Section 27

ARAV Master Classes and Roundtables

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Gross Lesion Recognition for the Reptile Clinician

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Session #008

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Reptile veterinarians in clinical practice, by nature of their specialty, often have to select diagnostic procedures that will provide the best and least expensive treatment options. A familiar course is establishing a viable differential diagnostic list during clinical exam or necropsy based on the gross appearance of the lesions. Gross lesion recognition (and photography), like other imaging modalities, is a bit of an art form. Vast zones of grey may confront the investigator when considering what the lesion is, what it could be, and what to do with it. And nobody is good at all of it: A seasoned livestock pathologist could possibly clear an entire days' necropsies without the help of a single histology slide. A bee keeper would recognize immediately the maggot stages that parasitize his colony. A dog-cat pathologist in a busy private laboratory or a companion animal clinician may not have a clue about gross lesions in livestock or bees. Historically, our attempts at accurately diagnosing disease by gross lesion recognition have been a humbling experience, and that has been the impetus for all further diagnostic specialties. Gross lesions, or lack thereof, are the first visual indication of what may be wrong with the patient (or cadaver, as the case may be). These are the lumps, the effusions, the asymmetrical oddities, the discolorations, the odiferous clues to disease that (hopefully) stimulate a "scientific" thought process culminating in a list of differential diagnoses. The purpose of this 2-hour interactive workshop is to present clear and not-so-clear images of common and not-so-common gross lesions in live and necropsy specimens, and in a participatory manner, establish a differential diagnosis and means for establishing a definitive diagnosis.

Recommended Bibliography

1. King JM, Dodd DC, Roth L, Newson ME. *The Necropsy Book*. Charles Louis Davis Foundation; 2002. (Mammals)

2. Reimschuessel R. Postmortem examination. In: Stoskopf MK, ed. *Fish Medicine*. Philadelphia, PA: W.B. Saunders:160-165. (Fish)

3. Garner, MM. Overview of biopsy and necropsy techniques. In: Mader DR, ed. *Reptile Medicine and Surgery*. 2nd ed. St. Louis, MO: Elsevier Science;2006:569-580. (Reptiles)

4. Nichols DK. Necropsy. In: Wright KM, Whitaker BR, eds. *Amphibian Medicine and Captive Husbandry*. Malabar, FL: Krieger Publishing Co.;2001:331-332. (Amphibians)

5. Latimer KS, Rakich PM. Necropsy examination. In: Ritchie BW, Harrison GJ, Harrison LR, eds. *Avian Medicine: Principals and Application*. Lake Worth, FL: Wingers Publishing, Inc.;1994:356-376. (Birds)

6. Campbell TW, Ellis C. *Avian and Exotic Animal Hematology and Cytology*. Ames, IA: Iowa State University Press;2007.

In-Depth Review of the Cranial Coelom (Thorax) of Reptiles: Normal Anatomy

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Session #349

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The reptilian thorax is defined here as the cranial coelomic cavity and its surrounding skeleton. Major structures are located cranial to the duodenum. Because no species has a diaphragm partitioning the pleuoperitonal cavity, the designation used here is based on structural and functional relationships. The esophagus, stomach, trachea, bronchi and lungs, liver, heart and great vessels, as well as major supporting mesenteries, occupy the cranial coelomic cavity. For the purposes of this review, the liver is discussed superficially because its major functions align more closely with caudal coelomic structures.

Skeleton

The trunk vertebrae, ribs (or cranial carapace), sternum and interclavical (or cranial plastron) and their muscles surround the thorax. In squamates (snakes and lizards), crocodilians and tuataras, the cranial aspects of the coelom are bounded by the trunk vertebrae (sometimes termed thoracic vertebrae) and ribs. Chelonians (turtles and tortoises) vary greatly as to how far the caudally the cranial organs extend dorsally so carapacial landmarks tend to be less useful than plastral landmarks. The cranial coelom can extend to the hyoplastron bones (usually demarked by the humeral and abdominal scutes). Normal trunk vertebrae in lizards, snakes and crocodilians have procoelous joints articulating vertebral bodies. Procoelous vertebrae each have cavity on the cranial aspect and a convex surface on the caudal end.[1,2] In all turtles and tortoises, the trunk vertebrae, ribs and ventral ribs (gastralia, abdominal ribs) are modified to form the shell's carapace and plastron.[3,4] The trunk vertebrae are neural bones and these overlie vertebral bodies, each with amphicoelous articulating ends. The ribs are flattened and expanded as pleural bones. In tuataras, the vertebrae are amphicoelous (having cavities at both ends), similar to those of fish.[5]

Many lizards, crocodilians and tuataras have ribs associated with the trunk vertebrae, with more cranial ribs joining the interclavicle and sternum via cartilaginous joints. Most posterior ribs are floating. These taxa also have ventral ribs that can extend sufficiently far cranially to reach the cranial coelom. All snakes lack an interclavicle, sternum and gastralia. Chelonian ribs are modified as flattened pleural bones and that articulate with vertebrae forming the carapace.

Tuataras are distinctive among reptiles in having short uncinate processes arising roughly orthogonally along the caudal edge of each rib. Uncinate processes are similar to those found in birds and some dinosaurs.[1] Tuataras also have floating ribs that do not attach to the sternum.

Divisions of the Coelom

The thorax is partitioned into the pleuroperitoneal cavity and pericardial cavity. The latter contains the heart and the roots of the heart with the roots of the great vessels. Both are surrounded by the pericardial sac, which is part of the transverse septum. The pleuroperitoneal cavity extends to the pericardial sac ventrally; it extends more cranially dorsal to the pericardium (Fig 1). The pleuroperitoneal cavity, surrounded by the peritoneum (coelomic membrane) contains the other visceral organs except for the kidneys, which are retroperitoneal. Pleural cavities form secondarily in chelonians, crocodilians and some lizards via a nonmuscular, thin fold of tissue that grows from the median mesentery supporting the gut to the peritoneum.[6]

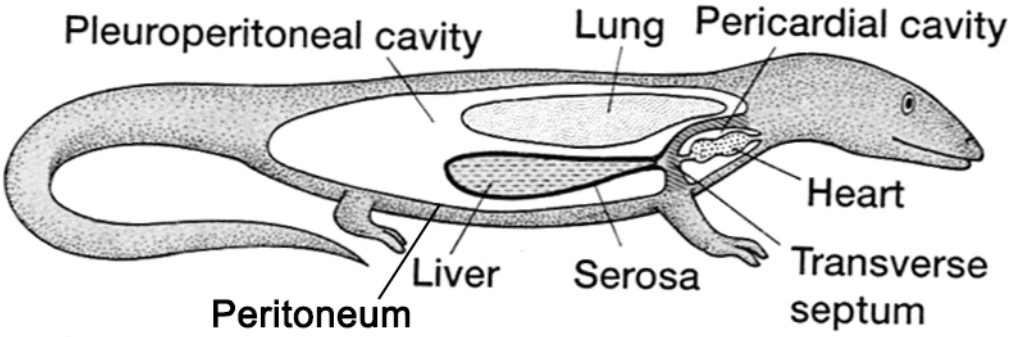

Figure 1. Body cavities of a generalized reptile. The serosa of the liver is formed from the posterior wall of the transverse septum (modified from Kardong, 2014 with permission).

The liver fills much of the cranial aspects of the pleuroperitoneal cavity, ventral to the lungs, with left and right lobes that may or may not join one another via a bridge of hepatic tissue. The liver attaches to the caudal pericardium via a short coronary ligament (homologous to the falciform ligament of mammals). The paired ventral abdominal veins (in chelonians) or single abdominal vein (squamates, crocodilian and *Sphenodo*n) travels through the peritoneum and passes into the liver through the coronary ligament.

Lungs, Trachea and Bronchi

The trachea, caudal to the larynx, passes through the thoracic inlet and soon bifurcates into right and left extrapulmonary bronchi in chelonians, lizards, crocodiles and *Sphenodon*. Snake trachea and bronchi may be extrapulmonary or pulmonary (within the lung) depending on species. For example, crotalids (rattlesnakes) have a single tracheal lung whereas boids (boas and pythons) have paired short extrapulmonary bronchi. In lizards and *Sphenodon*, no intrapulmonary bronchi are present and the extra-pulmonary bronchi, when present, tend to be short. Chelonians have bronchi that extend well into the lung in most species. Crocodilians have well-developed branched bronchi that direct airflow unidirectionally.[7] The trachea is formed of c-shaped cartilaginous rings. Bifurcation into bronchi is distal and initially hidden (grossly) within the tracheal tube. The lining of the trachea and bronchi is formed of ciliated, secretory and basal epithelium.[8] The lungs lie dorso-caudal to the heart and dorsal to the cranial parts of liver and stomach except on snakes.

Esophagus and Stomach

The esophagus passes into the cranial thorax centrally and as it passes caudally may lie dorsal to the trachea or slightly to one side. In cheloniid sea turtles and some freshwater turtles, the esophagus takes an s-shaped bend to the left before joining the stomach. The length of the esophagus is often related to the neck length except in snakes, legless lizards, and leatherback sea turtles (*Dermochelys coriacea*). It is particularly long in monitor lizards, snakes and some turtles (such as softshelled turtles, long-necked Australian pleurodires and mata matas [*Chelys fimbriata*]). In the leatherback sea turtle, the esophagus is particularly long, extending to the pelvic area then turning into the left and travelling cranially to the level of the axilla where it then turns medially and joins the stomach near the heart. The lining of the esophagus is smooth or folded and usually lined by stratified squamous epithelium with ciliated cells and goblet cells,[8] except in sea turtles that have large keratinous papillae lining the esophagus. These reduce dramatically in size at the gastroesophageal junction. The gastro-esophageal junction has a sphincter. The esophagus is primarily a transport organ. However, the mucosal wall contains lymphoid tissue patches, termed tonsils (gut-associated lymphoid tissue) that are particularly obvious in boid snakes.[8] The esophagus is very thin in snakes, particularly cranially. It has more pronounced muscular layers in tortoises and sea turtles.[8,9] Distally, in all reptiles, the esophagus has muscular layers near the stomach.

The stomach and esophagus tend to join to the left of the heart and often along the dorsal surface of the liver's left lobe. In all reptiles, the stomach is located on the left side of the body and attaches to the left lung (when present) by a gastropulmonary ligament. In snakes, the stomach is fusiform whereas in most lizards and turtles it is sac-like and c-shaped. The stomach of crocodilians has two parts: a large fundic portion that receives the esophagus and a smaller pyloric portion that connects proximally to the fundic part by a large orifice and distally to the duodenum.

The stomach is primarily a chemical digestion organ, except in crocodilians that also have a muscular pyloric region that functions in physical digestion. In snakes, the stomach is rather short and the gastroesophageal junction is located at approximately the caudal one-quarter point of the body and along the left side of the liver. In chelonians, the stomach is sac-like, usually c-shaped, and usually lies along the heart's left side and curves to the right. The stomach ends in a muscular pyloric sphincter, the pyloroduodenal junction.

The lining of the stomach is glandular and its surface mucosa can be very dynamic in shape and thickness.[9] In episodic feeders such as many snakes and some crocodilians, the lining of the stomach may be pale and folded during periods of fasting. After feeding, the mucosa of the stomach and intestine become pink and the epithelial cells elongate, thickening the stomach's mucosa and increasing area for absorption. When full, the stomach's muscular wall can be stretched thin.

Heart and Major Vessels

The heart is located medially in the pericardial cavity, at the level of the shoulder girdle in most lizards, just caudal to the shoulders in chelonians, approximately midway between the bases of the fore- and hindlimbs in crocodilians and with the second quarter of the length of snakes, caudal to the head.[10] The heart is connected caudally via the ascending vena cava to the liver and cranially via the external jugular veins from the head and neck.

The hearts of turtles, lizards, and snakes are grossly similar. In ventral view, the heart appears to be a three-chambered pump composed of two muscular atria and one ventricle; however, dorsally the large, thin-walled sinus venosus is found which is part of the heart.[10-12] The sinus venosus receives venous blood returning from

the head, limbs, and body that then flows to the right atrium. Three great vessels emerge from the cranioventral aspect of the ventricle. From left to right, these are the left and right aorta and the pulmonary trunk. Crocodilians have a heart structure that differs from the other reptiles. The ventricle is divided into pulmonary and systemic sides. The left aorta and pulmonary artery arise from the right ventricle; the right aorta drains the left ventricle. Crocodilians have a connection near the bases of the two aortae, the Foramen of Panizza, which appears to function like a central shunt.[6]

The major vessels passing through the cranial coelom include (but are not limited to) the left and right aortae, pulmonary arteries and veins, celiac artery, subclavian arteries and veins, proximal parts of the carotid arteries, post cava, azygos vein, caudal parts of the external jugular veins, hepatic veins, and cranial part of the hepatic portal vein.

Thyroid, Thymus, Parathyroid and Ultimobranchial Glands

The cranial coelomic cavity houses several endocrine glands. All develop as diverticula from the pharyngeal folds. The thyroid gland is a single structure in snakes (round or oval) and lizards (U-shaped) and is located ventral and lateral to the trachea and cranial to the heart; near the brachiocephalic trunk artery in chelonians (round gland). In crocodilians, glands are found along the ventrolateral trachea near the bronchi as a bilobed gland or as or two separate lobes. In Nile crocodiles (*Crocodylus niloticus*) they are found along the bronchii depending on species.[6,13]

Thymus glands are lobulated glands that are paired in chelonians and squamates, and usually single in crocodilians. In turtles they are associated with the common carotid and subclavian arteries.[8,10] In lizards and snakes, the thymus glands are associated with the common carotid arteries, jugular veins and vagus nerve.[8] In crocodilians, the main body of the thymus is a singular structure just cranial to the heart and has a cranial extremity that extends to the cervical region near the base of the skull.[8]

The parathyroid glands and ultimobranchial bodies are small, antagonistic glands that regulate blood calcium. There are one, two or three pairs of parathyroid glands (raises blood calcium) in reptiles and, usually, a single pair of ultimobranchial bodies (lowers blood calcium).[6] They are usually associated with the carotid arteries. In chelonians and crocodilians one of two pair(s) can be found near the heart. In turtles and tortoises another pair(s) may be associated with the thymus and sometimes may be palpated as dense bodies along the thymus' connective tissue.[8] In lizards, the pairs of parathyroids tend to be associated with the carotid arties near the heart. In snakes, these glands are often distributed along the carotid arteries, distal to the thymus.[8]

The ultimobranchial bodies are difficult to find and are usually near the thyroid gland. Jacobson (2007) notes that many lizards, crocodilians, and snakes have just one ultimobranchial body on the left side; the right does not persist.[8] Chelonians and, at least sand boas (*Eryx johnii*), have both right and left glands.[8]

References

1. Romer AS. The axial skeleton. In: *Osteology of the Reptiles*. Chicago, IL: University of Chicago Press;1956.

2. Molnar JL, Pierce SE, Bhullar B-AS, Turner AH, Hutchinson JR. Morphological and functional changes in the vertebral column with increasing aquatic adaptation in crocodylomorphs. *R. Soc Open Sci*. 2015;2(11):150439:1-22. http://dx.doi.org/10.1098/rsos.150439.

3. Pritchard PCH. Evolution and structure of the turtle shell. In: Wyneken J, Godfrey MH, Bels V, eds. *Biology of Turtles: From Structures to Strategies of Life*. Boca Raton, FL: CRC Press;2008:45-84.

4. Rice R, Kallonen A, Cebra-Thomas J, Gilbert SF. Development of the turtle plastron, the order-defining skeletal structure. *Proc Natl Acad Sci* USA. 2016;113(19):5317. doi:10.1073/pnas.1600958113

5. Gadow H. *Amphibia and Reptiles*. London: Macmillan and Co. Limited;1901.

6. Kardong, KV. *Vertebrates: Comparative Anatomy, Function, and Evolution*, 7th ed. New York, NY: McGraw-Hill;2014.

7. Farmer C, Sanders K. Unidirectional airflow in the lungs of alligators. *Science*. 2010;327(5963): 338-340.

8. Jacobson, ER. *Infectious Diseases and Pathology of Reptiles: Color Atlas and Text*. Boca Raton, FL: CRC Press Taylor & Francis Group;2007.

9. Parsons, TS, Cameron JE. Internal relief of the digestive tract. In: Gans C, Parsons, TS, eds. *Biology of the Reptilia*. Vol 6. Morphology E. New York, NY: Academic Press;1977:159-223.

10. Wyneken. J. *Guide to the Anatomy of Sea Turtles*. NMFS Tech Publication. 2001;NOAA Tech Memo NMFS-SEFSC-470.

11. Wyneken J. The structure of cardiopulmonary systems of turtles. In: Wyneken, J, Godfrey M, Bels V, eds. *The Biology of Turtles*. Boca Raton, FL: CRC Press Taylor & Frances Group;2008:213-224.

12. Wyneken J. Normal reptile heart morphology and function. *Vet Clin North Am Exot Anim Pract*. 2009;12(1):837-853.

13. Huchzermeyer FW. *Crocodiles: Biology, Husbandry and Diseases*. Cambridge, MA: CABI;2003.

In-Depth Review of the Cranial Coelom (Thorax) of Reptiles: Disease Conditions

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Session #199

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Abstract: This paper will cover the disease conditions of the reptilian cranial coelom (thorax). The organs include the thyroid, thymus, heart, trachea, lungs, esophagus, and stomach. Common abnormalities in these organs include developmental anomalies, degenerative lesions, obstructions, traumatic injuries, infectious disease agents, and neoplasms. The paper is divided by disease categories and location. A short discussion of these disease conditions in reptiles is found in each section. For the purposes of this paper, chelonia will refer to all of the shelled reptiles. The term tortoise will be used to describe the large land animals that have stout elephantine feet. Few references to marine turtles and crocodilian species are included, as these are uncommonly seen in most clinical practices.

Non-Infectious Diseases

Developmental anomaly

Developmental anomalies of the cardiovascular system are reported to be common in reptiles[1] and are usually associated with deaths of juveniles.[2] Incomplete development of the atrioventricular valves has been described, leading to incomplete valve closure.[3] A secundum atrial septal defect was described in a Komodo dragon (*Varanus komodoensis*).[4] The lesion was on the craniodorsal portion of the septum. It led to chronic congestion of several organs. Two juvenile ball pythons (*Python regius*) with bifid ventricles were reported.[5] All cardiac chambers were enlarged, the lesion disrupted the ability to separate pressures within the ventricles. An interventricular septal defect was also reported in an alligator (*Alligator mississipiensis*).[6]

A number of congenital disorders of the digestive tract and rarely the respiratory tract are recognized in reptiles even from the early Cretaceous age (dicephaly).[7] Ophidian and chelonian dicephaly (craniodichotomy) is presumably the result in incomplete separation of identical twins and is presumed to be the cause in other reptiles.[8,9] Rarely dicephaly has even been used in experimental studies on causes of satiety.[8] Tracheal duplication and malformation has been described in an adult (7-year-old) bicephalic Honduran milk snake (*Lampropeltis hondurensis*). This snake also had two esophagi and two stomachs that joined at a common duodenum.[10]

In a spur-thighed tortoise (*Testudo graeca ibera*) the two heads and necks were independent, with duplicated esophagus, stomach, liver, gallbladder, trachea, and two asynchronous hearts.[9] The proposed causes reported are exposure to environmental toxins, genetic defects, anoxia of the embryo, elevated environmental temperatures during incubation, and rapid thermal changes during internal incubation.[8,9]

Degenerative

An incidental finding of oxalate crystals in thyroid colloid has been described in wild desert tortoises, (*Gopherus agassizi*). The crystals are birefringent radiating aggregates and not associated with inflammation as those identified in some of the tortoise kidneys. It was felt the origin of the calcium oxalates was from the plants eaten.[11]

Few diseases are reported involving the thymus of reptiles. Thymic hyperplasia in subadult captive-bred Galapagos tortoises (*Geochelone nigra*) resulted in significant cervical swelling near the thoracic inlet.[12] Although thymic enlargement can occur normally in reptiles as a result of seasonal hormonal changes, these were felt to be excessive given the mass lesion.

Aortic aneurysm rupture leading to cardiopulmonary arrest was described in a Burmese python (*Python molurus bivittatus*) and the exact cause was not determined.[13] Arteriosclerosis has been documented in green iguanas (*Iguana iguana*)[14] and dystrophic mineralization of arteries is seen in various lizards. Mineralization of arteries is most likely due to secondary nutritional hyperparathyroidism due to a calcium deficiency. A mole king snake (*Lampropeltis calligaster rhombomaculata*)[15] and a Deckert's rat snake (*Elaphe obsoleta deckertii*)[16] were diagnosed with cardiomyopathy of undetermined cause. The lesions in the king snake were primarily collagen proliferation and osteoid-like material, while the rat snake had degeneration and necrosis of myocardial fibers. Aortic stenosis with associated dilatation of the right atrium and the ventricle were found in an iguana.[17] Histologically both aortic arches had narrow lumens with thickened intimas, and the myocardial fibers were attenuated. Although the cause was not determined the authors considered a chronic congenital lesion as one possibility.

Myocardial degeneration due to vitamin E deficiency has been seen in several reptile species.[18] Grossly and microscopically the lesion is similar to that described in mammals. The heart will have mottled white to grey myocardium and histologically there will be multifocal to coalescing loss of myocytes replaced with dense fibrous stroma. Visceral gout can lead to severe pericardial lesions,[19] as well as changes in the myocardium. These can include thickening of the pericardial sac with a deposition of the urate crystals as well as crystals forming within the myocardium. Primary disease can be nutritional or toxic.[20]

It has been reported that the most common noninfectious cause of respiratory disease in chelonians results from trauma to the carapace.[21] Though not well documented in the literature, pulmonary mineralization is occasionally seen secondary to osteodystrophy from any cause. In some cases, this is limited to pulmonary blood vessels, but basement membranes may also be affected. Pleural urate deposition can be seen in cases of visceral gout.[22] Inhalation of foreign material can lead to serious lung lesions and predispose to bacterial infections.[23]

Obstructive lesions

The most common cause of gastrointestinal obstructions is from the ingestion of foreign material.[24,25] Generally the foreign material is of inappropriate environmental substrates.

A variety of foreign objects have resulted in obstructive lesions in the digestive tract of snakes.[24,25] A black rat snake (*Elaphe obsoleta obsolete*) ingested a golf ball which lodged within the stomach.[26] It was successfully removed surgically and the snake returned to the wild.

An unusual case of parasitic impaction was reported in an adult make milk snake (*Lampropeltis triangulum*).[27] The snake was anorexic for two months and had regurgitated a partially digested mouse. Several fluid-filled coelomic masses were identified by radiographs and ultrasound. A gastric impaction was due to a firm solid white mass containing the eggs of the nematode *Kalicephalus* sp and the coelomic masses were granulomatous inflammatory foci secondary to the nematode eggs.

A review of post-mortem examinations on three free-living green turtles (*Chelonia mydas*) in the United Arab Emirates identified a duodenal volvulus and stomachs full of fresh seagrass. Based on a lack of other findings, the duodenal volvulus was suspected to be dietary in origin from over-fermentation of ingesta.[28]

Gastric impaction due to food material has been described in captive crocodiles (*Crocodilus niloticus*).[29] Five crocodiles fed only guinea pigs for 4 days died and in the stomach was a compacted mass of matted guinea pig hair.

Infectious Diseases

Heart

Infectious agents affecting the heart are usually secondary to systemic illness.[30] West Nile virus infection led to myocardial degeneration and necrosis in a farmed American alligator (*Alligator mississippiensis*).[31]

Bacterial disease has been reported in conjunction with endocardial thrombosis,[32] endocarditis,[33] and myocarditis.[34,35] *Chlamydophila* sp. infection is a cause of heart disease in reptiles. Granulomatous pericarditis and myocarditis was found in puff adders (*Bitis arietans*)[36] and necrotizing myocarditis in green turtles (*Chelonia mydas*).[37] Organisms could be demonstrated in macrophages in sections of paraffin-embedded heart. In emerald tree boas (*Corallus caninus*), there were histiocytic granulomas in the heart as well as in other organs. Small, basophilic organisms were seen on hematoxylin and eosin stained sections. Transmission electron microscopy of an intestinal granuloma demonstrated developmental stages of the chlamydial organisms.[38]

Mycoplasma infection of the heart was seen in American alligators (*Alligator mississippiensis*).[39] Rapidly growing mycoplasmas were identified by culture and polymerase chain reaction. The alligator isolate is a novel species in the mycoplasma family.

Spirorchid fluke (Digenea: Spirorchidae) infection was seen at necropsy of 96 stranded green turtles (*Chetonia mydas*).[40] Cardiovascular lesions included mural endocarditis, arteritis, and thrombosis, frequently accompanied by aneurysm formation. Spirorchiid trematode eggs were noted in the heart and other organs in black turtles (*Chelonia mydas agassizii*).[41] Seventy-five adult *Learedius learedi* were recovered from the heart of one of the turtles. During a 5-year period, 16 freshwater turtles (*Trachemys scripta elegans* and *Chrysemys picta*) died spontaneously.[42] Necropsy lesions included subcutaneous edema, hepatic necrosis, pancreatic necrosis, splenic necrosis, and intestinal parasites. Histologically, trematode eggs were seen within the myocardium, and in other tissues, associated with granulomatous lesions. The size and distribution of the eggs were consistent with *Spirorchis* sp. infection. Fluke infections can result in parasites within the three chambers of the heart and in major vessels (right aortic arch and brachiocephalic artery), attached to the walls, or free in the lumen.[43]

Whiptail lizards (*Cnemidophorus* spp.) from Texas and Colorado (USA), were examined for *Mesocestoides* sp. tetrathyridia and eleven (5%) were infected. Free tetrathyridia were found in the body cavity of lizards and encapsulated tetrathyridia were observed in the heart, liver, and stomach.[44]

Trachea and lungs

Infectious diseases have been considered the most commonly reported pulmonary diseases in reptiles in several surveys.[45-47]

Several viral diseases are associated with pneumonia. A paramyxovirus related to parainfluenza 2 (PI2) virus was recovered from the lungs of two dead Ottoman vipers (*Vipera xanthena xanthena*). Histologically there was interstitial pneumonia and degeneration and hyperplasia of bronchial and atrial epithelia. Scattered vacuoles, some of which contained eosinophilic inclusion bodies, were seen in the cytoplasm of several cells of affected epithelial tissues. Viral hemagglutination was inhibited by PI2 virus antiserum, but not by antisera to PI1, PI3,

respiratory syncytial, or canine distemper viruses. Indirect immunofluorescence with PI2 viral antiserum specifically stained inclusions in the epithelial cells of respiratory tissues and infected cell cultures.[48]

In one study investigating respiratory disease in 80 boid snakes (family Boidae and Pythonidae), Ferlavirus (formerly known as ophidian paramyxovirus)-RNA was detected only in pythons. Inclusion body disease (IBD) was rarely seen in pythons but often in boas.[49] Eosinophilic intracytoplasmic inclusion bodies within the epithelial cells of the many tissues is diagnostic for inclusion body disease (IBD) of Boid snakes, an arenavirus.[50] This disease can be responsible for clinical signs including chronic regurgitation, incoordination, loss of righting reflexes, paresis, and an increased incidence of secondary infections such as stomatitis and pneumonia. Clinical signs referable to the central nervous system are more prominent in the Pythoninae subfamily.

Nidovirus infection resulted in pneumonia, tracheitis and esophagitis in ball pythons (*Python regius*). The histologic findings were of marked hyperplasia of epithelial cells lining air exchange areas (pneumocytes) with significant subacute interstitial inflammation and epithelial necrosis. Similar inflammatory and hyperplastic changes were also present in the trachea, esophagus and oral cavity.[51]

An adult male gopher tortoise (*Gopherus polyphemus*) had signs of upper respiratory disease. On necropsy a severe, extensive necrotizing ulcerative tracheitis, multifocal necrotizing pneumonia, and multifocal necrotizing ulcerative pharyngitis and esophagitis were seen. Intracytoplasmic basophilic inclusions occurred within necrotic epithelial cells. On transmission electron microscopy virions and cytoplasmic inclusions were morphologically similar to those of the Family Iridoviridae.[52] In other animals with iridovirus (Russian tortoise [*Testudo horsfieldii*], and a box turtle [*Terrapene carolina*]) no lesions were described; however, a captive Burmese star tortoise (*Geochelone platynota*), a wild gopher tortoise, Eastern mud turtle (*Kinosternon subrubrum*) and 5 Eastern box turtles (*Terrapene carolina carolina*) were found to be infected with ranavirus. Several animals had varying degrees of multicentric vasculitis or thrombosis, necrosis of hematopoietic tissues, as well as multifocal necrotizing tracheitis. In some cases, basophilic intracytoplasmic inclusion bodies were observed within epithelial cells of the oral mucosa, esophagus, stomach, and trachea.[53,54]

Herpesvirus-associated rhinitis, stomatitis, glossitis, tracheitis and bronchopneumonia has been described in a number of tortoises (California desert tortoise [*Gopherus agassizii*],[55] spur-thigh tortoises [*Testudo graeca*],[56] Argentine tortoises [*Chelonoidis chilensis*], Mediterranean land tortoises [*Testudo* species], Hermann's [*Testudo hermanni*] and four-toed tortoises [*Testudo horsfieldii*]). This herpesviral infection is frequently complicated by secondary bacterial infections and generally results in severe debilitation of the tortoise. The transmission is most likely from infected carriers although the pathogenesis has not been fully described.

Herpesvirus infection was also diagnosed in 14 juvenile (15- to 20-month-old) green turtles (*Chelonia mydas*) with clinical signs of respiratory tract disease. Gross lesions included periglottal necrosis, tracheitis with intraluminal caseous and laminated necrotic debris, and severe pneumonia. Microscopically, the turtles had fibrinonecrotic inflammation around the glottal opening, tracheitis, and severe bronchopneumonia and interstitial pneumonia. Multifocally, tracheal epithelial cells adjacent to areas of necrosis were karyomegalic and had amphophilic intranuclear inclusions. A secondary bacterial infection was noted. Ultrastructurally, intranuclear viral particles (88-99 nm in diameter) were seen. The particles most closely resembled those of herpesviruses.[57]

Atlantic loggerhead sea turtles (*Caretta caretta*) recovered by a rescue group had gross and histologic evidence of a herpesviral infection consisting of oral, respiratory, cutaneous, and genital lesions characterized by necrosis, ulceration, syncytial cell formation, and intranuclear inclusion bodies. By nested polymerase chain reaction two unique herpesviral sequences referred to as loggerhead genital-respiratory herpesvirus and loggerhead orocutaneous herpesvirus were identified.[58]

Paramyxoviruses (PMVs) respiratory disease have only been documented in tortoises in a very few cases. A leopard tortoise (*Geochelone pardalis babcocki*) presented with lethargy, respiratory disease, and large amounts of mucopurulent discharge from the nares and oral cavity. On gross pathology there was severe diffuse consolidation of the lungs which were pale, firm, and filled with a thick serous exudate. By molecular methods (polymerase chain reaction [PCR]) several tissues were positive for Paramyxoviruses. Sequence analysis of the PMV amplicons revealed that the tortoise was coinfected with at least two different squamatid PMV species and not with a chelonian host-specific PMV.[59]

Infection with West Nile virus (WNV) in addition to myocardial degeneration with necrosis produced a mild interstitial pneumonia and lesions in many other organs in alligators.[31] Immunohistochemistry identified WNV antigen in the tissues.

In one review gram-negative bacterial infections were considered the most common cause of pneumonia in reptiles.[45] Bacterial pneumonia can lead to systemic complications. Poor husbandry conditions are an important trigger for the development of respiratory signs and pneumonia in snakes. Different bacterial pathogens are commonly isolated in almost all snakes with pneumonia, with Salmonella species being the most common in boid snakes.[49] In a group of sea kraits (*Laticauda* spp.), nine died of sepsis secondary to necrotizing enteritis or pneumonia. Based on the clinical picture, it was considered that stress, such as transport, captivity, or possible concurrent viral infection, resulted in a septic event and death.[61]

Bacterial pneumonia was one of the most frequently encountered clinically significant lesions in Kemp's ridley sea turtles (*Lepidochelys kempii*).[62]

In a group of California desert tortoises (*Gopherus agassizii*), 5 of 7 with respiratory disease had mycoplasmosis based on the presence of chronic proliferative rhinitis and positive serologic tests and/or isolation of *Mycoplasma* species.[63] Mycoplasma has also caused proliferative lymphocytic tracheitis and pneumonia in a Burmese python (*Python molurus bivittatus*). Polymerase chain reaction analysis of the 16S rRNA gene sequence indicated 0.90 similarity to *Mycoplasma agassizii*, an organism previously shown to cause respiratory disease in reptiles.[64] Pneumonia has been seen as a part of systemic disease due to *Chlamydophilia* sp. infection in puff adders (*Bitis arietans*).[36]

Mycobacterial infection can also cause pneumonia in reptiles.[23,45,65,66] It is considered a sporadic disease in captive collections, usually seen as a chronic disease. A variety of mycobacterial genera have been isolated, most of which are generally environmental microbes.[65] These infections are commonly disseminated with the portal of entry suspected to be oral or through defects in the skin (trauma). The lesions are typical for mycobacterium with granulomatous inflammation.

Mycotic pneumonia has been considered an occasional but uncommon diagnosis in reptiles,[23] with a variety of organisms isolated. Suboptimal temperatures[21] can lead to mycotic pneumonia in captive chelonians. Phaeohyphomycosis (pigmented fungi) resulted in obstructive tracheitis in stranded green sea turtles, *Chelonia mydas*.[67]

Other reported causes include mycotic pneumonia associated with *Aspergillus* sp. in green anacondas (*Eunectes murinus*),[68] and chronic pneumonia in a desert tortoise with respiratory disease.[63] In snakes, most cases of systemic mycosis begin in the lungs and then disseminate.[69]

Protozoal infections of the lung can occur in reptiles. Microsporidia, several cases having *Encephalitozoon*-like morphology, have been reported in bearded dragons (*Pogona vitticeps*).[70,71] These lizards presented with nonspecific signs of illness. On histology clusters of light basophilic intracytoplasmic microorganisms are present

and free microbes in areas of necrosis. The microorganisms were within cytoplasmic vacuoles in hepatic, renal, adrenal gland, splenic, pulmonary, enteric and gastric epithelial cells, capillary endothelial cells, and ventricular ependymal cells in the brain. The microbes were associated with granulomatous inflammation. The microorganism is Gram's stain positive, acid fast stain positive, and has a small polar granule that stained using the periodic acid-Schiff reaction. Electron microscopy revealed merogonic and sporogonic stages of a protozoa compatible with members of the phylum Microspora.[71] Nucleotide sequencing revealed high similarity with published *E. cuniculi* sequences in two cases.

An intranuclear coccidia has been seen in the lung of several species of captive tortoises in the United States, leading to severe proliferative pneumonia in some cases.[21]

Several types of reptile metazoan parasites have at least a portion of their life-cycle in the lung.[23] Nematodes of the genus *Rhabdia* lay eggs within the pulmonary parenchyma. If the eggs rupture, there can be a marked inflammatory response.

Lungworm infection was seen in loggerhead sea turtles (*Caretta caretta*). The lungworms may not have been the primary cause of illness, and they may be only contributory or incidental. Lesions in those that died included tracheal and bronchial epithelial hyperplasia and goblet cell hyperplasia. Lesions caused directly by the parasites seem to be restricted to the upper respiratory tract; debris produced by the worms may lead to changes in the lungs.[72]

Pentastomids (phylum Pentastomida) comprise a highly specialized taxon of arthropod-like parasites that are suspected to share a common ancestry with branchiuran crustaceans. Pentastomids are long-lived, often large parasites that probably became adapted to the lungs of amphibians and reptiles early in their evolutionary history. The majority of adult pentastomids feed on blood in the lungs and trachea of the definitive reptilian hosts and cause minimal damage. After mating once, the females deposit a large number of embryonated eggs. The eggs are carried up the trachea by the ciliary action of the mucosal lining to the oral cavity. The eggs are then swallowed and pass in the feces. In the typical life cycle, an invertebrate (e.g., cockroach) or vertebrate intermediate host (e.g., rodents, herbivores, carnivores, and primates) ingests the eggs. The larvae hatch and encyst within tissues where they will molt several times to an infective nymph. When the definitive reptile host eats the intermediate host, the nymph completes the life cycle by developing into an adult in the lung. In aberrant hosts and in the normal hosts under stress, the parasites can be associated with inflammation. If there is significant inflammation, air flow obstruction as well as secondary bacterial infections can develop.

Two Bosc's monitor lizards (*Varanus exanthematicus*) developed clinical signs of pentastomiasis. One died of chronic parasitic pneumonia associated with adult pentastomids of an undescribed *Sambonia* sp. Eggs and immature pentastomids were also seen in histologic sections of the lungs and liver. The other animal had fragments of pentastomid larvae in a laryngeal biopsy, and the animal recovered after treatment with ivermectin and supportive therapy.[73]

Several genera of trematodes can cause lesions in the lower respiratory tract of reptiles.[23] Identification of eggs from lung or tracheal washes is necessary for an exact etiologic diagnosis.

Esophagus and stomach

Adenovirus infections are documented in at least twelve different species of reptiles. In contrast to their mammalian and avian counterparts, reptilian adenoviruses are not well characterized as to their pathogenic potential and their ability to cause primary disease.

Adenoviral infections of bearded dragons are common and this viral infection has become established in certain US lizard breeding groups of the genus *Pogona* (bearded dragons).[74-76] The common clinical signs in young dragons are a progressive weakness, anorexia, circling, and head tilt.[74] The lesions are of severe acute coagulative hepatocellular necrosis and the large intranuclear basophilic inclusion bodies in the hepatocytes and enterocytes. It is speculated that there is possible vertical transmission through the egg in utero or at time of oviposition. The adenovirus infections are also reported to be associated with a second virus, Dependovirus.[75] This virus is defective and appears to need the adenovirus to replicate. It is not associated with a disease condition.

Adenovirus was associated with proliferation of tracheal and esophageal mucosa in a 6-month-old Jackson's chameleon (*Chamaeleo jacksoni*). The clinical signs included opisthotonus and anorexia. Histologically, eosinophilic intranuclear inclusion bodies found within ciliated epithelial cells of the esophageal and tracheal mucosa. The inclusions were morphologically consistent with adenovirus by electron microscopy.[77]

A group of seven juvenile California mountain kingsnakes (*Lampropeltis zonata multicincta*) developed severe acute gastroenteritis. The clinical signs included emesis, dehydration, and sudden death. Histopathology demonstrated segmental acute mucosal necrosis and hyperplasia. The enterocytes had intranuclear inclusions consistent with adenovirus.[78]

Esophageal adenovirus-like infection was described in a palm viper (*Bothriechis marchi*) with a concurrent inclusion body-like disease using electron microscopy. The adult snake was captive-born and found dead. The lesions included a stomatitis and esophagitis.[79]

Inclusion body disease (IBD) of boid snakes can be histologically recognized in lining mucosal epithelial and lymphoid cells aggregates of the esophagus, as well as within the mucosal cells of the stomach and less frequently in the intestines.[80] The eosinophilic intracytoplasmic inclusions have been described in boas and pythons (family Boidae), palm vipers (*Bothriechis marchi*) and corn snakes (*Elaphe guttata*).[81-83] It is important to note that these inclusions do not always confirm IBD, as the inclusions in the corn snakes did not have consistent morphology when examined by electron microscopic.[82] This disease can be responsible for clinical signs including chronic regurgitation, incoordination, loss of righting reflexes, paresis, and an increased incidence of secondary infections such as stomatitis and pneumonia. Clinical signs referable to the central nervous system are more prominent in the Pythoninae subfamily.[84,85] Antemortem diagnostics include CBC's and biopsies of the esophagus (especially of lymphocytic stromal aggregates or tonsils), gastric mucosa, and liver.[64] The etiologic agent of IBD is an arenavirus.[50]

Cryptosporidiosis is a well-known gastrointestinal disease of snakes and lizards. Hypertrophic or proliferative gastritis is a common manifestation of *Cryptosporidium* infections in snakes, and atropic gastritis has been described in lacertas. Polymerase chain reaction (PCR) has detected *Cryptosporidium varanii* (*saurophilum*) in corn snakes (*Pantherophis guttatus*) and in leopard geckos (*Eublepharis macularius*). *Cryptosporidium serpentis* was found in other leopard geckos. A *Cryptosporidium* sp. lizard genotype was reported in one leopard gecko and one corn snake.[86] Pseudoparasitic cryptosporidian species from those ingested with the prey (pseudoparasites) have been described in snakes.[86]

Two Russian tortoises (*Agrionemys* [*Testudo*] *horsfieldii*) and a pancake tortoise (*Malacochersus tornieri*), all from separate collections had histologic evidence of intestinal cryptosporidiosis and one with gastric cryptosporidiosis. A mild inflammation was associated with the infections; however, only one case of the intestinal case had mucosal hyperplasia. Consensus *Cryptosporidium* sp. PCR and sequencing was used to characterize the *Cryptosporidium* sp. present in these three tortoises. This may be an under-recognized problem in tortoises.[87]

The coccidia *Isopora amphiboluri* is most common in bearded dragons (*Pogona vitticeps)* and can be associated with clinical signs.[88] It has a direct life cycle and can predispose to other more serious diseases. An adverse synergistic effect of the coccidiosis with the adenoviral infection may be responsible for increased morbidity and mortality.[88]

Penned green turtles (*Chelonia mydas*) became infected with a larval nematode (*Anisakis sp.* Type I) most likely from the food source, sardine (*Harengula ovalis*).[89] The larvae were associated with hemorrhagic ulcers in the pyloroduodenal junction of the alimentary tract.

Neoplasia

Thyroid adenomatous hyperplasia and follicular adenomas have been reported in lizard, snake, and turtle species,[90-93] whereas thyroid carcinomas in reptiles are rare. Reports of thyroid carcinomas have been described in a Chinese crocodile lizard (*Shinisaurus crocodilurus*),[94] a red-eared slider (*Trachemys scripta elegans*),[95] an Indian black turtle (*Melanochelys trijuga*), and a rough and smooth knob-tail geckos (*Nephrurus amyae* and *N. levis*).[90] The clinical signs in the lizards included an intraoral mass, ventral neck swelling, oral hemorrhage, and weight loss. The thyroid carcinoma metastasized to the liver and lungs in the rough knob-tail gecko (*Nephrurus amyae*).[90] The functional aspects of the tumor are rarely described either by blood hormone levels or clinical signs.[90] One iguana (*Iguana iguana*) with a thyroid adenoma presented with polyphagia, aggression, and tachycardia. The elevated total T4 (30.0 nmol/L) resolved to a euthyroid state after surgical removal.[91]

A C cell carcinoma of the thyroid gland in a crocodile lizard (*Shinisauris crocodilurus*) metastasized to the liver and mesentery.[96]

Primary tumors of the reptile cardiovascular system are not common. Among those reported are a rhabdomyosarcoma, in a boa constrictor (*Boa constrictor*), and a fibrosarcoma in a Gaboon viper (*Bitis gabonica*).[97] Other tumors that may be primary or be part of a disseminated process include lymphoblastic malignant lymphoma and lymphoid leukemias in several species of reptiles.[97,98] Thirty-nine percent of green turtles (*Chelonia mydas*) with fibropapillomatosis (FP) had disseminated internal tumors, most of them in the lung, kidney, and heart.[99] Fibrosarcomas of low-grade malignancy were most frequently noted in the heart, and heart tumors had a predilection for the right atrium. A disseminated mast cell tumor diagnosed in an eastern kingsnake (*Lampropeltis getulus getulus*) involved the heart and numerous other organs.[100] A disseminated chondrosarcoma involved the heart in a corn snake (*Elaphe guttata*).[101] Large areas of myocardium had been replaced by neoplastic tissue. A metastatic oviductal adenocarcinoma has also been reported in the heart of a corn snake.[102]

In one survey[97] two primary adenocarcinomas of the respiratory tract were listed, one of tracheal origin and the other a bronchogenic carcinoma. Tracheal chondromas seem to have a predilection for ball pythons (*Python regius*).[103-105] Adult ball pythons generally present for severe dyspnea. Partial obstructions of the tracheal lumen can be identified radiographically and/or visualized. Histologically, the lesions are tracheal ring chondromas and appear to be benign. Surgical removal and tracheal resection was successful in several cases.

Disseminated tumors such as lymphoma can involve the lung,[97] and a variety of metastatic tumors have been reported. These include fibromas,[99] oviductal carcinoma,[102] chondrosarcoma,[101] squamous cell carcinoma,[106] fibrosarcoma,[107] and a plasma cell tumor.[108]

The majority of the adenocarcinoma/carcinoma gastrointestinal tract tumors are reported in snakes and rarely in lizards and chelonians.[109-112] These tumors will result in variable clinical signs of the digestive tract including

constipation, regurgitation, and progressive enlargement of the coelom. In snakes the differential should include gastric cryptosporidia infections.

There are several reports of a highly malignant gastric neuroendocrine carcinomas in young bearded dragons (*Pogona vitticeps*).[113,114] These tumors readily metastasize to the liver. The common clinical signs are anorexia, vomiting, hyperglycemia, and anemia. The author has identified metastases from gastric carcinomas into the intestine and liver of a carpet python (*Morelia spilota*) and in the liver of two bearded dragons (*Pogona vitticeps*).

References

1. Mitchell MA. Reptile cardiology. *Vet Clin North Am Exot Anim Pract*. 2009;12:65-79.

2. Rush EM, Donnelly TM, Walberg J. Aneurysm and subsequent cardiopulmonary arrest in a Burmese python (*Python molurus bivittatus*). *Proc Annu Conf Am Assoc Zoo Vet*. 1999;134-138.

3. Kik MJL, Mitchell MA. Reptile cardiology: a review of anatomy and physiology, diagnostic approaches, and clinical disease. *Semin Avian Exot Pet Med*. 2005;14:52-60.

4. Pizzi R, Martinez Pereira Y, Feltner Rambaud Y, et al. Secundum atrial septal defect in a Komodo dragon (*Varanus komodoensis*). *Vet Rec*. 2009;164:472-473.

5. Jensen B, Wang TW. Hemodynamic consequences of cardiac malformations in two juvenile ball pythons (*Python regius*). *J Zoo Wildl Med*. 2009;40:752–756.

6. Brockman HL, Kennedy JP. Interventricular septal defect in *Alligator mississipiensis* Daudin. *Tex Rep Biol Med*. 1962;20:719-720.

7. Buffetaut E, Li J, Tong H, Zhang H. A two-headed reptile from the Cretaceous of China. *Biol Lett*. 2007;3(1):80-81.

8. Andreadis PT, Burghardt GM. Feeding behavior and an oropharyngeal component of satiety in a two-headed snake. *Physiol Behav*. 1993;54(4):649-658.

9. Palmieri C, Selleri P, Di Girolamo N, Montani A, Salda LD. Multiple congenital malformations in a dicephalic spur-thighed tortoise (*Testudo graeca ibera*). *J Comp Pathol*. 2013;149(2-3):368-371.

10. Orós J, Rodriguez JL, de Los Monteros AE, Rodríguez F, Herráez P, Fernández A. Tracheal malformation in a bicephalic Honduran milk snake (*Lampropeltis hondurensis*) and subsequent fatal Salmonella arizonae infection. *J Zoo Wildl Med*. 1997;28(3):331-335

11. Jacobson ER, Berry KH, Stacy B, Huzella LM, Kalasinsky VF, Fleetwood ML, Mense MG. Oxalosis in wild desert tortoises, *Gopherus agassizii*. *J Wildl Dis*. 2009;45(4):982-988.

12. Fleming GJ, Heard DJ, Uhl EW, Johnson CM. Thymic hyperplasia in subadult Galapagos tortoises, *Geochelone nigra*. *J Herpetol Med Surg*. 2004;14(1):24-27.

13. Rush EM, Donnelly TM, Walberg J. Aneurysm and subsequent cardiopulmonary arrest in a Burmese python (*Python molurus bivittatus*). *Proc Annu Conf Am Assoc Zoo Vet*. 1999;134-138.

14. Schuchman SM, Taylor DO. Arteriosclerosis in an iguana (*Iguana iguana*). *J Am Vet Med Assoc*. 1970;157:614-616.

15. Barten S. Cardiomyopathy in a king snake (*Lampropeltis calligaster rhombomaculata*). *Vet Med Small Anim Clin*. 1980;75:125-129.

16. Jacobson ER, Seely JC, Novilla MN, et al. Heart failure associated with unusual hepatic inclusions in a Deckert's rat snake. *J Wildl Dis*. 1980;15:75-81.

17. Clippinger TL. 1993. Aortic stenosis and atrio/ventricular dilatation in a green iguana (*lguana iguana*). *Proc Annu Conf Am Assoc Zoo Vet*. 1993;342-344.

18. Frye FL. Common pathologic lesions & disease processes. In: Frye FL, ed. *Biomedical and Surgical Aspects of Captive Reptile Husbandry*. Malabar, FL: Krieger Publishing;1991:560.

19. Jacobson ER. Gentamicin related visceral gout in two boid snakes. *Vet Med Small Anim Clin*. 1976;71: 361-363.

20. Montali RJ, Bush M, Smeller JM. The pathology of nephrotoxicity of gentamicin in snakes. *Vet Pathol*. 1979;16:108-111.

21. Origgi FC, Jacobson ER. Diseases of the respiratory tract of chelonians. *Vet Clin North Am Exot Anim Pract*. 2000;3:537-549.

22. Schmidt RE, Hubbard GB. Respiratory system. In: Schmidt RE, Hubbard GB, eds. *Atlas of Zoo Animal Pathology*. Boca Raton, FL: CRC Press;1987:19.

23. Murray MJ. Pneumonia and lower respiratory tract disease. In: Mader DR, ed. *Reptile Medicine and Surgery*. Philadelphia, PA: WB Saunders;2006:865-877.

24. Benson KG. Reptilian Gastrointestinal diseases. *Semin Avian Exotic Pet Med*. 1999;8(2):90-97.

25. Mitchell MA, Diaz-Figueroa O. Clinical reptile gastroenterology. *Vet Clin North Am Exot Anim Pract.* 2005;8(2):277-298.

26. Adams CJ, Sleeman JM. Clinical challenge. The coelomic cavity consistent with a foreign body. *J Zoo Wildl Med*. 2005;36(4):731-734.

27. Klaphake E, Cross C, Patton S, Head L. Gastric impaction in a milk snake, *Lampropeltis triangulum*, caused by *Kalicephalus* sp. *J Herpetol Med Surg*. 2005;15(1):21-23.

28. Hasbun CR, Lawrence AJ, Samour JH, al-Ghais S. Duodenal volvulus in free-living green turtles from coastal United Arab Emirates. *J Wildl Dis*. 1998;34(4):797-800.

29. Rogers EP, Windsor RS. Gastric impaction in captive crocodiles (*Crocodilus niloticus*). *J S Afr Vet Assoc*. 1982;53(4):254.

30. Murray MJ. Cardiology. In: Mader DR, ed. *Reptile Medicine and Surgery*. Philadelphia, PA: WB Saunders;2006:181-195.

31. Jacobson ER, Ginn PE, Troutman JM, et al. West Nile virus infection in farmed American alligators (*Alligator mississippiensis*) in Florida. *J Wildl Dis*. 2005;41:96-106.

32. Obendorf DL, McManus CJ. *Vibrio damsela* infection in a stranded leatherback turtle (*Dermochelys coriacea*). *J Wildl Dis*. 1987;23:666-668.

33. Jacobson ER, Homer B, Adams W. Endocarditis and congestive heart failure in a Burmese python (*Python molurus bivittatus*). *J Zoo Wildl Med*. 1991;22:245-248.

34. Innis CJ. Myocardial abscess and hemopericardium in a green iguana (*Iguana iguana*). *Proc Annu Conf Assoc Rept Amph Vet*. 2000;185-188.

35. Gray TZ. Cranial body swelling in a boa constrictor, *Boa constrictor constrictor*. *J Herpetol Med Surg*. 2004;14(4):22-24.

36. Jacobson ER, Gaskin JM, Mansell J. Chlamydial infection in puff adders (*Bitis arietans*). *J Zoo Wildl Med*. 1989;20:364-369.

37. Homer BL, Jacobson ER, Schumacher J, Scherba G. Chlamydiosis in mariculture-reared green sea turtles (*Chelonia mydas*). *Vet Pathol*. 1994;31:1-7.

38. Jacobson E, Origgi F, Heard D, Detrisac C. Immunohistochemical staining of chlamydial antigen in emerald tree boas (*Corallus caninus*). *J Vet Diagn Invest*. 2002;14: 487-494.

39. Clippinger TL, Bennett RA, Johnson CM, et al. Morbidity and mortality associated with a new mycoplasma species from captive American alligators (*Alligator mississippiensis*). *J Zoo Wildl Med*. 2000;31:303-314.

40. Gordon N, Kelly WR, Cribb TH. Lesions caused by cardiovascular flukes (Dogemea:spirorchidae) in stranded green turtles (*Chetonia mydas*). *Vet Pathol*. 1998;35:21-30.

41. Cordero-Tapia A, Gardner SC, Arellano-Blanco J, Inohuye-Rivera RB. Learedius learedi infection in black turtles (*Chelonia mydas agassizii*), Baja California Sur, Mexico. *J Parasitol*. 2004;90:645-647.

42. Johnson CA, Griffith JW, Tenorio P, et al. Fatal trematodiasis in research turtles. *Lab Anim Sci*. 1998;48: 340-343.

43. Glazebrook JS, Campbell RS, Blair D. Studies on cardiovascular fluke (Digenea: Spirorchiidae) infections in sea turtles from the Great Barrier Reef, Queensland, Australia. *J Comp Pathol*. 1989;101:231-250.

44. McAllister CT, Cordes JE, Conn DB, Singleton J, Walker JM. Helminth parasites of unisexual and bisexual whiptail lizards (Teiidae) in North America. V. *Mesocestoides* sp. tetrathyridia (Cestoidea: Cyclophyllidea) from four species of Cnemidophorus. *J Wildl Dis*. 1991;27:494-497.

45. Barker IK, Goltz JP. Pneumonia in reptiles: Clinico-pathologic correlations. *Proc Annu Conf Am Assoc Zoo Vet*. 1980;60-63.

46. Keymer IF. Diseases of chelonians: (1) necropsy survey of tortoises. *Vet Rec*. 1978;103:548-552.

47. Keymer IF. Diseases of chelonians: (2) necropsy survey of terrapins and turtles. *Vet Rec*. 1978;103:577-582.

48. Potgieter LN, Sigler RE, Russell RG. Pneumonia in Ottoman vipers (*Vipera xanthena xanthena*) associated with a parainfluenza 2-like virus. *J Wildl Dis*. 1987;23:355-360.

49. Schmidt V, Marschang RE, Abbas MD, Ball I, Szabó I, Helmuth R, Plenz B, Spergser J, Pees M. Detection of pathogens in Boidae and Pythonidae with and without respiratory disease. *Vet Rec*. 2013;172(9):236.

50. Stenglein MD, Jacobson ER, Chang L-W, Sanders C, Hawkins MG, Guzman DS-M, Reavill DR, et al. Widespread recombination, reassortment, and transmission of unbalanced compound viral genotypes in natural arenavirus infections. *PLoS Pathog*. 2015;11(5): e1004900.

51. Uccellini L, Ossiboff RJ, de Matos REC, Morrisey JK, Petrosov A, Navarrete-Macias I, Jain K, Hicks AL, Buckles EL, Tokarz R, McAloose D, Lipkin WI. Identification of a novel nidovirus in an outbreak of fatal respiratory disease in ball pythons (*Python regius*). *Virol J*. 2014;11:144

52. Westhouse RA, Jacobson ER, Harris RK, et al. Respiratory and pharyngo-esophageal iridovirus infection in a gopher tortoise (*Gopherus polyphemus*). *J Wildl Dis*. 1996;32:682-686.

53. Winzeler ME, Hamilton MT, Tuberville TD, Lance SL. First case of ranavirus and associated morbidity and mortality in an eastern mud turtle *Kinosternon subrubrum* in South Carolina. *Dis Aquat Organ*. 2015;114(1):77-81.

54. Johnson AJ, Wellehan JFX, Pessier AP. Iridovirus infections of turtles and tortoises. *Proc Annu Conf Am Assoc Zoo Vet*. 2004;11-12.

55. Pettan-Brewer KC, Drew ML, Ramsay E, et al. Herpesvirus particles associated with oral and respiratory lesions in a California desert tortoise (*Gopherus agassizii*). *J Wildl Dis*. 1996;32:521-526.

56. Muro J, Ramis A, Pastor J, Velarde R, Tarrés J, Lavin S. Chronic rhinitis associated with herpesviral infection in captive spur-thighed tortoises from Spain. *J Wildl Dis*. 1998;34(3):487-495.

57. Jacobson ER, Gaskin JM, Roelke M. Conjunctivitis, tracheitis, and pneumonia associated with herpesvirus infection in green sea turtles. *J Am Vet Med Assoc*. 1986;189:1020-1023.

58. Stacy BA, Wellehan JFX, Foley AM, Coberley SS, Herbst LH, Manire CA, Garner MM, Brookins MD, Childress AL, Jacobson ER. Two herpesviruses associated with disease in wild Atlantic loggerhead sea turtles (*Caretta caretta*). *Vet Microbiol*. 2008;126(1-3):63-73.

59. Papp T, Seybold J, Marschang RE. Paramyxovirus infection in a leopard tortoise (*Geochelone pardalis babcocki*) with respiratory disease. *J Herpetol Med Surg*. 2010;20(2-3):64-68.

60. Jacobson ER, Ginn PE, Troutman JM, et al. West Nile virus infection in farmed American alligators (*Alligator mississippiensis*) in Florida. *J Wildl Dis*. 2005;41:96-106.

61. Chinnadurai SK, Brown DL, Van Wettere A, et al. Mortalities associated with sepsis, parasitism, and disseminated round cell neoplasia in yellow-lipped sea kraits (*Laticauda colubrina*). *J Zoo Wildl Med*. 2008;39:626-630.

62. Innis C, Nyaoke AC, Williams CR 3rd, et al. Pathologic and parasitologic findings of cold-stunned Kemp's ridley sea turtles (*Lepidochelys kempii*) stranded on Cape Cod, Massachusetts, 2001-2006. *J Wildl Dis*. 2009;45:594-610.

63. Homer BL, Berry KH, Brown MB, et al. Pathology of diseases in wild desert tortoises from California. *J Wildl Dis*. 1998;34:508-523.

64. Penner JD, Jacobson ER, Brown DR. A novel *Mycoplasma* sp. associated with proliferative tracheitis and pneumonia in a Burmese python (*Python molurus bivittatus*). *J Comp Pathol*. 1997;117:283-288.

65. Jacobson ER. Bacterial diseases of reptiles. In: Jacobson ER, ed. *Infectious Diseases and Pathology of Reptiles*. Boca Raton, FL: CRC Press;2007:461-526.

66. Woodhouse SJ, Fitzgerald SD, Lim A, Bolin SR. Disseminated *Mycobacterium haemophilum* infection in an ASSAM trinket snake (*Elaphe frenata*). *J Zoo Wildl Med*. 2014;45(4):966-969.

67. Origgi FC, Jacobson ER. Diseases of the respiratory tract of chelonians. *Vet Clin North Am Exot Anim Pract*. 2000;3:537-549.

67. Donnelly K, Waltzek TB, Wellehan JFX, Jr., Sutton DA, Wiederhold NP, Stacy BA. Phaeohyphomycosis resulting in obstructive tracheitis in three green sea turtles *Chelonia mydas* stranded along the Florida coast. *Dis Aquat Organ*. 2015;113(3):257-262.

68. Miller DL, Radi ZA, Stiver SL, et al. Cutaneous and pulmonary mycosis in green anacondas (*Euncectes murinus*). *J Zoo Wildl Med*. 2004;35:557-561.

69. Jacobson ER. Mycotic diseases of reptiles. In: Jacobson ER, ed. *Infectious Diseases and Pathology of Reptiles*. Boca Raton, FL: CRC Press;2007:527-570.

70. Richter B, Csokai J, Graner I, Eisenberg T, Pantchev N, Eskens HU, Nedorost N. Encephalitozoonosis in two inland bearded dragons (*Pogona vitticeps*). *J Comp Pathol*. 2007;148(2-3):278-282.

71. Jacobson ER, Green DE, Undeen AH, et al. Systemic microsporidiosis in inland bearded dragons (*Pogona vitticeps*). *J Zoo Wildl Med*. 1998;29:315-323.

72. Manire CA, Kinsel Jm, Anderson ET, et al. Lungworm infection in three loggerhead sea turtles, *Caretta caretta*. *J Zoo Wildl Med*. 2008;39:92-98.

73. Flach EJ, Riley J, Mutlow AG, et al. Pentastomiasis in Bosc's monitor lizards (*Varanus exanthematicus*) caused by an undescribed Sambonia species. *J Zoo Wildl Med*. 2000;31:91-95.

74. Kim DY, Mitchell MM, Bauer RW, Poston R, Cho D-Y. An outbreak of adenoviral infection in inland bearded dragons (*Pogona vitticeps*) coinfected with dependovirus and coccidial protozoa (*Isospora* sp.). *J Vet Diagn Invest*. 2002;14:332-334.

75. Jacobson ER, Kopitf W, Kennedy A, Funk RS. Coinfection of a bearded dragon, *Pogona vitticeps*, with Adenovirus- and Dependovirus-like viruses. *Vet Pathol*. 1996;33:343-346.

76. Moormann S, Seehusen F, Reckling D, Kilwinski J, Puff C, Elhensheri M, Wohlsein P, Peters M. Systemic Adenovirus infection in bearded dragons (*Pogona vitticeps*): histological, ultrastructural and molecular findings. *J Comp Pathol*. 2009;141:78-83.

77. Jacobson ER, Gardiner CH. Adeno-like virus in esophageal and tracheal mucosa of a Jackson's chameleon (*Chamaeleo jacksoni*). *Vet Pathol*. 1990;27(3):210-212.

78. Wozniak EJ, DeNardo DF, Brewer A, Wong V, Tarara RP. Identification of Adenovirus- and Dependovirus-like agents in an outbreak of fatal gastroenteritis in captive born California mountain kingsnakes, *Lampropeltis zonata multicincta*. *J Herpetol Med Surg*. 2000;10(2):4-7.

79. Raymond JT, Garner MM, Murray S, Nordhausen R. Oroesophageal Adenovirus-like infection in a palm viper, *Bothriechis marchi*, with inclusion body-like disease. *J Herpetol Med Surg*. 2002;12(3):30-32.

80. Garner MM, Raymond JT. Methods for diagnosing inclusion body disease in snakes. *Exotic DVM*. 2004;6(3):90-92.

81. Vancraeynest D, Pasmans F, Martel A, Chiers K, Meulemans G, Mast J, Zwart P, Ducatelle R. Inclusion body disease in snakes: a review and description of three cases in boa constrictors in Belgium. *Vet Rec*. 2006;158(2):757-760.

82. Fleming GJ, Heard DJ, Jacobson ER, Buergelt C. Cytoplasmic inclusions in corn snakes, *Elaphe guttata*, resembling inclusion body disease of boid snakes. *J Herpetol Med Surg*. 2003;13(2):18-22.

83. Raymond JT, Garner MM, Nordhausen W, Jacobson ER. A disease resembling inclusion body disease of boid snakes in captive palm vipers (*Bothriechis marchi*). *J Vet Diagn Invest*. 2001;13:82-86.

84. Schumacher J, Jacobson ER, Homer BL, Gaskin JM . lnclusion body disease in boid snakes. *J Zoo Wildl Med*. 1994;25:511-524.

85. Carlisle-Nowak MS, Sullivan N, Carrigan M, Knight C, Ryan C, Jacobson ER. Inclusion body disease in two captive Australian pythons (*Morelia spilota variegata* and *Morelia spilota spilota*). *Aust Vet J*. 1998;76(2):98-100.

86. Richter B, Nedorost N, Maderner A, Weissenbock H. Detection of Cryptosporidium species in feces or gastric contents from snakes and lizards as determined by polymerase chain reaction analysis and partial sequencing of the 18S ribosomal RNA gene. *J Vet Diag Invest*. 2011;23(3):430-435.

87. Griffin C, Reavill DR, Stacy BA, Childress AL, Wellehan Jr. JFX. Cryptosporidiosis caused by two distinct species in Russian tortoises and a pancake tortoise. *Vet Parasitol*. 2010;170:14-19.

88. Kim DY, Mitchell MM, Bauer RW, Poston R, Cho D-Y. An outbreak of adenoviral infection in inland bearded dragons (*Pogona vitticeps*) coinfected with Dependovirus and coccidial protozoa (*Isospora* sp.). *J Vet Diagn Invest*. 2002;14:332-334.

89. Burke JB, Rodgers LJ. Gastric ulceration associated with larval nematodes (*Anisakis* sp. type I) in pen reared green turtles (*Chelonia mydas*) from Torres Strait. *J Wildl Dis*. 1982;18:41-46.

90. Hadfield CA, Clayton LA, Clancy MM, Beck SE, Mangus LM, Montali RJ. Proliferative thyroid lesions in three diplodactylid geckos: *Nephrurus amyae*, *Nephrurus levis*, and *Oedura marmorata*. *J Zoo Wildl Med*. 2012;43(1):131-140.

91. Hernandez-Divers SJ, Knott CD, MacDonald J. Diagnosis and surgical treatment of thyroid adenoma-induced hyperthyroidism in a green iguana (*Iguana iguana*). *J Zoo Wildl Med*. 2001;32(4):465-475.

92. Garner MM, Hernandez-Divers SM, Raymond JT. Reptile neoplasia: a retrospective study of case submissions to a specialty diagnostic service. *Vet Clin North Am Exot Anim Pract*. 2004;7(3):653-671.

93. Frye FL. Common pathologic lesions and disease processes. In: *Biomedical and Surgical Aspects of Captive Reptile Husbandry*. Malabar, FL: Krieger Publishing;1991:529-619.

94. Whiteside DP, Garner MM. Thyroid adenocarcinoma in a crocodile lizard, *Shinisaurus crocodilurus*. *J Herpetol Med Surg*. 2001;11(1):13-16.

95. Gál J, Csikó G, Pásztor I, Boleskey-Molnár A, Albert M. First description of a papillary carcinoma in the thyroid gland of a red-eared slider (*Trachemys scripta elegans*). *Acta Vet Hung*. 2010;58:69-73.

96. Sykes JM, Trupkiewicz JG. Reptile neoplasia at the Philadelphia Zoological Garden, 1901-2002. *J Zoo Wildl Med*. 2006;37(1):11-19.

97. Mauldin CN, Done LB. Oncology. In: Mader DR, ed. *Reptile Medicine and Surgery*. Philadelphia, PA: WB Saunders;2006:299-322.

98. Bezjian M, Diep AH, de Matos R, Schaefer D. Chinese box turtle (*Cuora flavomarginata*) with lymphoid leukemia characterized by immunohistochemical and cytochemical phenotyping. *Vet Clin Pathol*. 2013;42(3):368-376.

99. Work TM, Balazs GH, Rameyer RA, et al. Retrospective pathology survey of green turtles *Chelonia mydas* with fibropapillomatosis in the Hawaiian Islands, 1993-2003. *Dis Aquat Organ*. 2004;62:163-176.

100. Schumacher JR, Bennett A, Fox LE, et al. Mast cell tumor in an eastern kingsnake (*Lampropeltis getulus getulus*). *J Vet Diagn Invest*. 1998;10:101-104.

101. Schmidt RE, Reavill DR. Metastatic Chondrosarcoma in a corn snake (*Pantherophis guttatus*). *J Herpetol Med Surg*. 2012:22(3-4):67-69.

102. Pereira ME, Viner TC. Oviduct adenocarcinoma in some species of captive snakes. *Vet Pathol*. 2008;45: 693-697.

103. Diethelm G, Stauber E, Tillson M, Ridgley S. Tracheal resection and anastomosis for an intratracheal chondroma in a ball python. *J Am Vet Med Assoc*. 1996;209:786-788.

104. Drew ML, Phalen DN, Berridge BR, Johnson TL, et al. Partial tracheal obstruction due to chondromas in ball pythons (*Python regius*). *J Zoo Wildl Med*. 1999;30:151-157.

105. Greenacre CB, Ritchie BW, Latimer KS. Treatment of cartilaginous hyperplasia in ball pythons. *Compend Contin Educ Vet*. 1999;21(7):633-637.

106. Orós J, Tucker S, Fernández L, Jacobson ER. Metastatic squamous cell carcinoma in two loggerhead sea turtles *Caretta caretta*. *Dis Aquat Organ*. 2004;58:245-250.

107. Orós J, P Monagas P, Andrada M, Calabuig P, et al. Metastatic fibrosarcoma in a captive Saharan horned viper (*Cerastes cerastes*) with high hepatic levels of cadmium. *Vet Rec*. 2009;164:690-692.

108. Schmidt RE. Plasma cell tumor in an East Indian water lizard (*Hydrosaurus amboinensis*). *J Wildl Dis*. 1977;13:47-48.

109. Jacobson ER, Ackerman N. What is your diagnosis? Snake swelling. *J Am Vet Med Assoc*. 1981;179(11): 1311-1312.

110. Martin JC, Schelling SH, Pokras MA. Gastric adenocarcinoma in a Florida indigo snake (*Drymarchon corais couperi*). *J Zoo Wildl Med*. 1994;25(1):133-137.

111. Schlumberger HG, Lucke B. Tumors of fishes, amphibians, and reptiles. *Cancer Res.* 1948;8:657-754.

112. Hernandez-Divers SM, Garner MM. Neoplasia of reptile with an emphasis on lizards. *Vet Clin North Am Exot Anim Pract*. 2003;6:251-273.

113. Ritter JM, Garner MM, Chilton JA, Jacobson ER, Kiupel M. Gastric neuroendocrine carcinomas in bearded dragons (*Pogona vitticeps*). *Vet Pathol*. 2009;46:1109-1116.

114. Lyons JA, Newman SJ, Greenacre CB, Dunlap J. A gastric neuroendocrine carcinoma expressing somatostatin in a bearded dragon (*Pogona vitticeps*). *J Vet Diagn Invest*. 2010;22:316-320.

Diagnosis and Treatment of Diseases of the Reptile Cranial Coelom

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Abstract: The diagnosis and treatment of disease conditions of the reptilian cranial coelom (thorax) is reviewed. By organ, the review includes the thyroid, thymus, trachea, lungs, esophagus, and stomach. This academic review extends well beyond the scope of standard reptilian practice and has the goal of improving reptile practice, and to point out areas for needed research, based on standards and information adapted from that known in human and companion mammals suffering similar diseases. Many reptile diagnostics indicated for disease diagnosis in other species lack basic normative clinicopathologic baseline data required for diagnosis based on hormonal or other analytes. Clinical signs, imaging and histopathologic diagnosis often remain required for definitive diagnoses in reptilian medicine.

Introduction and Overview

This review of the diagnosis and treatment of diseases of the reptile cranial coelom will be based on diseases commonly encountered and diagnostics documented in reptiles and the appropriate therapeutics. Some organs within the cranial coelom, but extending past this region (the trachea, the stomach) will be included and some glands which may or may not be located in the cranial coelom, based on species, will also be included. Diagnostics reviewed will include both those indicated for diagnosis in reptiles based on literature review as well as those successfully used in reptiles. Applicable antemortem diagnostics that have only been reported postmortem will be included. Diagnostics and treatment applicable to the vasculature and heart will not be included in this review.

Trachea

Common diseases of the reptile trachea include trauma, foreign body obstruction, inflammation based on infection (tracheitis) and neoplasia. Uncommon diseases include pulmonary tracheal bronchial prolapse and death in a giant New Caledonian Gecko *(Rhacodactylus leachianus)* and tracheal obstruction caused by fungal granuloma in sea turtles. Many serious viral, bacterial, and fungal diseases (mycoplasmosis, herpesvirus, iridovirus, nidovirus, coronavirus, adenovirus, ferlavirus [OPMV]) have necrotizing tracheitis as a sequella; etiologic agents may proliferate in tracheal mucosa. Based on literature review, tracheitis and obstruction secondary to intubation trauma has not been reported in reptiles unlike in avian species. Vitamin A deficiency also may contribute to tracheitis.

Diagnosis of tracheal disease in reptiles can be challenging based on currently available imaging and other diagnostics. In children, bacterial tracheitis can result in mucopurulent exudates that may acutely obstruct the upper airway and definitive diagnosis remains visualization of the trachea via bronchoscopy.[1] In reptiles, diagnosis of tracheitis, as in other species, generally extends from clinical examination and history. Unlike in companion mammals, because of lack of diaphragm, a cough is not generally appreciated in affected reptiles; however,

irritation, caseated tracheal phlegm and dyspnea may be present. Copious discharge may obstruct the tracheal lumen resulting in open mouth breathing and vertical positioning of the head and neck.[2] Affected snakes may extend or lift the cranial portion of the body to facilitate movement of air through the trachea, despite the lumen narrowing based on mucous accumulation. [3,4]

Direct endoscopic examination may be performed, but direct visual guidance of the scope is necessary to avoid damage to tracheal mucosa. Divers reports that complete tracheal occlusion for several minutes is well tolerated[5]; however, preoxygenation would definitely be recommended. Parasites, abscesses, granulomas, trauma, mucosal defects, chondromas and other neoplasms may be seen. Swab samples for cytology, culture and PCR are indicated for lesions consistent with infectious or inflammatory disease. Radiographs may also be supportive in the diagnosis of chondroma in ball pythons (*Python regius*); as CT imaging advances, this modality may become useful for the diagnosis of tracheal disease in reptiles.

Tracheal wash (often erroneously termed transtracheal wash) of reptiles is generally performed via sterile vascular or urinary catheter placed through the glottis. Sedation may or may not be necessary based on the species anatomy and patient temperament for best sample collection. Lidocaine may facilitate glottal relaxation. Methodical care to avoid glottal and oral contamination during catheter placement is necessary. Based on the relatively open lung structures of the reptile, tracheal washes, dependent upon the reptiles positioning, often represent lung washes and procedures for BAL are unnecessary but tracheal wash could be termed bronchofaveolar lavage (BFL).[6] The technique is perhaps best described for the American alligator which was performed endoscopically: Sterile saline is instilled cranial to the tracheal bifurcation, and the animal is slightly tipped ventrally as fluid is aspirated back into a syringe. Calculation of appropriate volumes to instill for reptile tracheal wash appear adapted from mammalian recommendations, and range from 1-10 ml/kg. Samples obtained may be used for PCR, culture and cytology. Bacterial flora and cytology of tracheal wash determined in American alligators consisted of small amounts of mucus, low numbers of ciliated columnar epithelium, cuboidal epithelium, and keratinized squamous epithelial cells in 90% of samples collected.[7] All samples lacked bacteria, parasites and seasonal variation. To my knowledge, specific clinicopathologic blood analyte abnormalities have not been associated with tracheal disease in reptiles; however, serology and PCR for select diseases known to affect the trachea and culture and sensitivity are indicated in cases of suspected viral or bacterial infection.

Treatment of tracheitis/bronchitis should be based on diagnosis and removal of underlying causes. In human pediatric patients suffering viral and or bacterial tracheitis, management includes close observation and monitoring, early initiation of broad spectrum antibiotics, pain management and aggressive airway clearance techniques. Similarly, general treatment recommendations for ferlaviral infection (OPMV), which is associated with purulent tracheal exudate likely based on secondary opportunistic bacterial infection, includes supportive care to include antimicrobial therapy, fluid therapy and nutritional support.[3,4] Systemic treatment may be of less efficacy for treatment of tracheitis than of pneumonia based on the relatively poor blood supply to the trachea; nebulization may be better suited for tracheal delivery of therapeutics. Provision of adequate humidity and hydration and POTZ to facilitate best function of mucociliary apparatus is essential. Expectorants, proteolytics, mucolytics, and bronchodilators (N-acetyl-L cysteine, mucomyst; *guaifenesin*, mucinex, terbutaline, silbutamol) may be of use (Table 1); however, these agents have yet to be specifically assessed for efficacy or safety in reptile patients. Analgesia and/or anti-inflammatory agents deserve consideration in these cases. Infectious disease precautions, as those used for *Salmonella* spp. and infectious agents which may be transmitted via aerosol, should be provided for these patients.

Table 1. Therapeutics for consideration in reptiles with tracheal, lung, esophagus or stomach disease.

Drug	Indication	Dose	Comments and cautions
Acetylcysteine	Mucolytic, thins loosens mucus, bronchitis, pneumonia.	Pediatric[a] nebulized: 1-10 ml of 20% soln, or 2-20 ml of 10% soln, q2-6h.	Very unpleasant taste and odor.
Aminophylline	Acute respiratory disease.	Aminophylline 2.5 mg/ml + sterile saline followed by nebulization with antimicrobials q12h[22]. 50-75 mg/snake 0.7-1.7 kg + 100 ml sterile water nebulize for 30 min q12h[2,9].	Decreases sedative effects of propofol, can lead to theophylline toxicity. Diuretic, central nervous system and cardiac stimulant.
Guaifenesin	Expectorant, thins loosens mucus in airways, clears congestion, cough suppressant, decongestant.	Pediatric[a] immediate release form: 12 mg/kg/day orally, 6 divided doses Sustained release form: 300 mg q12 NTE 600 mg q24h.	Should be administered with fluids in hydrated patient.
Terbutaline	Bronchodilator, bronchospasm prevention.	0.01-0.02 mg/kg IM[3,4] pediatric, acute asthma[a] SC: 0.005-0.01 mg/kg, max dose 0.4 mg, q15-20 min for 2 doses, nebulization: 0.01-0.03 mg/kg min-max dose 0.1-0.25 mg, dilute in 1-2 ml saline q4-6h, continuous IV infusion: 0.08-6 µg/kg/min.	Side effects: anxiety, tachycardia, tremors, nervousness, headache, hyperglycemia, hypokalemia, hypotension, pulmonary edema (rare).
Salbutamol Albuterol	Alleviation of bronchospasm, short-acting β_2 adrenergic receptor agonist that relaxes airway smooth muscles.	Pediatric, acute asthma:[3,4] albuterol: 0.05-0.15 mg/kg q4-6h, subsequent doses titrated based on clinical response.	Use with caution based on cardiovascular side effects, hypokalemia.
Orajel PM, Colgate Orabase Soothe-N-Seal, Zilactin, ORA5, Rincinol PRN	Oral ulceration, barrier products, local wash sheet or gel placement, provides pain relief, bioactive barrier.	Topical application PRN[b]	Avoid overdose of benzocaine in small animals.

Drug	Indication	Dose	Comments and cautions
Sodium alginate Sodium polymannuronate Gaviscon® Advance Peppermint SA 100 mg/ml, KHCO3 20 mg/ml	Esophageal ulceration, mucoadhesive polymer sodium alginate with esophageal adhesion properties, bioadhesive upon hydration, capable of self-repair or readherence to the next contact point if dislodged, suspension reacts with gastric acid to form a raft of alginic acid gel of near-neutral pH, floats on stomach contents, impedes gastro-esophageal reflux up to 4 h, protects esophagus from acid, pepsin and bile, in severe cases, raft may be refluxed into esophagus and exert a demulcent effect.	0.1 ml/kg PO PRN up to q6h[15]. Modified adult dose, may not be useful in species without adequate gastric acid secretion for activation.	Use with caution in Na, K, congestive cardiac failure, renal failure or drugs that increase potassium CACO3, hypercalcemia, nephrocalcinosis, recurrent calcific renal calculi.
Sucralfate 100 mg/ml suspension	Oral, gastric or duodenal ulceration.	40-80 mg/kg PO q6-24h,[a] modified pediatric dose.	Renal failure, use with caution, aluminum salt may accumulate.
Viroxyn (benzalkonium chloride)	Herpetic oral ulcers, 0.13 percent benzalkonium chloride, disrupts viral lipid coating and kills on contact.	Single topical application to affected area.[b]	

[a]Suggested, adapted dosages based on drug insert information as found in www.drugs.com accessed June 12, 2016.
[b]Suggested dosages and usage based on http://www.rdhmag.com/articles/print/volume-24/issue-12/feature/treating-oral-ulcers.html.

Tracheal obstruction based on chondroma, cartilaginous granuloma, lymphoma or mucoid tracheal and pulmonary exudate have been treated with tracheal resection and anastomosis and lung cannulation in snakes.[2-4, 8-10] Tracheal resection and anastomosis has been performed in snakes, secondary to removal of obstructive chondroma, and tracheal surgery appears well tolerated.

Esophagus

Compared to domestic mammals, esophageal disease of reptiles appears rare. Congenital anomalies affecting the esophagus, megaesophagus or other esophageal motility disorders appear nonextant. In humans, characteristic symptoms of esophagitis include odynophagia (retrosternal pain on swallowing), dysphagia, nausea and sub-

sternal burning pain. Clinical signs may be lacking or could include inanition, regurgitation or, rarely, drooling; however, sea turtles have suffered esophageal impaction with crude oil tar balls and in all aquatic turtles, fish hook and other gear ingestion and lodging within the esophagus is not uncommon.[11] Plaques and erosions in the esophagus commonly occur based on a variety of infectious diseases (ranaviruses adenovirus and others). Based on reported pathology findings, viral particles or inclusions bodies could be found based on swab or biopsy samples, respectively which could be assessed by cytology, culture and PCR. As a widely distensible potential space, indirect imaging may have limited diagnostic capability in the palpably normal esophagus. Endoscopy with gas or liquid insufflation is indicated for lesion visualization and sampling; Intubation is required and downward patient positioning is recommended to avoid aspiration pneumonia. As in humans, no hematologic or biochemical analyte abnormalities are specific to the diagnosis of esophageal disease; however, in reptiles with persistent regurgitation, electrolyte alteration would be a supportive finding.[12]

In humans esophageal infection is rare and associated with immunocompromise. Within this population, Herpes simplex, cytomegaloviral and candidal infections and idiopathic esophageal ulceration are common. Recent human studies of these populations provide data relevant to reptiles (especially tortoises) suffering esophageal ulceration. Human symptoms characteristic of esophagitis include odynophagia (retrosternal pain on swallowing), dysphagia, nausea and substernal burning pain.[13] In a prospective endoscopic study of these lesions, brush samples (for cytology or culture) were more sensitive for diagnosis of candidal or viral infection than biopsy for histology. Blind brushing of the esophagus via esophageal tube has a reasonable sensitivity for each disease at 84 and 75%, respectively.[14] Treatment of esophageal disease is aimed at etiology identification and systemic treatment; however, systemic analgesics and liquid topical antiulcer medications (sucralfate and sodium alginate) also deserve consideration in these cases.[15]

Lungs

Lung disease is commonly recognized in reptilian species based on infectious and traumatic causes, although disseminated or primary neoplasia and nutritional disease (hypovitaminosis A) may also affect the lungs. Acute pulmonary edema, chronic obstructive pulmonary disease and asthma remain unreported in reptiles; however, severe obesity can occur in reptiles and could result in similar signs (Pickwickian tortoise). Clinical signs may be lacking or may include obvious damage based on trauma, increased respiratory rate or effort, abnormal respiratory sounds, or, in aquatic species, abnormal buoyancy. In chelonians, the author finds percussion more useful than auscultation. The challenging diagnosis and occult nature of pneumonia in reptiles is similar to the disease in geriatric humans, in which arterial blood gas and lactate are often used to facilitate diagnosis.[16]

Imaging may be accomplished via direct or indirect methods. Horizontal beam radiographs 3 or 2 view as dictated by species are indicated for diagnosis of respiratory disease; however, CT and MRI are more sensitive although also more expensive for detection of respiratory tree abnormalities and normal snake lung images have been determined.[17-20] Approaches for endoscopic examination and biopsy of the lung generally requires pulmonoscopy; the approach is determined based on species anatomy and lesion localization from indirect imaging. Air sac pulmonoscopy of snakes or a carapacial or prefemoral lung assessment approach in chelonia has been described. Sample collection via biopsy, wash and brush may be performed for evaluation of cytology, histology, and culture and PCR. Blood gases and hemoximetry may be useful to diagnose respiratory acidosis, oxygenation status, and acid base status, should normal values be available from the same species and preferably the same analyzer or monitor. These analytes would likely prove most useful in evaluating a patient's response to therapeutic interventions and for monitoring severity and progression of disease.[21] Leukocytosis is likely to occur in patients with pneumonia of infectious etiology; however, one would expect a more delayed response in the leukogram than in blood gases when assessing disease progression or patient response to intervention.

Treatment of pneumonia and other inflammatory or infectious diseases of the lung of reptiles may have a prolonged course prior to resolution. Viral, parasitic, bacterial or fungal etiologies are expected etiologies. A combination of nebulization and systemic treatment as well as excellent supportive care are recommended. In the author's experience, lung biopsy of chelonians may result in lateral coelomic skin extension "puffing out" with slow resolution not to be confused with actual coelomitis. Similarly, the placement of pneumonic catheters for direct pulmonary therapy is not without potentially life threatening consequence.

Stomach

Common diseases of the reptile stomach include foreign body ingestion/ impaction/ bloat, gastric ulceration (green iguanas, *Iguana iguana*) cryptosporidial infection, neoplasia (gastric adenoma of bearded dragons, *Pogona* spp.), coccidiosis, viral, fungal and bacterial infections and idiopathic atrophic gastritis. Clinical signs of gastric disease in reptiles may include anemia, lethargy, anorexia, vomiting and melena.[23,24]

Diagnosis of gastric disease via plain radiography in most reptiles is challenging based on lack of normal values for gastric positioning and size, lack of coelomic fat to outline internal organs and the natural position of most organs within the coelom which lie in a similar plane to the stomach. Contrast radiography (barium sulfate or iodinated contrast media may be used) may be helpful, but GI transit time may be prolonged in many of these species.[23] CT or MRI combined with intravenous administration of iodinated contrast media are recommended to provide greatly enhance detail for these structures; For chelonians CT scans repeated at 1, 3 and 10 minutes after contrast injection may improve the diagnostic quality of the images. In CT images, the stomach is easy to recognize when gas is present, but can otherwise be difficult to differentiate from surrounding soft tissue unless oral contrast media is administered.[23]

Ultrasound is likely best used when the GI tract is fluid filled or empty and may be less useful in the herbivorous reptile based on gas production. Thus carnivorous reptiles suffering ileus or obstruction may be the best clinical choice to obtain ultrasound diagnosis; however, the stomach was well evaluated via ultrasound in a study of young green iguanas.[23] In the ball python, a left approach revealed the stomach with the pylorus identified medial and dorsal to the gall bladder.[25]

As in evaluation of the esophagus, stomach endoscopy is performed with insufflation of air or saline, and facilitates gross evaluation of lesions, foreign body retrieval and obtaining samples for cytology, histology, culture, microscopic observation of parasites and PCR. In contrast to the delicate esophagus of some species, especially those suffering erosions and necrotic lesions, the stomach is generally thicker walled and more amenable to biopsy. Stomach wash via blind placement of a red rubber catheter may be a less invasive and expensive way to obtain wash samples for similar testing, but does not allow for histology, object retrieval or direct observation of gastric lesions.

Normal gastrointestinal flora is not well characterized in most reptiles. Unlike in mammalian species, specific clinicopathologic plasma biomarkers for gastrointestinal function remain lacking in reptiles. Anemia of chronic disease or from gastrointestinal blood loss and/or electrolyte abnormalities may be present, but the author finds a lack of clinicopathologic abnormalities to be the most common finding in these cases, followed by leukopenia and anemia.[12]

Thyroid

In reptiles, the thyroid regulates ecdysis, reproduction, tail regeneration, growth, endocrine function, and metabolic rate.[26] The role of external factors in thyroid function of reptiles including light cycles, hibernation, and thermal gradients must be considered in suspect cases.[23] Diseases of the thyroid appear not uncommon in reptiles and if neoplasia is included, abnormal thyroid glands have been reported in all classes of reptiles. As in cats, lizard antemortem diagnosis may be based on clinical signs and increased total T4.[27] Clinical signs reported in lizards include those linked to the mass itself: Intraoral mass, ventral neck swelling, palpably enlarged thyroid gland, oral hemorrhage and behavioral changes associated with hormonal changes such as polyphagia, aggression, weight loss, tachycardia. Increased ecdysis with response to methimazole treatment prompted the diagnosis of hyperthyroidism in an older corn snake, but thyroxine concentration were not determined.[28] Total T4 Values have been reported in a number of other reptilian species but vary seasonally in some species and relatively low "normal" values may make diagnosis challenging via hormonal assay.[26] Although rare, The likelihood of metastasis in cases of thyroid carcinoma dictates that radiographs or advanced imaging be a part of the diagnostic work up of these cases.

Goiter (enlarged thyroid) has been reported to affect tortoises on iodine deficient diets or diets with dietary goitrogens: therefore these should be eliminated or minimized in the herbivorous reptile diet: bok choy, broccoli, cabbage, cauliflower, kale, mustard seed, rapeseed, soy bean sprouts, and turnips.[26] Oxalate crystals were found in free-living desert tortoises with thyroid enlargement but without other apparent disease consequence.[29] The diagnosis of hypothyroidism in reptiles via thyroid hormones and TSH remains poorly validated, but the disease appears rare and controversy exists regarding differentiation of obesity and hypothyroidism in tortoises.

Thymus

True thymic disease appears rare in reptiles, with reports limited to a single case in young Galapagos tortoises (*Chelonoidis niger*).[30] Rule outs of expected seasonal change, or generalized lymphatic proliferation from infectious agents or other inflammatory diseases should be considered in these cases. In humans, tumors of the thymus (thymomas and thymic carcinomas) are rare intrathoracic tumors of a variety of cell type origins; a correlation with autoimmune disorders exists in about 1/3 of human cases (Myasthesia gravis, red cell aplasia and hypogammaglobulinemia). Thoracic CT imaging is considered similar or superior to MRI in these cases. In humans, thymoma is more likely to be associated with autoimmune disease while thymic carcinoma cases typically have nonspecific local symptoms. Thus clinicopathologic workup in humans includes complete blood cell count, reticulocyte count and serum protein electrophoresis, as well as anti-acetylcholine receptor and anti-nuclear antibody tests.[31] Relevant differential diagnoses include lymphomas, germ-cell tumours (teratoma or seminoma/non-seminomatous tumours),and primary lung carcinoma.[31] In reptiles, biopsy is recommended prior to resection or other treatment and remains the best option for definitive diagnosis, but may still result in equivocal results.

Parathyroid Glands

While nutritional and renal secondary hyperparathyroidism (NSHP, RSHP) remain common diagnoses in reptilian medicine, primary parathyroid gland disorders appear rare and remain undescribed antemortem likely based on lack of validated laboratory assay for PTH and few normative values for ionized calcium in apparently healthy reptiles.[26] Treatment of NSHP and RSHP is common and beyond the scope of this manuscript. In humans, primary hyperparathyroidism is extremely rare and generally found based on finding hypercalcemia during routine

blood screen or in elderly patients with confusion, and dehydration. Of human patients with hypercalcemia on blood testing, approximately 55% are then diagnosed with primary hyperparathyroidism (PHP), based on radioimmunoassay.[32]

Ultimobranchial Glands

Diagnosis and treatment of primary disease arising from the ultimobranchial bodies of reptiles remains undescribed.

References

Trachea

1. Graf J, Stein F. Tracheitis in pediatric patients. *Semin Pediatr Infect Dis*. 2006;17(1):11-13.

2. Myers DA, Wellehan JF, Isaza R. Saccular lung cannulation in a ball python (*Python regius*) to treat a tracheal obstruction. *J Zoo Wildl Med*. 2009;40(1):214-216.

3. Schumacher J. Respiratory diseases of reptiles. *Semin Avian Exot Pet Med*. 1997;6(4):209-215.

4. Schumacher J. Reptile respiratory medicine. *Vet Clin North Am Exot Anim Pract*. 2003;6(1):213-231.

5. Divers SJ. Reptile diagnostic endoscopy and endosurgery. *Vet Clin North Am Exot Anim Pract*. 2010;13(2):217-242.

6. De la Navarre BJ. Common procedures in reptiles and amphibians. *Vet Clin North Am Exot Anim Pract*. 2006;9(2):237-267.

7. Lafortune M, Göbel T, Jacobson E, Heard D, Brown D, Alleman R, Vliet K, Harr KE, Hernandez J. Respiratory bronchoscopy of subadult American alligators (*Alligator mississippiensis*) and tracheal wash evaluation. *J Zoo Wildl Med*. 2005;36(1):12-20.

8. Diethelm G, Stauber E, Tillson M, Ridgley S. Tracheal resection and anastomosis for an intratracheal chondroma in a ball python. *J Am Vet Med Assoc*. 1996;209(4):786-788.

9. Drew ML, Phalen DN, Berridge BR, Johnson TL, Bouley D, Weeks BR, Miller HA, Walker MA. Partial tracheal obstruction due to chondromas in ball pythons (*Python regius*). *J Zoo Wildl Med*. 1999;1:151-157.

10. Summa NM, Guzman DS, Hawkins MG, Grosset C, Chen VS, Goldsmith D, Keel K, Woolard K, Young AC, Bucy DS, Steffey MA. Tracheal and colonic resection and anastomosis in a boa constrictor (*Boa constrictor*) with T-cell lymphoma. *J Herpetol Med Surg*. 2015;25(3-4):87-99.

Esophagus

11. Orós J, Torrent A, Calabuig P, Déniz S. Diseases and causes of mortality among sea turtles stranded in the Canary Islands, Spain (1998-2001). *Dis Aquat Org*. 2005;63(1):13-24.

12. Mitchell MA, Diaz-Figueroa O. Clinical reptile gastroenterology. *Vet Clin North Am Exot Anim Pract*. 2005;8(2):277-298.

13. Baehr PH, McDonald GB. Esophageal infections: risk factors, presentation, diagnosis, and treatment. *Gastroenterol*. 1994;106(2):509-532.

14. Bonacini M, Young T, Laine L. The causes of esophageal symptoms in human immunodeficiency virus infection: a prospective study of 110 patients. *Arch Intern Med*. 1991;151(8):1567-1572.

15. Smart JD, Dunkley S, Tsibouklis J, Young S. An evaluation of the adhesion of solid oral dosage form coatings to the oesophagus. *Int J Pharm*. 2015;496(2):299-303.

Lungs

16. Wunderink RG, Waterer GW. Community-acquired pneumonia. *N Engl J Med*. 2014;370(6):543-551.

17. Pees M, Kiefer I, Thielebein J, Oechtering G, Krautwald-Junghanns ME. Computed tomography of the lung of healthy snakes of the species *Python regius*, *Boa constrictor*, *Python reticulatus*, *Morelia viridis*, *Epicrates cenchria*, and *Morelia spilota*. *Vet Radiol Ultrasound*. 2009;50(5):487-491.

18. Pees MC, Kiefer I, Ludewig EW, Schumacher JP, Krautwald-Junghanns ME, Oechtering GU. Computed tomography of the lungs of Indian pythons (*Python molurus*). *Am J Vet Res*. 2007;68(4):428-434.

19. Pees M, Kiefer I, Oechtering G, Krautwald-Junghanns ME. Computed tomography for the diagnosis and treatment monitoring of bacterial pneumonia in Indian pythons (*Python molurus*). *Vet Rec*. 2008;163(5).

20. Pees M, Kiefer I, Ludewig E, Westphal S, Schmidt V, Krautwald-Junghanns ME, Oechtering G. Comparative use of modern imaging techniques for the diagnosis of pneumonia in three Indian pythons (*Python morulus*). *Tierärztliche Praxis Kleintiere*. 2006;34(4):275-282.

21. Davis MD, Walsh BK, Sittig SE, Restrepo RD. AARC clinical practice guideline: blood gas analysis and hemoximetry: 2013. *Respir Care*. 2013;58(10):1694-1703.

22. Raiti P. Administration of aerosolized antibiotics to reptiles. *Proc Annu Conf Assoc Rept Amph Vet*. 2002; 119-123.

Stomach

23. Mans C. Clinical update on diagnosis and management of disorders of the digestive system of reptiles. *J Exot Pet Med*. 2013;22(2):141-162.

24. Kubisch U, Fischer I, Hatt JM. Gastric ulcer in green iguanas (*Iguana iguana*). *Tierärztliche Praxis Kleintiere*. 2006;34(1):50-53.

25. Banzato T, Russo E, Finotti L, Milan MC, Gianesella M, Zotti A. Ultrasonographic anatomy of the coelomic organs of boid snakes (*Boa constrictor imperator*, *Python regius*, *Python molurus molurus*, and *Python curtus*). *Am J Vet Res*. 2012;73(5):634-645.

Thyroid

26. Rivera S, Lock B. The reptilian thyroid and parathyroid glands. *Vet Clin North Am Exot Anim Pract*. 2008;11(1):163-175.

27. Hernandez-Divers SJ, Knott CD, MacDonald J. Diagnosis and surgical treatment of thyroid adenoma-induced hyperthyroidism in a green iguana (*Iguana iguana*). *J Zoo Wildl Med*. 2001;32(4):465-475.

28. Frye FL. Biomedical and Surgical Aspects of Captive Reptile Husbandry. Malabar, FL: Krieger Publishing, Co.;1991.

29. Jacobson ER, Berry KH, Stacy B, Huzella LM, Kalasinsky VF, Fleetwood ML, Mense MG. Oxalosis in wild desert tortoises, *Gopherus agassizii*. *J Wildl Dis*. 2009;45(4):982-988.

Thymus

30. Fleming GJ, Heard DJ, Uhl EW, Johnson CM. Thymic hyperplasia in subadult Galapagos tortoises, *Geochelone nigra*. *J Herpetol Med Surg*. 2004;14(1):24-27.

31. Girard N, Ruffini E, Marx A, Faivre-Finn C, Peters S. Thymic epithelial tumours: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015;26(suppl 5):v40-55.

Parathyroid

32. Mundy G, Cove D, Fisken R. Primary hyperparathyroidism: changes in the pattern of clinical presentation. *Lancet*. 1980;315(8182):1317-1320.

Chelonian Conservation-Breeding: Health and Husbandry

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Session #325

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Abstract: Reptile health depends upon good husbandry, and the best captive care is based upon knowledge about each species' individual native microhabitat and seasonality. This masterclass will describe health and husbandry challenges and successes over the 10-year history of a large colony of threatened tortoise and freshwater turtle species at an Association of Zoos and Aquariums-certified facility. Descriptive data are presented at the population level as well as individual unique cases and outbreaks.

History

In 2004, John L. Behler (1943-2006), then curator of reptiles at the Bronx Zoo in New York City, sought out conservationist Eric Goode to ask whether he would be willing to provide a permanent home for the zoo's long-standing collection of endangered tortoises being kept at St. Catherine's Island in Georgia. Goode, a lifetime turtle enthusiast, rose to the challenge and transformed his Southern California retreat into the Turtle Conservancy, a non-profit facility certified by the Association of Zoos and Aquariums (AZA) and dedicated to protecting the most endangered turtles and tortoises and their habitats worldwide. In 2006, we named the new campus the Behler Chelonian Center to honor John's life of dedicated service to turtle and tortoise conservation.

Since that time, the Turtle Conservancy has become one of the most successful conservation-breeding centers in the world. We have hatched over 1,500 offspring from 21 different species, 99% of which are threatened with extinction, according to the Tortoise and Freshwater Turtle Specialist Group of the IUCN. Eighteen of the 44 species we have cared for over the years are critically endangered, 13 are endangered and 2 are vulnerable. Experienced turtle keepers would agree that several of these species are difficult to keep alive, let alone breed in captivity. Examples include the impressed tortoise (*Manouria impressa*), forest hinge-back tortoise (*Kinixys erosa*), Chaco tortoise (*Chelonoidis chilensis*), Okinawa leaf turtle (*Geoemyda japonica*), Madagascar flat-tailed tortoise (*Pyxis planicauda*) and all three subspecies of spider tortoise (*Pyxis arachnoides*). Such delicate species require a peaceful existence with minimal human contact to thrive, and we can focus on husbandry at the Turtle Conservancy, instead of attendance and entertainment like most zoos, because we are not open to the public.

Captive-Bred Animals

The Turtle Conservancy takes its mission seriously, protecting species from the threats that drive them toward extinction. Our animals come from other zoological institutions, wildlife authorities that have confiscated illegally trafficked animals around the world, and from private individuals who donate their animals for conservation. Our policy is to not buy wild-caught animals; therefore, we do not contribute to one of the greatest threats to endangered turtles and tortoises. We strongly encourage hobbyists and pet owners to demand captive-bred reptiles instead of supporting the global wildlife trade.

The number of pet turtles in the United States is growing, and commercial trade in wild-caught reptiles is one of the factors driving many species toward extinction. Alternatively, when you choose to purchase captive-born reptiles from local or regional breeders instead of buying wild-caught animals, you not only reduce pressure on wild populations, but if you breed them, you also might help prevent their extinction. The African spurred tortoise (*Centrochelys sulcata*) is just one example of how an endangered species can become one of the most common pet reptiles and be at lower risk of extinction because of the work of hobbyists and private breeders. Many other species might also be helped in this way, and the Turtle Conservancy is setting an example for the zoological community by offering a limited number of our captive-bred offspring to qualified private individuals for this very reason. All proceeds from these adoptions directly support our in range global conservation programs. Together, we can ensure these species live on.

International Conservation

The offspring we hatch at the Turtle Conservancy participate in programs that help save their species from extinction. Accordingly, the best possible outcome is to repatriate our captive-born offspring to their native habitats. This is more difficult than one might imagine, and amazingly, with the five yearling golden coin turtles (*Cuora trifasciata*) we sent to Hong Kong for a reintroduction program in January 2013, the Turtle Conservancy became the first organization to return foreign-born, captive-bred turtles to their native country for conservation.

We also share offspring with other conservation centers to create multiple assurance colonies around the world, providing a safety net in case of natural disasters, political uprisings or insufficient funding. For example, in May 2014 we sent Burmese star (*Geochelone platynota*) and Burmese black mountain tortoises (*Manouria emys phayrei*) to initiate colonies at Richard Branson's conservation center on Necker Island in the British Virgin Islands. In addition to these programs, a few of our offspring will remain in AZA institutions to satisfy the needs of their Species Survival Plan programs and ensure genetic variability in the professionally managed captive populations.

The Turtle Conservancy's in-country conservation programs include habitat protection and stewardship, turtle and tortoise reintroduction and education, curbing the illegal wildlife trade, and a seed grant program. Our work currently spans the globe and includes 14 countries. For example, we are protecting one of the last strongholds of the Critically Endangered geometric tortoise (*Psammobates geometricus*) in South Africa with an assemblage of adjacent tracts of intact fynbos ecosystem that now exceeds 800 acres and contains the largest known population of this species. We work with partners to protect the Critically Endangered ploughshare tortoise (*Astrochelys yniphora*) in Madagascar by engaging in community education programs and by marking the shells of wild tortoises with an indelible inscription to thwart poachers.

You Can Help

The Turtle Conservancy's work protecting species from extinction can only be sustained through generous public support, and even small donations help feed the animals and keep them healthy. Our goals are not just to be a last line of defense against extinction, like Noah's Ark, but also to actively refine husbandry techniques, enhance reproduction and protect native habitats to ensure that wild populations are self-sustaining.

What can you do? First, know the laws and report illegal animal trafficking activities to the authorities, especially the U.S. Fish and Wildlife Service. Second, buy reptiles that were captive-born in your region at facilities such as The Turtle Conservancy. Third, volunteer with local, regional and international programs that help protect

species from extinction. And fourth, donate generously to organizations that protect threatened species. Learn more about our work at TurtleConservancy.org and follow us on Twitter @turtletweets

On-Site Greenhouses

The Turtle Conservancy's formula for husbandry success relies heavily on knowledge about each species' unique natural history. We personally undertake field expeditions to the native range of species under our care to fill in the gaps in available scientific evidence regarding seasonal and daily variations in temperature, humidity, rainfall, fog, water sources, canopy, diet, shelter, substrates and activity. At our conservation-breeding center, we continually define and redefine the best possible husbandry techniques to provide species-specific microhabitat needs with a limited number of indoor environments and the Mediterranean climate of California's central coast. We provide as much outdoor time as possible with fresh air, sunshine and natural browse. Although we avoid mixing multiple species in a single enclosure, we do group species from similar climates together in the same building.

We have 2 greenhouses that provide excellent exposure to natural light cycles, but which can be challenging to heat and cool. One simulates a cloud forest and is kept very humid with moderate temperatures, 29°C (84°F) during the day, 22°C (72°F) at night, and the other simulates a lowland hot climate with moderate humidity and a broad diurnal temperature fluctuation, 32°C (90°F) day/20°C (68°F) night. Both employ multiple heating, cooling and moisture systems coordinated by an advanced climate control computer manufactured for commercial greenhouses.

The cloud forest environment primarily houses impressed tortoises and Okinawa leaf turtles. The hot lowland environment houses an array of species, including Sulawesi forest turtles (*Leucocephalon yuwonoi*), Forsten's tortoises (*Indotestudo forstenii*), spiny hill turtles (*Heosemys spinosa*), Arakan forest turtles (*Heosemys depressa*), Oaxaca wood turtles (*Rhinoclemmys rubida rubida*) and Burmese star tortoises, which spend much of their time outdoors.

Background heating systems include circulating warm water in the concrete floor and hot water radiators on the walls. Automated ridge vents open with cooling needs, and when temperatures really heat up, they close almost completely so fresh cool air can freely enter from the air conditioner in the cloud forest greenhouse or the commercial evaporative cooler in the lowland hot greenhouse. Horizontal shade-cloth curtains draw closed to reduce excess radiant heat on sunny days and at night to hold warmth inside. Humidity is maintained with commercial ultrasonic and centrifugal humidifiers.

We provide locally high humidity, condensation and artificial rain inside enclosures using irrigation or fine-mist systems to meet species-specific needs. Basking spots are provided using ultraviolet-B (UVB)-emitting mercury vapor lamps on cloudy days and in enclosures with insufficient sunlight. Some of the greenhouse panels are Acrylite OP-4 acrylic (CYRO Industries, Parsippany, NJ), which transmits some UVB radiation, and others are either Plexiglas or tempered glass, which both block nearly all UVB. We provide compact fluorescent UVB-emitting bulbs for non-basking species that are generally more active at dusk and for those from dense forests with little direct sunlight. Lights are timed to meet the individual needs of each species, including seasonality and basking behavior. The intensity of UVB emitted by each lamp is tested every six months using a meter with peak response at 290 nm, and lamps are replaced when UVB emission falls below 10 microWatts/cm^2 at the basking site.

Other Optimal Environments

In addition to the greenhouses, we constructed two semi-enclosed environments that we call cold frames, with removable translucent panels that constrain temperature extremes and manage humidity. The first of these simulates coastal dry zones with 90% relative humidity at night and 20% during the day. Species housed here include the Egyptian tortoise (*Testudo kleinmanni*), spider tortoise and ploughshare tortoise, which all retreat beneath shrubs or bushes at night and during the hot, dry hours of the day to take advantage of focal moderate humidity.

The second cold frame simulates southern hemisphere scrub habitats with generally cooler temperatures and low to moderate humidity. The species housed here include Chaco tortoises, parrot-beaked tortoises (*Homopus areolatus*) and angulated tortoises (*Chersina angulata*), which all retreat into subterranean burrows at night and during the heat of the day to find moderate humidity and temperature.

We also utilize two well-heated, well-insulated and biologically isolated houses to provide safe environments for hatchlings, animals under quarantine and the most temperature-sensitive species during the winter. These houses employ insulated wood-frame construction with screened windows and skylights that provide natural ambient light cycles and insect-proof ventilation.

In addition, there are four large tortoise houses for hardy species such as radiated tortoises (*Astrochelys radiata*) and Galapagos tortoises (*Chelonoidis nigra* complex). Circulating warm water in the floors and radiators is used for background heating, and basking sites are illuminated as needed. Materials and instruments such as pens, notebooks, scales, hoses, nozzles, scrub brushes, tools, mops, buckets and watering cans are dedicated to each house, and footbaths are used at all entrances and exits. Disposable, biodegradable nitrile gloves are used on individual enclosures, not just in these houses but throughout the center as part of the Turtle Conservancy's comprehensive biosecurity plan.

Ponds, pools and aquariums are managed with recirculating biological filtration with UVB sterilizers in the larger systems. One pond is approximately 110 cm deep and allows for outdoor hibernation of Pan's box turtle (*Cuora pani*). We monitor water quality parameters with a multi-test kit and perform partial water changes as often as twice a week in systems with numerous turtles.

The Casitas

Twelve small houses called casitas provide hardy outdoor species, such as radiated tortoises and Burmese black mountain tortoises, with a warm, dry space on cool nights and during brief periods of winter rain. These small houses are approximately 120 cm long, 90 cm wide and 75 cm tall. They are insulated with R-10 rigid foam insulation beneath the concrete slab and between layers of plywood on the walls, top and doors. Cracks are sealed with weather-stripping to prevent drafts, heat is provided by thermostat-controlled ceramic heat emitters, and daytime light is provided by timer-controlled compact fluorescent bulbs. Interior height is designed so electrical fixtures are high enough to protect the tortoises from thermal burns.

Bedding in the casitas consists of grass hay and rice hulls for radiated tortoises, and sphagnum moss for Burmese black mountain tortoises. We open casita doors in the morning and encourage the tortoises to exit the houses within a few hours. Flexible, translucent vinyl door strips are hung inside the doorframes to retain heat when a door is open on relatively cool days, and most tortoises pass through them readily.

Hatchlings and Juveniles

Six floor-to-ceiling incubators provide enough space for more than 500 eggs at one time. We incubate 130-150 clutches and hatch out 250-300 turtles and tortoises per year. Each incubator is set for temperatures that meet the needs of the species being incubated. High temperature incubators (29°C to 32°C) have a thermostat that allows them to cool down to 24-25.5°C at night, which simulates the daily fluctuations found in natural nests. Incubators for forest and temperate species are kept at relatively cooler temperatures (25-27.5°C) without daily fluctuations because the microhabitats in these regions have more constant diurnal temperatures.

Incubation protocols are based on the natural history of each individual species. Over time we refine them according to our successes and failures. For example, we started incubating parrot-beaked tortoise eggs in their natural nest in the substrate of the enclosure, and we hatched two of six eggs. Then we incubated the next eight eggs in an incubator at either 27.8°C or 30°C because other people reported success at those temperatures, but none hatched. With that new knowledge, we began incubating at 31.7°C; since then seven out of seven parrot-beaked tortoise eggs have hatched. The next step is to define what temperatures produce each sex.

We house juvenile tortoises in tables constructed from common lumber and marine-grade plywood. A table approximately 70 cm wide and 150 cm long can accommodate up to 30 hatchling tortoises with plates, dishes, plants and décor for hiding, browsing and environmental enrichment. We drill holes in the bottom for drainage and paint interior surfaces with floor epoxy to delay moisture damage. A hinged top of hardware cloth excludes predators, especially rats.

A table depth of 35 cm can accommodate a few inches of gravel, a layer of weed cloth and sufficient topsoil substrate for plants to root and tortoises to dig. We also provide a layer of rice hulls mixed with sand to allow juvenile Burmese star tortoises and spider tortoises to bury themselves. At night we cover outdoor tables with thermostat-controlled heating pads and insulated blankets whenever temperatures are predicted to be less than 18°C (65°F). We water the plants once or twice a week, and use a fine mist to cool and moisturize the air on very hot, dry days. We strategically place palm fronds to provide additional patches of shade on sunny days when ambient temperatures exceed 32°C (90°F) because the substrate in direct sunlight can reach in excess of 60°C (140°F).

Keeping records is a major part of managing such a large collection. Each specimen is photographed regularly because individual markings change with age. No identification system is perfect, so at the Turtle Conservancy we use redundant systems including radio frequency identification (aka PIT tags), marginal marks, color coding and painted numbers. Information is recorded in the International Species Information System database using the online software known as Zoological Information Management System. Each species is managed to meet reproductive and demographic needs of its conservation programs. Thefts have occurred at conservation breeding centers around the world, so we maintain a multi-layered security system including infrared motion detectors and video cameras with 24/7/365 monitoring.

Health Concerns in a Large Collection

The individual circumstances that allow pathogens to cause disease differ for each organism, and result from interactions among the environment, the pathogen, and the host. The study of these factors is a field known as "disease ecology." Species-appropriate environmental conditions are paramount for a healthy immune system in reptiles. An "emerging disease" has either appeared recently in a population, is rapidly increasing in incidence, changing in geographic range, or threatens to increase in the near future. Intranuclear coccidiosis, cryptospo-

ridium, ranavirus, and adenovirus are emerging infectious diseases of chelonians. Mycoplasmosis, herpesvirus, and systemic amoebiasis, though no longer emerging, do continue to be important causes of disease in collections. Other parasites include an array of nematodes, flagellated protozoa, and rarely, flukes (*Falcaustra* sp.) and pentastomids. In addition to infectious diseases, several non-infectious problems occur with some frequency. Necropsy is performed on every animal that dies at the Turtle Conservancy and the etiologic categories are shown for 473 mortalities with 70 histopathologic examinations in Table 1.

Table 1. Cause of death for 473 animals at the Turtle Conservancy, 2005-2015.

Etiologic category	Count
Parasites	177
Husbandry related	114
Developmental	34
Bacterial	13
Iatrogenic	8
Metabolic	8
Predator attack	6
Toxic	6
Unknown	106

Preventive medicine

Intestinal parasites can become pathogenic in captive animals because, even though many of the parasite species are not harmful in the wild, captive conditions increase the transmission rate, particularly for species transmitted via the fecal-oral route. Preventive measures are not intended to eliminate nematodes or protozoa, but to keep the intestinal load below the threshold of disease. The antiparasitic agents used most commonly at the Turtle Conservancy include benzimidazoles, metronidazole, and praziquantel.

Intranuclear coccidiosis of testudines

Intranuclear coccidiosis of Testudines (TINC) was first reported over 20 years ago and has been increasingly reported in numerous species in Europe, North America, and possibly Asia. It may be the most important disease currently affecting chelonians, and should be considered for every terrestrial chelonian with systemic illness or clinical signs involving multiple organ systems. Early diagnosis and treatment is essential, and PCR performed on conjunctival, oral/choanal mucosa, and cloacal tissue appears to be the most useful antemortem diagnostic tool. Effective treatment includes providing optimal environmental conditions and either ponazuril or toltrazuril 15 mg/kg PO q48h × 30 days. This prolonged treatment is necessary because these drugs have no effect on oocytes.

Cryptosporidiosis

Cryptosporidium-related disease has recently been described in a number of chelonians globally. Infection is sometimes associated with chronic diarrhea, anorexia, pica, decreased growth rate, weight loss, lethargy, or passing undigested feed. A consensus PCR followed by sequencing can be performed on feces to identify the species of *Cryptosporidium*. No treatments have been shown to clear infection, but paromomycin did eliminate clinical signs of disease in a group of *Testudo hermanni*. Concurrent infection with other pathogens may enhance disease progression.

Ranavirus

Ranaviral infection recently caused mass mortality events in wild North American box turtles (*Terrapene* spp.) and has been found in some captive chelonians that are allowed outdoors. Although the virus has been shown to be widespread in stable, healthy populations, when fulminant infection occurs it is often fatal. Clinical signs are similar to many other systemic diseases, and can include signs of upper respiratory disease, oral ulceration, cutaneous abcessation, subcutaneous edema, anorexia, and lethargy. Ranaviral infection is not acquired orally, and disease may require stress coupled with a vector or open wound. Clinically ill chelonians can be tested for ranavirus via PCR of oral and cloacal swabs, or whole blood.

Adenoviruses

Since 2009, adenoviruses have been identified as the cause of several mortality events in captive chelonians. Disease has involved liver, gastrointestinal tract, spleen, and brain. It affects young or immunocompromised animals such as the recent group of 105 illegally imported Sulawesi tortoises that all died with systemic *Siadenovirus* infection despite aggressive supportive care at several zoological institutions. PCR of cloacal swabs and plasma samples have shown promise for antemortem detection, although treatment has proven unsuccessful to date.

Mycoplasmosis

Upper respiratory tract disease (URTD) can be caused by a number of bacteria and viruses in chelonians. *Mycoplasma agassizii* was first described in 1995, and *M testudineum* was described in 2004. Both organism, as well as other species of *Mycoplasma* can be found in both healthy and ill tortoises. Clinical signs of disease appear with stress (e.g., territorial encroachment, habitat perturbation, relocation, capture) or poor environmental conditions (e.g., insufficient heat, poor nutrition, poor ventilation, overcrowding). Clinical signs of disease include nasal discharge, blepharal edema, and blepharoconjuctivitis observed as bubbly or wet nose, puffy eyelids, and reddened conjunctiva. Debris adhering to the rostrum is a common sign of nasal discharge. Pet tortoises are often presented for decreased appetite, most likely resulting from reduced sense of smell. Affected tortoises often wipe their rostrum with their forearms, which may be covered with wet or dry mucus, often with adherent debris or substrate. These clinical signs are not specific, and the list of differential diagnoses include foreign material (nasal/conjunctival), inhaled/aerosolized irritants, hypovitaminosis A, neoplasia, herpesvirus, ranavirus, opportunistic bacteria, and *Pasteurella testudinis*. Newly infected individuals may not exhibit clinical signs (prepatent period) for up to one year, and signs can persist for months to years. Mycoplasmal organisms are found in healthy tortoises, and many species rarely exhibit clinical signs of illness. In addition to North American tortoises, *Gopherus* spp., the most susceptible species include star tortoises (*Geochelone elegans* and *Geochelone platynota*), Chaco tortoises, *Indotestudo* spp., *Testudo* spp., and Russian tortoises (*Agrionemys horsfieldii*). Serology is available for some species and can help identify newly infected individuals, but chronically infected tortoises can be seronegative. Consensus PCR is the most useful test in a tortoise with clinical signs of illness. Treatment controls clinical signs of illness, but does not eliminate infection. Treat mild cases by retrograde nasal flush with 1:10 enrofloxacin:water (2.27% injectable solution:tap water) q24h for at least 3 days after clinical signs have resolved. More severe cases usually respond well to systemic clarithromycin or azithromycin. Some clinicians report that danofloxacin by injection also resolved clinical signs.

Systemic amoebiasis

Various amoebae normally reside in the intestinal tracts of turtles and tortoises as commensals. Some can become opportunistic pathogens, and *Entamoeba* can be an important pathogen under certain host and environmental conditions. *Entamoeba invadens* is most pathogenic at 25°C (77°F), and is inhibited at 16°C (61°F) and 33°C (91°F). Disease usually occurs within a week or two after a period of hypothermia, when the environmental

temperature of a reptile was not maintained in the optimal range. Clinical signs are nonspecific and include changes in daily activity patterns, spending more time than usual in the water dish, decreased appetite, and diarrhea. Death often occurs within a few days after the first subtle signs of illness occur. The infective stage of *Entamoeba invadens* is the quadrinucleate cyst, which is shed in the feces and can persist in the environment for extended periods. Ingested cysts develop into invasive trophozoites in the intestinal tract and can, like the cysts, can be seen under light microscopy. PCR is necessary to distinguish whether amoeba found on fecal examination should be treated or not. Typical gross postmortem signs of systemic amoebiasis include right sided to diffuse, grey-green, friable liver; fibrinous peritonitis, and petechial hemorrhages in the myocardium and kidneys. Cage mates of the deceased animal should be treated immediately with metronidazole (25 mg/kg PO q24h × 5 days) and broad-spectrum antibiotics because secondary bacterial infections are common. If appropriate for the species, the environmental temperature should be maintained at least 5-10°F above 25°C (77°F) during the treatment period. The environment of chelonians that have been diagnosed with a pathogenic amoeba infection should be thoroughly cleaned with hot (>65°C [150°F]), soapy water and disinfected with 1:5 household bleach:cool water solution for 20 minutes of contact time. Substrate, plants, cage furniture, and water dishes should be discarded.

Bacterial gastroenteritis/colitis

On occasion, bacterial colitis is diagnosed during the postmortem examination without identifying additional infectious agents. One reasonable explanation for bacterial infection of the gastrointestinal tract is an abnormally ineffective immune response, which allows normally commensal organisms to become pathogenic. Environmental conditions that may contribute to a weakened immune system include shipping, overcrowding, insufficient environmental resources (basking areas and shelters), and inadequate environmental complexity including tactile and visual components.

Non-infectious diseases

Non-infectious diseases at the Turtle Conservancy have included congestive heart failure in a Wolf Volcano Galapagos tortoise (*Chelonoidis nigra becki*), cloacal prolapse, beak overgrowth, and gout, both visceral and articular. Congestive heart failure, characterized by severe edema (anasarca), has been reported and one possible cause is an improper diet. The Wolf Volcano tortoise experiences extreme wet and dry seasons, so it is deprived of water under natural conditions for at least half of the year. Such water deprivation is rare in captivity, but could be an important physiologic need of this species.

Cloacal prolapse can occur in any species, and is most common in Burmese black mountain tortoises and radiated tortoises. In most cases the prolapsed cloaca can be reduced with granular sugar and steady digital pressure, and a purse-string suture around the vent is sufficient to prevent re-prolapse in the short term. A few cases have recurred more than one year after the first incident. Beak overgrowth, both rhinothecal and gnathothecal, is not rare in Burmese star tortosies, Burmese black mountain tortoises, and Chaco tortoises. Excessive beak can be trimmed in a manner similar to that employed with psittacine birds, using a stone bit on a high-speed rotary tool. Two of these tortoise species are known to eat a varied diet in the wild, and providing whole boiled eggs to the diet of Burmese black mountain tortoises and earthworms to the diet of Burmese star tortoises seems as if it might reduce or slow the reoccurrence of overgrowth. Gout is a somewhat enigmatic condition in reptiles. Young, growing Burmese star tortoises are particularly susceptible at the Turtle Conservancy, and visceral gout occasionally occurs together with cystic calculi.

Protozoal Parasitology for the Reptile Practitioner

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Session #220

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When is a parasite not truly a parasite? Rather often in the field of herpetological medicine. The definition of a parasite is "an organism that lives in or on another organism (its host) and benefits by deriving nutrients at the host's expense." Presence of one organism on or within another organism does not define it, as the key portion of the definition is that the parasite creates an expense to the host with its presence. Cases where significant virulence or innate pathogenicity is identified should pursue treatment. How, then, should a patient be treated if there is no clear direct relationship of an expense to the host caused by the secondary organism in question?

This form of organism cohabitation is one of the most common health issues in reptile medicine and it is not common to find cases so simple that presence or cohabitation equals disease. All aspects of the health and condition of the patient must be considered including a review of the specific organism of concern as well as an attempt to define how the invader is affecting the host. In an effort to meet the standard of care, this needs to be performed before a need for treatment can be determined, let alone select specific therapies.

Evidence-based-medicine is critical to herpetological medicine, and to apply it in these situations the practitioner needs to form appropriate questions to determine if there is virulence involved with an infestation. These questions might include:

- Is the protozoan identified as a species known to have pathogenicity in the species of reptile involved?
- Is there physical damage to the host?
- Does the protozoan disrupt the host's energy and nutritional balance?
- Are there changes in the host's immunologic system?
- Is the host affected in other ways, in other systems (hematologic, renal, etc)?
- Is the presence of the protist associated with behavior abnormalities in the host?

These questions often have no well-defined answer, or at least not easily obtained answers. The identification of parasite species is often not answered, and there have been very few studies of protist-host relationships in reptiles. To further compound clear answers, in many cases the identified protist is also considered normal flora and the pathology seen is a consequence of high protozoal population densities rather than simple presence within the host.

Determination of a protozoan inhabitant as a pathogen or as a commensal is the first step to appropriate treatment, and this is anything but a well-defined process. As pathogenicity is specifically an innate function of the agent (the "pathogen") to cause damage to the host organism, it does not necessarily apply in reptile/protozoal relationships. Virulence is a more accurate term; it implies that the pathology can be variable depending on other factors, and that the invader does not have an innate pathology. Both commensalism and opportunism fit well

under this definition. Thus, before a need for treatment can be assessed, the virulence of the identified protozoal agent needs to be determined, with a high virulence organism considered a parasite while a low virulence organism being commensal.

In reptilian host-protozoal relationships, the line between commensalism and parasitism often becomes rarefied. To have a true commensal relationship, there must be no detriment to the host organism, which is very uncommon and has been argued that it may not be possible. With alimentary protozoa, there may be situations where the organism is providing a benefit to the host (i.e. digestion of cellulose within the gut) and there is a net increase of positive effects to the host compared to the cost to the host by the inhabitation. However, the comparison between adverse effects and benefits is rarely direct, as increasing energy supply cannot be considered the opposite to the detriment caused by a vitamin deficiency. Thus, each situation must be examined independently as to the virulence displayed by the protist.

Superinfections appear to play a significant role in the virulence of protozoal flora in reptiles. In many species of reptiles, particularly herbivores and omnivores, protozoa live in limited, apathogenic relationships within the host's alimentary tract that benefits both the reptile as well as the protozoa. A superinfection would be defined by marked increases in protozoal population density. One method of the generation of superinfections is associated with repeated reinfections in less than a generation time frame. This occurs much more commonly in captive animals than in wild populations because of the nature of how these animals are housed. When animals are isolated in an enclosure in which they eat, drink, and defecate within a confined space, the result is that they ingest and repopulate their system with protozoa at higher densities than their wild counterparts would typically encounter.

Stress is another established etiology of superinfections, and it may be primary or in conjunction with reinfection rates. In any animal, changes in (captive) husbandry, poor environmental conditions, reproductive activity, seasonal cycles, underlying health conditions (infection, neoplasia, nutritional deficiencies), conspecific stress and competition, and many other factors all can cause immunosuppression and changes in metabolism. The corresponding physiologic changes can allow a commensal protist to replicate, cause pathology, and expand their tissue tropism, thus increasing their virulence. In short, when the populations of normal protozoa increase above a threshold, these organism's virulence damage the host, thus becoming parasites. Therefore, superinfections and the corresponding increase in virulence demonstrate the need for treatment despite not having an innately highly pathogenic organism as the root cause.

Determining a need for treatment must also involve evaluating the condition of the patient. It is exceptionally easy to identify a protist in a sample collected from a clinically healthy, normal patient, and decide that its presence equates a pathologic process of disease and thus make the decision for treatment. However, this approach must be questioned.

It is more difficult to examine a sample from a clinically ill reptile, identify the presence of protozoa, and determine whether or not the pathologic process is a result of said presence; however, as clinicians, we must be treating a patient, not a laboratory finding. This is the value of reviewing a complete anamnesis, having a good working knowledge of the natural history for that species, and selecting and properly executing appropriate diagnostic tools.

Although reptiles are well known for not showing overt symptoms in cases of disease, often the signs that the animal is presented for give us the clues that are needed to determine pathology. A thorough knowledge of a reptile's natural history, captive husbandry, behavior, and metabolism is necessary to effectively assess the health of these patients.

Ancillary testing can play a role in the determination of virulence and thus the need for treatment. Routine serum/plasma biochemistry testing is of limited value in specifically determining virulence, but can show changes in markers that indicate tissue damage. Furthermore, if the infestation is directly affecting specific organs, the biochemistry profile may reflect these effects.

Specific biochemical marker and function tests may be more indicative of damage to the host, and thus virulence. Serum electrophoresis has been demonstrated to provide more information on reptile immune system activity and can be an effective indicator of the effects of increased virulence. Urine gamma-glutamyl transferase (GGT) levels have been demonstrated to be a much more sensitive marker of early renal disease in birds and mammals and may be of similar value in reptiles. Serum and fecal biliverdin levels, while not commonly measured, may potentially be of higher diagnostic value in determining liver function than most other routinely run tests.

A complete blood count (CBC) can be very helpful in assessing virulence. Not uncommonly, a single CBC is performed on a clinically ill reptile patient and the results are reported as "within normal limits", causing many practitioners to put the value of hematologic evaluation low in their diagnostic plan. Granted, a single CBC performed at almost any time during the disease process has low diagnostic and prognostic value. However, the value of white blood cell assessments is in serial evaluations and monitoring trends and changes, rather than in a single static set of values. Additionally, specific cell type values that might be within reference ranges for a species can trend in directions that provide understanding of both pathologic processes as well as prognosis to the intuitive clinician.

Although non-invasive diagnostic techniques are preferential in most cases, adept reptile clinicians should pursue histopathologic diagnosis in complex, difficult and/or chronic cases. There are many excellent retrospective and review articles demonstrating virulence in protozoal infestations, and the results can be exceptionally useful both for planning treatment strategies as well as for understanding the prognosis of the patient.

Speciation of the purported pathogen may be necessary for effective management. If for no other reason, it may be necessary to determine if the protozoa found is an inhabitant or a pseudoparasite that is merely passing through the reptile's GI tract. Even true parasitism can be difficult to identify; *Sarcocystis* not only has sporozoites that can be difficult to identify, but an understanding of whether the reptile is playing the role of a primary or intermediate host, or if the organism is merely a pseudoparasite, needs to be assessed before appropriate treatment can be initiated. There are well-documented cases of reptile feces containing coccidian sporozoites that were parasites of their prey, but not known to inhabit the predator other than through GI transit.

Although most commonly associated with gastrointestinal infestation, protozoal parasites can inhabit and cause pathology in all tissues of a reptilian host. Treatment of protozoal parasites requires a careful identification of the parasite type, tissue tropism, associated pathology, and possible therapies. Therapeutic options must consider the overall health and condition of the animal as well as species sensitivities to particular medications, pharmacokinetics, and drug metabolism before appropriate therapy can be started. Every reptile clinician must take into account the entire host before selecting a chemotherapeutic agent.

Reptile Surgery and Endoscopy

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Session #155

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Abstract: Reptile surgery is a field in quick development. In recent years, new techniques have been developed for diagnosis and treatment of reptiles. In this masterclass we will illustrate current advances in surgical and endoscopic procedures in reptiles.

Skin Surgery

Reptile skin has the tendency to invert after incision, especially in squamates. Therefore, a slightly everting suture pattern (e.g., horizontal mattress) is recommended to ensure first intention wound healing. Healing of the skin can be accelerated if reptiles are maintained at the upper end of their preferred temperature range. Often, definitive skin healing with disappearance of the scab occurs after the first or the second ecdysis. Current recommendation for closure of coelomic breaches is to employ absorbable synthetic monofilament suture material (e.g., poliglecaprone 25, polyglyconate).

Surgery and Endoscopy of Chelonians

Aural abscesses

Aural abscesses are a common clinical presentation in aquatic turtles characterized by unilateral or bilateral swelling of the tympanic membranes. An association between aural abscesses and hypovitaminosis A has been proposed but could not be proven and the etiology of aural abscesses is more likely to be multifactorial. Surgical treatment consists of incision of the tympanic membrane and surgical debridement of the tympanic cavity under general anesthesia. A single vertical, two cross incisions, or a circular incision (180-360º) are made on the tympanum. By use of a cotton tip, debris and caseous material in the tympanum is gently removed. Culture and sensitivity testing may be performed from the debris or the tympanum. The tympanic cavity is lavaged, packed with an antimicrobial ointment and managed as an open wound to allow healing by second intention. Appropriate changes in management and diet are crucial to avoid recurrences.

Esophagostomy tube placement

Esophagostomy tube placement is typically recommended in anorectic chelonians, given the difficulty of oral administration of food and/or medication. Curved mosquito hemostats are inserted in the mouth and pushed on the side of the neck. Gentle pressure favors displacement of the jugular vein and carotid artery. A small skin incision is made over the tip of the mosquito exposing the external muscular layer of the esophagus. The esophageal wall is incised and the tip of the forceps is exposed. The feeding tube is grasped and passed through the incision and directed cranially. Once visualized through the mouth, the tube is gently curved and pushed into the esophagus towards the stomach up to the level of the predetermined length of the tube. A standard roman sandal suture

(syn. Chinese finger knot) with an optional purse-string pattern around the tube is performed. Confirmation of the proper positioning of the tube is usually not necessary, but may be performed by administration of contrast media through the tube.

Celiotomy

In chelonians, the two main approaches to the coelom are plastron osteotomy and prefemoral fossa coeliotomy. With the increased availability of laparoscopic and endosurgical equipment, prefemoral fossa coeliotomy has gained popularity due to the reduced invasiveness compared to plastron osteotomy. However, in certain species and for certain surgeries, plastron osteotomy is still required as it permits a better maneuvering of cranial coelomic organs.

Prefemoral fossa coeliotomy

Prefemoral fossa celiotomy (syn. prefemoral coeliotomy) is currently considered the surgical access of choice for most reproductive surgeries and for diagnostic endoscopy. In particular, in semiaquatic and aquatic turtles, this approach avoids prolonged post-surgical dry-docking, which is necessary following plastron osteotomy. It should also be considered for urinary bladder and intestinal surgery. This approach is particularly indicated in species with a relatively small plastron and in semiaquatic and aquatic species. The chelonian is placed in ventral, dorsal or lateral recumbency depending on species, size, and indications for surgery. The skin, subcutaneous tissue, and the transverse and oblique abdominal muscles, and coelomic membrane are transected and the coelomic cavity accessed. The use of ring retractors is extremely useful to enhance access and visibility. Care should be taken to avoid trauma to the urinary bladder upon entering the coelom. Cystocentesis should be performed if the bladder is distended and impairs surgical access to the coelom. Closure of the body wall should be performed in 2-3 layers. It may not always be possible to close the coelomic membrane. Closure of the muscles and skin are routine. If a substantial amount of subcutaneous tissue is present, then additional subcutaneous sutures should be considered. The healing times after prefemoral coeliotomy (approximately 4 weeks) are substantially shorter than after a plastron osteotomy (approximately 1 to 2 years). In aquatic and semi-aquatic chelonia, early return to the aquatic environment is critical to allow normal behaviour, food intake and defecation. The prefemoral approach significantly shortens the post-surgical duration, during which time the turtle must be maintained out of water. Therefore, the prefemoral approach should be considered the preferred surgical approach to the coelom in aquatic and semi-aquatic chelonian species.

Plastron osteotomy

The size of the plastron osteotomy (syn. plastrotomy) is dependent on the indication for surgery (e.g., size of eggs, bladder stones, GI foreign bodies, etc.) and is limited cranially by the heart and caudally by the pelvic girdle. The animal is placed in dorsal recumbency. Various instruments (e.g., a rotary tool equipped with a cutting circular blade, an oscillating sagittal saw, etc.) may be employed to create the incision into the plastron. Three sides of the flap should be incised at a 45-degree angle to obtain slightly beveled incisions. The fourth side is only partially incised and is used as a hinge. A periosteal elevator is used to complete the three full-thickness incisions of the plastron and to elevate the flap. The flap is reflected cranially or caudally and covered with moist gauze. The coelomic membrane is visualized and a ventral midline incision is performed taking care to avoid the ventral abdominal veins. After plastron osteotomy, the coelomic membrane is gently sutured with a fine monofilament absorbable suture in a simple interrupted or continuous pattern. The lack of suturing of the coelomic membrane has been suggested to be associated with increased risk of postoperative adhesions. The bony flap is repositioned and sutured (in young or demineralized chelonians) or stabilized by means of epoxy resins, fiberglass mesh, or metal plates and screws. Plastron osteotomy is generally associated with prolonged surgical procedure time, prolonged recovery and is thought to be significantly more painful compared to the prefemoral soft tissue ap-

proach. The plastron flap may become a sequestrum and provide temporary protection to the developing bone. Eventual post-surgical complications are usually serious, require prolonged treatment and include: lack of revascularization of the bone flap and consequent necrosis, infection with consequent coelomitis, and dehiscence of the bone flap margins. A further limitation of the technique is the need for dry-docking of aquatic turtles. Even with application of a fiberglass patch postoperative leakage may occur, leading to infection. Healing times of the bone flap are variable, but are considered to be around 1 to 2 years.

Endoscopy-assisted prefemoral oophorectomy and salpingectomy

A technique for exteriorization and excision of ovaries and oviducts of chelonians through the prefemoral fossa has been described. The technique relies on the assistance of standard endoscopic equipment (e.g., 2.7-mm, rigid endoscope). Depending on species, size and individual morphology, oophorectomy, salpingectomy, and/or salpingotomy may be performed through the prefemoral approach. Chelonians are preferably placed in dorsal recumbency, although ventral, or lateral recumbency may be indicated in particular instances (e.g., unilateral egg dystocia). A rigid endoscope is introduced into the coelom following a standard prefemoral coeliotomy approach. The reproductive tract is identified and gently grasped with atraumatic grasping forceps preferably by an avascular connective area of the ovary. Care must be taken to avoid rupture of ovarian follicles. Once all ovarian follicles are exteriorized and the mesovarium is visible, the ovarian vasculature in the mesovarium is ligated and transected. Hemostasis and complete excision of the ovarian tissue should be confirmed with the endoscope. If the oviduct is diseased and surgical removal is intended, the ipsilateral oviduct is exteriorized, ligated and transected through the same prefemoral incision. The procedure is then repeated for the contralateral ovary and oviduct. Often the procedure may be performed for the contralateral ovary via the same prefemoral incision. However, performing salpingectomy or salpingotomy of the contralateral oviduct is usually not possible, and coelomic access is required through the contralateral prefemoral fossa. Retained eggs in the oviduct and ectopic eggs free within the coelom can also be removed using this technique.

Cystotomy

In general, to resolve the presence of cystic calculi or ectopic eggs in the urinary bladder in chelonians, the surgical procedure should be elected in the following order: (1) Transurethral endoscopic retrieval, mechanical destruction (eggs) or lithotripsy (calculi); (2) Pre-femoral fossa coeliotomy and cystotomy; (3) Standard coeliotomy and cystotomy. Removal of uroliths through a prefemoral fossa approach is not always feasible. In a case series of 10 desert tortoises (*Gopherus agassizii*) good candidates for the prefemoral fossa approach were large tortoises (over 15 cm in carapace length), with small calculi (less than twice the length of the fossa) that did not have a laminated radiographic appearance. Note that in the pre-operative evaluation, radiographs, computed tomography, ultrasound and cystoscopy are essential to reach a definitive diagnosis. In rare instances, (e.g., neoplasms, chronic prolapses of the urinary bladder) partial cystectomy may be indicated.

Prefemoral fossa orchiectomy

Prefemoral endoscopic orchiectomy has been recently described in turtles and tortoises. Briefly, the testis is visualized and grasped, distending the mesorchium. Ligation clips or radiosurgical instruments are employed for hemostasis and dissection of the ligament. The testis is removed while the epididymis is left in situ. Orchiectomy may be also performed through the prefemoral fossa approach without endoscopic guidance. An incision is performed in the prefemoral fossa and the musculature is dissected. Urinary bladder, intestine and lungs are manipulated in order to expose the testes. The testis is grasped using a curved hemostat, the spermatic cord is dissected and ligation clips are placed around the spermatic cord and testicular artery. The spermatic cord and the testicular artery are dissected distal to the ligation clips. The same technique may be performed under endoscopic assistance with exteriorization of the testes. The testis is visualized through prefemoral fossa coelioscopy.

The testis is grasped and exteriorized. Ligation clips or radiosurgery are employed to excise the mesorchium preventing bleeding. The epididymis is controlled with the endoscope for hemorrhages. The coelomic access is routinely closed.

Cystoscopy in chelonians

Historically, cloacoscopy and cystoscopy have been used for evaluation of intrinsic disorders of the cloaca, urinary bladder, and accessory bladders. More recently, a novel diagnostic application of cystoscopy has been described utilizing the transparency of the organ to visualize the coelom. The value of cystoscopy as a diagnostic tool relies on two main features of the chelonian urinary bladder, the large size and its transparency. Chelonians may be positioned in dorsal or ventral recumbency for cloacoscopy. Chelonian cystoscopy is indicated for chelonians with a non-specific illness, as an initial screening technique to identify the diseased organ. It can be used in chelonians with dystocia, in order to screen for ectopic eggs in the bladder and chelonians with uroliths, for diagnosis and treatment, and in chelonians that underwent trauma, to evaluate bladder integrity, as well as in selected species of chelonians lacking sexual dimorphism or for selected immature chelonians, in order to identify the sex.

Technique: A rigid or flexible endoscope is gently inserted in the vent and directed cranially in order to reach the cloaca. The cloaca is a sac-like cavity into which the ureters, gonadal ducts, colon, and bladder(s) empty. In some aquatic turtles, paired sac-like structures, the accessory bladders, open into the cloaca dorsal to the colon. The endoscope, once in the cloaca, is directed toward the urethral opening, which is located ventral to the rectum. Access to the urethral sphincter is gained by gentle ventral pressure. Warm (30°C) fluids are infused (1 drop every 3-4 seconds) during the entire procedure and to allow distension of the urethral opening. As an alternative, air can be insufflated. Care should be paid to avoid over-distension of the bladder in smaller chelonians. Gentle pressure and fluid infusion are usually sufficient to gain access to the urinary bladder.

Coelioscopy in chelonians

A standard left-sided approach is performed positioning the animal in right lateral recumbency and holding it by means of a vacuum positioning aid or sand bags. The left hindlimb is retracted and taped caudally in order to expose the left prefemoral fossa. Surgical scrub is performed on the prefemoral fossa and the chelonian is draped. A 3- to 4-mm skin incision is performed in a cranio-caudal direction in the skin in the center of the prefemoral fossa. The subcutaneous fat and connective tissues are bluntly dissected and the coelomic membrane is gently stabbed with a hemostat. The endoscope is inserted at a 30-45º angle and directed cranially to visualize the liver, gallbladder, stomach, pancreas, duodenum, heart. The testes (or ovaries), epididymides and adrenals are visualized by dorso-caudal direction of the endoscope. The kidneys lay in the retroceolom and are visualized after dissection with an endoscopic forceps or with laser of the coelomic membrane, right after the epididymis in male chelonians. Diagnostic samples can be obtained by use of an endoscopic biopsy forceps. After inspection of all the organs of interest, the coelom is gently sutured with an interrupted pattern and the prefemoral skin is routinely sutured.

Surgery of Squamates

Phallectomy

Male snakes and lizards have paired copulatory organs (i.e., hemipenes) that lie in respective sacs caudal to the cloaca in the ventral tail. Phallectomy is employed to resolve hemipenile disorders. Hemipenile prolapse is a

very common presentation. If the tissue is already necrotic or the prolapse is recurrent, amputation is required. Amputation is performed *in toto*, as hemipenes do not contain the urethra. In squamates, amputation of a single hemipene does not preclude reproduction, while in chelonians phallectomy precludes reproduction. The reptile is positioned in dorsal recumbency and the hemipene is surgically prepped. In small individuals, two transfixing ligatures are placed at the base of the hemipene. The tissue is excised distal to the ligatures. The hemipenile stump can be closed with a simple continuous or a purse-string suture, in particular in larger species.

Limb amputation

Indications for amputation of limbs in lizards and chelonians are severe trauma or infection, severe joint infections, which are refractory to treatment, as well as non-healing chronic fractures or neoplasia. In general, chronic infected distal limb wounds and joint infections, in particular with associated osteomyelitis usually do not respond to medical therapy and wound management, and instead limb amputation should be considered. Amputation of the forelimbs should be performed through the scapulohumeral joint and amputations of the hindlimbs through the coxofemoral joint. Midshaft amputation of the humerus or femur are not recommended, since it is very likely that the remaining limb stump will be traumatized by attempted ambulation. In female reptiles used for breeding purposes or planned to be released in the wild, amputation of the hindlimb might interfere with successful reproduction, since digging of nesting sites may be impaired. Limb amputation is performed using the same techniques as in mammals. Complications are uncommon.

Tail amputation

Amputation of an infected, traumatized or necrotic tail is a common problem in lizards. Surgical amputation of the tail is recommended in order to achieve primary wound healing following surgical resection of the diseased distal tail and closure of the amputation site. Radiographs should be taken prior to surgical amputation in order to evaluate for underlying bone involvement. Amputation of a significant portion of the tail in arboreal lizards (e.g., green iguanas, *Iguana iguana*), has substantial effects on their ability to balance and climb. Therefore, owners should be informed that the enclosure may require adjustment following tail amputation and that changes in locomotion are possible. The patient is anesthetized and placed in ventral recumbence. The tail amputation site is surgically prepped and a tourniquet is placed. The location of the hemipenes should be considered in cases of proximal tail amputation. Symmetric wedge incisions of the skin are made on lateral aspects of the tail in lizards with laterally flattened tails (e.g., iguanas) or on the dorsal and ventral aspect in lizards with dorso-ventrally flattened tails (e.g., bearded dragons, *Pogona* spp.). The skin incision should be made distal enough to allow for tension free wound closure following amputation, but at the same time be proximal enough to avoid incomplete excision of diseased tissue. The soft tissue and bone are transected. The ventral tail vein may require ligation in larger lizards. The amputation site is lavaged and assessed for hemorrhage following release of the tourniquet. The muscles should be apposed over the vertebrae with simple interrupted sutures. The skin edges are trimmed and closed with horizontal mattress sutures. The amputation site can be bandaged for the first few days following surgery, in order to aid in absorption of wound discharge. Suture removal is recommended 4-6 weeks following amputation. In species that performs tail autotomy, tail amputation can be performed exploiting the natural line of fractures on the vertebrae by use of quick manual latero-lateral movements. Regrowth of the tail is possible after surgical amputation, but not commonly seen.

Coeliotomy

Surgical approaches to the coelom greatly vary depending on the species. Appropriate preparation of the reptile patient for coeliotomy is mandatory. Sterile scrub brushes may be employed to provide effective cleaning of reptiles. Once anesthetized, the patient is instrumented, placed in the recumbency indicated for the surgical pro-

cedure and surgically prepped. In general, the size of the incision will depend on the indications for coeliotomy. During the opening of the coelomic membrane, the surgeon should watch for signs of free gas or liquid in the coelom, associated with gastrointestinal (GI) tract and urinary bladder perforation, respectively. After entering the coelom, all of the organs that are visible should be carefully inspected.

Lizards: In lizards, there are three main approaches to the coelom including paramedian, median and flank approaches. The paramedian and median approaches are generally indicated in lizards that are dorsally compressed (e.g., families *Iguanidae, Agamidae, Gekkota*). In chameleons and other lizards that are laterally compressed (e.g., basilisks), the coelom may be easily approached through the flank. The paramedian approach is generally preferred over the median approach to avoid the ventral midline abdominal vein, a large vessel that runs just over the linea alba. An incision of the skin is made parallel to the midline. The incision may be made with a scalpel blade or dissecting devices (e.g., lasers, radiosurgical and electrosurgical equipment). In a study conducted on green iguanas, radiosurgery and laser both produced bloodless incisions, but radiosurgery caused significantly less collateral tissue damage in the skin and the muscle. The distance of the incision from the midline will depend on the size of the lizard. The incision is extended cranially and caudally using scissors, taking care to avoid the ventral abdominal vein. In some instances, the ends of the incision may need to be extended at a 90º angle, forming a sort of "L" or "H". The skin is retracted and the musculature is dissected by use of blunt scissors, cotton-tipped applicators, laser or electrosurgical equipment. Sharp dissection of the musculature should be avoided in order to minimize bleeding.

Snakes: In snakes, a paraventral coeliotomy is indicated for most surgeries. Obviously, a single incision does not permit access to all the organs. Therefore, an incision needs to be made at the level of the organ of interest. The skin is incised between the first and second row of lateral scales, and the resulting surgical wound is scalloped. This incision avoids distortion of the ventral scutes once the skin is sutured in an everting pattern.

Closure of the coelom in snakes and lizards: In squamates, the coelomic membrane and muscular wall are fragile and do not hold sutures. Therefore, closure of the coelom relies on the skin sutures. In small to medium sized squamates, the coelomic membrane, muscularis and subcutis are sutured together in a simple continuous suture pattern with monofilament absorbable sutures. The skin is sutured with fine monofilament nonabsorbable sutures (e.g., nylon) in an everting pattern as previously discussed.

Cystotomy

Cystotomy may be indicated for removal of cystic calculi and ectopic eggs from the urinary bladder. In recent years alternative techniques to routine cystotomy, in particular in chelonians, have been reported, which are less invasive. These less-invasive techniques include transurethral endoscopic techniques (cystoscopy) or prefemoral endoscopy-assisted cystotomy. However, if such approaches fail (e.g., when dealing with cystic calculi of large size) cystotomy may be required. Access to the coelom is routinely performed through a paramedian incision in lizard. In small-to medium species, manipulation and exteriorization of the urinary bladder should be reduced to the minimum, due to the risk of trauma of the thin bladder wall. Stay sutures on the urinary bladder wall and moist gauze around the surgical site minimize urine leakage into the coelom. In lizards, a longitudinal incision is made in the ventral aspect of the bladder. The bladder stones or eggs present in the urinary bladder are removed. Retrograde use of an endoscope may be useful to ascertain the removal of all the material. The urinary bladder wall is closed with a monofilament suture in a single or double inverting layer. A rounded, atraumatic needle and minimal traction are mandatory to avoid rupture of the urinary bladder wall during suture in small-to-medium sized reptiles.

Nephrectomy

In snakes, neoplasms or degenerative disorders of the kidneys are not uncommon, and unilateral nephrectomy is associated with an acceptable prognosis if the remaining kidney is functional. Often, a swelling of the caudal third of the snake is present. Standard coeliotomy is performed at the level of the affected kidney. The contralateral kidney should be grossly inspected. The affected kidney is isolated from the surrounding tissue. The renal vein and the renal arteries are ligated. The ureter is ligated distally and the kidney removed. A unilateral gonadectomy may need to be performed as the vas deferens in males and the oviduct in females are in contiguity with the kidney.

References

1. Bronson E, Pereira M, Sanchez C, et al. Iridophoroma in a veiled chameleon, *Chameleo calyptratus*. *J Herp Med Surg*. 2006;16:58-60.

2. Di Girolamo N, Mans C. Reptile soft tissue surgery. *Vet Clin North Am Exot Anim Pract*. 2016;19:97-131.

3. Funk RS. Early neutering of male green iguanas, Iguana iguana—an experiment. *Proc Annu Conf Assoc Rept Amph Vet*. 2000;147-149.

4. Govett PD, Harms CA, Linder KE, et al. Effect of four different suture materials on the surgical wound healing of loggerhead sea turtles, *Caretta caretta*. *J Herp Med Surg*. 2004;14:6-11.

5. Hannon DE, Garner MM, Reavill DR. Squamous cell carcinomas in inland bearded dragons (*Pogona vitticeps*). *J Herp Med Surg*. 2011;21:101-106.

6. Innis CJ, Feinsod R, Hanlon J, et al. Coelioscopic orchiectomy can be effectively and safely accomplished in chelonians. *Vet Rec*. 2013;172:526.

7. Innis CJ, Hernandez-Divers S, Martinez-Jimenez D. Coelioscopic-assisted prefemoral oophorectomy in chelonians. *J Am Vet Med Assoc*. 2007;230:1049-1052.

8. Mans C, Sladky KK. Diagnosis and management of oviductal disease in three red-eared slider turtles (*Trachemys scripta elegans*). *J Small Anim Pract*. 2012;53:234-239.

9. Mans C, Sladky KK. Endoscopically guided removal of cloacal calculi in three African spurred tortoises (*Geochelone sulcata*). *J Am Vet Med Assoc*. 2012;240:869-875.

10. McFadden MS, Bennett RA, Kinsel MJ, et al. Evaluation of the histologic reactions to commonly used suture materials in the skin and musculature of ball pythons (*Python regius*). *Am J Vet Res*. 2011;72: 1397-406.

11. McFadden MS. Suture materials and suture selection for use in exotic pet surgical procedures. *J Exot Pet Med*. 2011;20:173-181.

12. Myers DA, Wellehan JF Jr, Isaza R. Saccular lung cannulation in a ball python (*Python regius*) to treat a tracheal obstruction. *J Zoo Wildl Med*. 2009;40:214-216.

13. Selleri P, Di Girolamo N, Melidone R. Cystoscopic sex identification of posthatchling chelonians. *J Am Vet Med Assoc*. 2013;242:1744-1750.

14. Smith DA, Barker IK, Allen B. The effect of ambient temperature and type of wound on healing of cutaneous wounds in the common garter snake (*Thamnophis sirtalis*). *Can J Vet Res*. 1988;52:120-128.

Getting to Grips with the Reptile Environment

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Session #184

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Abstract: The majority of reptile diseases are either directly caused or predisposed by poor captive management. Many aspects of captive husbandry have a direct effect on the health and welfare of reptiles. Unfortunately, welfare legislation for this group of vertebrates in the U.S. lags behind many other nations. This roundtable provides an opportunity for the presentation and discussion of various issues facing the provision of appropriate environments for reptile patients.

Introduction

The majority of reptile disease presentations are either directly or indirectly caused or exacerbated by poor husbandry and nutrition.[1-3] In addition, poor environment has both immediate and delayed effects on health and welfare.[4,5] A veterinarian has little chance of successfully treating reptiles if he/she does not appreciate the importance of the environment, and the modern approach to providing such.[6]

Species

The importance of identifying the species (and subspecies) cannot be overemphasized. In certain cases, even locality may cause diet and environmental preferences within the same species. Different species from different geographic locations must never be mixed. Ideally, only a single species should be kept in any enclosure, and care must be exercised to avoid competition for resources such as food, basking areas and retreats. Some snakes may eat cagemates (e.g., kingsnakes), many male lizards are territorial and aggressive (e.g., leopard geckos, bearded dragons), while some species (*Chameleo* spp) are so territorial that individual isolation is often essential.

The Vivarium Enclosure

The type of enclosure (arboreal, terrestrial, subterranean and aquatic) should be appropriate for the species. Arboreal species (e.g., green iguana, *Iguana iguana*; emerald tree boa, *Corallus caninus*) prefer to move in a vertical plane while terrestrial species (e.g., skinks, *Tiliqua* spp; rat snakes, *Elaphe* spp) prefer a more down to earth existence. The size of the enclosure is also important and although many breeders and retailers may be able to intensively manage stock the author prefers to advise clients on minimum enclosure sizes and the importance of providing the largest enclosure possible. There are very few guidelines available for minimum space requirements.

Vivarium construction

Glass aquaria are commonly used but the greater visualization perceived as an advantage to the owner may serve as a stressful reminder of open exposure and poor security to the inmate. Glass is a poor insulator and greater heat loss may lead to dramatic temperature fluctuations. Even if the entire top of the enclosure is covered by mesh, ventilation may be severely reduced due to a lack of ventilation along the sides and back wall. Plastic-coated wooden, plastic or fiberglass enclosures are popular because they are more versatile, permitting the incorporation of additional ventilation panels, front access via sliding glass doors and greater security for the inhabitants.

Cage furnishings

In most cases what is placed inside the vivarium is for the aesthetic enjoyment of the owner rather than meeting the biologic and ethologic demands of the reptile. Particulate floor substrate is often changed infrequently due to their relative high cost. Some materials may cause contact dermatitis, acute stomatitis or intestinal impaction is ingested. The provision of hideouts or retreats are often overlooked and when provided may be too spacious to provide adequate security. Newspaper, Astroturf and commercial organic particulates (e.g., bark chips) are suitable materials, but they must be replaced regularly. Soil, sand, natural leaf litter can also be used but care is required to maintain hygiene and not to introduce disease. Sterilization of such material can be easily accomplished by baking in an oven. Gravel and pebbles are not recommended as they are difficult to clean and often ingested. Other essential items include a water bowl (large enough for reptile to bathe), and secure branches (non-toxic fruit trees) for arboreal species.

Vivarium hygiene

It is an unfortunate fact that many owners will decide that it is time to clean the vivarium once a foul odor becomes obvious, and therefore poor hygiene. Some reptiles will naturally excrete, or can be trained to excrete in one area. Effective hygiene requires the removal of organic debris prior to disinfection. All chemicals, even if considered safe, should be thoroughly rinsed away before replacing the reptile(s).

The Captive Environment

Captive environments may be considered as tropical, temperate, desert, rainforest, scrubland etc. These ecosystems can be notoriously difficult to reproduce in captivity. In certain areas the local weather may permit the keeping of reptiles in outdoor enclosures for at least some of the year. Theft, predators and wildlife carriers of disease should be considered.

Heating

There are a variety of heaters that may be employed including incandescent bulbs, infrared ceramics, heating pads or mats, warming cables, tubular heaters, radiators, convector heaters and natural sunlight radiation. From a clinical perspective there are several factors that must be considered. Is the heater screened from the animal to prevent burns? Is the heating equipment sufficient to provide the preferred optimum temperature zone (POTZ) for the reptile? Is the heating provision suitable to the species in question? Most arboreal species thermoregulate in a vertical plane and therefore heating should be positioned in the roof of the enclosure. Terrestrial species will tend to move in a more horizontal plane and so all heating should be concentrated at one end and not evenly distributed throughout the length of the vivarium. Finally, captive aquatic environments seldom permit the establishment of a water temperature gradient but acceptable water temperatures should be provided using

a dedicated double-insulated water heater. Electrical cords must be protected from the powerful bites of aquatic chelonians as electrocution is not only fatal to the kept but presents serious threats to the keeper as well.

Temperature control: High wattage heaters must be controlled as at times only a fraction of their output may be required to maintain the desired thermal gradient. Many thermostats have the ability to carefully regulate temperature using a dimming system of gradual wattage reduction. Thermostats with nighttime drop facilities are useful to create a natural diurnal temperature fluctuation. Thermostat failure can be disastrous, especially if the power to the heater(s) is maintained after failure.

Temperature gradient: Although the temperature readings on modern-day thermostats are generally trustworthy, it is still advisable to measure the temperature gradient within the enclosure using a dual read-out digital thermometer, preferably with memories for maximum and minimum temperatures. A single temperature is meaningless in most cases; the thermal gradient within the vivarium is what is important. The daytime basking temperature (hottest daytime temperature), daytime air temperature gradient and nighttime air temperature gradient should be elucidated during the consultation; however, be aware that many owners will reel off the known requirements for their particular species without actually having measured the temperature with an accurate thermostat. It is important not to lead the client when asking husbandry questions, nor to force an inaccurate answer.

Environmental Lighting

Most snakes do not have any significant requirement for broad or full-spectrum lighting; however, many diurnal lizards and chelonia do require quality light and in particular wavelengths of around 295 nm (UVB) for the production of vitamin D_3 that plays an essential role in calcium metabolism. There is also some indication that natural light has behavioral and psychologic benefits. By far the best source of quality full-spectrum lighting is unfiltered sunlight; however, there is an increasing number of fluorescent and even LED lights commercially available but the spectral quality varies dramatically between different makes. More worrisome is that several lights specifically marketed as full-spectrum lights for reptiles are anything but, and this can create confusion for both veterinarians and clients alike. In general, for basking species the use of mercury halide lamps can provide light and heat up to a distance of 1 meter. Fluorescent strip-lights, compact fluorescents, and LEDs that specifically indicate a significant UVB output can provide light (without significant heat) over shorter distances of up to 30 cm.

Most transparent plastic and glass barriers filter out UVB wavelengths and therefore placing a reptile in a greenhouse or conservatory is not acceptable unless specialized UVB-transmitting materials are used. In order to determine whether the lighting is suitable for a diurnal reptile, the owner must be quizzed as to the exact photoperiod, age, position, make and model of light being used within the vivarium. In many cases, captive lighting is deficient in one or more of these facets.

Humidity

This is often an overlooked aspect of the captive environment with even the most enthusiastic pet reptile keeper failing to install a hydrometer within the vivarium. The importance of humidity is also poorly appreciated, for example, the high incidence of renal disease in adult iguanas may, in part, be related to chronic dehydration from prolonged inadequate humidity. Humidity is seldom directly controlled, although the advent of dedicated humidifiers and sprinkler systems is changing this perception. Instead humidity tends to be a function of tem-

perature, water surface area and ventilation. The major problem in most cases is the view that ventilation must be decreased to increase humidity. Such actions result in stagnant air and an increase in bacterial and fungal infections, especially of the integument and respiratory system. Far better to maintain ventilation but maintain humidity by providing a greater water surface area (shallow water containers, water drip systems, sprinkler systems, regular spraying) or an increase in water temperature by placing a heat mat under a shallow water container.

References

1. Divers SJ. Reptiles: Husbandry, history taking and clinical examination. *In Pract*. 1996;18:51-65.

2. Mader DR. *Reptile Medicine and Surgery.* Second ed. Philadelphia, PA: WB Saunders Co;2006.

3. Raiti P, Girling S. *Manual of Reptiles.* 2nd ed ed. Cheltenham, England: British Small Animal Veterinary Association;2004.

4. Warwick C, Arena P, Lindley S, Jessop M, Steedman C. Assessing reptile welfare using behavioral criteria. *In Pract*. 2016;35:123-131.

5. Warwick C, Frye FL, Murphy JB. *Health and Welfare of Captive Reptiles.* London:Chapman & Hall;2004.

6. Barten SL, Fleming GJ. Current herpetological husbandry and products. In: Mader DR, Divers SJ, eds. *Current Therapy in Reptile Medicine and Surgery*. St Louis, MO: Elsevier;2014:2-12.

Overview of Reptile Clinical Pathology

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Session #185

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Abstract: Reptile clinical pathology is as much an art form as it is a science! Indeed, when you start correlating your pathology and histopathology findings to hematology and plasma chemistry changes you might be tempted to throw in the towel. This roundtable focuses on the appropriate collection, processing, and interpretation of samples, and specifically tries to bring some of the recent research to light.

Introduction

The accumulation of species-specific reference ranges continues to be slow for most reptiles. Unfortunately, most publications fall short of the standardized requirements necessary to establish true reference ranges, and some fail to take into account the significant effects of gender, age, season, reproductive status, diet and feeding schedules.[1] As zoological practitioners we must adhere to the established procedures in domestic animal clinical pathology, particularly regarding consistent collection, handling and laboratory practices, and deviate only when proven necessary to do so.

Despite the recent interest and reviews in reptile clinical pathology, much detailed data can still only be found outside of the veterinary literature.[2,3] Given the frequency with which the clinician may be forced to evaluate a clinical pathology report without an appropriate reference range, referring to some of these older but detailed chapters can still be rewarding.[4-6] Due to necessary space constraints, this chapter is an abbreviated summary, and reference to more detailed clinical pathology texts is suggested.[2,3,7,8]

Sample Collection

The blood volume of reptiles varies between 4 and 8% of bodyweight, and it is generally accepted that 10% of total blood volume (i.e., 0.4-0.8 ml/kg) can be safely collected from healthy reptiles.[9] Lymphatic contamination is a distinct possibility during reptile venipuncture. Complete or selective hematologic and biochemical evaluations are possible but the accuracy of results is often adversely affected by poor venipuncture, handling or laboratory skills. The production of poor quality blood data can be useless or even worse, misleading to the clinician. Venipuncture is generally a blind technique in reptiles and as a general guide up to 0.4-0.5 ml/100 g may be safely collected from any healthy reptile – less in debilitated animals. The jugular vein of certain tortoises may be visible particularly following temporary caudal occlusion of the vessel but in any case anatomic knowledge of the position of veins is vital. It is wise to aseptically prepare the venipuncture site, as infection and abscessation following venous access is a potential complication. Given the variability in published ranges, more reliance should be placed upon establishing an individual's observed range and using serial sampling to monitor the progress of hematologic and biochemical changes rather than relying on a single result. The following descriptions indicate the most humane and practical venipuncture sites.

Snakes

The two common sites for venipuncture in snakes are the caudal (ventral tail) vein and the heart. The caudal vein is accessed caudal to the cloaca, between 25 and 50% down the tail. It is wise to avoid the paired hemipenes of males (that may extend up to 14-16 subcaudal scales down the tail), and the paired cloacal musk glands of females (that may extend up to 6 subcaudal scales). The needle is angled at 45-60^{o} and positioned in the ventral mid line. The needle is advanced in a craniodorsal direction, while maintaining slight negative pressure. If the needle touches a vertebral body it is withdrawn slightly and redirected more cranially or caudally. This vessel is most easily entered in larger snakes and lymphatic contamination is possible. With the snake restrained in dorsal recumbency, the heart is located approximately 22-33% from snout to vent. Sedation is preferred. The heart is palpated and immobilized. The needle is advanced at 45^{o} in a craniodorsal direction into the apex of the beating ventricle. Blood often enters the syringe with each heartbeat. It is wise to maintain digital pressure for 30-60 seconds following this technique. This technique appears to be safe in snakes of all sizes and is less likely to result in lymphatic contamination. It has been employed in snakes of all sizes from 10-g neonates to 100-kg constrictors. Nevertheless, good restraint is essential if significant cardiac trauma is to be avoided.

Lizards

The most clinical useful vessel is the caudal (ventral tail) vein. The needle is positioned in the ventral midline between 20%-80% down the tail and advanced at 45-90^{o} in a craniodorsal direction, while maintaining slight negative pressure. If a coccygeal vertebra is encountered the needle is withdrawn slightly and redirected either further craniad or caudad. An alternative approach that is particularly useful for the larger or more ventrodorsally compressed species, is to insert the needle from the lateral midline. The needle is advanced at 45-90^{o} in a craniomedial direction, aiming just ventral to the lateral processes of the coccygeal vertebrae. Lymphatic contamination is more likely with the lateral approach. Lizards possess a large ventral abdominal vein that runs within a suspensory ligament just below the linea alba. This vessel is most easily entered in the mid-coelomic region. The needle is positioned in the ventral midline and advanced in a craniodorsal direction. It is difficult to apply pressure to this vessel, which makes post-venepuncture hemorrhage a potential complication.

Tortoises, turtles and terrapins

The most clinically useful vessels are the jugular, subcarapacial, and dorsal coccygeal veins. The left and right jugular veins are preferred because of the greatly reduced risk of lymphatic contamination. The regional anatomy varies with species but the vessel is generally located laterally and may even be visible if temporarily occluded by digital pressure at the base of the neck. The needle is positioned caudal to the tympanum, and directed in a caudal direction. Post-venepuncture pressure should be applied to prevent hematoma formation.

A subcarapacial site is also available and formed by the venous communication between the most craniad intercostal vessels arising from the paired azygous veins and the caudal cervical anastomosis of the left and right jugular veins. This sinus can be accessed with the chelonian's head either extended or retracted, making it useful for uncooperative or aggressive individuals. Depending upon the species and conformation of the carapace, the needle may be bent up to 60^{o} and positioned in the mid-line just caudal to the skin insertion on the ventral aspect of the cranial rim of the carapace. The needle is advanced in a caudodorsal direction maintaining slight negative pressure. If a vertebra is encountered the needle is withdrawn slightly and redirected further craniad or caudad. Lymph contamination appears to be a common complication. The dorsal coccygeal vein is probably the most commonly employed of the tail veins. The needle is angled at 45-90^{o} and placed, as craniad as possible, in the dorsal mid line of the tail. The needle is advanced in a cranioventral direction while maintaining slight negative pressure. If the needle encounters a vertebra it is withdrawn slightly and

redirected more craniad or caudad. The exact position, size and even presence of this vessel may vary between species and there is a significant risk of lymphatic contamination.

Crocodilians

The most appropriate venepuncture sites are the caudal (ventral tail) vein in small to medium crocodilians and supravertebral vein in medium to large specimens. The supravertebral vein is approached with the needle positioned in the dorsal midline, just caudal to the occiput and perpendicular to the surface of the skin. The needle is slowly advanced while maintaining slight negative pressure. Excessive penetration may lead to spinal trauma. The technique for caudal vein access is as described for lizards. The heart is located on the ventral midline, approximately 11 scale rows caudal to the forelimbs. Cardiocentesis in crocodilians is not as safe as it is in snakes because the heart cannot be stabilized.

Sample Handling

Traditionally, the anticoagulant of choice has been heparin because of the perceived risks of lysis caused by EDTA; however, studies have demonstrated the superiority of EDTA in numerous species of lizards and snakes, and even some chelonians.[10,11] Therefore, species-specific anticoagulant preferences should be considered. A fresh blood smear should be made at the time of venipuncture using blood that has not been contaminated by anticoagulant (i.e., the last drop in the syringe). Of the various techniques that have been employed to create blood smears coverslip-to-slide and bevel-edge slide technique have been found to create a preferred monolayer with reduced numbers of smudge cells.[12] Blood for hematologic evaluation should ideally be processed within 1 hour. Delays can be accommodated by refrigerating the sample, but deterioration is inevitable. Blood smears should not be refrigerated as water condensation on the glass may damage cell morphology. Blood should never be frozen as this will result in cell lysis.[13] For biochemical analyte determinations, heparin is generally preferred over serum because (a) it results in a greater amount of sample per unit of blood, (b) can be processed immediately without waiting for 30-60 minutes or more for clot formation, and (c) prevents the formation of fibrin clots that can interfere with many automated analyzers. A recent study that evaluated the differences between serum and plasma in green iguanas demonstrated that statistically significant differences did exist between certain analytes.[13] Total protein, albumin, and globulin levels were all higher with plasma compared to serum. In addition, AST was also higher with plasma, but ALT was lower. The paper concluded that these differences were unlikely to be clinically important and that plasma provided reasonable approximations for serum biochemical values. Small blood tubes containing lithium heparin with plasma separators are especially useful in reptile practice as they come in a variety of sizes, as small as 400 µl.

Hemolysis is known to cause changes in biochemical results and clinicians should be vigilant to evaluate for any hemolysis before laboratory submission. In the green iguana, moderate hemolysis increases phosphorus levels, while marked hemolysis significantly increases phosphorus, potassium, total protein and AST.[13]

Samples should ideally be processed immediately; however, when delays of 1-48 hours are anticipated, plasma should be separated immediately from the cellular fraction, and kept refrigerated or, in field conditions, kept on ice. If delays exceed 2 days, then freezing at -70ºC will preserve most chemical constituents.[14] Frozen-thawed plasma should be thoroughly mixed prior to processing.

References

1. Friedrichs KR, Harr KE, Freeman KP, et al. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Vet Clin Pathol.* 2012;41(4):441-453.

2. Campbell TW. Clinical pathology. In: Mader DR, Divers SJ, eds. *Current Therapy in Reptile Medicine and Surgery*. 2nd ed. St Louis, MO: Elsevier;2014:70-92.

3. Campbell TW. Hematology of reptiles. In: Thrall MA, ed. *Veterinary Hematology and Clinical Chemistry*. London: Lippincott Williams and Wilkins;2004:259-276.

4. Dessauer HC. Blood chemistry of reptiles: physiological and evolutionary aspects. In: Gans C, Parsons TS, eds. *Biology of the Reptilia*. Vol 3. *Morphology C*. London: Academic Press;1970:1-72.

5. Girons MCS. Morphology of the circulating blood cells. In: Gans C, Parsons TS, eds. *Biology of the Reptilia*. Vol 3. *Morphology C*. London: Academic Press;1970:73-92.

6. Duguy R. Numbers of blood cells and their variation. In: Gans C, Parsons TS, eds. *Biology of the Reptilia*. Vol 3. *Morphology C*. London: Academic Press;1970:93-110.

7. Strik NI, Alleman AR, Harr KE. Circulating inflammatory cells. In: Jacobson ER, ed. *Infectious Diseases and Pathology of Reptiles - Color Atlas and Text*. London: Taylor and Francis;2007:167-218.

8. Campbell TW. Clinical chemistry of reptiles. In: Thrall MA, ed. *Veterinary Hematology and Clinical Chemistry*. London: Lippincott Williams and Wilkins;2004:493-498.

9. White FN. Circulation. In: Gans C, Dawson WR, eds. *Biology of the Reptilia*. Vol 5. *Physiology A*. London: Academic Press;1976:275-334.

10. Hanley CS, Hernandez-Divers SJ, Bush S, Latimer KS. Comparison of dipotassium EDTA and lithium heparin on hematologic values in the green iguana (*Iguana iguana*). *J Zoo Wildl Med.* 2004;35(3):328-332.

11. Mayer J, Knoll J, Innis C, Mitchell MA. Characterizing the hematologic and plasma chemistry profiles of captive Chinese water dragons, *Physignathus cocincinus*. *J Herpetol Med Surg.* 2005;15(3):45-52.

12. Perpinan D, Hernandez-Divers SM, Hernandez-Divers SJ. Comparison of three different techniques to produce blood smears from green iguanas, *Iguana iguana*. *J Herpetol Med Surg.* 2006;16(3):99-101.

13. Finkelstein AD, DiGesualdo CL, Hoover JP. Comparison of biochemical profile values from paired plasma and serum samples of green iguanas, *Iguana iguana*, and red-tailed boa constrictors, *Boa constrictor*. *J Herpetol Med Surg.* 2003;13(3):5-9.

14. Weiser G. Sample collection, processing, and analysis of laboratory service options. In: Thrall MA, ed. *Veterinary Hematology and Clinical Chemistry*. London: Lippincott Williams and Wilkins;2004:39-44.

Preventive Health Habits of Effective Herpetoculturists

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Roundtable Discussion Points: ExoticsCon 2016

1. Redefining success in herpetoculture: Cultural value of reproductive success over longevity and low incidence of preventable diseases. “Big box breeding” and concerns for individual health.

2. Teaching your clients to manage their collection like a zoo:

- Record-keeping for trend recognition
- Establishing a quarantine protocol
- Taking parasites seriously
- Building a good relationship with your vet
- Dealing with death in the collection; the value of necropsies
- Risk management in husbandry techniques