publishers, London, 1997 (in press)

Baseman, **J.B.,** and J.G. Tully: Mycoplasmas: sophisticated, re-emerging and burdened **by** their notoriety. Emerg. Infect. Dis. 3:in press, **1997.**

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28 October 1996

Consensus Statement on Current Understanding of Upper Respiratory Tract Disease (URTD) in the Desert Tortoise from the URTD research group at the University of Florida, Gainesville, Florida.

Over the last several decades, many wildlife populations across the North American continent have experienced severe and often sudden declines that in some cases have lead to extirpation. In different ecosystems different factors have been suggested or identified which may have contributed to these declines. These have included epidemics, over-harvesting, habitat changes, introduction of exotic species, and manmade contaminants. Upper respiratory tract disease has been associated with declines in some desert tortoise populations.

This statement reviews the pertinent accumulated information on URTD as studied by the University of Florida group over the past 7 years and attempts to provide a scientifically sound perspective on the known effects of this disease.

1. We are certain of the following:

a) *Mycoplasma agassizii* causes URTD
b) Light microscopic lesions are confined to upper respiratory tract (URT) and eyes
c) *M. agassizii* causes hyperplastic and dysplastic lesions in the URTD
d) Clinical signs vary in onset, severity, and duration
e) In adults, disease is chronic and may be clinically silent
f) *Pasteurella testudinis* does not cause URTD by itself
g) *M. agassizii* elicits antibody response
h) Antibody response can be detected by serologic test (ELISA)
i) Antibody response is reliably detectable, 8 weeks after first: exposure to M.a.

j) Colonization of URT with *M. agassizii* is detectable by culture and PCR
k) isolation of *M. agassizii* from nasal lavage requires up to 6 weeks
l) Individual host recognition of *M. agassizii* varies
m) It is a horizontally transmissible disease
n) Tortoises become "sicker quicker" on repeat exposure

2. The following are areas of uncertainty:

a) Effect of URTD on individual survival
b) Relationship among infection rates, transmission rates, population size, and clinical expression
c) Effects on population dynamics and viability
d) Burrow/fomite transmission
e) Protective immunity
f) vertical transmission
g) Other mycoplasma strains (mysterioso, others)
h) Association of *M. agassizii* infection and hemosiderosis in liver
j) Systemic effects

3. Suggested areas of further research

a) Effects on individual reproduction and behavior
b) Spread of URTD: large scale, under semi-natural conditions, through a population
c) Transmission via burrows/fomites
d) Seasonal expression of clinical signs of URTD
e) Systemic effects
f) Defining short-term **vs** long-term effects

4. What Needs to be done now

a) Developing recommendations/guidelines on how research animals should be handled, disposition of clinically ill tortoises, disposition of seropositive clinically healthy tortoises, and how can we better manage wild populations with current knowledge and tools
b) development of decision tree for management of captive tortoises

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