Section 23

ARAV Master Classes

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Clinical Approach to Tortoises and Turtles

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Session #081

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Abstract: Chelonians remain one of the most popular types of reptiles kept in captivity, whether it be as a companion animal or a zoo/wildlife exhibit. Their characteristic shell can be a hindrance to veterinary evaluation and medical/surgical intervention. This masterclass focuses on the veterinary approach to these interesting animals and includes an introduction to the species, relevant anatomy and physiology, physical examination, sedation and anesthesia, diagnostic imaging, diagnostic sample collection and therapeutic routes for drug delivery including central line placement.

Introduction

Tortoises, turtles and terrapins are vertebrates with similar organ systems to mammals. However, they are ectothermic and rely on environmental temperature and behavior to control their core body temperature. They possess both renal and hepatic portal circulations, and predominantly excrete ammonia, urea, or uric acid depending upon their evolutionary adaptations (Table 1). They possess nucleated red blood cells and their metabolic rates are lower than those of mammals. Like all reptiles they exhibit ecdysis – a normal process by which the outer integument is periodically shed throughout life. Most are diurnal but may be crepuscular and secretive in their habits. The species require broad spectrum light for psychological and, in the case of UVB (290-300 nm), vitamin D3 synthesis and calcium homeostasis. Fertilization is internal, and females produce eggs (oviparous). Most species exhibit temperature-dependent sex determination during egg incubation. The longevity of some selected species have been cited but the true life-span of most species have not been determined, largely because deficiencies in captivity invariably lead to premature death while good captive care without predators or exposure to disease can result in extreme geriatric ages of over 100 years. This article focuses on certain veterinary aspects of tortoises, and more complete reviews are listed in the references.[1-4] In addition, space restrictions prevent the inclusion of basic anesthesia and surgery details which will be covered in the lecture.

Table 1. Relative nitrogenous waste products excreted by different chelonians.

	Relative % excretion		
Taxa	Ammonia	Urea	Uric acid
Aquatic turtles	20-50	20-50	0-10
Semi-aquatic turtles	5-15	40-70	15-25
Rainforest tortoises	5-10	30	60-70
Desert tortoises	<5	10-30	50-70

Common Species

Of the over 8000 species of reptiles, there are only around 300 species of chelonians (tortoises, turtles and terrapins). However, almost half of these are threatened or endangered, while several have become popular pet species, or revered exhibit animals. Captive breeding is common and the chelonian pet market is active, therefore, veterinarians are frequently asked to provide professional services to this unique group on vertebrates.

European species (*Testudo* species)

- Spur-thighed tortoise (*T. graeca*) – Mediterranean countries, North Africa, and the Middle East.
- Hermann's tortoise (*T. hermanni*) – Southern Europe
- Horsfield's tortoise (*T. horsfieldii*) – Afghanistan to north-western China, through southern Russia, Iran, and Pakistan.
- Egyptian tortoise (*T. kleinmanni*) – Critically endangered; smallest tortoise in the northern hemisphere.
- Marginated tortoise (*T. marginata*) – Greece, Italy and the Balkans in southern Europe; Largest European tortoise, reaching a weight of up to 5 kg

Box turtles (*Terrapene* species)

- Ornate box turtle (*T. ornata*) – Omnivores with a very varied diet; Midwest US to the Gulf of Mexico, and from Louisiana to Colorado
- Eastern box turtle (*T. carolina*) – Omnivores with a very varied diet; South-Central, Eastern and South Eastern parts of US, and Mexico

Miscellaneous species (*Geochelone* species)

- African spurred tortoise (*Geochelone sulcata*) – Sahara Desert and North Africa; 60-90 cm, 45 - 91 kg
- Red-foot tortoise (*Geochelone carbonaria*) – South American forests; plants, vegetables and fruits
- Galapagos tortoise (*Chelonoidis nigra*) – Galapagos islands; largest living species of tortoise, reaching weights of over 400 kg
- Radiated tortoise (*Astrochelys radiata*) – Native to Madagascar; critically endangered
- Aldabra tortoise (*Aldabrachelys gigantea*) – Islands of the Aldabra Atoll in the Seychelles

Anatomy and Physiology

Tortoises are characterized by a hard shell, formed dorsally by the carapace and ventrally by the plastron. The shell is composed of bony plates overlaid by offset keratin scutes, and each area has a specific name. Tortoises possess a common cloaca which receives the lower gastro-intestinal, reproductive, and urinary tracts. In addition,

lungs are simpler and composed of vascular pockets, more like a cavitated sponge than alveoli. The internal organs are not dissimilar in distribution but separated by two thin membranes. The heart is located within a cardiac membrane, while the lungs are dorsad and separated from the remaining viscera by a post-pulmonary membrane (or septum horizontale). Chelonians have complete tracheal rings, and males possess a single copulatory phallus.

Tortoises rely on environmental temperature and behavior to maintain their body temperature within their preferred optimum temperature zone (POTZ). Within, this species-specific POTZ, a tortoise is able to achieve the preferred body temperature (PBT) for specific metabolic activities, which may vary diurnally and seasonally, and by age and gender. The metabolic rate of reptiles is lower than for mammals and birds, and consequently the k constant in determining energy expenditure, nutritional requirements and even calculating allometric drug doses are related to the equation; Basal Metabolic Rate (BMR) = 10 x W $kg^{0.75}$ kcal/day.

The chelonian heart is composed of 3 anatomical chambers but arguably 5 functional divisions; left and right atria, and a single ventricle formed by the cavae venosum, arteriosum, and pulmonale. Despite the single ventricle, oxygenated and deoxygenated blood are kept functionally separate and there are both pulmonary and systemic circulations (similar to mammals). Peripheral blood cell types include thrombocytes, erythrocytes, heterophils, eosinophils, basophils, lymphocytes, and monocytes (including azurophils). Renal and hepatic portal circulations exist.

The skin varies by location from thin and keratinized to think and heavily armored by large scales. The chelonian shell composed of both dermal bone plates and epithelium. Reptiles do not have extensive skin glands and their skin is essentially dry. All reptiles shed their skin. The frequency of ecdysis is dependent upon species, age, nutritional status, environmental temperature and humidity, reproductive status, parasite load, hormonal balance, bacterial/fungal skin disease, and skin damage.

Most species require some form of conditioning prior to breeding (eg, hibernation or seasonal cooling of temperate species). Sexual dimorphism in chelonians is usually obvious in adults with males often having a concave plastron and a longer tail. Overzealous and unrelenting males may ardently pursue females causing repeated harassment. Fertilization is internal, and reproduction is oviparous (hard shelled eggs). Gender determination is related to incubation temperature, often with elevated temperatures producing males and lower temperatures females.

Physical Examination

Small to medium tortoises (eg, *Testudo* species) are not difficult to handle, although their strength and uncooperative nature can hinder the examination and cause frustration. A little patience with the tortoise head held down often persuades a shy individual to protrude the head from the shell, at which time the thumb and middle finger can be placed behind the occipital condyles to prevent retraction of the head back into the shell. However, in the larger species (eg, leopard tortoise, *Geochelone pardalis*), keeping a strong individual from pulling free may be physically impossible. In such cases, sedation or the use of a neuromuscular blocking agent may be necessary.

Steady distractive pressure to the maxilla and mandible can open the mouth, and once the mouth is open, the index finger (in small gentle specimens) or a mouth gag (in larger specimens) can be inserted into the mouth to prevent closure. This method enables the handler to keep the mouth open with one hand, leaving the other free to examine the head and take samples for laboratory investigation. Examination of the head should include the nostrils for any discharges and the beak for damage and overgrowth. The eyelids should be open and not obviously distended or inflamed, and the eyes should be clear and bright.

Conjunctivitis, corneal ulceration, and opacities are frequent presentations. The retina can often have degeneration as a consequence of freezing during hibernation, and ophthalmic examination is warranted in any anorectic animal. The tympanic scales should be examined for signs of swelling associated with aural abscessation. Verification of a tympanic abscess can often be made with observation of exudate emanating from the eustachian tube openings within the lateral walls of the pharynx. The integument should be free of damage that is often caused by aggressively courting males and subcutaneous swellings that are usually abscesses. The buccal cavity must always be examined, particularly for evidence of inflammation, infection, gout, and foreign bodies. Stomatitis can quickly lead to a generalized esophagitis, and examination down the pharynx and into the esophagus with a rigid endoscope is advisable. Note should also be made of the mucous membrane coloration, which is normally pale pink. Hyperemic membranes may be associated with septicemia or toxemia. Icterus is rare but may occur with biliverdinemia from severe liver disease. Pale membranes are often observed in cases of true anemia. Pale deposits within the oral membranes may represent infection or urate tophi associated with visceral gout. The glottis may be difficult to visualize, being positioned at the back of the fleshy tongue; however, one must check for any inflammation and glottal discharges that may be consistent with respiratory disease.

The withdrawn limbs can also be extended from the shell of small to medium chelonians with steady traction. The coelomic space within the shell is restricted, and therefore, gently forcing the hindlimbs into the shell often leads to partial protrusion of the forelimbs and head, and vice versa. The more aggressive species, especially the terrapins and turtles, should be held at the rear of the carapace. Larger species can deliver an extremely powerful bite, and so great care is necessary at all times. Certain species also possess functional hinges at the front or back (or both) of the plastron or carapace, and care should be exercised not to trap a finger when the hinge closes. A wedge or mouth gag can be used to prevent complete closure of a hinge, and no chelonian will close a hinge on its own extended limb. The integument should be examined for parasites, particularly ticks and flies; dysecdysis; trauma; and infection that may arise from rodent, dog, or wildlife attacks. Aggressive conflicts and courting trauma must also be considered in the communal environment. Limb fractures are less commonly reported in chelonia but are often caused by rough handling, with a greater incidence reported in those individuals with secondary nutritional hyperparathyroidism. Focal subcutaneous swellings are usually abscesses, but grossly swollen joints or limbs are more often cases of fracture, osteomyelitis, or septic arthritis.

The prefemoral fossae should be palpated with the chelonian held head-up. Gentle rocking of the animal may then enable the clinician to palpate eggs, cystic calculi, or other coelomic masses. The shell should be examined for hardness, poor conformation, trauma, and infection. Soft poorly mineralized shells are usually a result of secondary nutritional hyperparathyroidism from dietary deficiencies of calcium, excess phosphorus, or a lack of full spectrum lighting. Pyramiding of the shell, historically, has been linked to dietary excesses of protein, although the cause may be multifactorial it appears to be primarily linked to environmental humidity. Shell infection may present as loosening and softening of the scutes with erythema, petechiae, purulent or caseous discharges, and a foul odor. Deep infections usually involve the bones of the shell and cause osteomyelitis.

Prolapses through the vent are obvious, but determination of the structure involved is necessary. Prolapses may include cloacal tissue, shell gland, colon, bladder, or phallus. Internal examination with digital palpation and an endoscope is recommended. Male chelonians can be differentiated from females by their longer tails and the position of their cloaca caudal to the edge of the carapace. Other sexually dimorphism characteristics may also be obvious, including the concavity of the male plastron in many species.

Anesthesia and Sedation

Regional anesthesia and local block

Inadequate knowledge of precise nerve positions is probably responsible for the current lack of regional nerve blocks in reptiles; however, nerve locators may help alleviate this problem.[5] Certainly the judicious use of local anaesthetics or spinal blocks have been used, and although inadequate for major surgery, they may be useful for decreasing general anaesthetic requirements.[6,7] Recently, an epidural technique was developed that was effective for permitting field-based phallectomy of hybrid Galapagos tortoises (*Geochelone*), and has subsequently been investigated in red eared sliders (*Trachemys*).[8] The author used 1ml lidocaine per 25 kg injected into the dorsal tail into the coccygeal spinal cord to induce complete relaxation of the cloaca and phallus. Mild hindlimb paresis was noted in some animals but resolved within 2-3 hours.

Anesthesia with injectable drugs

Intravenous or intraosseous propofol (3-10 mg/kg) or alphaxalone (5-10 mg/kg) provides a rapid, controlled mode of induction.[9] They are relatively non-toxic and there is reduced risk of thrombophlebitis if injected perivascularly - this is of particular concern since intravenous access may be relatively difficult, especially in active animals undergoing elective procedures. Alphaxalone also has the added advantage of being effective when administered intramuscularly (10-20 mg/kg).[10]

If intravenous access is impractical or dangerous to attempt, intramuscular agents can be used to induce sufficient chemical restraint for intubation. For intramuscular injections in lizards and chelonia the forelimb musculature is preferable, whilst for snakes, the epaxial muscles are used. Recently, an intramuscular combination of ketamine (10-30 mg/kg), medetomidine (0.1-0.2 mg/kg) or dexmedetomidine (0.05-0.1 mg/kg), and morphine (1.5 mg/kg) or hydromorphone (0.5 mg/kg) has proven effective for a variety of chelonians, and can be readily reversed using atipamezole (0.5-1 mg/kg), and, if necessary, naloxone (0.2 mg/kg).

Anesthesia with volatile agents

Isoflurane or sevoflurane are the agents of choice for maintenance of anesthesia. These volatile agents have faster modes of action, are more controllable, and facilitate faster recoveries than most alternatives. Furthermore, their lack of reliance on hepatic metabolism or renal excretion further reduces the anaesthetic risk to debilitated reptiles or those with questionable renal or hepatic function. Prolonged breath holding is common with chelonians and crocodilians, and gas induction is not recommended. Intubation of conscious patients has been suggested following local lidocaine spray, but cannot be recommended given the stress and risks of trauma to reptile and staff.

Many chelonians have a powerful bite and a strong mouth gag is required for protection during intubation and throughout anesthesia. For most pet reptiles, small gauge endotracheal tubes or catheters are inserted through the glottis immediately caudal to the tongue; this may be aided by forcing the tongue up and forward by pressing a finger into the intermandibular space from under the jaw. The reptilian glottis is actively dilated, and therefore its movement will often be abolished once anesthetized. A guiding stylet can therefore be useful in facilitating endotracheal tube placement. The bifurcation of the trachea may be sited far craniad in some chelonia and therefore a short uncuffed endotracheal tube should be used and securely taped into position.

Ventilation

Chelonians lack a functional diaphragm, instead relying on limb movements for ventilation. The action of these muscles is abolished at surgical anesthetic planes, and intermittent positive pressure ventilation is essential for all reptiles that are anesthetized for prolonged periods. Ventilation rates should initially mirror pre-anesthesia evaluations, and then adjusted to maintain end-tidal capnography readings of above 10 mmHg, and ideally 15-25 mmHg. The use of electrical ventilators enables precise and consistent ventilation rates and pressures to be maintained which has removed some of the variables associated with manual ventilation and unstable anesthetic depths. Large reptiles are prone to ventilation-perfusion mismatch if placed into lateral or dorsal position, and therefore it is wise to achieve a surgical plane of anesthesia before surgical positioning.

Monitoring

Monitoring anesthesia can be very different compared to that of mammals. Palpebral and corneal reflexes are generally reliable. Corneal reflexes are abolished at excessive depth, while pupillary diameter may bear little relation to the depth of anesthesia (unless fixed and dilated which indicates excessive anesthetic depth or brain anoxia and death). Jaw tone and withdrawal reflexes (tongue, limb or tail) are useful, becoming abolished only at a surgical plane. This also correlates with full loss of righting reflex, loss of spontaneous movement, and complete muscle relaxation. Cloacal tone is lost at excessively deep levels. Temperature should be monitored as metabolism of drugs is directly related to core temperature with decreases commonly associated with protracted recoveries. Pulse oximetry, using either an oesophageal or cloacal reflectance probe is useful for monitoring pulse rate, and strength. In addition, although the spO_2 readings are often low and have not been conclusively validated for reptiles, monitoring the trend in spO_2 is often helpful. Doppler is often more reliable than pulse oximetry when placed over the carotid or aimed towards the heart. End-tidal capnography has proven useful, and should be maintained above 10 mmHg and ideally between 15-25 mmHg. Excessive ventilation and low $ETCO_2$ readings tend to correlate with a delayed return to spontaneous respiration and slower recoveries.[9]

Arterial lines are difficult to place in reptiles and require a surgical cut-down procedure. A recent study in green iguanas has demonstrated poor correlation between indirect and direct blood pressure measurements in reptiles.[11] The same researchers also concluded that only norepinephrine (0.3-0.5 mcg/kg/min) resulted in a significant increase in blood pressure when used in hypotensive iguanas (unpublished data). Blood gas estimations are often affected by intracardiac or pulmonary shunts, especially in aquatic species.

Recovery and post-operative care

Towards the end of surgery, the anesthetic gas should be discontinued while maintaining ventilation for a further 5-10 minutes to facilitate drug excretion. At this point, oxygen should be discontinued in favor of ventilation using room air delivered by an ambu(lance) bag as this will help stimulate spontaneous respiration. Once breathing spontaneously, the reptile can be extubated, and returned to an incubator to fully recover. Continued monitoring remains essential until righting reflexes return and the animal is ambulatory. It is not unusual for a recovering reptile to revert back to unconsciousness and apnea. Additional analgesia and fluid support should be provided as indicated.

Diagnostic Imaging[12]

Radiography

Anesthesia facilitates accurate positioning and better diagnostic films. For vertical beam dorsoventral radiographs, most conscious individuals will remain motionless long enough to permit exposure. Ideally, the head and limbs should be extended from the shell in order to reduce superimposition of the limb musculature on the coelomic viscera. For lateral horizontal beam radiographs the chelonian is best placed on a central plastron stand. By lifting the animal clear of the ground the limbs and head will be encouraged to extend but the tortoise will remain immobile. Both left and right lateral projections should be taken with the lateral edge of the shell touching (or as close as possible to) the cassette. The third basic coelomic view is the horizontal craniocaudal (or anterior-posterior) view. Again the chelonian is positioned on a central plastron stand, with the caudal edge of the carapace touching (or as close as possible to) the cassette, with the head facing the x-ray tube and the beam centered on the midline of the cranial rim of the carapace.

Radiology of the head and limbs will require their exteriorization from the shell and this will require general anesthesia. The use of sandbags, foam, and tape will aid positioning. Standard interpretation requires that orthogonal views should always be taken. Even slight rotation makes interpretation difficult.

Ultrasonography

Ultrasound has gained popularity, particularly with regard to examining tissue parenchyma, guiding biopsy needles and, with color flow doppler, investigation of cardiac disease. The giant species will require a 5MHz probe while a 7-14 MHz probe will suffice for most pet animals. When dealing with very small specimens (or for the ultrasound examination of eyes) a 20+MHz probe is more appropriate, but increasingly expensive. Good contact and imaging generally require copious quantities of gel or a water bath. It is helpful to try and maintain the animal in a normal position or, failing that, at least appreciate the complications associated with organ displacement. When using a water bath, variable stand-off distance is easily achieved but, without a bath, a suitable stand-off and copious gel may be required. There is no doubt that the interpretation of a two-dimensional grey scale image takes time and experience to master, but with practice ultrasound can be a useful adjunct to radiography. The authors find the use of ultrasound most rewarding for the assessment of (i) reproductive function and disease, especially ovarian activity and distinguishing between pre-ovulatory ova stasis and post-ovulatory egg stasis; (ii) liver and gall bladder, (iii) kidneys and (when present) bladder; (iv) any soft tissue mass; (v) ocular and retrobulbar disease; and (vi) cardiac disease (using color flow doppler).

It has been stated that ultrasound can be used to guide biopsy collection, and although this is theoretically true, when dealing with small exotic patients the use of endoscopy offers superior, direct visualization with less collateral damage, and has proved more valuable for accurate and safe biopsy collection.

Computed topography

Computed topography (CT), offers excellent high-resolution, detailed images. Potentially, it would be the diagnostic imaging technique of choice for tissue-air interfaces (eg, respiratory tract) and skeletal structures. Magnetic Resonance Imaging (MRI) is preferred for most soft tissue evaluations especially the CNS.

Diagnostic Sample Collection[13]

A definitive diagnosis relies upon the demonstration of a host pathological response (eg, paired rising titers, cytology, histopathology) and the causative agent (eg, cultures, parasitology, PCR, toxicology). Consequently, the ability to collect diagnostic material is of paramount importance.

Blood collection

The most clinically useful vessels appear to be the jugulars, subcarapacial sinus, and dorsal coccygeal veins. The left and right jugular veins are preferred because of the 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 caudodorsal direction. 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 is certainly possible, but uncommon. To access the dorsal coccygeal vein the needle is angled at $45\text{-}90^{o}$ 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 appears to be a greater risk of lymphatic contamination.

Shell and skin biopsies

A combination of local, regional, or general anesthesia and sharp excision or a skin punch biopsy instrument is effective. A single suture closes the deficit. Biopsy of the chelonian shell requires general anesthesia and cortical biopsy devices.

Lung lavage

The simplest method of obtaining a representative sample from the lower respiratory tract is by lavage performed in the sedated or anesthetized patient. A sterile catheter of appropriate size is placed through the glottis (ideally through a sterile endotracheal tube) taking great care not to touch the oral membranes. The catheter is advanced down the trachea and into the lung. The catheter is advanced to a mid-coelomic position. Once in place, 5 ml of sterile saline per 1 kg bodyweight can be infused. Sample recovery is often aided by rotating the animal and repeatedly aspirating. The submission of fresh and fixed lavage material, multiple air-dried smears, and a microbiologic swab permits detailed investigation.

Cloacocolonic lavage

This can be performed on most conscious reptiles and provides the clinician with a diagnostic sample where defecation is infrequent. A sterile lubricated round-tipped catheter is inserted into the cloaca and craniad into the colon. A relatively large catheter should be used as this helps prevent kinking of the tube and perforation of the thin intestinal wall. On no account should the catheter be forced. Once in place, 10ml per kg bodyweight should

be gently infused through the catheter and repeatedly aspirated until a sample is obtained. The direct collection of fecal material from the distal colon using a lubricated gloved hand offers another practical option in large reptiles.

Gastric lavage

A relatively large, round-tipped catheter can be inserted into the stomach of most conscious reptiles. It is wise to use a mouth gag to prevent damage to the tube. The catheter should pass to the mid-coelomic region before instilling 5 - 10 ml sterile saline per kg bodyweight.

Urinalysis

Urinalysis is less helpful in reptiles than mammals. The reptilian kidney cannot concentrate urine and so urine specific gravity is of limited use in the assessment of renal function. Furthermore, renal urine passes through the urodeum of the cloaca before entering the bladder (or posterior colon in those species that lack a bladder). Bladder urine is therefore not necessarily sterile. The clinical picture is further complicated by the fact that electrolyte and water changes can occur across the bladder. Despite these biochemical drawbacks, urine samples are useful for cytological assessments of inflammation, infection, and for the identification of renal casts.

Visceral biopsy

Diagnostic imaging and clinicopathology can often indicate visceral disease, which can only be definitively diagnosed by tissue biopsy. Samples may be collected via standard (transplastron or prefemoral) coeliotomy, percutaneously (with or without ultrasound guidance), or endoscopically. Biopsies can be collected using ligation or wedge techniques, biopsy needles, and endoscopic instruments. Endoscopic techniques are typically less invasive, permit closer examination of more of the organ and enable the collection of multiple biopsies.[14] Correlation between biochemical tests and histopathology are generally lacking but serial blood sampling and biopsy currently offers the best diagnostic and monitoring approach to hepatic disease.

Necropsy

A detailed necropsy should be undertaken whenever possible as they often provide definitive answers. When dealing with a disease outbreak in a population, elective euthanasia and necropsy of one or more individuals is often the most efficient and cost-effective means to a diagnosis. A thorough and systematic approach is essential as, unlike a physical examination of a live animal, it can never be repeated. Fresh necropsies can provide organ biopsies, blood and other bodily fluids for laboratory examination. Microbiology, histopathology and electron microscopy all take time, but cytology can provide an almost immediate working diagnosis until complete results are obtained. The submission of microbiology samples, especially bacteriology, from reptiles that have died and remained within a heated enclosure must be interpreted with caution. Following the necropsy and the submission of tissues/fluids, samples of major organs should also be frozen in case there is a future need to assess potential toxic or viral involvement.

Therapeutics

There are few drug preparations approved for use in reptiles. Drugs authorized for use in other species or for humans may be administered at the recommendation of the veterinarian. All reptiles should be accurately weighed before being medicated to avoid overdosage, and during treatment to monitor response.

As ectotherms, temperatures outside their POTZ can have profound influences on drug distribution, metabolism, excretion, and hence elimination half-life. Some therapeutic regimens state a fixed temperature at which the reptile should be held during treatment. The advantage of this approach is that where pharmacokinetic evidence exists the elimination of the drug will be known and constant. However, if this stated temperature is below or above the POTZ for the species being treated then stress and debilitation may ensue. In addition, where the stated therapeutic temperature is within the POTZ for the species being treated, constant exposure to a fixed temperature is likely to cause stress and maladaption over a period of time.

Reptiles have a well-developed renal portal system where blood from the caudal half of the body passes through the kidneys before reaching the systemic venous circulation. Therefore drugs that are injected into the caudal half of the body may have a significantly reduced half-life if excreted via tubular secretion; however, studies have demonstrated that these effects are unlikely to be clinically significant. Of potential concern is the injection of nephrotoxic drugs into the caudal limb or tail which may reach renal tissue in high concentration.

Medications can be given by a variety of routes including by mouth or stomach tube (PO), subcutaneous (SC), intramuscular (IM), intravenous (IV), intracardiac (IC), intracoelomic (ICe), intraosseous (IO), intrasynovial (IS) or intratracheal (IT) injection. Certain drugs can be applied topically, given per cloaca, by inhalation (nebulisation) or by direct intralesional administration.[15] Central line catheters can be placed early on and are useful for facilitating repeated sedation and continued intravenous administration. They are particularly useful for large animals that are less tractable.

References

1. Mader DR. *Reptile Medicine and Surgery.* Philadelphia, PA: W.B. Saunders Co;1996.

2. Raiti P, Girling S. *Manual of Reptiles.* 2nd ed ed. Cheltenham, England: Br Small Anim Vet Assoc;2004.

3. McArthur S, Wilkinson R, Meyer J, Innis C, Hernandez-Divers SJ. *Medicine and Surgery of Tortoises and Turtles.* Oxford, London: Blackwell Publishing Ltd;2004.

4. Mader DR, Divers SJ. *Current Therapy in Reptile Medicine and Surgery*. St. Louis, MO: Elsevier;2014.

5. Wellehan JF, Gunkel CI, Kledzik D, Robertson SA, Heard DJ. Use of a nerve locator to facilitate administration of mandibular nerve blocks in crocodilians. *J Zoo Wildl Med.* 2006;37(3):405-408.

6. Hernandez-Divers SJ, Stahl SJ, Farrell R. Endoscopic gender identification of hatchling Chinese box turtles (*Cuora flavomarginata*) under local and general anesthesia. *J Am Vet Med Assoc.* 2009;234(6):800-804.

7. Knafo SE, Divers SJ, Rivera S, Cayot LJ, Tapia-Aguilera W, Flanagan J. Sterilisation of hybrid Galapagos tortoises (*Geochelone nigra*) for island restoration. Part 1: endoscopic oophorectomy of females under ketamine-medetomidine anaesthesia. *Vet Rec.* 2011;168(2):47.

8. Rivera S, Divers SJ, Knafo SE, et al. Sterilisation of hybrid Galapagos tortoises (*Geochelone nigra*) for island restoration. Part 2: phallectomy of males under intrathecal anaesthesia with lidocaine. *Vet Rec.* 2011;168(3):78.

9. Divers SJ. Anaesthesia of zoological species (exotic pets, zoo, aquatic, and wild animals). In: Clarke KW, Trim CM, Hall LW, eds. *Veterinary Anaesthesia.* 11th ed: Elsevier;2013:535-569.

10. Shepard MK, Divers S, Braun C, Hofmeister EH. Pharmacodynamics of alfaxalone after single-dose intramuscular administration in red-eared sliders (*Trachemys scripta elegans*): A comparison of two different doses at two different ambient temperatures. *Vet Anaesth Analg.* 2013;40(6):590-598.

11. Chinnadurai SK, DeVoe R, Koenig A, Gadsen N, Ardente A, Divers SJ. Comparison of an implantable telemetry device and an oscillometric monitor for measurement of blood pressure in anaesthetized and unrestrained green iguanas (*Iguana iguana*). *Vet Anaesth Analg.* 2010;37(5):434-439.

12. Hernandez-Divers SM, Hernandez-Divers SJ. Diagnostic imaging of reptiles. *In Practice.* 2001;23(7): 370-391.

13. Hernandez-Divers SJ. Diagnostic techniques. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Elsevier;2006:490-532.

14. Divers SJ. Diagnostic endoscopy. In: Mader DR, Divers SJ, eds. *Current Therapy in Reptile Medicine and Surgery.* St. Louis, MO: Elsevier;2014:154-178.

15. Hernandez-Divers SJ. Pulmonary candidiasis due to Candida albicans in a Greek tortoise (*Testudo graeca*) and treatment using intrapneumonic amphotericin. *Br J Zoo Wildl Med.* 2002;32:352-359.

Reptile Cranial Structures and Functions

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Session #330

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Abstract: The cranial anatomy of reptiles is represented by great diversity in skull design, dentition, jaw form, and integument. The circulatory, muscular, and nervous systems are some what more conservative so gross patterns in structure are more similar than not. Taxonomic diversity has resulted in species-specific differences that are superimposed upon major structural patterns. Here that major structures and their patterns are described while specific taxonomic specializations are left for other more specialized venues.

Introduction

The cranial anatomy of reptiles is diverse in many aspects, yet many features are shared by all taxa. At the level of the integument species are scaled, have scutes, or may have secondarily lost their scales so that the heads is covered with scaleless skin. Reptilian skulls are highly complex structures of bones and cartilages that can be kinetic (in many lizard and snakes) or akinetic in as in turtles, crocodilians and tuataras. Both dermal and endochondral bones occur in the skull.[1,2] The hyoid skeleton is part of the skull but is physically separate from the cranium and jaws and is mobile; it supports the tongue and pharyngeal muscles. This hyoid skeleton mobility allows considerable expansion of the throat may occur during feeding, display, taste or olfaction. The skull provides the attachment sites for extensive musculature associated with jaw depression and elevation and for neck muscles. All reptiles differ from mammals in having a single ear bone (stapes or columella) that located just caudal to the jaw joint.

The skull provides protections for the brain, sensory structures and nerves. The braincase houses a relatively small tubular brain formed of several vesicles. The brain is housed fully within the skull and is protected by skull bones as well as extensive muscles.

The jaws are composite structures formed of several bony elements.[1-3] The jaw joint is formed by the quadrate and articular bones. Extant turtles and tortoises are the only reptiles to lack teeth normally; instead the mandibular margins are covered with keratinous rhamphothecae. Snakes, lizards, crocodilian, amphisbaenians and tuataras have teeth that range from simple acrodont structures to polyphydont teeth that may be attached as thecodont or pleurodont.[1]

The details of the major head structures are discussed in the following sections.

Integument

The integument of the head includes skin and scales osteoderms, scutes, other kinds of dermal armor, specialized structures such as crests or dewlaps, rhamphothecae, mucus and waxy secretions, and pigment.[1,3-6] It is a composite structure formed of an outer epidermis and an inner dermis. A loosely organized superficial fascia

(the hypodermis) connects the integument to the underlying muscles or skeleton.[5] The integument contributes to the shape and color of the animal and serves multiple roles. The single most significant role of the integument is the barrier between the environment and the organism. This separation is a barrier to pathogens and allows for animals to differ in osmotic state from the environment in which they live.[6-8]

Reptile skin may be differentiated as having scales or the skin may be scaleless. Scale form, color, patterns and species-specific scale patterns that are important in species identification. Scales may be smooth or keeled, overlapping or abutting tightly, large or small.[4] Cranial scales that form bony scales of crocodilians are called scutes. The scutes overly bone, cartilage and fibrous connective tissue. The cranial integument also can form a number specialized structures used for defense, species or mate recognition and display (crests, keels, horns, spines, dewlaps, or barbles).[1,4]

Integumentary glands are few in the head. Some turtles and tortoises as well as crocodilian have mental glands ventral to the lower jaw.[6] Some lizards (eg, chameleons) have a small gland at the angle of the jaw.

Special sensory receptors can be found in the integument of some species. In some boid and viperid snakes, the integument may form pits that house infrared sensors, a type of special sense organ.[1,6] Pits on the jaws of crocodilians house wave-sensing structures. In several lizard taxa and the tuatara, an integumentary scale located dorsal to the parietal organ functions in specialized light transmission associated with circadian rhythm.[1]

Cranial Skeleton

The reptilian skull is composed of the cranium (often termed the "braincase"), jaws, and hyoid apparatus. Bones and cartilages that have different developmental origins form these three parts of the skull: *chondrocranium, dermatocranium,* and *splanchnocranium*. The braincase is a composite of parts of the chondrocranium roofed by dermatocranial bones.[1-3] Chondrocranial bones are endochondral (cartilage-replacement) in origin.[3] They encase much of the brain and form the posterior skull including the parietal bones. Most of the endochondral skull bones are deep within the skull housing the brain and inner ear. Most reptiles have a ring of endochondral bones in each eye (scleral ossicles) and hyaline cartilage within the sclera supporting the back of each eyeball. The exceptions are snakes and crocodilians.[6,8,9]

The dermatocranial (dermal) bones tend to be thin and cover many of the chondrocranial and splanchnocranial bones and cartilages.[2,3] Bones of the dermatocranium form as intramembranous bone and often they arise from neural crest ectoderm rather than ectoderm or mesoderm.[1] They are often flat and make up the outer casing and roof of the skull, superficial bones of the face, jaws and palate. Facial dermatocranial bones include premaxillae, maxillae, postorbitals, prefrontals, parietals, jugals, quadratojugals, and squamosals. The bones of the lower jaw (mandible) are dermatocranial in adults. These include the large dentary, surangular, angular, and splenial bones. Dermal bones of the palate include the buccal surfaces of the premaxillae, maxillae, vomer, palatines and pterygoids.[1,3,7,8] These palatal bones are important in species identification; they form the primary palate and partial secondary palate (when one is present), and the secondary palate of crocodilians.[2]

The splanchnocranial bones and cartilages contribute to the jaws, ear and hyoid apparatus. They include the bones of the jaw joint (quadrate, articular) ear ossicles (stapes = columella) with their supporting structures (extracolumella), as well as the hyoid apparatus.[1-3] By the time of hatching or birth, upper and lower jaws are composites of several dermatocranial bones and the splanchnocranial elements are reduced. The hyoid apparatus is formed boney and cartilaginous parts (ceratohyal, hyoid body, and horns of the hyoid),[1,2,4] and serves as a site for muscle attachments in the jaws, throat, and tongue.

The hyoid apparatus (the hyoid body and paired ceratohyal bones and cartilages) is attached to the lower jaw, tongue and throat muscles and is located between and ventral to the two rami of the lower jaw.[10] Part of the hyoid may be modified, particularly in lizards, to support dewlaps for display or as part of the tongue projections system of chameleons.[1,5]

Muscles

Skeletal muscles of the head include three major muscle groups: *branchiomeric muscles* (many of the larger jaw and face muscles), *hypobranchial muscles* (primarily ventrally located throat and neck muscles) and *extrinsic eye muscles* (the muscles that move the eyes).[1-3] Some body muscles (*hypaxial* and *epaxial* muscles) act as stabilizers or fixators of the head or jaw apparatus.[1] These muscles can be identified by their innervation patterns (Table 1).

Table 1. Head and neck muscles and their innervations.

Muscle Groups	Innervation
Neck muscles	
Epaxial neck muscles (transversospinalis, longissimus group, illiocostalis group)	Dorsal ramus of spinal nerves
Hypaxial neck muscles (tranversus, longus colli)	Ventral ramus of spinal nerves
Extrinsic eye muscles	
Superior rectus, medial rectus, inferior rectus, inferior oblique:	Oculomotor III
superior oblique	Trochlear IV
Lateral rectus	Abducens VI
Hypobranchial muscles	
Rectus cervicis, sternohyoid, omohyoid, genioglossus, & geniohyoid	Hypoglossal XII
Branchiomeric muscles	
Adductor mandibulae, pterygoideus, intermandibularis	Trigeminal V
Depressor mandibulae, branchiohyoideus, interhyoideus, Constrictor colli (part)	Facial VII
Trapezius, sternomastoid, intrinsic pharyngeal muscles	Vagus X and glossopharyngeal IX

Branchiomeric muscles are associated with movement of parts of the splanchnocranium, including mandibular, hyoid and more dorsal and lateral pharyngeal arch derivatives.[1,3,10]

Hypobranchial muscles are associated with other structures derived from the pharyngeal arches: Hyoid, tongue, glottis.[1,3,10]

Muscles tend to have conservative patterns of formation so that muscle "blocks" form similarly across vertebrates and subdivide into homologous muscles in closely related taxa. In taxa that are closely related, similarly positioned muscles tend to share both innervation and function. In species that are more distantly related, muscle homologies can be traced through their innervation (cranial nerves), which is quite conservative, rather than by function or location alone.[1]

Sense Organs

Eyes

Reptilian eyes are anatomically similar to those of other vertebrates. The eyeball tends to be round and is formed of structural and sensory layers surrounding fluid. Reptilian lenses are usually round or oval.[11-13] The eye has as a series of 3 chambers. The *anterior chamber* is the fluid-filled space located between the cornea's innermost surface and the iris. The *posterior chamber* is small and located posterior to the iris and anterior to the lens; it bordered by the *ciliary body* or ciliary muscles. The anterior and posterior chambers are filled with *aqueous humour*. The *vitreous* chamber is the third chamber and the largest located between the retina and the lens and is filled with a viscous liquid, the *vitreous* humour.[11-15]

Each eyeball sits within a bony orbit. In reptiles, the two orbits are separated from one another by a cartilaginous *interorbital septum* in lizards, crocodilians, tuataras, and turtles, or by bones and cartilages (frontal, parietal, and parasphenoid bones) in snakes.[1,3,10,14,16]

Ocular adnexa

Ocular adnexa include the eyelids and their parts, conjunctiva, orbital glands, and extrinsic eye muscles (Table 2).[11,14,15]

Table 2. Extrinsic eye muscles, their innervations and actions.

Muscle[a]	Innervation[b]	Action[c]
Medial rectus	Cranial nerve III (*Oculomotor*)	Draws gaze nasally
Lateral rectus	Cranial nerve VI (*Abducens*)	Draws gaze temporally
Superior rectus	Cranial nerve III (*Oculomotor*)	Draws gaze temporally and dorsally
Inferior rectus	Cranial nerve III (*Oculomotor*)	Draws gaze nasally and ventrally
Inferior oblique[d]	Cranial nerve III (*Oculomotor*)	Draws gaze temporally and ventrally
Superior oblique[d]	Cranial nerve IV (*Trochlear*)	Draws gaze nasally and dorsally

[a]The eye muscles are organized functionally and are listed sequentially as agonist-antagonist pairs.
[b]Some innervation is thought to cross from one side of the brainstem to the other to coordinate the movements of these pairs of eye muscles in both eyes.
[c]Muscle actions are given in general terms because of species-specific differences.
[d]The oblique muscles together are responsible for rotation of the eyes so that the eyes return to the correct vertical and horizontal position when the head is tilted.

Eyelids: All reptiles have external eyelids. In all turtles, tuataras, crocodilians, and most lizards, both upper and lower eyelids are present.[1] The lower lid of lizards contains a cartilaginous support, the *tarsus*. Lids are modified in a number of species so that they are partially fused, as in chameleons, leaving a circular opening the diameter of the cornea, or fused and clear as in many geckos and snakes.[11,12] In snakes, the eyelids fuse during development and form the *spectacle* (*brille*). Some gecko species and some skink species have a secondarily derived spectacle. The spectacle does not move. It is shed when the skin is shed. Some skinks, lacertid, and iguanine lizards have a transparent lower eyelid formed by clear scales. In general, the upper lid has mostly smooth muscle and is less mobile than the lower lid, which has striated muscle. In crocodilians, the upper lid contains a bony plate; the lower lid lacks bone or cartilage, but moves up to close the eye.[12]

The borders of the upper and lower lids are often rich in secretory goblet cells, which are important in corneal lubrication. The eyelids cover a poorly cornified *nictitating membrane* (*nictitans*) along the nasal surface of the eye. Nictitating membranes are highly developed in crocodilians and turtles but absent in snakes and many lizards.[11,12,15] The "lid-less" lizards, with a clear spectacle covering the cornea, lack a nictitans. Chameleons also lack a nictitans. The nictitans, an extension of the conjunctiva, is cartilage-supported in non-burrowing lizards and crocodilians. The nictitating membrane may be pigmented. It is usually largest toward the medial (nasal) part of the eye and may have folds. Depending upon the species, it may cover all or part of the eye. The *pyramidalis* muscle draws the nictitans across the eye. The nictitans acts to mechanically protect and cleanse the cornea and moisten its surface.[1,15,16]

*Orbital glands***:** Orbital glands are lubricatory to the cornea and their secretions often drain into the mouth. Lizards and crocodilians usually have three orbital glands (*lachrymal glands*, *Harderian glands, conjunctival glands*), which may be compact or have extensions around the eyeball.[1,11] Most lizards have well-developed *lachrymal glands,* located posterior, dorsal and ventral to the eye. They are absent in chameleons, calotes, some geckos, and Australian snake-lizards. *Harderian glands* are located ventral or anterior to each eyeball and drain via a duct onto the inner surface of the nictitating membrane; the duct empties into the palate.[12,15] A small mucous producing *conjunctival gland* opens onto the outer surface of the nictitating membrane, when present.[11,12]

Snakes have well-developed Harderian glands, located dorsally and nasally that lubricate the spaces between the spectacle and the cornea.[6,11] They lack lachrymal glands. The nasohardarian duct drains this fluid from the *subspectacular space* into the *Jacobson's organ* (VNO) in the palate of the oral cavity. Tuataras, too, have only Harderian glands that lubricate the cornea and the conjunctiva.[12]

In turtles, lachrymal and Harderian glands are well developed. They vary greatly in size with taxon. Dorsally positioned lachrymal glands are very large in marine turtles but small in freshwater and tortoise species.[11,12,17] The Harderian gland is present dorsally and nasally in all turtles. There are no reported *nasolachrymal ducts* in turtles, however some species have the bony opening suggesting duct or its remnant occurs in the floor of the orbit.

In crocodilians, the elongated lachrymal gland is small relative to the size of the eyeball and located dorsally within the orbit. The Harderian gland is large, triangular and located anterior and medial to the eye. It secretes lubricating fluid via two ducts that drain between the nictitating membrane and the eye.[12,16] The conjunctival gland is located at the junction of the conjunctiva and the eyeball within the lower lid.

Nasal structures and function

Reptiles have nasal sacs that are functionally, and sometimes structurally, separated into an anterior vestibule and a posterior nasal chamber. Lateral walls of the nasal chamber usually have folds, conchae (turbinals).[1] Air enters through the nares via vestibule, passes across the conchae in the nasal chamber, then exists into the pharynx via the choanae (internal nares). The nasal epithelium is chemosensory in both aquatic and terrestrial species.[1,3,4]

The vomeronasal organ (Jacobson's organ) is present as a pair of pits into which the tongue transfers odors in snakes and lizards.[1] It is thought to be absent in crocodilians and turtles appear to have VNO sensory cells scattered across the dorsal nasal epithelium. Tuataras have a vomeronasal duct in the choanae (not in the oral cavity); it is thought that VNO sensory cells may detect air-borne chemosensory cues.[18]

Oral structures and function

Mouth or buccal cavity includes the lips, cheek walls, teeth, tongue, glottis, and oral glands.[1] In reptiles both teeth and tongue function in prey capture or prehension and food transport, as well as in display. The teeth

function to catch and hold prey, in handling and cutting food but not in chewing per se. The teeth also are use in aggression and defense. Turtle lack teeth so the rhamphothecae serve similar functions to teeth.[1,17] The tongue functions in odor detection, taste, food prehension, manipulation and transport. The tongues of all snakes and some lizard are bifurcated anteriorly. The tongues of chelonians, tuataras, many lizard species and crocodilian lack bifurcation and are fleshy[1, 6]. Many are not protrusible. Oral glands include salivary, lubricatory, salt excretion, venom glands in some species.[1]

Circulatory Structures

The general pattern of reptilian arteries of the head follows. The *dorsal aortae* gives rise to paired *common carotid arteries* that are parallel to the esophagus. Each gives off a relatively small *external carotid artery* that supplies the soft tissues of the throat and ventral tongue, and the remaining large vessels continue to the head as the *internal carotid arteries*. The internal carotids enter the skull, passing along the ventrolateral edge of the braincase, medial to the middle ear. The internal carotid arteries divide into a large dorsal *temporomandibularis branch* (*stapedial artery*) near the ear and a smaller *inferior internal carotid* that gives rise to the *palatine artery*. The temporomandibularis branch gives off a large *mandibular artery* to the jaw adductors and temporal muscles, it then proceeds anterodorsosally, giving off an *inferior orbital artery* to the base of the orbit and a s*uperior orbital artery* along the dorsal medial orbit that supplies eye muscles and eventually the nasal cavity.[1,2,19] These three branches are associated with the trigeminal nerve branches. There are variations on this general pattern among taxa and within individuals. The most common taxonomic differences are described below.

The venous system of the head can be variable and there are many thin walled venous spaces or sinuses draining cranial structures. They are best known from the tuatara.[2,3] There are three major routes of venous drainage in reptiles. A single medial dorsal vein (*longitudinal cerebral vein = median dorsal longitudinal sinus*), a pair of longitudinal *lateral head veins* on the sides of the head that drain the facial and dorsal cranial structures. They drain to the *anterior vena cava* (*anterior cardinal veins = superior vena cava*). A pair of large *orbital sinuses* drains blood from the head muscles.[2,19] Several smaller paired venous sinuses drain via these three main routes for blood to leave the head. The small sinuses include *the nasal, palatine, transverse and* longitudinal.[2] A large *maxillary vein* drains into the orbital sinus on each side of the head. The two orbital sinuses are connected together at their posterior ventral extent and each also drains into the *lateral head veins* along with the *pterygoid veins* and the *occipital vein* to the *anterior vena cava*.[2,19]

Lizards

The *left common carotid* artery arises first and gives off the *external carotid artery* to the lower jaw then extends cranially as the *internal carotid artery* to supply the left side of the head. The *right common carotid artery* continues cranially as the *internal carotid artery* and supplies the right side of the head[2]. The inferior internal carotids each give rise to an *ophthalmic artery* that runs with the optic nerve to the eye. The *palatine arteries* arise next and supply the roof of the mouth.[1-3]

Snakes

The right jugular vein receives blood from the following organs: trachea, esophagus, right thymus gland, thyroid gland, fat body, epigastric vein, tongue muscles, and head. The left jugular vein originates in the head and courses along the left ventral surface of the esophagus to the head. It carries blood from the esophagus and is the first of a series of veins, which differ in number, from the esophagus to the left jugular vein.[2,19]

In snakes and lizards there are three sets of transverse veins (*anterior cerebral, medial cerebral, and posterior cerebral*), which flow into the *internal jugular veins*. The superficial circulation of the head and the internal jugular veins drain into the *external jugular* veins that flow into *common jugular* segments that drain into the right *anterior vena cava*. A pair of *tracheal veins* run along the sides of the trachea and drains the lower jaw, pharynx, tongue, thyroid esophagus and trachea. Snakes have a large maxillary sinus that extends to the neck.[2] The left tracheal vein connects to the right distally and drains into the right *anterior vena cava*.[19]

Crocodilians

Three major arteries from the dorsal aortae and extend along the ventral neck to the head. These are left and right collateral colli arteries and a single common carotid artery.[2,8,10,16,19] Lateral head veins are absent.[2]

Turtles

The *brachiocephalic trunk* from the left dorsal aorta supplies the right and left *common carotid* arteries. Each supplies a small branch to the thymus on each side then continues without branching the length of the neck to supply the head, entering at the base of the skull.[2,19,20] Each common carotid becomes an *internal carotid artery* supplies each side of the head.[2,17,19] Each *internal carotid* artery gives off a *temporomandibularis branch* (*stapedial) artery*, travels anterior of the stapes and continues into the brain case as the *inferior internal carotids*. There each give rise to an *orbital artery* that runs with the oculomotor nerve to the eye. A *palatine artery* and usually, a *cerebral carotid artery* branch from the remaining inferior internal carotid artery.[20,21] The *mandibular artery* (to the jaw adductors and temporal muscles) can arise from the *internal carotid, palatine* or *temporomandibularis branch (stapedial) arteries*. A *pseudopalatine artery* is present in softshell turtles (Trionychidae).[20,21]

Brain and Braincase

The reptilian CNS is tubular and organized linearly in all species. The forebrain is formed of the *telencephalon* and *diencephalon*. The *tectum*, including *optic lobes*, forms the mesencephalon (midbrain). The hindbrain is composed of the *metencephalon* part of medulla oblongata and cerebellum) and the *myelencephalon* (most of the medulla oblongata). The brain is located midsagittally has some degree of dorsoventral flexure along its length.[9,22] It is housed with in a tubular braincase bounded rostrally by the ethmoid cartilages, laterally by the otic bone series, ventrally by the basisphenoid and laterosphenoid bones, and caudally by the occipital bone series. The braincase is roofed by the supraoccipital, parietal and frontal bones.[1,2] There are subdural (beneath the dura mater) and epidural (above the dura mater) spaces within the brain case. There is substantial endocranial space between the brain and the walls of the braincase in many lizards, aquatic turtles, and tuataras; moderate endocranial space in tortoises and crocodilians, and minimal endocranial space in snakes.[9,22]

When viewed dorsally, the most rostral portions form the telencephalon and include the olfactory tracts from the olfactory sacs to the olfactory bulbs. They are continuous with the relatively large paired cerebral hemispheres. Paired lobes of the mesencephalon, the tectum, are found caudal to the cerebral hemispheres and epiphysis. The unpaired cerebellum, part of the hindbrain is a single structure that integrates touch, proprioception, vision, hearing, and motor input and has a role in maintaining postural equilibrium.[1,9] It is organized and functions similarly in all vertebrates. As in mammals and birds, it is important in coordinating and modifying motor actions.[1]

The cranial nerves (Table 3) arise from the developing brain roughly linearly; elaboration of the parts of the brain may obscure some of this linear arrangement.[1,22,23]

Table 3. Reptile cranial nerves and their functions.

Nerve	Function
0: Nervus Terminalis	Innervates vasculature of nasal epithelium; chemosensory for gonadotropin releasing hormones
I: Olfactory (including the vomeronasal nerve branch [VNO])	Olfaction, carries sensory information from the nasal sacs and VNO
II: Optic	Vision, carries sensory information from the retina to the thalamus and optic tectum
III: Oculomotor	Controls movement of eye, tends to pull eye in or fix gaze; controls iris and ciliary body
IV: Trochlear	Controls movement of eye; draws gaze anteriorly and dorsally.
V: Trigeminal 3 branches: ophthalmic, maxillary, and mandibular nerves	Sensory from skin around eye, and mouth. Sensory pits of pit vipers and boids. Controls jaw adductor muscles, muscles of skin around teeth-bearing bones in snakes, and the intermandibularis muscle (in floor of mouth).
VI: Abducens	Controls movement of eye; draws gaze posteriorly
VII: Facial	Sensory from skin and muscle around the ear, upper jaw and pharynx. Controls superficial neck muscles and mandibular depressors.
VIII: Statoacoustic = Acoustic, = auditory	Balance and hearing: sensory from the inner ear.
IX: Glossopharyngeal	Taste and sensation in the pharynx. Controls tongue muscles.
X: Vagus	Sensory and motor to glottis, heart, and viscera
XI: Spinal accessory	Controls trapezius and sternomastoid muscles
XII: Hypoglossal	Controls hyoid muscles and tongue.

The pineal complex (epiphysis or pineal gland and the parietal eye) arises just caudal to the cerebrum via a thin stalk; it extends to the dorsal skull at the region of the pineal scale[1,3,6,22,23] The epiphysis is located deep to the pineal eye scale in iguanine lizards and *Sphenodon* and deep to the pink spot of leatherback sea turtles (*Dermochelys coriacea*).[1,9,17] In other taxa, an external landmark does not as clearly demark its position. The pineal gland is both sensory and secretory and is important in regulating circadian rhythms in many animals. It is not well developed in snakes and crocodilians.[1,16]

Two meninges cover the reptilian brain; an outer dura mater that is tough and largely avascular and an inner leptomenix is the more delicate, vascular and lies directly on the brain's surface. CSF is found between the dura mater and leptomenix.[22,23]

References

1. Kardong KV. *Vertebrates: Comparative Anatomy, Function, Evolution.* Boston, MA: McGraw-Hill Science;2006.

2. Romer AS. *Osteology of the Reptiles.* Chicago, IL: University of Chicago Press;1956.

3. Wake MH. *Hyman's Comparative Vertebrate Anatomy.* Chicago, IL: University of Chicago Press; 1979.

4. Hildebrand M. *Analysis of Vertebrate Structure.* New York, NY: John Wiley & Sons, Inc;1974.

5. Alibardi L. Proliferation in the epidermis of chelonians and growth of the horny scutes. *J Morphol.* 2005;265(1):52-69.

6. Jacobson ER. *Infectious Diseases and Pathology of Reptiles: Color Atlas and Text.* Boca Raton, FL: CRC Press;2007.

7. Cooper JE. Dermatology. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Saunders Elsevier; 2006:196-216.

8. Reese AM. *The Alligator and Its Allies.* New York, NY: G Putman's Sons;1915.

9. Wyneken J. Reptilian neurology: Anatomy and function. *Vet Clin N Am Exot Anim Pract.* 2007;10(3):837-853.

10. Schumacher GH. The head muscles and hyolaryngeal skeleton of turtles and crocodilians. In: Gans C, Parsons T, eds. *Biology of the Reptilia.* Vol. 4 (Morphology D). New York, NY: Academic Press; 1973:101-199.

11. Walls GL. *The Vertebrate Eye and Its Adaptive Radiation.* Bloomfield Hills, MI: Cranbrook Institute of Science; 1942.

12. Underwood G. The eye. In: Gans C, Parsons T, eds. *The Biology of the Reptilia.* New York, NY: Academic Press; 1970;1-97.

13. Schwab IR. *Evolution's Witness: How Eyes Evolved.* New York, NY: Oxford University Press; 2012.

14. Franz-Odendaal TA, Vikaryous MV. Skeletal elements in the vertebrate eye and adnexa – morphological and developmental perspectives: Review for a special issue on craniofacial development. *Dev. Dynam.* 2006;235:1244-1255.

15. Lawton MPC. Reptilian opthalmology. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Saunders Elsevier;2006:323-342.

16. Richardson, KC, Webb G, Manolis SC. Crocodiles: Inside Out. Chipping Norton, Australia: Surrey Beatty;2002.

17. Wyneken J. The Anatomy of Sea Turtles. Miami, FL: U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470;2001.

18. Schwenk K. Morphology of the tongue in the tuatara, *Sphenodon punctatus* (Reptilia: Lepidosauria), with comments on function and phylogeny. *J Morphol.* 1986;188(2):129-156.

19. Bruner HL. On the cephalic veins and sinuses of reptiles, with description of a mechanism for raising the venous blood-pressure in the head. *Am J Anat* 1907;7(1):1-117.

20. Jamniczky HA. Turtle carotid circulation: a character analysis case study. *Biol J Linnean Soc* 2008;93(2): 239-256.

21. Jamniczky HA, Russell AP. Carotid circulatory development in turtles: using existing material to seek critical developmental stages that localize establishment of clade-specific patterns. *Amphibia-Reptilia* 2008;29(2):270-277.

22. Starck D. Craniocerebral relations in recent reptiles. In: Gans C, Northcutt RG, Ulinski P, eds. *Biology of the Reptilia.* Vol. 9 (Neurology A). New York, NY: Academic Press;1979:1-38.

23. Kappers, CU, Huber GC, Crosby EC. The Comparative Anatomy of the Nervous System of Vertebrates, Including Man. New York, NY: The Macmillan Co;1936.

Clinical Conditions Affecting the Head of Reptiles

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Abstract: There are a myriad of clinical problems that affect the head of reptiles. Conditions and causes such as congenital abnormalities, trauma, neoplasia, infectious disease and nutritional disorders can all have impacts on the cephalic region that result in pathology. Diagnosis of these conditions requires a thorough history, physical examination, laboratory testing, imaging and if warranted biopsies and histopathology. Treatment depends on various factors such as type of patient and cause of the disease. Prognosis also varies from excellent to grave depending on the specific pathology.

Introduction

Clinical conditions affecting the head of reptiles could easily fill an entire chapter or full day seminar.[1-11] Typical pathology falls under the various categories of dermatology, oncology, ophthalmology, neurology, otorhinolaryngology and dentistry. Likewise, causes are numerous. This paper will review the most common presentations of clinical conditions affecting the cephalic region of reptile patients.

Congenital Defects

Head

The most pronounced congenital defect in reptiles is bicephaly: having two heads. This has been reported in lizards, snakes and turtles. In most cases, both heads are independently functional. Although not to believe that these animals fair well in the wild, in captivity some live relatively normal lives.

It is felt that bicephaly develops as a consequence of aberrant temperatures during incubation.

Central nervous system

Meningoencephalocoele and hydrocephalus have been reported in crocodilians and anecdotally in water dragons. Most congenital neuropathies lead to embryonic or early death in the animal.

Eyes

Unilateral and bilateral anophthalmia can occur, but is not as common as microphthalmia. The latter, being either uni- or bilateral, can be minimal or severe, often with the globe never being seen as the small organ develops under the scales with no visible palpebral or ocular fissure.

As with the bicephaly, this is believed to be an incubation consequence.

Animals with either anophthalmia or microphthalmia can survive in captivity. However, affected animals may also have other occult congenital anomalies that may have direct negative impact on the animal's survival.

Ears

No congenital aural defects have been reported in reptiles.

Nose

Occasionally congenital defects of the oral cavity will incorporate the rostrum or nasal cavity. See below for more detail.

Oral cavity

Congenital defects of the oral cavity and facial bones are commonplace. Brachygnathism, both mandibular and maxillary, are seen in lizards, chelonians and crocodilians. Cleft lips (cheiloschisis) and cleft palates (uranoschisis) have been reported in crocodilians, snakes, chelonians and lizards. These can be unilateral or bilateral. Severity can range from minor disfiguration of the lip to complete absence of the rostrum or mandible.

These defects can be attributable to aberrations in incubation temperatures, genetic defects, toxins and nutritional deficiencies of the gravid females.

Trauma

Head

Trauma is probably the most common clinical condition affecting the head. In this category, husbandry related trauma has to top the list. Rostral trauma from captives rubbing against or striking the glass or screen can lead to severe damage to the scales, facial bones and soft tissues. Secondary infection and osteomyelitis is common. Anorexia secondary to the injury leads to further deterioration of the patient with death often as the endpoint.

Trauma from conspecifics, predators, prey is also encountered.

Thermal burns can fall under the category of trauma. Heat damage from light bulbs and other heat sources are not uncommon in caged reptiles.

Crushing injuries, lawnmower encounters, boat propellers and automobiles are all potential sources of head trauma.

Central nervous system

Any severe head trauma has the potential of damaging the brain and CNS.

Freeze damage has been seen in tortoises emerging from brumation. Etiology is not clear but suspected to be related to either post brumation fatty liver syndrome or bacterial micro-abscesses forming in the brain.

Eyes

As above, bites from conspecifics, prey and predators are not uncommon. Likewise, burns can be seen on or around the eyes. Abrasions from difficult sheds, rubbing on cage furniture or squeezing into small hide places can all damage the spectacle and eye.

Any penetrating wound or foreign body can cause panophthalmitis. Damage to the Harderian duct can reduce drainage from the subspectacular space, thus putting pressure on the globe.

Freeze damage (seen during improper hibernation) has been reported in all taxa and can result in either reversible or permanent cataract formation.

In animals without protective spectacles, corneal damage such as abrasions and ulcers are not uncommon.

Ears

As above, any of the previously mentioned scenarios can cause traumatic injury to the external, middle or inner ear.

Nose

Rostral trauma is common in captive reptiles, either from rubbing on screen or constant abrasions from contact with glass or other barriers. Metal screen is the worst culprit as it can literally wear the skin and soft tissue down into the bone. Even in the presence of severe, self-induced lesions, the self-destructive behavior seems to continue. This can lead to deep infections, including cellulitis, osteomyelitis and sloughing of the maxillary bone. Although some can be treated if the underlying cause can be eliminated, the damage that results can be permanent, resulting in an animal becoming an obligate mouth breather.

Oral cavity

Iatrogenic trauma, such as forcing the mouth open during an examination, can damage teeth and bones. Teeth can be injured from biting into hard objects or during missed strikes.

Soft tissues of the oral cavity are also subject to trauma. Bites from live prey can damage the gingiva, glottis and tongue. Force feeding by inexperienced handlers can damage the jaw, hinge structure, soft tissues and tongue. Lacerations in the esophagus are not uncommon during rough "force" feeding attempts in snakes.

Foreign bodies, such as string, hair (human, animal), large scales, bones, substrates and more can become lodged in the oral cavity and cause serious health problems.

Nutrition

Head

One of the most common conditions that affect captive reptiles is nutritional secondary hyperparathyroidism (NSHP). The hallmark is foreshortening of the bones of the mandible, but, when the condition affects young, growing animals, as it often does, all of the flat bones of the head tend to be affected. Normally long skulls develop foreshortened with a more "round" appearance. NSHP can have anywhere from mild to severe effects on a patient's cosmetics even if the underlying deficiencies can be corrected. Mandibular or maxillary brachygnathism often results in the extreme, whereas chronic malocclusion with exposure gingivitis is the more common outcome.

Central nervous system

Toxic ingestion of heavy metals such as zinc or lead can cause CNS derangements. Certain nutrients, such as avidin, found in raw, unfertilized eggs, can induce biotin deficiencies in egg eating reptiles. Reptiles that eat fertilized eggs do not experience this problem. Clinical signs include muscle tremors and generalized weakness.

Ingestion of toxins such as organophosphates and pyrethrins, as well as disinfectants such as chlorhexidine can all cause nervous system signs such as paralysis and death.

Hypocalcemia, a common finding in captive reptiles, can impact nerve conduction (more of a PNS involvement) which results in generalized tetany.

Similar symptoms are seen in crocodilians suffering from hypoglycemic stress.

Thiaminase, which is found in certain frozen feeder fish, can induce leukoencephalopathy. This results from the thiaminase destroying the thiamine in the eaten fish. Clinical signs include muscle twitching, incoordination, blindness, seizures and potentially death.

Eyes

Arcus lipoides cornea, which manifests as infiltration of cholesterol crystals in the peripheral cornea is not uncommon in aging *Testudo* species. Corneal cholesterol dystrophy and corneal lipidosis also are seen in reptiles fed high polysaturated fat diets.

Hypovitaminosis A can lead to squamous metaplasia, which can have many consequences. Of importance here is that it can lead to blockage of the nasolacrimal ducts, especially in aquatic chelonians, which ultimately causes blepharoedema, giving the appearance of bilaterally swollen eyes.

Ears

Aural abscesses in chelonians, especially common in terrapins, have been associated with Vitamin A deficiencies. Although suggested, these abscesses are seen in wild animals and animals on proper diets.

Nose

No specific nutrition-related nasal conditions have been reported. That stated, cases of severe NSHP can develop facial deformities that may affect the nasal cavity.

Oral cavity

As stated, the most common nutritional problem in reptiles, NSHP, can lead to severe facial deformities that can have direct effects on the oral cavity.

It has been suggested that a vitamin C deficiency can be associated with gingival bleeding in the Boidae. However, studies in garter snakes show that at least in that species, they make enough vitamin C in their kidneys it is not required in the diet.

In chelonians hypovitaminosis A has been associated with abnormal growth of the rhamphotheca.

Although not directly related to nutritional intake per se, dental calculi are seen in several species of lizards, most notably bearded dragons.

Infections: Bacterial, Viral, Fungal and Parasitic

Head

Any breach in the skin can result in bacterial or fungal infections of the cephalic region. Cephalic cellulitis, a generalized swelling of the head, has been associated with gram negative septicemia. Viremic animals, especially those that are immunosuppressed from the pathogen, are likely to become secondarily affected by bacteria or fungi.

Chrysosporium anamorph of *Nannizziopsis vriesii* (CANV) and other fungi can affect the skin and soft tissues of the head.

Papillomavirus and pox viruses have been associated with skin lesions on the head.

Subcutaneous parasites and parasitic cysts may be found in the skin and muscles around the head region.

Central nervous system

Bacterial, viral, fungal and parasites are all known to affect the CNS in reptiles. Acanthamebic meningoencephalitis, toxoplasmosis, viral and bacterial meningitis are all reported.

Paramyxovirus and arenavirus are two common agents causing viral meningitis in snakes. West Nile virus has been implicated in crocodilians with neurological symptoms.

Eyes

Bacterial, viral, fungal and parasitic pathogens can all affect the eyes, from the globe to the extraocular tissues.

Ectoparasites, such as mites, ticks and leaches can all attach to the periocular tissues for protection and feeding. In heavy numbers these can cause severe damage to the globe and adnexa.

Nematodes of the genus *Serpentirhabdias*, have been reported throughout the periocular tissue, the subspectacular space and the subcutaneous tissues of the head in ball pythons.

Ears

As with the eyes, bacteria, viruses, fungi and parasites have all been associated with pathology of the middle and inner ears. Aural abscesses in aquatic turtles, as mentioned previously, are often colonized by a mix of bacteria and fungi. Cryptosporidiosis has been associated with middle ear infections in the iguana.

Nose

Secondary infection of the nasal cavity can occur with bacteria or fungi, especially in cases of trauma.

Oral cavity

"Mouth rot," the lay term for "infectious stomatitis," is the most common malady of the head in snakes. It is not a disease, per se, but rather a consequence of a combination of many factors: malnutrition, improper husbandry, immunosuppression, trauma, neoplasia and much more. Similar lesions are seen in lizards, and to a certain extent, chelonians.

Although Gram-positive bacteria are the most prevalent organisms in the oral cavity, bacterial stomatitis is most commonly associated with Gram negative bacteria. *Pseudomonas, Aeromonas, Salmonella, Morganella, E. coli,* and *Proteus* have all been associated with stomatitis and oral abscesses. Anaerobic bacteria such as *Bacteroides*, *Clostridium, Fusobacterium* and *Peptostreptococcus* have also been isolated in cases of oral cavity disease.

Mycobacteria have been reported as pathogens associated with granulomatous and non-granulomatous lesions in snakes.

Several viruses have been linked to oral cavity disease. Herpesviruses, ranavirus, adenoviruses, papillomaviruses, arenaviruses, reoviruses and picornaviruses have all been associated with stomatitis and oral lesions.

Fungal stomatitis is usually considered secondary to other disease conditions and an immunocompromised patient. Patients with fungal stomatitis usually have systemic fungal disease.

Metazoan parasites are not common causes of pathology in the oral cavity. Encysted helminths may be seen under the mucosa and nematodes, such as *Kalicephalus* species, may accumulate the oral cavity.

Neoplasia

Head

Several viruses have been associated with neoplastic lesions seen on or around the head. Papillomavirus, reoviruses and fibropapilloma-associated turtle herpesvirus (FPTHV) can all cause large, neoplastic masses on the skin surface of the head.

Papillomas, keratomas, squamous cell carcinomas, melanomas, lymphosarcomas, fibrosarcomas have also been reported.

Eyes

Herpesvirus, specifically FPTHV, produces papillomas on the corneal surface and surrounding ocular tissue. A sarcoma has been reported in the eye of a milksnake. Meibomian gland tumors have been seen from the palpebra of green iguanas.

Ears

Nothing reported.

Nose

A finding of a papilloma was reported in the "nasal area" in an American alligator, but actual location was not specified.

Oral cavity

FPTHV masses have been reported in the esophagus and stomach in green turtles, but not in the oral cavity.

Squamous cell carcinomas, transitional cell carcinomas, rhabdomyosarcomas, adenoameloblastomas, lymphoma, fibroma, adenocarcinomas, lymphosarcomas and osteosarcomas have all been reported from the oral cavity and mandible in snakes, lizards and chelonians.

Diagnostics

Pathology of the head should be evaluated no differently from any other body location. A thorough history is imperative. A complete physical examination from nose to tail is crucial.

Supportive diagnostics should include laboratory testing, including CBC, chemistries, bacterial culture and sensitivity testing, cytology, fine needle aspirates, biopsies, radiographs may all be necessary. In complicated cases advanced imaging such as ultrasound and CT/MRI may be needed.

Therapeutics

Treatment of cephalic pathology will depend directly on the diagnosis, type of patient and experience of the clinician. Topical and systemic (oral and or parenteral) therapy may be warranted. Physical therapy, cold laser therapy and other modalities may be incorporated as needed. In cases of neoplasia, adjunctive therapies such as radiation treatment may be required. Surgical resection of masses, abscesses or orthopedic implants are indicated in certain conditions.

Summary

A myriad of conditions can affect the cephalic region of reptiles. Detailed evaluation may be required to elucidate the cause(s). Treatment may be anywhere from a simple topical application of an antibiotic to complex surgical, chemotherapy and radiation therapy. Prognosis depends on cause and length of time the condition has been ongoing.

References

1. Youngprapakorn P, Ousavaplangchai L, Kanchanapangka S. *A Colour Atlas of Diseases of the Crocodile.* Bangkok, Thailand: Style Creative House Co;1994.

2. Housmann J, Mans C, Dreyfus J et. al. Subspectacular nematodiasis caused by a novel serpentirhabdias species in ball pythons (*Phython regius*). *Proc ICARE.* 2015;286.

3. Marschang RE, Divers SJ. Reptile viruses. In: Mader DR, ed. *Current Therapy in Reptile Medicine and Surgery.* St. Louis, MO: Elsevier;2014:368-381.

4. Mehler SJ, Bennett RA. Upper alimentary tract disease. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Elsevier;2006:924-930.

5. Maudlin GN, Done LB. Oncology. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Elsevier;2006:299-322.

6. Bennett RA, Mehler SJ. Neurology. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Elsevier;2006:9239-250.

7. Donoghue A. Nutrition. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Elsevier;2006:251-298.

8. Lawton MPC. Reptilian ophthalmology. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Elsevier;2006:323-342.

9. Greiner EC, Mader DR. Parasitology. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Elsevier;2006:343-364.

10. Mader DR. Perinatology. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Elsevier;2006:365-375.

11. Cooper JE. Dermatology. In: Mader DR, ed. *Reptile Medicine and Surgery.* 2nd ed. St. Louis, MO: Elsevier;2006:196-216.

Diseases of the Head of Amphibians and Reptiles

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Abstract: The gross and microscopic lesions of tissues making up the head of amphibians and reptiles are reviewed and described. Tissues from the integument, musculoskeletal system, nasal cavity and oropharynx, special senses (eye and ear) and central nervous system are included. Etiologic categories include infectious, noninfectious and neoplastic disease.

Introduction

Diseases of the head can affect a number of organ systems including skin, upper gastrointestinal and respiratory systems, musculoskeletal system, special senses, and central nervous system. A variety of infectious, noninfectious, and neoplastic causes are possible. This presentation will cover the gross and histologic features of common conditions seen in a diagnostic pathology practice. General disease categories are infectious, noninfectious, and neoplasia. Infectious disease may be due to viruses, bacteria, fungi, and parasites. Noninfectious conditions include trauma and toxins. Reported neoplasms include epithelial, mesenchymal, and melanomas/chromatophoromas.

Skin

Infectious disease

Viral disease: A number of viral infections have been reported to involve the skin of amphibians and reptiles. These include herpesvirus in both, poxvirus in both, papillomavirus possibly in both and iridovirus/ranavirus in both amphibians and reptiles.

Herpesvirus: Cutaneous herpes infection has been reported in Italian frogs.[1] Grossly small variably-sized, white to gray vesicles are seen. Histologically there is epidermal hyperplasia, epithelial cell karyomegaly and intranuclear inclusion body formation.

In reptiles, grey patch disease (Chelonid herpesvirus 1) affects cultured green turtle hatchlings. It was reported to cause mortality in 5-20% of affected animals. Grossly there are circular papular skin lesions that coalesce into diffuse grey lesions with superficial epidermal necrosis.[2] Histologically lesions were characterized by hyperkeratosis and acanthosis. Epidermal cells contained basophilic intranuclear inclusions and marginated chromatin.

Another herpesvirus, Chelonid herpesvirus 5, is associated with fibropapillomas and fibromas in marine turtles in tropical waters.[3] Gross lesions are present on the epidermis, eyes, carapace and plastron, and in severe cases on the serosal surface of internal organs. The tumors may vary from smooth to verrucous, and may be light pink to dark gray. Fibropapillomatosis is a major chronic disease of juvenile green turtles. Histologically fibropapillomas have a fibrovascular matrix that supports acanthotic epithelium. Ballooning degeneration may be noted. Inclusion bodies may be seen.

Other herpesviruses that may affect structures of the head of reptiles include: herpesvirus infections in tortoises that lead to conjunctivitis and central nervous system involvement. Elapid herpesvirus (Indian cobra herpesvirus) is associated with degeneration and necrosis of glandular epithelial cells in the venom gland of Siamese cobras (*Naja naja kaouthi*). Herpesviruses have also been detected in lizards: green lizard herpesvirus was identified in green lizard papillomas. Herpesviruses were also identified in lizards with stomatitis and were named Varanid herpesvirus 1 or gerrhosaurid herpesviruses.[1-3]

Ranavirus: Infections with frog virus 3 (Ranavirus, Iridoviridae) has been shown to cause dermal erosion and hemorrhage as well as systemic disease in adult frogs.[1,4] In tadpoles and metamorphosing frogs there may be subcutaneous hemorrhage and edema as well as hemorrhage in skeletal muscle. In salamanders, iridovirus was the cause of systemic disease and pale, raised foci in the skin that may progress to erosions and ulcers. Histologically the lesions began with edema and progressed to ballooning degeneration and vesicle formation. Cytoplasmic inclusions may be seen in affected cells.

Ranavirus may cause skin lesions in reptiles[5] and will also be discussed with lesions of the upper digestive tract (oral cavity and pharynx).

Poxvirus: Poxvirus infection has been reported in European frogs (*Rana temporaria*). Grossly the skin is red and there is dermal ulceration progressing to necrosis of digits or legs. Histologically the epidermis is hyperplastic with areas of necrosis. Cytoplasmic inclusions are present.

Poxvirus infections in reptiles have been reported in caimans, crocodiles, a Hermann tortoise, and in tegus. Infections are not common in reptiles, and mortality is usually low. Grossly, raised, discolored ulcers have been seen, and histologically there is epithelial cell hyperplasia, ballooning degeneration and intracytoplasmic inclusions.

Reptile epidermal cells may contain keratohyalin, which in some animals may appear similar to poxvirus inclusions histologically and these cytoplasmic bodies must be differentiated from viral inclusions.

Papillomavirus: Cutaneous papillomas in Japanese newts are suspected of being caused by papillomavirus, but the virus has not been conclusively identified. Grossly lesions are solitary and histologically they are similar to other papillomas. They may spontaneously regress. Papular lesions with typical histology but no inclusion bodies have been seen in side-necked turtles, a Russian tortoise, Green lizards, sea, green and loggerhead turtles, and a diamond python.[6] Virus particles were seen with electron microscopy.

Bacterial disease: The most common condition affecting the skin of amphibians is 'red-leg', which classically is considered to be due to Aeromonas hydrophila.[1] There is septicemia with generalized capillary dilatation leading to edema, hyperemia, hemorrhage and possibly ulcers in the skin. Although most commonly involving the hind legs, lesions can be anywhere in the skin. In addition to Aeromonas, a number of other bacteria have been associated with the disease. Grossly there is reddening of the skin and edema of the skin and subcutis. Histologically early lesions may have only a minimal disseminated inflammatory cell infiltrate, but as the lesion progresses necrosis and inflammatory cell infiltration become more prominent. In reptiles bacterial septicemias can lead to skin changes grossly similar to those seen in amphibians.

Blister disease in reptiles has been considered by some to be due to Staphylococcus aureus infection. Pustules and blisters are noted and often precede ulcers. Microscopically acute dermatitis is associated with hyperemia, edema, hemorrhage, necrosis and a disseminated cellular infiltrate. With chronicity multiple to coalescing abscesses may be seen. Organisms can be found in these lesions in some cases. Mycobacteriosis and fungal infections are differential diagnoses.

Mycobacterial infections are seen in both amphibians and reptiles. They are usually chronic conditions with slowly growing nodules seen grossly. Histologically lesions are similar to those of other bacteria with special stains needed to see the organism. PCR sequencing is needed for an exact identification of the organism.

The most frequently identified species of chlamydial organisms reported to cause disease in amphibians are *Chlamydia psittaci* and *Chlamydia pneumoniae*. Skin lesions seen in the disease vary from depigmentation to necrosis and sloughing and the condition in amphibians can closely resemble bacterial 'red-leg.'[1] *Chlamydia pneumoniae* is a possible cause of granulomas in reptiles. In both cases gross lesions are nonspecific and histologic changes are only specific if chlamydial intracytoplasmic basophilic inclusions are seen. PCR is the diagnostic method of choice for specific identification of the organism.

Mycotic disease: Several fungi cause disease of the skin of reptiles and amphibians. In amphibians, superficial infections are often caused by *Basidiobolus* or *Batrachochytrium* (chytrid fungus). Mixed infections can occur. Severe lesions lead to skin changes which in turn cause generalized disease.

Batrachochytrium (chytrid fungus) causes variable inflammation and marked epidermal proliferation/acanthosis leading to thickened skin. Organisms are usually present in the thickened epidermis.[7]

Basidiobolus may occur as a primary entity or in conjunction with chytrid infection. Primary changes include hyperkeratosis, acanthosis, necrosis, and inflammatory cell infiltrate. Organisms (hyphae) are usually seen in the lesion.

Chromomycosis (phaeohyphomycosis) has been a problem in toads and frogs.[8] It may cause superficial or deep mycosis. There are several possible causative organisms usually from an environmental source. Animals are usually presented due to skin lesions which can vary in severity grossly. Ulceration and possible pigmentation are noted. The histologic lesions depend on the duration of the condition. Early there is inflammation, necrosis, and edema. Organisms may be present. With chronicity, fibroplasia may also be noted. In early lesions and areas exposed to the air, hyphae predominate. As the infection progresses the lesion becomes an organized granuloma containing pigmented spores that may occur in clumps. Granulomas can become fibrous spores are usually pigmented, but can be stained by methods for fungi.

Fungal diseases of the skin of reptiles are often secondary to/associated with improper humidity and/or temperature, poor diet, or other improper husbandry procedures. A variety of fungi have been isolated including *Candida*, *Mucor*, *Fusarium*, and *Pacilomyces*. These usually have nonspecific lesions grossly and histologically. Fragments of hyphae may be seen in the hyperkeratotic crusts.

Dermatophytosis is uncommon in reptiles however there was a recent report of *Trichophyton* species infection in a Tenerife lizard.[9]

Chromomycosis can be seen in reptiles and the lesion is similar to that in amphibians. Grossly there may be granulomas with focal pigmentation and histologically there is chronic inflammation associated with pigmented fungi.

Trichosporon has been considered a yeast as it produces hyphae and pseudohyphae. It has been reported in chelonians and banded rock rattlesnakes, and *T cutaneum* has caused dermatitis in a spiny-tailed lizard. In anoles, granulomas may be seen and histologically numerous organisms can be present.

The 'former' *Chrysosporium* anamorph *of Nannizziopsis vriesii* (CANV) is associated with disease in snakes, lizards, and crocodilians.[10] The condition is called 'yellow fungus disease.' The organisms making up this

complex have recently been given novel genera based on molecular studies.[11,12] The disease is progressive and often fatal. It is spread via contact or fomites. The area around the mouth is often affected. Diagnosis is by culture, histopathology and/or PCR. The lesions may extend into deeper tissues.

Grossly CANV can begin as a hyperkeratotic plaque followed by necrosis, hemorrhage, and sloughing of skin. The skin may have a yellowish color. The histologic appearance depends on the stage of the disease. Early lesions are hyperkeratotic and necrotic, and organisms are usually seen. With chronicity the lesion becomes pyogranulomatous.

*Parasitic disease:*External parasites such as mites and ticks may be found on the skin. Nematodes and cestodes in the subcutis may lead to nodular lesions that 'move'.

Noninfectious disease

Trauma: A variety of insults may lead to traumatic lesions. Physical trauma and burns are most common, and detailed history is usually necessary as the gross and/or histologic lesions are often not specific.

Nutritional and metabolic disease

Mineralization and urate deposition can occur, often as part of a systemic problem, although localized chronic irritation may also lead to mineralization. Grossly the skin is roughened and may feel gritty. The lesions can be distinguished histologically.

Neoplastic disease

Tumors can be epithelial, mesenchymal, and chromatophore origin. These tumors are more often seen/reported in reptiles. Most tumors are not grossly specific, but those of chromatophore origin may be pigmented. Epithelial tumors can be benign (papilloma) or malignant (squamous cell carcinoma). Histologic criteria for malignancy are similar to those of mammals.

Mesenchymal tumors can also be benign or malignant, and the types seen/reported are similar to mammals. They are also more common in reptiles than amphibians. Grossly they are not specific, diagnosis being made histologically or with immunochemistry.

Chromatophoromas can originate from xanthophores, erythophores, iridophores, and melanophores. Chromatophoromas of several types have been reported in a variety of reptiles[13]; however, melanophoromas seem to be the only type seen in amphibians. Grossly they may be pigmented and histologically pigment cells of varying degrees of differentiation may be seen. Differentiation of the pigment on routine H&E sections is usually not possible.

A thorough review of the diseases and lesions of the reptile integument is covered in the ARAV Specialty exam preparation session in the 2009 proceedings.[14]

Musculoskeletal System

Any of the conditions affecting the skin can become severe enough to affect underlying skeletal muscle and bone. The lesions will have similarities to those in the skin and subcutis, with differences depending on the way muscle and bone react to insults.[1]

There are no infectious diseases specific to the muscle or bone of the head, and although carnivorous reptiles are potentially at risk for vitamin E deficiency, lesions affecting the muscles of the head are not well documented. Vitamin E deficiency is also not well documented in amphibians.

Neoplastic disease

Although any type of neoplasm associated with skeletal muscle or bone could occur in the head, there are few reports. Osteosarcoma, fibrosarcoma, and chondrosarcoma have been seen.[2,3]

Metabolic bone disease (MBD)

The most common problem involving the musculoskeletal system of the head of reptiles and amphibians, is 'metabolic bone disease'. This is usually fibrous osteodystrophy in reptiles and amphibians, but rickets, osteomalacia, and osteoporosis/osteopenia also fall into the category of metabolic bone disease. The underlying problem is excessive production of parathormone by the parathyroid glands.

Primary hyperparathyroidism-neoplasia or hyperplasia: There are very few reports of tumors in reptiles and amphibians.

Secondary hyperparathyroidism-renal or nutritional: The most common cause of fibrous osteodystrophy in reptiles and amphibians is nutritional.[4,5] Anything that decreases the concentration of serum ionized calcium, or leads to an improper ratio of Ca:P can be the cause. That includes a Ca deficiency, P excess or vitamin D deficiency. Ca decrease or Ca:P imbalance leads to parathormone production which stimulates release of Ca from bone to rectify the blood levels.

There is one report in amphibians (*Leiopelma* species) indicating that excessive fluoride intake can lead to osteofluorosis which may complicate MBD.[6]

The basic lesion is osteoclastic resorption of bone and replacement by poorly defined fibrosseous tissue, leading to weak bone, fractures, and deformities. The mandible and maxillary areas are commonly affected.

Nasal Cavity and Oropharynx

These areas are considered part of the upper GI and upper respiratory systems. Several infectious and noninfectious diseases affect these areas, and several types of neoplasia have been reported. Lesions seen in these tissues may be solitary or part of a generalized disease process.

Infectious disease

Inflammation of the oral cavity (stomatitis/mouth rot) can be due to bacteria, fungi, parasites, and viruses. These conditions have been reported more often in reptiles.

Bacterial stomatitis is usually due to gram-negative bacteria and can present grossly with necrosis and hemorrhage and/or proliferative lesions, depending on extent and duration of the condition. Histologic changes vary from acute necrosis, hemorrhage and, inflammatory infiltrate, to granuloma formation. Mycobacteria may cause similar granulomas.

The bacterium, *Devriesea agamarum* has been isolated and characterized from *Uromastyx* species with dermatitis and/or septicemia.[1] This facultative pathogenic bacterium is able to cause dermatitis in agamid lizards (*Agama impalearis, Pogona vitticeps, Uromastyx geyri*, and *Uromastyx acanthinura*) when the integrity of the skin is breached. The lesions that develop are a proliferative dermatitis and/or cheilitis. The bacterium is a Gram positive small rod that by comparative analysis of 16S rRNA gene sequences was identified as a strain in the new taxon within the class Actinobacteria. In further studies *D. agamarum* was found to be part of the oral microbiological flora in *Pogona vitticeps*.

Mycotic stomatitis: Gross lesions are similar to those of bacterial infections and mixed infection can occur. *Candida* is a common cause. Histologic changes are necrotizing and granulomatous. Organisms are usually seen histologically, but as with bacterial infections, culture is necessary for a specific diagnosis.[2]

Parasitic stomatitis: Most of the lesions are due to transient/incidental infections. Grossly these are similar to other causes of proliferative granulomas. Specific identification is due to finding organisms grossly or histologically. Both protozoa and nematodes have been identified.

Viral stomatitis: Herpesvirus causes stomatitis in several species of tortoises.[3] Necrotizing and proliferative changes are seen grossly with histologically variable inflammation and necrosis. Intranuclear inclusions and syncytial giant cells are present.

Mycoplasma rhinitis of desert tortoises is due to *Mycoplasma agassizii*.[4] Grossly the mucous membranes are reddened and there is an exudate that varies from serous to almost caseous. The latter may lead to blockage of the nasal passages. Histologically the epithelium may be proliferative and necrotic, with a variable inflammatory infiltrate whose character depends somewhat on the duration of the disease.

Noninfectious disease

Hypovitaminosis A is the cause of oral cavity lesions in both amphibians and reptiles.[5,6] In amphibians fed insect diets, squamous metaplasia of the oral and lingual mucosa is seen. In reptiles, the disease is seen mostly in chelonians, however it has been reported in iguanas and cheilitis has been seen in chameleons and anoles.

Gross lesions in the oral cavity usually consist of nodules or foci that may look almost caseous and that must be differentiated from primary infections. Affected lips will be swollen and variably necrotic. Histologically the primary change is squamous metaplasia involving non-squamous mucosa and glands. There may be secondary infection.

Neoplastic disease

Oral cavity tumors are rarely reported in amphibians. One unusual tumor of the oral cavity of axolotls has been seen in several animals. Although present in the oral cavity it is considered to be a neuroepithelioma/neuroblastoma of olfactory origin. Grossly the tumor is roughened and polypoid, and histologically there are lobules, rosettes, and trabeculae separated by variable amounts of stroma.[7,8]

Both carcinomas and sarcomas have been reported in several reptile species.[9,10] Fibromas[11] have also been seen. Sarcomas include fibrosarcoma[12] and malignant lymphoma. Grossly the masses are not diagnostic and histologically they are similar to those seen in other species. We have also seen a tumor with the histologic features of an ameloblastoma in a gecko.

Special Senses

Eye

Corneal lipidosis (lipid keratopathy) is seen in both amphibians and reptiles.[1-3] The etiology/pathogenesis are not well-defined. There appears to be an association with high fat diets and hypercholesterolemia is seen in some affected animals. The condition may also occur as a sequela to other insults including trauma and infection.

Grossly the cornea may have opaque white foci, to more diffuse gray-white plaques. Histologically there is stromal degeneration and separation of lamellae. There may be some inflammation, and cholesterol clefts are seen.

Ocular and periocular infections are usually bacterial or mycotic, although wandering parasites can also be found in some inflammatory processes. Gross lesions can be suggestive but for a definitive diagnosis observation of the organism or culture is necessary.[4-6] Histologically the reactions depend on the duration of the lesion and are similar to those seen in other tissues. The exact morphologic diagnosis depends on what portion(s) of the eye are affected.

Miscellaneous ocular lesions include a variety of incidental lesions have been seen in the eyes of amphibians and reptiles. These include retinal degeneration, occasionally with cholesterol cleft formation, mineralization, and cataract formation. Vitamin A deficiency can lead to squamous metaplasia of the Harderian glands with resultant infection/infection that leads to subconjunctival exudate.[7,8]

Ocular neoplasia: Both sarcomas and carcinomas, as well as melanomas, can occur in the orbit and ocular adnexa. A tumor of the spectacle has also been reported.[9-12] Most melanomas will be pigmented, but the gross appearance of other tumors is not specific. Primary tumors of the eye are infrequently seen, but in frogs, we have seen a tumor that was morphologically consistent with medulloepithelioma. Grossly these tumors may fill the eye and histologically they are comprised of poorly differentiated neuroepithelial cells that form sheets and poorly defined rosettes. The tumor is histologically similar to tumors seen in birds and mammals.

A thorough review of the diseases and lesions of the reptile eye and ocular adnexa is covered in the ARAV Specialty exam preparation session in the 2012 proceedings.[13]

Ear

Aural infections are seen in both amphibians and reptiles. Gram negative bacteria are a common cause. Grossly there may be swelling that includes the outer ear, and an exudate is possible. Histologically the reaction is similar to that in other tissues and organisms must be visualized for a specific diagnosis. Ticks can be found in the outer ear in reptiles and amphibians. Grossly the parasite may be seen and histologically reaction is often minimal. Mites can colonize the outer ear canal and may only be seen histologically in some cases. There is often no appreciable reaction.

Cryptosporidial infection has been reported in the ear of iguanas.[14] Grossly there were polyps that protruded from the ear canal. Histologically nests of cystic glands and abundant fibrous connective tissue were present. Hyperplastic cuboidal to pseudostratified columnar epithelium are colonized by cryptosporidial organisms. Electron microscopy revealed that the majority of organisms were trophozoites.

Otitis media in box turtles have been attributed to dietary imbalances and possible underlying organochlorine pesticides.[15] A mixture of bacteria and rarely yeast have been isolated from these inflammatory lesions.

Central Nervous System

Infectious disease

Viral disease: Amphibians and reptiles may be infected and play a part in the transmission cycle of viruses such as Eastern Equine Encephalomyelitis, Japanese Encephalitis and West Nile Virus. Proved clinical infections are rarely reported. Clinical signs are not specific, gross lesions absent and histologic lesions not diagnostic unless inclusion bodies are present.[1-3]

Nonspecific clinical signs such as head tilts may be due to encephalitis, but inner ear infections have to be ruled out. Histologic lesions are typical, consisting of perivascular cuffs and gliosis. Inclusion bodies may be suggestive, but PCR or other diagnostics are needed to get a definitive diagnosis.

'Inclusion body disease' of Boid snakes has long been considered a viral disease, and recently an arenavirus has been suggested as the etiology.[4,5] Affected snakes may have histologic lesions in the brain, but no gross changes are seen.

Other viruses reported to cause CNS lesions include an adenoviral-like infection leading to degenerative encephalomyelopathy in a kingsnake, and meningoencephalitis in a python due to paramyxovirus.[6,7]

Bacterial disease: A variety of bacteria have been isolated from meningoencephalitis in amphibians and reptiles. Gross lesions include meningeal exudate and possible abscess formation in the brain. Histologic changes are typical of bacterial infections in any tissue. Specificity depends on visualizing and/or culturing organisms.

Mycotic disease: Mycotic infections and wandering metazoan parasites may cause brain lesions.[8] A myxozoan was found in the axons of dead frogs that had characteristic systemic lesions in the liver.[9]

Neoplastic disease: With the exception of the olfactory neuroblastoma,[10] and a glioma in the spinal cord,[11] tumors of the CNS are not reported. We have seen one tumor apparently arising in the meninges of a snake. Grossly it was not specific and histologically was comprised of poorly differentiated cells. It was considered a sarcoma morphologically.

Miscellaneous lesions: Hydrocephalus is infrequently seen. The affected brain has variably enlarged ventricles filled with fluid. There is compression of adjacent tissue. Cholesterol granulomas are occasionally found involving the brain/meninges.[12]

References

Skin

1. Reavill DR. Amphibian skin diseases. *Vet Clin N Am.* 2001;4:413-440.

2. Jacobson ER. Diseases of reptiles. Part II. Infectious diseases. *Compend Contin Educ Pract Vet.* 1981;3:195-200.

3. Stacy BA, Wellehan JF, Foley AM, et al. Two herpesviruses associated with disease in wild Atlantic loggerhead sea turtles (*Caretta caretta*). *Vet Microbiol.* 2008;126:63-73.

4. Forzán MJ, Jones KM, Vanderstichel RV, et al. Clinical signs, pathology and dose-dependent survival of adult wood frogs, *Rana sylvatica*, inoculated orally with Frog Virus 3 (*Ranavirus* sp, Iridoviridae). *J Gen Virol.* 2015 Jan 15. pii: vir.0.000043 (Epub ahead of print).

5. Stöhr AC, Blahak S, Heckers KO, et al. Ranavirus infections associated with skin lesions in lizards. *Vet Res.* 2013;44:84.

6. Gull JM, Lange CE, Favrot C, et al. Multiple papillomas in a diamond python *Morelia spilota spilota*. *J Zoo Wildl Med.* 2012;43:946-949.

7. Martel A, Spitzen-van der Sluijs A, Blooi M, et al. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. *Proc Natl Acad Sci USA.* 2013;110:15325-15329.

8. Schmidt RE. Amphibian chromomycosis. In: Hoff GL, Frye FL, Jacobson ER, eds. *Diseases of Amphibians and Reptiles.* New York, NY: Plenum Press;1984:169-181.

9. Orós J, Hernández JD, Gallardo J, et al. Dermatophytosis caused by *Trichophyton* spp. in a tenerife lizard (*Gallotia galloti*): an immunohistochemical study. *J Comp Pathol.* 2013 Aug;149:372-375.

10. Mitchell MA, Walden MR. *Chrysosporium* anamorph *Nannizziopsis vriesii*: An emerging fungal pathogen of captive and wild reptiles. *Vet Clin N Am Exot Anim Pract.* 2013;16: 659-668.

11. Sigler L, Hambleton S, Paré JA. Molecular characterization of reptile pathogens currently known as members of the *Chrysosporium* Anamorph of *Nannizziopsis vriesii* complex and relationship with some human-associated isolates. *J Clin Microbiol.* 2013;51:3338–3357.

12. Dolinski AC, Allender MC, Hsiao V, Maddox CW. Systemic *Ophidiomyces ophiodiicola* infection in a plains garter snake (*Thamnophis radix*). *J Herp Med Surg.* 2014;24:7-10.

13. Heckers KO, Aupperle H, Schmidt V, Pees M. Melanophoromas and iridophoromas in reptiles. *J Comp Pathol.* 2011;8:1-11.

14. Reavill D, Schmidt R. Pathology of the reptile integument. *Proc Annu Conf Assoc Rept Amph Vet.* 2009;93-106.

Musculoskeletal system

1. Gartrell BD, Hare KM. Mycotic dermatitis with digital gangrene and osteomyelitis, and protozoal intestinal parasitism in Marlborough green geckos (*Naultinus manukanus*). *N Z Vet J.* 2005;53:363-367.

2. Schmidt RE, Reavill DR. Metastatic chondrosarcoma in a corn snake (*Pantherophis guttatus*). 2012;22:67-69.

3. Janert B. A fibrosarcoma in a Siamese crocodile (*Crocodylus siamensis*). *J Zoo Wildl Med.* 1998;29:72-77.

4. Hoby S, Wenker C, Robert N, et al. Nutritional metabolic bone disease in juvenile veiled chameleons (*Chamaeleo calyptratus*) and its prevention. *J Nutr.* 2010;140:1923-31.

5. Anderson MP, Capen CC. Fine structural changes of bone cells in experimental nutritional osteodystrophy of green iguanas. *Virchows Arch B Cell Pathol.* 1976;20:169-184.

6. Shaw SD, Bishop PJ, Harvey C, et al. Fluorosis as a probable factor in metabolic bone disease in captive New Zealand native frogs (*Leiopelma* species). *J Zoo Wildl Med.* 2012;43:549-565.

Nasal cavity and oropharynx

1. Martel A, Pasmans F, Hellebuyck T, Haesebrouck F, Vandamme P. *Devriesea agamarum* gen. nov., sp. nov., a novel actinobacterium associated with dermatitis and septicaemia in agamid lizards. *Int J Syst Evol Micr.* 2008;58:2206–2209.

2. Cheatwood JL, Jacobson ER, May PG, et al. An outbreak of fungal dermatitis and stomatitis in a free-ranging population of pigmy rattlesnakes (*Sistrurus miliarius barbouri*) in Florida. *J Wildl Dis.* 2003;39:329-37.

3. 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.

4. Brown MB, Schumacher IM, Kleinet PA, et al. *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. *Infect Immun.* 1994;62:4580–4586.

5. Miller EA, Green SL, Otto GM, Bouley DM. Suspected hypovitaminosis A in a colony of captive green anoles (*Anolis carolinensis*). *Contemp Top Lab Anim.* 2001;40:18-20.

6. Rodríguez CE, Pessier AP. Pathologic changes associated with suspected hypovitaminosis A in amphibians under managed care. *Zoo Biol.* 2014;33:508-15.

7. Shioda C, Uchida K, Nakayama H. Pathological features of olfactory neuroblastoma in an axolotl (*Ambystoma mexicanum*). *J Vet Med Sci.* 2011;73:1109-1111.

8. Brunst VV, Roque AL. Tumors in amphibians. I. Histology of a neuroepithelioma in Siredon mexicanum. *J Natl Cancer Inst.* 1967;38:193-204.

9. Steeil JC, Schumacher J, Hecht S, et al. Diagnosis and treatment of a pharyngeal squamous cell carcinoma in a Madagascar ground boa (*Boa madagascariensis*). *J Zoo Wildl Med.* 2013;44:144-151.

10. Wilhelm RS, Emswiller BB. Intraoral carcinoma in a Burmese python. *Vet Med Small Anim Clin.* 1977;72:272-273.

11. Idowu AL, Golding RR, Ikede BO, et al. Oral fibroma in a captive python. *J Wildl Dis.* 1975;11:201-204.

12. Salinas EM, Arriaga BO, Lezama JR et al. Oral fibrosarcoma in a black iguana (*Ctenosaura pectinata*). *J Zoo Wildl Med.* 2013;44: 513-5166.

Special sense

1. Shilton CM, Smith DA, Crawshaw GJ, et al. Corneal lipid deposition in Cuban tree frogs (*Osteopilus septentrionalis*) and its relationship to serum lipids: an experimental study. *J Zoo Wildl Med.* 2001;32: 305-19.

2. Wright K. Cholesterol, corneal lipidosis, and xanthomatosis in amphibians. *Vet Clin N Am Exot Anim Pract.* 2003;6:155-67.

3. Lawton MPV. Common ophthalmic problems seen in chelonia. *Proc Annu Conf Assoc Rept Amph Vet.* 1997.

4. Sutton DA, Marín Y, Thompson EH, et al. Isolation and characterization of a new fungal genus and species, *Aphanoascella galapagosensis*, from carapace keratitis of a Galapagos tortoise (*Chelonoidis nigra microphyes*). *Med Mycol.* 2013;51:113-120.

5. Collete BE, Curry OH. Mycotic keratitis in a reticulated python. *J Am Vet Med Assoc.* 1978;173:1117-1118.

6. Cooper JE, McClelland MH, Needham JR. An eye infection in laboratory lizards associated with an *Aeromonas* sp. *Lab Anim.* 1980;14:149-151.

7. Donoghue S. Nutrition. In: Mader DR. *Reptile Medicine and Surgery* 2nd ed. St. Louis, MO: Saunders/Elsevier;2006:289.

8. Miller EA, Green SL, Otto GM, Bouley DM. Suspected hypovitaminosis A in a colony of captive green anoles (*Anolis carolinensis*). *Contemp Top Lab Anim*. 2001;40:18-20.

9. Gál J, Demeter Z, Palade EA, et al. Harderian gland adenocarcinoma in a Florida red-bellied turtle (*Pseudemys nelsoni*) - case report. *Acta Vet Hung.* 2009;57:275-281.

10. Kottwitz J, Zehnder AM, Wyre N, Aquino S. Lacrimal cystadenoma in a Chinese box turtle (*Cuora flavomarginata*). *J Zoo Wildl Med.* 2008;39:103-106.

11. Hardon T, Fledelius B, Heegaard S. Keratoacanthoma of the spectacle in a boa constrictor. *Vet Ophthalmol.* 2007;10:320-322.

12. Brooks DE, Ginn PE, Miller TR, Bramson L, et al. Ocular fibropapillomas of green turtles (*Chelonia mydas*). *Vet Pathol.* 1994;31:335-339.

13. Reavill D, Schmidt R. Pathology of the reptile eye and ocular adnexa. *Proc Annu Conf Assoc Rept Amph Vet.* 2012;87-97.

14. Uhl EW, Jacobson E, Bartick TE, et al. Aural-pharyngeal polyps associated with *Cryptosporidium* infection in three iguanas (*Iguana iguana*). *Vet Pathol.* 2001;38:239-242.

15. Tangredi BP, Evans RH. Organochlorine pesticides associated with ocular, nasal, or otic infection in the eastern box turtle (*Terrapene carolina carolina*). *J Zoo Wildl Med.* 1997;28(1):97-100.

Central nervous system

1. Graham SP, Hassan HK, Chapman T, et al. Serosurveillance of eastern equine encephalitis virus in amphibians and reptiles from Alabama, USA. *Am J Trop Med Hyg.* 2012;86:540-544.

2. Dauphin G, Zientara S, Zeller H, Murgue B. West Nile: worldwide current situation in animals and humans. *Comp Immunol Microbiol Infect Dis.* 2004;27:343-355.

3. Ariel E. Viruses in reptiles. *Vet Res.* 2011;42:100. http://www.veterinaryresearch.org/content/42/1/100. Accessed June 21, 2015.

4. Hetzel U, Sironen T, Laurinmäki P, et al. Isolation, identification, and characterization of novel arenaviruses, the etiological agents of boid inclusion body disease. *J Virol.* 2013;87:10918-10935.

5. Hepojoki J, Kipar A, Korzyukov Y, et al. Replication of boid inclusion body disease-associated arenaviruses is temperature sensitive in both boid and mammalian cells. *J Virol.* 2015;89:1119-1128.

6. Raymond JT, Lamm M, Nordhausen R, Latimer K, et al. Degenerative encephalopathy in a coastal mountain kingsnake (*Lampropeltis zonata multifasciata*) due to adenoviral-like infection. *J Wildl Dis.* 2003;39:431-436.

7. West G, Garner M, Raymond J, et al. Meningoencephalitis in a Boelen's python (*Morelia boeleni*) associated with paramyxovirus infection. *Zoo Wildl Med.* 2001;32:360-365.

8. Olias P, Hammer M, Klopfleisch R. Cerebral phaeohyphomycosis in a green iguana (*Iguana iguana*). *J Comp Pathol.* 2010;143:61-64.

9. Hartigan A, Sangster C, Rose K, et al. Myxozoan parasite in brain of critically endangered frog. *Emerg Infect Dis.* 2012;18:693-695.

10 Shioda C, Uchida K, Nakayama H. Pathological features of olfactory neuroblastoma in an axolotl (*Ambystoma mexicanum*). *J Vet Med Sci.* 2011;73:1109-1111.

11. Craig LE, Wolf JC, Ramsay EC. Spinal cord glioma in a ridge-nosed rattlesnake (*Crotalus willardi*). *J Zoo Wildl Med.* 2005;36:313-315.

12. Kummrow MS, Berkvens CN, Paré JA, Smith DA. Cerebral xanthomatosis in three green water dragons (*Physignathus cocincinus*). *J Zoo Wildl Med.* 2010;41:128-132.

Reptile Virology: Understanding the Methods and Their Interpretation

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Session #142

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Abstract: There are increasing numbers of viruses detected in reptiles. Methods and tests available for virus detection in reptiles also are increasing. Understanding the possible clinical importance of viral infections as well as diagnostic methods and their interpretation can be challenging. In many cases, data is still needed to help optimize clinical and laboratory interpretation of results. On the other hand, international trade of reptiles has led to high prevalence of some viruses in captive reptiles and also increases the possible importance of infectious agents and the need for quarantine exams. Interpretation of diagnostic tests requires an understanding of what the test detects and how it does so.

Introduction

Diagnostic methods used in virology can be divided into two distinct categories: methods for the direct detection of viruses (or parts of viruses) and methods for the detection of the immune response to viruses. Methods from each category answer different questions. What method is chosen will depend on many different factors. In situations in which herd health management or quarantine is of interest, different methods may be chosen from situations of acute disease processes. In some cases it may also be helpful to combine virologic diagnostic methods with other methods such as histopathology, cytology, and/or hematology in order to help interpret the results.

The quality of the results obtained from virologic testing will also depend on various factors, and it is always a good idea to contact your diagnostic laboratory of choice before sample submission. In addition to the choice of test, choices of samples or tissues submitted, time of collection, method of collection, and handling of samples will all play a role in the reliability of the final results.

The following should be considered when choosing samples:

- Animal species and size, clinical status (alive, dead, healthy, acutely or chronically ill)
- Morphologic changes observed in tissues
- Time between sampling and transportation to laboratory (and storage options)

Lists of select viruses found in turtles and tortoises as well as in squamates can be found in Tables 1 and 2. These also include the most common tests used for the detection of each virus and the samples in which viruses have most often been detected.

Table 1. Select viruses of turtles and tortoises.

Virus	Species	Clinical importance[a]	Clinical and pathologic signs	Common diagnostic methods[b]	Samples for diagnostics
Poxviridae	Hermann's tortoise	(+)	Skin lesions around the eye	PCR, EM	Material from lesions
Iridoviridae: *Ranavirus*	Wide range of species, viruses not species specific	+++	Upper respiratory tract disease, pneumonia, tracheitis, esophagitis, pharyngitis, hepatitis, enteritis	PCR	Oral swabs, liver, tongue, blood
Herpesviridae	All testudines should be considered susceptible, diverse herpesviruses present	+-+++	Rhinitis, conjunctivitis, stomatitis and glossitis, in diverse species, fibropapillomatosis in sea turtles and box turtles	PCR Serology: VNT	Oral swabs, tongue, trachea and oesophagus, liver, intestine, kidney and brain
Adenoviridae	Diverse Testudines	+-+++	Anorexia, lethargy, mucosal ulcerations, nasal and ocular discharge, diarrhea	PCR, EM	Nasal flush, oral and cloacal swabs, liver
Papillomaviridae	Diverse testudines, best characterized in sea turtles	+	Skin lesions	PCR, EM	Skin samples
Tornovirus	Green sea turtles	?	Animals also had severe fibropapillomatosis (herpesvirus infection)	Metagenomics	
Reoviridae	Spur-thighed tortoise	(++)	Cachexia, epithelial necrosis of the tongue, splenomegaly	Virus isolation, RT-PCR, EM	Oral swab, tongue, lung
Paramyxoviridae: *Ferlavirus*	Spur-thighed, Hermann's and leopard tortoises	(+++)	Pneumonia, dermatitis described in one case	RT-PCR	Oral and cloacal swabs, tracheal wash, lung, intestine, liver, kidney, trachea
Picornaviridae (virus "x"), proposed genus "Topivirus"	Many tortoise species, including spur-thighed, marginated, Egyptian, and leopard tortoises	++	Softening of the carapace in young tortoises, diphtheroid-necrotizing stomatitis and pharyngitis, conjunctivitis, rhinitis, pneumonia, enteritis, ascites	Virus isolation, RT-PCR Serology: VNT	Oral swabs, tongue, intestine, liver, kidney

[a]+++ = highly pathogenic; - = no pathology associated with infection in available reports; clinical importance in parentheses: only individual report or reports available, virus appears to be uncommon in these animals
[b]EM = electron microscopy; PCR = polymerase chain reaction; VNT = virus neutralization test; RT-PCR = reverse transcriptase polymerase chain reaction.
Source: Adapted from Girling SJ, Raiti P. *BSAVA Manual of Reptiles.* 2nd ed. Quedgeley, Gloucester: Br Sm Anim Vet Assoc;2004.

Table 2. Select viruses of squamates.

Virus	Species	Clinical importance[a]	Clinical and pathologic signs	Common diagnostic methods[b]	Samples for diagnostics
Poxviridae	Flap-necked chameleon, tegu	(+)	Skin lesions	PCR, EM	Skin, spleen, liver
Iridoviridae: *Ranavirus*	Numerous squamate species, viruses not species specific	+++	Ulceration of nasal mucosa, hepatic necrosis, Granulomatous lesions and necrotizing inflammation of the pharyngeal submucosa, skin lesions	Virus isolation, PCR, EM	Oral swabs, oral mucosa, skin, liver
Iridoviridae: Invertebrate iridovirus (IIV)	Multiple insectivorous squamate species, particularly bearded dragons and chameleons	?	Cachexia, pneumonia, skin lesions	PCR, EM	Skin, liver
Iridoviridae: erythrocytic virus	Several squamate species	?	From no clinical signs to anemia and systemic disease	PCR, EM	Blood
Herpesviridae	Diverse squamate species	+-+++	Cutaneous papillomas, hepatic necrosis, proliferative stomatitis, pneumonia	PCR	Affected tissues
Adenoviridae	Diverse squamate species	++	Hepatitis, enteritis, nephritis, encephalitis	PCR, EM	Cloacal swabs, liver, intestine
Parvoviridae	Bearded dragons, kingsnakes	?	Found with adenovirus infections	PCR, EM	Intestine
Reoviridae	Diverse squamate species	+ - +++	Pneumonia, stomatitis, wasting, hepatitis, enteritis, CNS signs	Virus isolation, RT-PCR, EM	Oral swabs, lung, liver, brain
Bornaviridae	Various snake species	?	Not yet known	Metagenomics, (PCR)	Various tissues
Paramyxoviridae (PMV): *Ferlavirus*	Diverse squamate species, most common in snakes	+++	Proliferative pneumonia and central nervous system disorders	RT-PCR	Oral and cloacal swabs, tracheal washes, lung, liver, kidney, pancreas
Paramyxoviridae: Sunshine virus	Australian pythons	+ - +++	Respiratory and CNS-disease	RT-PCR	Oral and cloacal swabs, Lung, brain
Arenaviridae	Boas and pythons	+++	Inclusion body disease, immunosuppression	RT-PCR	Esophagus swabs, whole blood, brain, liver, pancreas
Nidovirales: Torovirinae	Pythons	+++	Stomatitis, Proliferative pneumonia	RT-PCR	Lung, Intestine
Rhabdoviridae	Australian house gecko, teiid lizards	?	Erythrocytic inclusions	RT-PCR, EM	Blood
Caliciviridae	Rattlesnakes	(++)	Enteritis, hepatitis	EM, virus isolation	Intestine, liver

[a]+++ = highly pathogenic; - = no pathology associated with infection in available reports; clinical importance in parentheses: only individual report or reports available, virus appears to be uncommon in these animals
[b]PCR = polymerase chain reaction; EM = electron microscopy; RT-PCR = reverse transcriptase polymerase chain reaction.
Source: Adapted from Girling SJ, Raiti P. *BSAVA Manual of Reptiles.* 2nd ed. Quedgeley, Gloucester: Br Sm Anim Vet Assoc;2004.

Direct Virus Detection

Direct virus detection methods look for the presence of a specific virus or parts of the virus (eg, viral proteins or, most commonly, parts of the viral genome). Samples collected for direct virus detection must contain virus or part of the virus, which requires some knowledge of the biology of the virus in order to choose the most appropriate sample for testing. A negative test in a specific sample does not mean that the animal tested is not infected, as there are many possible causes of false negative results. Direct virus detection methods include virus isolation, methods for genome detection (eg, polymerase chain reaction (PCR) or in situ hybridization (ISH)), methods for protein detection (eg, antigen detection ELISAs or immunofluorescence testing), as well as methods for direct visualization of virus particles (electron microscopy). A number of methods have been described or are commonly used to detect viruses in reptiles. Virus isolation was long used as a gold standard for virus detection in reptiles and can still be a helpful technique. PCR based techniques are now the most commonly used for standard virologic diagnostics.

Virus isolation in cell culture

This was one of the first methods used for direct virus detection and is still used for reptile virology in some labs (mostly in Europe). It requires the use of appropriate cell lines for specific viruses and appropriate growth conditions (eg, temperature) as well as the presence of live virus in a sample. Virus isolation in cell culture is not a very specific method, and numerous different reptilian viruses can be grown in cell culture (eg, some herpesviruses and picornaviruses of tortoises, many reoviruses of different reptiles, adenoviruses of various snakes and lizards, and ranaviruses in general). This method is slow and is only offered in a very few laboratories. It has the advantage that it is theoretically able to detect viruses for which no other method may yet be available (this is how some picornaviruses of tortoises were originally discovered), as well as that additional studies and development of additional tests (eg, virus neutralization tests, see below) become possible.

Polymerase chain reaction (PCR)

This is currently the most common method used for the detection of viruses in reptiles. Development of a PCR as a diagnostic test requires some knowledge of the expected genome of the virus. The test uses oligonucleotide primers that bind to a portion of the genome and an enzyme (polymerase) to copy the viral genome. In the case of RNA viruses, a preliminary step in which a complementary strand of DNA is made, is necessary. This is called reverse transcription and these PCRs are abbreviated as RT-PCRs. PCR products are defined by the primers used in the reaction and identification of a specific product generally starts with determination of the size of the product. Additional confirmation of specificity of the reaction is, however, generally necessary. Sequencing is the most commonly used and helpful method for this, especially since it also provides additional information about the detected DNA. Real-time PCRs are a further development in the use of this technology. In this case, an oligonucleotide probe is used which binds to the target sequence between the two primer sequences. The probe is attached to a marker dye and a color reaction indicates that the PCR product is correct. Real-time PCRs are often both more specific and more sensitive than conventional PCRs. Real-time PCR can also use unspecific dyes (eg, SYBR green), which does not increase the specificity of the assay.

Immunodiagnostic Methods

The detection of an immune response to a virus indicates that the animal has been in contact with that or a similar virus, but does not provide any information on the current infection status of that animal. In the case of viruses that cause life-long persistent infections, such as herpesviruses, detection of an immune response to the virus

does indicate current infection (although not necessarily disease or shedding). In the case of most other viruses that have been described in reptiles, this is not known, making interpretation of the results of serologic testing for clinical use or in quarantine somewhat difficult. The immune response to viral infections is complex and consists of humoral and cellular components. While cellular immunity may in many cases be more important for the outcome of an infection, the diagnostic testing available only looks at the humoral immunity, specifically the detection of antibodies against specific viruses. With these methods, it is important to understand that in some cases, there may be cross reactivity between different microorganisms, so that detection of an antibody reaction against a specific virus may be caused by contact with a different, but antigenically related virus. In other cases, related viruses may not cross react, so that only antibodies against a specific strain can be detected with a given test. This is for example the case for some testudinid herpesviruses. In reptile medicine it is also important to remember that antibody production is dependent on many factors, including host specific factors (eg, species, health status, age), pathogen specific factors (eg, strain), environmental factors (eg, time of year, temperature), and other factors (eg, time post infection, infection dose). Serologic methods used in reptile medicine include virus neutralization, ELISA, and hemagglutination inhibition (HI).

Virus neutralization

This test requires a virus that can be grown in cell culture. It measures antibodies in the serum of the animal that can prevent the virus from growing in cells (neutralization). It is therefore a measure not only of the presence of antibodies, but also for a function of these antibodies. This test is currently used for the detection of antibodies against the testudinid herpesviruses 1 and 3 in Europe and tortoise picornaviruses in the proposed genus "Topivirus," but is not available in the USA.

ELISA

Antibody detection ELISAs require viral antigen, which is used to coat plates. Serum from the patient is then added, and specific antibodies in the serum bind to the viral antigen. This reaction is detected using secondary antibodies against the host antibodies. These secondary antibodies are a limiting factor for the development and use of ELISAs for antibody detection in reptile medicine, as they are only available in specific laboratories. They are also generally species specific, although there is often cross reactivity with related species. Due to the limiting factor of the secondary antibodies, this test is only available in some laboratories in the USA for reptile serology.

Hemagglutination inhibition (HI)

This method can be used to detect antibodies against viruses that can be grown in cell culture in the lab and that hemagglutinate red blood cells. The test is relatively fast and easy to carry out. A specific amount of virus is added to serum from the patient, and antibodies in the sample prevent the virus from binding and agglutinating red blood cells. As for virus neutralization, this measures not only the presence of antibodies, but also a biologic (protective) function of the antibodies. In reptile medicine, this test is used for the detection of antibodies against ferlaviruses (family Paramyxoviridae).

Disclosure statement: Dr. Marschang is employed by a private lab (Laboklin) that offers diagnostic services for veterinarians.

Non-Steroidal Anti-inflammatory Drugs (NSAIDs) in Reptiles and Amphibians: A Review

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Session #020

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Abstract: Non-steroidal anti-inflammatory drugs are commonly used in veterinary medicine for managing acute and chronic pain and inflammation. Although they are used extensively in reptiles and amphibians, there are very few pharmacokinetic or pharmacodynamic studies of the use of these drugs in these species in the literature. This master class will review these drugs and their methods of action, and look at the safety, efficacy, toxicity, and recommended usage in reptiles and amphibians.

Cyclooxygenase and Prostaglandins

In 1971 it was discovered that aspirin inhibited the activity of a cyclooxygenase enzyme 1 (COX-1) that produced prostaglandins (PGs) involved in the pathogenesis of fever, pain, swelling, and inflammation.[50,57] In the following 30 years, 2 more enzymes also would be discovered, COX-2 and COX-3. Cyclooxygenase oxidizes arachidonic acid to various eicosanoids, including prostaglandins. The initial prostanoid formed is prostaglandin hydroperoxide (PGG_2), which is then converted to PGH_2. PGH_2 is then converted by prostaglandin E-synthetase to PGE_2, by prostaglandin D-isomerase to PGD_2, by prostaglandin F-reductase to PGF_2, by prostacyclin synthetase to PGI_2 (prostacyclin), and by thromboxane synthetase to thromboxane A_2 and B_2 (TXA_2, TXB_2). Arachidonic acid is also oxidized by 5-lipoxygenase (5-LOX) to form leukotrienes. Prostaglandins have a short half-life (4-6 min at 37^o C), so they are subsequently synthesized constantly and not stored by the body. They tend to act locally at the site of production.[50]

The COX enzymes each have different roles. COX-1 produces prostaglandins that are involved in mucosal defense, such as secretion of mucus and bicarbonate, attenuation of constriction of mucosal blood vessels, and regeneration of mucosal epithelium. It generates TXA_2, which is necessary for normal platelet function. It also has a cytoprotective function in some tissues, including the gastric mucosa, kidneys, reproductive tract, and central nervous system.[50] COX-2 generates prostaglandins that exert anti-inflammatory effects by the inhibition of leukocyte adherence, prevent mucosal erosions and promote healing of these lesions, and play a role in the protection and maturation of the kidneys.[50] COX-3 produces prostaglandins that initiate fever.[50] Depending on the NSAID that is selected, expected effects can not only include decreased inflammation, but also alteration of platelet function, modulation of vascular tone in the gastric mucosa and kidneys, cytoprotective functions within the gastric mucosa, smooth muscle contraction, and alteration of body temperature.[50]

Prostaglandins are important local mediators of pain and inflammation. Of the prostaglandins, PGE_2 and prostacyclin are the most potent mediators of pain and inflammation. They exert a hyperalgesic effect, and they enhance nociception produced by other factors, such as bradykinin. The COX-2 isoenzyme is upregulated in inflammatory states (up to 20 times its basal levels) and plays a key role in nociception, whereas COX-1 is produced constitutively and also plays an integral role in nociception.[50] Prostaglandins are also known to lower activation thresholds to mechanical, thermal, and chemical stimulation. It has also been shown that NSAIDs produce much of their analgesic effects by inhibiting COX activity centrally.[50]

Different NSAIDs have been noted to have different effects on COX enzymes. Most NSAIDs that inhibit COX will divert arachidonic acid to the 5-LOX pathway, subsequently increasing the synthesis of leukotrienes, which have been implicated in the creation of NSAID-induced gastric ulcers. Some newer NSAIDs will also inhibit 5-LOX.[50]

COX and Prostaglandins in Reptiles and Amphibians

Inflammation

Royal et al evaluated traumatized versus normal tissues for COX protein expression in eastern box turtles. They found that traumatized muscle tissue had statistically significantly increased COX-1 and statistically insignificant increased COX-2 compared to normal muscle. The highest levels of both COX-1 and COX-2 were noted in the liver and kidney, but these levels were not significantly different in traumatized or non-traumatized turtles. They suggest that NSAIDs that block both COX-1 and COX-2 might be more efficacious in eastern box turtles than COX-2 selective drugs, and that these drugs need to be evaluated further for potential liver and kidney toxicity.[105] Sadler et al looked at COX expression in normal and traumatized skin and muscle in ball pythons. They found that COX-1 expression was significantly increased in inflamed skin tissues compared to normal skin, but that there were not any significant changes in the expression of either COX-1 or COX-2 in inflamed muscle tissues compared to normal muscle.[107] They also suggest that a non-specific NSAID may be more effective in reptiles than a COX-2 selective drug.

Cardiovascular system

Robleto and Herman evaluated the cardiovascular effects of PGI_2 and $PGF_{2\alpha}$ in unanesthetized bullfrogs. They found that both PGs increased the heart rate (independent of the autonomic nervous system), but had differing effects on the mean arterial pressure; PGI_2 was hypotensive while $PGF_{2\alpha}$ was hypertensive.[102]

Urinary system

In mammals, it has been shown that prostaglandins normally cause vasodilation of the afferent arterioles of the glomeruli, thereby maintaining a normal glomerular perfusion and glomerular filtration rate (GFR).[50] However, this has not been studied in reptiles and amphibians. PGE_1 and PGE_2 are known to interfere with the water permeability effect of vasopressin in the toad urinary bladder and kidney.[87] Forrest and Goodman showed that PGE_2 inhibits the action of vasopressin in the toad urinary bladder.[35] In 1977, Zusman et al discovered that vasopressin stimulated PGE biosynthesis in the urinary bladder of Bufo toads, and that the water permeability response of the bladder to vasopressin was subsequently inhibited by PGE.[144] In 1978, Orloff and Zusman found that in the toad urinary bladder and kidney, vasopressin increases the release of arachidonic acid, and subsequently PGE_2.[87] Herman et al (1981) looked at the effects of prostaglandins and prostaglandin precursors on osmotic water flow in the anuran urinary bladder. They found that PGE_1, PGE_2, and PGI_2 inhibited water flow, as did arachidonic acid, which was converted to PGD_2 and PGE_2. Eicosapentaenoic acid was converted to PGD_3 and PGE_3, which had an opposite effect.[60] In 1982, Arruda showed that in turtle and toad bladders, high intracellular calcium inhibits water transport via prostaglandin release, but the inhibition of sodium or H^+ transport was independent of prostaglandins.[6] Also in 1982, Forrest et al found that PGE_2 is and no other PGs are responsible for acidification in the toad urinary bladder.[36] In 1985, Schlondorff and Satriano noted that in the toad urinary bladder, vasopressin stimulated PG synthesis in a cAMP-independent manner, and inhibited PG synthesis in a cAMP-dependent manner.[111] Sabatini (1986) found that the effect of PTH on water flow in the toad urinary bladder is mediated by an increased cellular uptake of Ca that stimulates PG release, and that PG

release, in turn, appears to mediate the inhibitory effect of PTH on vasopressin-stimulated water transport.[106] In 1991, Yorio et al demonstrated that in the toad urinary bladder, PGE_2 inhibited H^+ excretion at lower doses, but enhanced it at higher doses. He also found that toads that were maintained under chronic metabolic acidosis had enhanced H^+ excretion rates and a threefold increase in cellular PGE_2 concentrations.[142]

Reproductive system

Prostaglandins have extensive involvement in the reproductive system. They are associated with ovulation, oviposition, and luteal function. In reptiles, prostaglandins have been shown to stimulate oviducal contractions, which may be overridden by neural control, which can subsequently result in egg retention.[53] Jones et al demonstrated that a prostaglandin inhibitor (indomethacin) delayed FSH-induced ovulations, lowered PGE secretion, and inhibited preovulatory changes in the follicular wall in anoles.[67] Mahmoud et al showed that a single injection of $PGF_{2\alpha}$ in recently ovulated snapping turtles induced early luteolysis and a significant decrease in plasma progesterone levels.[73] Late pregnancy in common geckos, a viviparous species, could not be induced to give birth with high doses of $PGF_{2\alpha}$ unless the animal was pretreated with a β-adrenergic antagonist in vivo, whereas in vitro, administration of $PGF_{2\alpha}$ caused uterine contraction, which could be blocked by administration of a β-adrenergic agonist.[22] In 1992, Gobbetti et al showed that in the brains of crested newts, $PGF_{2\alpha}$ is involved in the post-reproduction processes through estradiol secretion, while PGE_2 was involved in the reproductive processes, possibly through androgen secretion.[45] In 1994, Gobbetti et al studied prostaglandins in the brains of Italian wall lizards during reproduction and found that $PGF_{2\alpha}$ levels were elevated during the refractory phase of the reproductive cycle, whereas PGE_2 levels were decreased. They also determined that levels of $PGF_{2\alpha}$ were increased after treatment with salmon GnRH, and decreased by substance P and acetylsalicylic acid.[48] Dubois and Guillette demonstrated that the uteri of alligators secrete PGE and $PGF_{2\alpha}$ at varying levels depending on the reproductive status of the animal.[29] In 1994, Gobbetti et al looked at prostaglandins in the reproductive cycle of male Italian wall lizards, and found that testicular androgen synthesis during the fighting phase is under the control of PGE_2, whereas 17β-estradiol synthesis during the refractory phase is regulated by $PGF_{2\alpha}$ in the testes, and by PGE_2 and $PGF_{2\alpha}$ in interrenal cells.[47] In 2006, Jones et al looked at the interactions between uterine tension, PGs, calcium and AVT in the uteri of anoles, and noted that indomethacin blocked the AVT-induced tonic contractions in stretched uteri, and that the interval between contractions was decreased by $PGF_{2\alpha}$ and PGE_2, an effect that was also blocked by indomethacin.[68]

In amphibians, Guillette et al noted that ovarian and oviducal $PGF_{2\alpha}$ acts like an endocrine hormone, coordinating oviducal contractions and CNS-controlled oviposition behavior.[53] Diakow and Nemiroff showed that PGE_2 and $PGF_{2\alpha}$ affect female mating behavior in leopard frogs, and that vasotocin acts in part through a mechanism that involves prostaglandin synthesis.[26] In 1992, Gobbetti and Zerani showed that testicular $PGF_{2\alpha}$ was lower in the post-reproduction phase in edible frogs, and that administration of mammalian GnRH increased its levels during the pre-reproduction and reproduction phases.[44] In crested newts, testicular $PGF^{2\alpha}$ levels were lower during the reproduction phase, and administration of GnRH increased its levels during the pre-reproduction and reproduction phases.[44] They also showed that $PGF_{2\alpha}$ levels were increased in ovulating edible frogs, and it may be involved in the control of egg deposition in this species.[43] In 1993, Gobbetti and Zerani showed that in edible frogs, a seasonal increase in plasma PGE_2 may inhibit breeding activity by stimulating ovarian androgen secretion, whereas a seasonal increase in plasma $PGF_{2\alpha}$ may inhibit breeding by stimulating ovarian estradiol secretion.[46] In 1995, Chang et al demonstrated that elevated levels of $PGF_{2\alpha}$ are associated with spontaneous hormone-induced ovulation, and that protein kinase C mediates gonadotropin induction of $PGF_{2\alpha}$ in the ovaries of *Rana sp*.[20] In leopard frogs, Schuetz noted that normal synchronization and maturation of oocytes may require the combined action of steroids and prostaglandins acting within different follicular compartments.[113] Ramos et al found that $PGF_{2\alpha}$ increased ovulation induced by a pituitary homogenate in toad ovaries, whereas PGE_1 had an inhibitory effect on ovulation.[97]

Gastrointestinal system

The roll of prostaglandins in the protection of the gastrointestinal (GI) mucosa is well known. In 1979, Garner et al showed that PGE_2, 16,16-dimethyl PGE_2, and PGI_2 all inhibited H^+ secretion from the gastric mucosa of the European frog, whereas only 16,16-dimethyl PGE_2 stimulated bicarbonate secretion, and it also prevented the inhibitory action of indomethacin on bicarbonate secretion.[41] Also in 1979, Garner and Heylings demonstrated that 16,16-dimethyl PGE_2 inhibited H^+ secretion and stimulated bicarbonate secretion in the fundus of the common frog, and that $PGF_{2\alpha}$ increased bicarbonate secretion in the fundus of salamanders, but had no significant effect on H^+ secretion.[40] Alkali transport by the proximal duodenum of the bullfrog showed a dose-dependent increase upon administration of PGE_2, 16,16-dimethyl PGE_2, and $PGF_{2\alpha}$, with an increased response to the E-type PGs than to $PGF_{2\alpha}$.[34] In 1982, Takeuchi et al found that in isolated American bullfrog gastric fundic mucosa, PGs at low concentrations had an inhibitory effect on histamine-stimulated H^+ secretion, but at higher concentrations, they stimulated H^+ secretion by releasing histamine from mast cells.[128] In 1995, the same group reported that ulceration of isolated bullfrog gastric mucosa in the presence of acid depends upon either a deficiency of endogenous PGs or a lack of nutrient bicarbonate.[129]

Integument

Page and Yorio showed that $PGF_{2\alpha}$ exhibited a dose-dependent inhibition of acidification of the abdominal skin of the southern leopard frog, by maintaining a low basal H^+ excretion rate and regulating intracellular pH. Administration of PGE_2 and $PGF_{1\alpha}$ showed no significant alteration in H^+ excretion rates.[90] In 1991, Yorio et al showed that the abdominal skin of southern leopard frogs pretreated with ibuprofen had enhanced H^+ excretion similar to frogs with chronic metabolic acidosis, and that this effect was inhibited by administration of $PGF_{2\alpha}$.[142]

Fever

The concept of behavioral fever has been well-documented in reptiles[16,58] and amphibians.[138] In 1981, Hutchison and Erskine discovered that injections of PGE_1 (a known pyrogen) into the third ventricle of the brain of common mudpuppies produced a long-lasting behavioral fever, with animals selecting locations with temperatures averaging nearly 5°C greater than baseline for up to 48 hours post-injection.[65] In 2002, Bicego et al showed that Rococo toads injected with indomethacin did not seek a warmer temperature compared to controls when injected with pyrogenic lipopolysaccharides.[13] Seebacher and Franklin found in 2003 that bearded dragons had a higher heartrate during warming than during cooling, and this effect was negated with administration of COX-1 and COX-2 inhibitors, and enhanced by prostacyclin and $PGF_{2\alpha}$ but not by thromboxane B_2.[114] However, in 2006 they noted that this same effect did not occur in saltwater crocodiles.[115] In 2005, Chingbin et al noted that this effect only occurred in Przewalski's toadhead agamas below 25°C.[70] It has been hypothesized that central processing of peripheral thermal information may involve COX enzymes that have a bearing on cardiovascular response, and that this response to heating and cooling in reptiles is at least in part mediated by the integration between the baroreflex and systemically-acting nitric oxide synthase and COX enzymes.[116]

Other prostaglandin effects

In 1981, Delarue et al found that exogenous prostaglandins can control corticosteroid production in marsh frogs, that endogenous prostaglandins are required for spontaneous biosynthesis of corticosteroids, and that endogenous prostaglandins are not involved in ACTH-induced steroidogenesis.[23] In 1983, Perroteau et al showed evidence that a physiological relationship between the kidney and interrenal gland may exist in frogs, and that the action of angiotensin II on both corticosterone and aldosterone production may be mediated by prostaglandins.[93]

In 1987, Ferrary et al found that the inner ear of the frog produces PGI_2 and PGE_2, and that prostaglandins could be involved in the physiology of the inner ear.[32]

In 1990, Ajayi and Okpako studied effluents from the lungs of rainbow lizards, and found high contents of a PGE_2-like prostanoid, the release of which was blocked by COX inhibitors.[39]

In 1997, Herman et al found that whole blood, purified erythrocytes, and leukocytes activated by clotting obtained from western rat snakes produced thromboxane, PGE_2, and 5-LOX, and that inhibition of clotting in this species was achieved with administration of indomethacin.[62]

Sharma and Suresh showed in 2008 that when the production of PGE_2 was blocked by COX inhibitors, tail regeneration after autotomy was significantly delayed in northern house geckos.[117] In 2015, Narayanan found that COX inhibitors not only blocked PGE_2, but also blocked fibroblast growth factor-2 (FGF_2), another contributing factor in the tail regrowth of house geckos.[85]

In 1985, Schmidt showed that administration of progesterone and arginine vasotocin concurrently with $PGF_{2\alpha}$ increased mating call phonotaxis in female American toads, whereas administration of $PGF_{2\alpha}$ alone had minimal effect.[112] In 2009, Gordon and Gerhardt repeated this work by assessing phonotaxis in female gray treefrogs, and found that concurrent administration of $PGF_{2\alpha}$- and progesterone-treated animals exhibited phonotaxis more often than untreated controls.[49]

Pain Perception in Reptiles and Amphibians

Although peripheral nociceptors have not been directly observed in most reptiles, they have been documented amphibians, as well as mammals, birds, and fish.[120] Thermosensitive and thermomechanosensitive nociceptors have been identified in the trigeminal ganglia of pit vipers, and mechaniconociceptors have been identified in the cutaneous plantar nerve in American alligators.[120] Substance P is a peptide that is present in peripheral nerves and the spinal cord, and is expressed with painful stimuli in mammals. It is highly conserved across vertebrate species, and has been documented in the nervous system of several species of turtles.[120]

Nociceptive pathways have not been extensively studied in reptiles and amphibians, but in mammals prostaglandins exert hyperalgesic effects and enhance nociception produced by other mediators, such as bradykinin.[50] The anti-nociceptive effects of NSAIDs are exerted both centrally and peripherally. Peripherally, NSAIDs penetrated inflamed tissues and have a local effect, while the central effect is at both the spinal and supraspinal levels, with contributions from both COX-1 and COX-2.[50] Reptiles and amphibians have pathways similar to the mammalian pathways that perceive pain and nociception, so the presumption that these pathways work in the same manner is sound, but there has been very little research done to confirm this.[120,126]

Behavioral and physiological parameters typically associated with pain in reptiles include the absence of normal behavior, a hunched posture, increased aggression, rubbing or scratching a specific area of the body, skin color changes (particularly darkening), lameness, decreased food consumption, decreased activity, changes in response to stimulation (increased or decreased), stinting on palpation, keeping eyes closed, ataxia or lameness, and changes in heart or respiratory rates.[14,120] Chelonians may keep their heads extended away from the body and ventrally directed.[14,120] Snakes may hold their bodies less coiled at the site of pain.[14] Amphibians may show increased aggression, immobility or lethargy, closed eyes, lameness or ataxia, an increased flight response, anorexia, color changes, rapid respiration, and they may flick their foot or bite at the affected area(s).[14]

Individual NSAIDs in Reptiles and Amphibians

Aspirin (acetylsalicylic acid)

Willow bark contains salicylic acid, and its anti-inflammatory effects have been documented as far back as Hippocrates (460-377 BC). Salicylic acid was first isolated in 1763, and acetylsalicylic acid (ASA) was first created in 1853. In 1900, Bayer marketed the first commercial aspirin product.[50,57] Aspirin inhibits COX-1, inhibiting the production of PGs and TXA_2, resulting in analgesic, antipyretic, and anticoagulative effects. Even though it does not directly inhibit COX-2, it can modify it to produce, in conjunction with 5-LOX, aspirin-triggered lipoxin (ATL), which appears to have a protective effect on the gastric mucosa. In mammals, aspirin can cause an irreversible reduction in platelet aggregation. It is primarily metabolized in the liver and excreted through the kidneys.[94]

Aspirin has been documented to have varying effects in reptiles and amphibians. In 1976, Bernheim and Kluger showed sodium salicylate injections in desert iguanas resulted in a dose-dependent attenuation of a febrile response.[11] Gobbetti et al noted that ASA decreased $PGF_{2\alpha}$, estradiol, and aromatase activity, and increased androgen production in the brain of male Italian wall lizards.[48] In 1999, Stewart and Hudspeth found that prolonged administration of ASA produced a marked but reversible suppression of spontaneous otoacoustic emissions in the ears of Tokay geckos.[127] In 2005, Ahmed et al showed that ASA induces hyperprolactinemia in Indian spiny-tailed lizards.[2]

In amphibians, it has been noted that aspirin potentiates the hydrosmotic effect of antidiuretic hormone (ADH) in the toad urinary bladder.[91] In 1898, aspirin was shown to have a negative inotropic effect on frog hearts.[57] In 1976, Hall et al showed that it decreased sodium transport across isolated common frog skin in vitro.[56] In 1977, Spenney and Bhown looked at the effects of ASA on the bullfrog gastric mucosa, and found that it caused changes in transmucosal resistance, mucosal permeability, and a reduction in mucosal ATP and phosphocreatine.[122,123] In 1986, Rowe et al showed that ASA causes inhibition of H^+ secretion in isolated gastric mucosa from American bullfrogs.[104] In 1989, Ashley et al found that under acidic conditions, aspirin increased the permeability of the gastric mucosa in mudpuppies.[7] ASA caused mucosal ulceration in isolated bullfrog stomachs, even when bathed in a bicarbonate-rich solution.[129] Aspirin reduced PGE_2 and PGI_2 production by ampulla and duct tissue isolated from the frog posterior semicircular canal.[32] In 1989, Puel et al found that ASA suppressed spontaneous activity, water motion evoked excitement, and the increase in afferent nerve activity in hair calls evoked by L-glutamate and kainic acid in a dose responsive manner in the lateral line of African clawed frogs. This response was similar to its effects on mammalian hair cells in the cochlea.[95] In the isolated lenses of leopard frogs, aspirin (which is believed to influence cataract development) was shown to slow the recovery of depolarization of membrane potential, the decrease in membrane resistance, and the increase in internal resistance caused by acidification.[7] In 1994, Nandi et al found that aspirin enhanced histamine-stimulated H^+ release in the gastric mucosa of frogs.[84] The critical effect environmental contamination concentrations of acetylsalicylic acid on aquatic animals, including amphibians, has been calculated.[33]

Acetaminophen

Acetaminophen was first marketed as Paracetamol in 1887. Its mechanism of action is not completely understood, but it produces analgesia and antipyresis by weak inhibition of COX-3. It does not have any significant anti-inflammatory effects, nor does it inhibit platelet function.[94] In animals, its toxic effects (methemoglobinemia, liver necrosis, keratoconjunctiva sicca (KCS), etc.) have been well-documented.[94]

In 1970, Urakabe et al documented that acetaminophen enhanced water permeability when applied on the serosal surface of Japanese common toad bladders in vitro.[118,133,134] Also in 1970, Shirai noted that acetaminophen had a theophylline- and vasopressin-like effect on the toad bladder.[119] In 1976, Hall et al found that acetaminophen decreased sodium transport across the skin of common frogs in vitro.[56] In 1991, Bhatt et al demonstrated that the addition of penetration enhancers increased transportation of acetaminophen through shed snake skin.[12]

Acetaminophen is considered a common wastewater contaminant, and its effects on amphibian larvae have been well documented. Fraker and Smith showed that it caused a decrease in the activity levels of leopard frog tadpoles when they were exposed to it concurrently with caffeine, and on African clawed frog tadpoles when combined with triclosan.[37,38] Smith and Burgett showed that had a negative effect on the activity level of American toad tadpoles, and at higher doses decreased survival.[121] In 2006, Richards and Cole showed that the maximum concentration of ibuprofen detected in surface waters had no significant toxicity, teratogenicity, or growth inhibition in *Xenopus* tadpoles.[100] The critical effect environmental contamination concentrations of acetaminophen on aquatic animals, including amphibians, has been calculated.[33]

Acetaminophen has been used to poison brown tree snakes in Guam. An 80 mg dose of acetaminophen administered to brown tree snakes resulted in 100% mortality.[108] It has subsequently been distributed in dead rodent baits, which have been successful in reducing snake populations while having a minimal effect on non-target feral and wildlife species.[66,108] It is being investigated for similar use to control invasive Nile monitors, Burmese pythons, and black spiny-tailed iguanas in Florida.[8,76]

Dipyrone (Metamizole)

Dipyrone was discovered in 1921. Its exact mechanism of action is unknown, but it is thought to behave similar to acetaminophen in that it has analgesic and antipyretic effects thought to be the result of blocking the formation of PGD and PGE, which are endogenous pyrogens, but it has a minimal anti-inflammatory effect.[98] It has been studied in amphibians, and has been found to cause a regression in curarimimetic effects in the skeletal muscle of frogs.[98]

Phenylbutazone

Phenylbutazone was discovered in 1949. It inhibits both COX-1 and COX-2, although its effects on COX-1 are significantly greater. It has primarily been used in horses and dogs for its anti-inflammatory, analgesic, antipyretic, and mild uricosuric properties.[94] In 1970, it was found to enhance the permeability of the toad urinary bladder when applied to the serosal surface in vitro.[118,119,133,134] Also in 1970, Breull and Karzel found that phenylbutazone had a calcium-like effect on the membrane resting potential of frog skeletal muscle fibers in vitro.[15] It was reported to cause a decrease in sodium transport across common frog skin in vitro.[56] In 1985, Madden and Van der Kloot found that phenylbutazone altered transmissions at the neuromuscular junctions of frogs.[72]

Ibuprofen

Ibuprofen was discovered in 1961. It is a non-selective COX inhibitor, with its anti-inflammatory, analgesic, and antipyretic effects thought to be due to inhibition of COX-2, while side effects, such as GI ulceration, are thought to be caused by its inhibition of COX-1.[57] In 1977, Zusman et al found that, in the urinary bladders of Bufo toads, ibuprofen had no effect on basal water flow or on cAMP-stimulated water flow, but it did block PGE synthesis and increased vasopressin-stimulated water flow.[144] In 1981, Rees et al found that ibuprofen inhibited fundic alkaline secretion in the stomachs of salamanders and bullfrogs, and that the effect was reduced with the addition of PGE_2.[99] In 1982, Arruda showed that ibuprofen significantly decreased the inhibitory effect of high

extracellular calcium on vasopressin-stimulated water flow in the toad urinary bladder, and that it blunts the effect of agents which increase intracellular calcium on water transport, but had no effect on sodium or H^+ transport.[6] Schlondorff and Satriano (1985) found that when toad bladder epithelial cells were prepared with ibuprofen, subsequent PGE_2 synthesis was enhanced sevenfold, whereas TXB_2 was not.[111] In 1986, Sabatini showed that ibuprofen inhibited the effects of PTH on vasopressin-stimulated water flow in the urinary bladder of toads.[106] In 1988, Herman and Martinez found that ibuprofen inhibited basal and epinephrine-stimulated PG synthesis in bullfrog lung tissues at 22ºC, but not at 5ºC.[61] In 1990, Page and Yorio found that the treatment of leopard frogs with ibuprofen at 30 mg/kg/day stimulated mucosal acidification of the abdominal skin that was similar to that found in an animal with chronic metabolic acidosis, and that this effect was inhibited by $PGF_{2\alpha}$,[90] and this effect was caused by enhanced H^+ secretion.[142] In 1991, Bhatt et al demonstrated that the addition of certain penetration enhancers increased transportation of ibuprofen through shed snake skin.[12] In 1992, Yakushiji et al showed that when combined with a fluoroquinolone antibiotic, ibuprofen and other NSAIDs inhibited GABA responses in bullfrog sensory neurons.[140] In 1994, Nandi et al found that ibuprofen enhanced histamine-stimulated H^+ release in the gastric mucosa of frogs.[84] In 2002, Carrasquer demonstrated that ibuprofen altered epithelial transport parameters in the bullfrog cornea.[18]

Toxic effects of ibuprofen have also been documented in reptiles and amphibians. In 2006, Richards and Cole showed that the maximum concentration of ibuprofen detected in surface waters had no significant toxicity, teratogenicity, or growth inhibition in *Xenopus* tadpoles.[100] In 2006, Gladden reported the successful treatment of a South American red-footed tortoise that had ingested 256 mg/kg ibuprofen.[42] In 2014, Veldhoen et al looked at the effects of ibuprofen on bullfrog tadpoles, and found that the LC50 of ibuprofen for this species was 41.5 mg/L, exposure to 15 mg/L altered mRNA transcripts in the liver and disrupted thyroid hormone function, and concentrations as low as 1.5 mg/L altered mRNA in tadpole tailfins.[136] The critical effect environmental contamination concentrations of ibuprofen on aquatic animals, including amphibians, has been calculated.[33]

Indomethacin

Indomethacin was discovered in 1963. It has been used extensively in research, including studies involving reptiles and amphibians. It also inhibits the motility of polymorphonuclear leukocytes.[94a] It is a nonselective COX inhibitor, and many adverse effects have been reported.[57,94]

Urinary tract: In 1971, Urakabe et al documented that indomethacin enhanced water permeability when applied on the serosal surface of Japanese common toad bladders in vitro.[134] In 1977, Zusman et al found that indomethacin had no effect on basal water flow in vitro in the bladders of Bufo toads, but it increased water flow in response to vasopressin by inhibiting PGE_2 synthesis. It also enhanced cAMP-stimulated water flow, which was determined to be unrelated to its inhibitory effects on PG synthesis.[144] In 1982, Arruda showed that indomethacin significantly decreased the inhibitory effect of high extracellular calcium on vasopressin-stimulated water flow in the toad urinary bladder but not in the turtle bladder, and that it blunts the effect of agents which increase intracellular calcium on water transport, but had no effect on sodium or H^+ transport.[6] In 1986, Sabatini showed that indomethacin inhibited the effects of PTH on vasopressin-stimulated water flow.[106]

GI tract: In 1979, Garner et al found that indomethacin was a potent inhibitor of bicarbonate production by the gastric mucosa of European Frogs, but had a minimal effect on H^+ secretion.[41] In 1980, Flemstrom found that indomethacin inhibited alkali transport in duodenal mucosa of bullfrogs.[34] In 1986, Rowe et all showed that indomethacin caused no significant changes in potential difference, resistance, or H^+ secretion on bullfrog gastric mucosa treated with histamine or metiamide (a precursor to cimetidine).[104] In 1994, Nandi et al found that indomethacin did not enhance histamine-stimulated H^+ release in the gastric mucosa of frogs.[84] Takeuchi et al reported in 1995 that indomethacin caused gastric mucosal ulceration in bullfrogs, even in the presence of a bicarbonate-rich solution.[129]

Reproductive tract: In 1981, Diakow and Nemiroff found that receptive behavior in the female leopard frog was potentiated by treatment with PGE_2 and $PGF_{2\alpha}$, and that this effect was blocked by indomethacin.[26] In 1990, Guillette et al showed that indomethacin has an inhibitory effect on oviposition and parturition in oviparous (eastern fence lizards) and viviparous lizards (Yarrow's spiny lizards).[51] Also in 1990, Jones et al found that indomethacin inhibited ovarian PGE secretion and gonadotropin-induced ovulation in anoles.[67] In 1991, this same group showed that indomethacin-delayed parturition disrupted the normal birth process in viviparous Yarrow's spiny lizards.[52] In 1995, Chang et al found that indomethacin suppressed ovulation from ovaries obtained during mid-hibernation but not late-hibernation in leopard frogs.[20] In 2006, Jones et al looked at the interactions between uterine tension, PGs, calcium and AVT in the uteri of anoles, and noted that indomethacin blocked the AVT-induced tonic contractions in stretched uteri, and that the interval between contractions was decreased by $PGF_{2\alpha}$ and PGE_2, an effect that was also blocked by indomethacin.[68] Ramos et al found that $PGF_{2\alpha}$ increased ovulation induced by a pituitary homogenate in toad ovaries, and that indomethacin produced a significant decrease in this effect.[97]

Endocrine system: In 1981, Delarue et al found that indomethacin caused a marked decrease in the spontaneous production of corticosterone and aldosterone from the interrenal cells from marsh frogs, but did not alter the stimulation of steroidogenesis induced by ACTH.[23] This effect was also documented by Perroteau et al in 1984.[93]

Nervous system: In 1985, Madden and Van der Kloot looked at the effects of indomethacin on transmission at the neuromuscular junctions of frogs, and found that at low doses it irreversibly decreased acetylcholine release, and at high doses it reversibly increased its release. It also decreased the latencies of evoked responses by increasing synaptic delays and increasing nerve action potential conduction times.[72] In 1992, Yakushiji et al showed that when combined with a fluoroquinolone antibiotic, indomethacin and other NSAIDs inhibited GABA responses in bullfrog sensory neurons.[140]

Other effects: In 1976, Hasll et al showed that indomethacin decreased sodium transport across isolated common frog skin in vitro, as well as increasing the sensitivity of the skin to PGE_1 at lower doses and decreasing it at higher doses.[56] In 1987, Ferrary et al documented that indomethacin decreased PGI_2 and PGE_2 synthesis in the frog inner ear.[32] In 1997, Herman et al discovered that indomethacin inhibited clotting in snake blood.[62] In 2001, Stevens et al looked at indomethacin as an analgesic in northern grass frogs, and found that it weak but noticeable analgesic effect using the acetic acid test.[125] In 2002, Bicego et al showed that indomethacin completely blocked the behavioral fever induced by lipopolysaccharides in Rococo toads.[13] In 2010, Staigmiller found that indomethacin had no effect on healthy leopard frog capillaries following a shear stress stimulus.[124] Also in 2010, Priebe found that in capillaries isolated from leaopard frogs, the gaps between endothelial cells get smaller when treated with indomethacin, resulting in a decreased flow of fluid out of the capillary.[94a] The critical effect environmental contamination concentrations of indomethacin on aquatic animals, including amphibians, has been calculated.[33]

Mefenamic acid

Mefenamic acid was discovered in the early 1970's. It is a nonselective COX inhibitor that has also been shown to decrease uterine contractions in humans.[57,94] In 1971, Urakabe et al found that mefenamic acid enhanced the permeability of the toad urinary bladder when applied to the serosal surface in vitro.[134] In 1976, Hall et al showed that mefenamic acid decreased sodium transport across isolated common frog skin in vitro.[56] In 1993, Morales et al found that mefenamic acid caused no appreciable electrophysiological changes in the function of frog cardiac pacemaker cells.[82a] In 2004, Ahmad et al looked at the effects of mefenamic acid on the erythrocytes of Uromastyx lizards, and found that it caused a dose-dependent decrease in red blood cell counts, and noted that this drug appears to induce red cell autoantibodies, causing an immune mediated hemolytic anemia-type

response.[1] In 2005, this same group looked at its effects on blood hemoglobin levels in Uromastyx lizards, and they found significantly higher hemoglobin levels in blood from treated animals when compared to control animals.[4] Also in 2005, this same group looked at the effects of mefenamic acid on osmotic fragility on lacertilian erythrocytes, and found that increased osmotic fragility was observed with increased drug dosage.[3] And in 2006, they looked at its effects on the hematocrits of Uromastyx lizards and found a dose-dependent reduction in mean packed cell volume, and indications of extravascular hemolysis due to destructive changes in the red cell membrane through an autoantibody mechanism.[5]

Ketoprofen

Ketoprofen first hit the market in 1972. It is a nonselective COX inhibitor, labeled to treat pain, inflammation, and fever.[94] In 1992, Yakushiji et al showed that when combined with a fluoroquinolone antibiotic, ketoprofen and other NSAIDs inhibited GABA responses in bullfrog sensory neurons.[140] In 2006, Tuttle et al looked at the pharmacokinetics of ketoprofen after IV and IM injections in green iguanas, and they found that it had a 2-compartment disposition when given IV and a 78% systemic absorption when given IM at 2 mg/kg. They recommend a less-frequent dosing interval than is commonly recommended in veterinary formularies (q24h).[132] In 2007, Manire and Norton reported that it could be effectively used in sea turtles at 2 mg/kg IM, but no dosing interval was noted. They did recommend that NSAID's should only be given for 3-5 days in these animals.[74] In 2014, Pathak et al noted that Indian bullfrogs that were injected with a hypertonic saline solution had increased eye blinking and buccal oscillations in response to pain. They tested multiple analgesic drugs on these frogs, looking for a reduction in these 2 parameters. Even though ketoprofen decreased the number of blinks, it was not significantly different than the controls.[92] In 2015, Yerasi et al used ketoprofen as one of several drugs used to evaluate the frog as an animal model to study the fraction of oral dose absorbed in humans, and found that the absorption rates were comparable.[141] A dose of 2 mg/kg IM,SQ q24h has been recommended for reptiles, but doses up to 4 mg/kg IM q24h have been reported.[88,94] The critical effect environmental contamination concentrations of ketoprofen on aquatic animals, including amphibians, has been calculated.[33]

Diclofenac sodium

Diclofenac was discovered in 1973. It is a non-specific COX inhibitor that may also have some inhibitory effects on LOX. It is primarily used as a topical cream or ophthalmic medication, but oral forms also exist.[57,94] It has also been used to induce mydriasis in cataract surgery.[94] In 1992, Yakushiji et al showed that when combined with a fluoroquinolone antibiotic, diclofenac and other NSAIDs did not inhibit GABA responses in bullfrog sensory neurons.[140] In 2003, Seebacher and Franklin noted that the heart rates of bearded dragons during heating were significantly faster than during cooling, and that administration of diclofenac blocked this effect, which might be considered an antipyretic effect.[114] In 2004, this same group looked at its effects on heartrate and blood pressure in saltwater crocodiles, and found that it had a similar effect on the heart as in the bearded dragon, but no effect on blood pressure was noted.[115] In 2006, Liu et al looked at this effect in Przewalski's toadhead agamas, and found that it did not significantly affect heart rate in this species.[70] In 2014, Pathak et al noted that Indian bullfrogs that were injected with a hypertonic saline solution had increased eye blinking and buccal oscillations in response to pain. They tested multiple analgesic drugs on these frogs, looking for a reduction in these 2 parameters. Even though diclofenac sodium decreased the number of blinks, it was not significantly different than the controls.[92] Also in 2014, Mescher amputated a single limb in African clawed froglets at stage 54 and 55 of metamorphosis, when the regenerative ability is initially diminished, and found that topical application of diclofenac at the site of amputation resulted in improved limb regeneration.[79] In 2015, Chae et al found that diclofenac can cause teratogenicity that results in morphological abnormalities in *Xenopus* embryos, but it does not the developmental tissue arrangement during embryogenesis.[19] The critical effect environmental contamination concentrations of diclofenac on aquatic animals, including amphibians, has been calculated.[33]

Naproxen

Naproxen was first marketed as a prescription drug in 1976. It is a nonselective COX inhibitor that is highly protein bound, and linked to GI ulceration, but not cardiovascular disease.[94] In 1977, Zusman et al found that, in the urinary bladders of Bufo toads, naproxen had no effect on basal water flow or on cAMP-stimulated water flow, but it did block PGE synthesis and increased vasopressin-stimulated water flow.[144] Schlondorff et al built on this work, and demonstrated that naproxen significantly enhanced the water flow response of toad bladders to hypertonicity.[110] In 1992, Yakushiji et al showed that when combined with a fluoroquinolone antibiotic, naproxen and other NSAIDs inhibited gamma-aminobutyric acid (GABA) responses in bullfrog sensory neurons.[140] In 1994, Nandi et al found that naproxen did not enhance histamine-stimulated H^+ release in the gastric mucosa of frogs.[84] Also in 1994, Vree et al described the glucuronidation of naproxen by red-eared sliders.[137] In 2014, Melvin et al evaluated naproxen as a potential environmental toxin to amphibians. They showed a dose-dependent toxicity of naproxen in striped marsh frog tadpoles, and the effect worsened when it was mixed with carbamazepine and sulfamethoxyzole.[78] In 2015, Yerasi et al used naproxen as one of several drugs used to evaluate the frog as an animal model to study the fraction of oral dose absorbed in humans, and found that the absorption rates were comparable.[141] The critical effect environmental contamination concentrations of naproxen on aquatic animals, including amphibians, has been calculated.[33]

Flunixin meglumine

Flunixin was first introduced into the pharmaceutical market in 1977. It is a potent non-specific COX inhibitor that is highly protein bound.[94] Historically, flunixin meglumine has been used extensively in reptiles and amphibians at doses ranging from 0.1-2 mg/kg IV, IM q12-24h in reptiles and 0.1-1 mg/kg IV, IM q12-24 in amphibians, but little research has been done on the use of this NSAID in these species.[9,14,17,75] In 1993, Morales et al found that flunixin caused a dose-dependent decrease and eventual cessation in the electrophysiological function of frog cardiac pacemaker cells.[82a] In 1996, Terril-Robb et al looked at the analgesic effects of flunixin in leopard frogs, and found that 25 mg/kg provided good analgesia for 2 to 4 hours.[130] In 2011, Coble et al determined that 25 mg/kg administered into the dorsal lymph sac of African-clawed frogs provided longer and more effective analgesia compared to controls and to frogs treated with morphine, xylazine, or meloxicam. However, one frog in this treatment group did die afterward, and renal lesions were noted on histopathology.[21]

Piroxicam

Piroxicam is an NSAID in the oxicam class that was introduced in 1977. It is a non-selective COX inhibitor, and in addition to its use in treating pain, inflammation, and pyrexia, it is also noted to have antineoplastic effects, particularly in the treatment of transitional cell carcinomas in dogs and cats.[94] In 1992, Yakushiji et al showed that when combined with a fluoroquinolone antibiotic, piroxicam and other NSAIDs did not inhibit GABA responses in bullfrog sensory neurons.[140] In 2014, Pathak et al found that the number of eye blinks and buccal oscillations increased with a painful stimulus in African clawed frogs, and that piroxicam decreased this response, but was not significantly different from controls.[92] Its use as an antineoplastic drug in reptiles and amphibians has not been reported. The critical effect environmental contamination concentrations of piroxicam on aquatic animals, including amphibians, has been calculated.[33]

Meclofenamic acid (meclofenamate)

Meclofenamic acid was introduced in 1980 as an NSAID for horses. It is a nonselective COX inhibitor.[57] In 1977, Zusman et al found that, in the urinary bladders of Bufo toads, meclofenamic acid had no effect on basal water flow or on cAMP-stimulated water flow, but it did block PGE synthesis and increased vasopressin-stimulated

water flow.[144] In 1980, Schlondorff et al built on this work, and demonstrated that meclofenamate significantly enhanced the water flow response of toad bladders to hypertonicity.[110] When applied to the corneas of Bufo toads in vitro, Bentley and McGahan found that meclofenamic acid inhibited the flux of chloride toward the tear surface.[10] Richter et al showed that meclofenamic acid had a similar effect in lung tissue isolated from African clawed frogs.[101] In 1985, McGahan et al showed that melittin, a compound isolated from bee venom, increased the short-circuit current across the skin and corneas of Bufo toads, and that this effect is inhibited by meclofenamic acid.[77] In 1986, Miller and Vanhoutte noted that in the isolated descending aortas from turtles, caymans, and bullfrogs, acetylcholine caused contraction-dependent relaxation of aortas in caymans and bullfrogs and contraction of aortas from turtles, and that meclofenamate did not inhibit this response.[80] Also in 1986, Duranti et al found that meclofenamate inhibited acid secretion through the skin of edible frogs.[30]

Carprofen

Carprofen was introduced to the human market in 1988, and the veterinary market in 1996. Unlike its predecessors, it has more COX-2 specificity, and is more sparing of COX-1. As a result, side effects tend to be fewer and much less severe.[82,94] There are few studies looking at carprofen in reptiles and amphibians, but many reports of its empirical use in these species. In 1994, Nandi et al found that carprofen did not enhance histamine-stimulated H^+ release in the gastric mucosa of frogs.[84] In 2007, Trnkova et al examined the effects of carprofen on blood profile parameters in green iguanas, and found that animals treated with carprofen had a decreased hemoglobin and hematocrit, and an increase in azurophils compared to controls. They also had significantly higher AST and ALT activity.[131] Empirically, carprofen has been used in a Greek tortoise at 2 mg/kg IM;[63] in a bluetongue skink at 4 mg/kg IM q24h for 5 treatments;[109] in a green sea turtle at 1.5 mg/kg PO q48h for 4 months;[55] in a White's tree frog at 4 mg/kg IM;[71] in red-eared sliders at 2 mg/kg IM and 4 mg/kg IM;[89,96] in a black water monitor at 3.33 mg/kg SQ, followed by 4 mg/kg PO q24h for 14 days;[83] in a diamond python at 2 mg/kg IM;[54] and in a monocellate cobra at 2 mg/kg IM q24h for 3 treatments.[135] No side effects attributed to carprofen use were documented in any of these case reports, including the monocellate cobra, which was being surgically treated for renal gout. Doses at 1-4 mg/kg IM, IV, SQ, or PO q24-72h have been reported for reptiles.[17,82]

Flurbiprofen

Flurbiprofen was introduced in 1988. Although an oral formulation has been utilized in humans, the topical ophthalmic formulation is typically used in veterinary medicine. It is a nonselective COX inhibitor that has also been shown to inhibit miosis.[94] There are few reports of its use in reptiles and amphibians. De Voe et al used it topically q12-24h to manage conjunctivitis in an eastern box turtle infected with ranavirus.[25] Dolinski et al used it topically q24h to treat a systemic fungal infection that also affected the eyes of a plains garter snake.[28]

Ketorolac

Ketorolac was introduced in 1989. Oral, parenteral, and ophthalmic human-label formulations are available. Ketorolac is a nonspecific COX inhibitor that is similar to aspirin, except without significantly affecting platelet function.[94] In 1998, Henson and Lewbart looked at ketorolac for management of post-operative pain in injured wild turtles, and found that turtles that received the drug following shell fracture repair began eating much sooner than controls.[59] In 2001, Stevens et al looked at ketorolac as an analgesic in northern grass frogs, and found that it weak but noticeable analgesic effect using the acetic acid test.[125] The critical effect environmental contamination concentrations of ketorolac on aquatic animals, including amphibians, has been calculated.[33]

Etodolac

Etodolac was introduced in 1991. It is considered to be a COX-1 sparing drug rather than a COX-2 selective drug.[94] There is only one published report of its use in reptiles. In 2010, O'Shea and Ball reported its use in a Komodo dragon at 5 mg/kg PO q72h for 30 days. Even though it was given concurrently with 4 mg/kg ketoprofen IM q24h, no side effects were reported.[88]

Meloxicam

Meloxicam was first introduced in 1995, although it was several years later before it was available in the US. It is considered to be COX-2 preferential but not COX-2 specific, because its specificity is diminished at higher dosages.[94] Due to its safety, ease of administration, and performance record, meloxicam has become the NSAID of choice in most exotic species.

Reptiles: Divers et al looked at the pharmacokinetics of meloxicam in green iguanas, and found that the administration of meloxicam at 0.2 mg/kg IV or PO would result in plasma concentrations >0.1 µg/ml for approximately 24 hours, and that oral overdose at 10-50 times the recommended dose for 2 weeks produced very high terminal plasma levels, but no evidence of toxicity based on hematology, biochemistry, and histopathology.[27,64] In 2007, Trnkova et al examined the effects of meloxicam on blood profile parameters in green iguanas, and found that animals treated with meloxicam had increased ALT activity and a decrease in blood calcium concentrations.[131] In 2008, Olesen et al looked at the effects of perioperative administration of meloxicam on the physiologic responses to surgery in ball pythons, and found that a dose of 0.3 mg/kg IM neither decreased the physiologic stress response nor provided an analgesic effect to treated ball pythons compared to controls. Response to therapy was based on hematological analysis, assays of plasma catecholamines and cortisol, changes in blood pressure and heart rate, and behavioral observations.[86] In 2009, Rojo-Solis et al looked at the pharmacokinetics of meloxicam administered IV, IM, or PO to red-eared sliders, and found that after IV administration of 0.22 mg/kg, plasma clearance was rapid, resulting in an elimination half-life of 7.57 hours. Administration of 0.5 mg/kg IM and PO resulted in a rapid absorption via both routes, but IM administration showed significantly higher bioavailability and maximum concentrations than PO administration, and subsequently more predictable clinical pharmacokinetic behavior; PO administration also showed marked individual variability.[103] In 2015, Olimpia et al investigated the pharmacokinetics of meloxicam administered at 0.1 mg/kg IM and IV in loggerhead sea turtles, and noted a rapid elimination time, with the plasma drug concentration dropping below analytical limits within 8 hours, and concluded that this dose is inadequate for this species.[69] Also in 2015, Deli et al looked at the pharmacokinetics of meloxicam after a single dose of 0.2 mg/kg IM, PO, and ICe in red-eared sliders, and found that after IV and ICe administration, plasma drug concentrations were 10 times that of PO administration.[24] Doses ranging from 0.1-0.5 mg/kg IV, IM, SQ, or PO q24-48h have been recommended.[17]

Amphibians: In 2011, Minter et al looked at the effects of IM meloxicam on PGE_2 in bullfrogs following tissue trauma by a punch biopsy, and found that 0.1 mg/kg IM q24h was effective in suppressing PGE_2 levels in this species.[81] In 2011, Coble et al determined that 0.2 mg/kg meloxicam administered into the dorsal lymph sac of African-clawed frogs provided analgesia similar to morphine and xylazine, but significantly less than flunixin meglumine.[21] Wright recommends a dose of 0.05-0.1 mg/kg PO q24-72h for the treatment of corneal lipidosis, but doses up 0.4 mg/kg IM,SQ, or ICe have been recommended.[17,139] The critical effect environmental contamination concentrations of meloxicam on aquatic animals, including amphibians, has been calculated.[33]

Celecoxib

Celecoxib is a selective inhibitor of COX-2. It was introduced to the human market in 1995. The most common veterinary usage is for management of inflammation in birds suffering from PDD. In 2008, Sharma and Suresh looked at its effects on tail regeneration in northern house geckos, and found that animals treated with celecoxib had delayed tail regrowth.[117] However, In 2014, Mescher amputated a single limb in African clawed froglets at stage 54 and 55 of metamorphosis, when the regenerative ability is initially diminished, and found that topical application of celecoxib at the site of amputation resulted in improved limb regeneration and digital patterning.[79] The critical effect environmental contamination concentrations of celecoxib on aquatic animals, including amphibians, has been calculated.[33]

Rofecoxib

Rofecoxib was discovered in 1995, and approved for human use in 1999. It is a selective COX-2 inhibitor.[57] There is limited information about its use in amphibians. In 2002, Carrasquer demonstrated that rofecoxib altered epithelial transport parameters in the bullfrog cornea.[18] In 2006, Zelarayán et al reported that rofecoxib inhibited frog pituitary meisosis resumption in *Bufo arenarum* follicles treated with hCG and PGE_1.[143] The critical effect environmental contamination concentrations of rofecoxib on aquatic animals, including amphibians, has been calculated.[33]

Etoricoxib

Etoricoxib is a COX-2 selective inhibitor that was introduced to the human market in 1999.[57] In 2008, Sharma and Suresh looked at its effects on tail regeneration in northern house geckos, and found that animals treated with etoricoxib had delayed tail regrowth, with a 71% reduction in growth rate during the 2-12 mm stage and a 54% reduction during the 12-24 mm stage of regeneration.[117] In 2015, Narayanan found that eroticoxib at 50 mg/kg inhibited PGE_2- and FGF_2-induced tail regeneration in northern house geckos.[85] The critical effect environmental contamination concentrations of etoricoxib on aquatic animals, including amphibians, has been calculated.[33]

Other NSAIDs utilized in veterinary medicine

Bromfenac, Deracoxib, Fenclofenac, Firocoxib, Mavacoxib, Napafenac, Robenacoxib, Suprofen, Tepoxaline, and Tolfenamic acid have been utilized in veterinary patients. No information about the use of these drugs in reptiles or amphibians could be found.

Key References

Below are select references cited in the text. A full reference list is available upon request from Dr. Hannon at hannondvm@msn.com.

17. Carpenter JW. *Exotic Animal Formulary.* St. Louis, MO: Saunders Elsevier;2013.

33. Fick J, Lindberg RH, Tysklind M, Larsson DGJ. Predicted critical environmental concentrations for 500 pharmaceuticals. *Regul Toxicol Pharm.* 2010; 58: 516-523.

50. Grimm KA, Lamont LA. Clinical pharmacology. In: West G, Heard D, Caulkett N, eds. *Zoo Animal and Wildlife Immobilization and Anesthesia.* 1st ed. Ames, IA: Blackwell Publishing;2007:22-28.

58. O'Malley B. *Clinical Anatomy and Physiology of Exotic Species.* Philadelphia, PA: Saunders Elsevier;2005.

94. Plumb DC. *Plumb's Veterinary Drug Handbook* 7th ed. Ames IA: Wiley-Blackwell;2011.

107. Sadler R, Schumacher J, Rathore K, et al. Cyclooxygenase enzyme (COX-1 and COX-2) expression in control and traumatized skin and muscle of ball pythons (*Python regius*). *Proc Assoc Rept Amphib Vet.* 2014.

116. Seebacher F, Franklin CE. Physiological mechanisms of thermoregulation in reptiles: a review. *J Comp Physiol* B. 2005;175:533-541.

120. Sladky KK. Analgesia. In: Mader DR, Divers SJ, eds. *Current Therapy in Reptile Medicine and Surgery.* St. Louis, MO: Saunders Elsevier;2014:217-228.

125. Stevens CW, Maciver DN, Newman LC. Testing and comparison of non-opiod analgesics in amphibians. *Contemp Top Lab Anim* 2001;40(4):23-27.

131. Trnkova S, Knotkova Z, Hrda A, Knotek Z. Effect of non-steroidal anti-inflammatory drugs on the blood profile in the green iguana (*Iguana iguana*). *Veterinarni Medicina.* 2007;52(11):507–511.

Managing Dystocia in Snakes

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Abstract: Breeding snakes for fun and/or profit is becoming commonplace for reptile enthusiasts. Complications with laying eggs or giving birth to young are common sequelae of breeding snakes. Dystocia in snakes can be multifactorial and may be the result of inappropriate nesting sites, stress, dehydration, malnutrition, obesity, salpingitis, malformed eggs and abnormal reproductive anatomy. The consulting veterinarian should be familiar with the literature on the common methods for initiating breeding activity and cycling, gestation, fecundity, and incubation techniques for the various species of snakes seen in practice. This paper will discuss the general clinical presentation and commonly used medical and surgical options for the treatment of dystocia in snakes.

Clinical Presentation

Dystocia is seen more commonly in the egg-laying (oviparous) snakes than in the live-bearing (ovoviviparous) snakes.[1-3] Oviparous snakes include pythons, colubrids (rat snakes, king snakes, milk snakes, hognose snakes, green snake, etc.) and elapids (cobras, coral snakes, etc.). Ovoviviparous snakes include boas, colubrids (garter snakes, water snakes, ring-neck snakes, etc.), rattlesnakes, copperheads, and other vipers (gaboon, tree, eyelash, etc.)

Historical Information

In oviparous species, the snake may have laid a clutch of eggs and the owner notes what appear to be eggs still in the snake. Or the snake has not laid any eggs but is past her due date and the owner can see or feel eggs in the snake.

In ovoviviparous species the owner may present the snake knowing the snake has been bred, noting that it appears gravid but is well past its due date. Or the snake may have produced an abnormally small clutch size and the owner is concerned there are still babies present. Often with live-bearing snakes owners may not be aware they are gravid and may present them for anorexia or another problem.

Clinical Assessment

In oviparous snakes, retained eggs are often palpable on physical examination. They may be distributed unevenly throughout the lower 1/3 of the body or they may be "bunched up" at the cloaca. The eggs may be misshapen on palpation, they may be free moving, or they may be immobile. Use caution when palpating not to force eggs to move. The oviduct is extremely fragile and may tear easily. Radiology or ultrasonography can be used to confirm egg numbers, abnormalities, and comparative egg size but may not be necessary, as eggs are usually readily palpable.

In ovoviviparous snakes it is more difficult to palpate developing fetuses in these live-bearing snakes and radiology and/or ultrasonography may be necessary for confirmation. Ultrasound can be very useful in determining whether fetuses are alive. In late gestation snakes can be seen moving (often in a tight coil) and a heartbeat may be seen. Sometimes determining a true dystocia versus a normal pregnancy is difficult in live-bearing snakes.

Management

Medical

In this author's experience the use of oxytocin for dystocia in snakes is limited. It is often difficult to determine if a dystocia is obstructive or non-obstructive and if obstructive oxytocin is contraindicated. Snakes are generally presented well past there "due date" and thus have likely become an obstructive dystocia. Ova or fetuses that are retained past normal oviposition or birth often become adhered to the oviductal mucosa. Once adhered, the use of oxytocin may result in torsion and or rupture of the oviduct. Therefore there is a small window of opportunity for oxytocin to be effective. If used for a non-obstructive dystocia where some portion of the eggs (fetuses) have been laid or obvious nesting or straining is occurring, the oxytocin is best initiated within 48-72 hours of such activity.[2,3] A dose range of 5-20 IU/kg intramuscularly is used by this author starting with the lower end of the dose range and increasing the dose on subsequent doses if no response is initiated. Dosing can be repeated in 6-12 hours. If two doses have been given with no response then medical therapy will likely not be effective. Arginine vasotocin, which is the natural reptile oxytocin-equivalent hormone, is likely a more useful drug than oxytocin but it is available only as a research drug at this time.[4]

Egg manipulation

General anesthesia with propofol (PropoFlo 10 mg/ml; Abbott Animal Health) at 5-10 mg/kg intravascularly (tail vein or intracardiac) and/or isoflurane gas may be utilized to achieve muscle relaxation which may allow eggs to be gently manipulated toward the cloaca and removed. In some cases, the egg can be manipulated to the cloaca but still remain lodged. In these cases if the egg can then be visualized through the cloaca with a speculum it may then be aspirated. Often the deflated egg then can be easily manipulated out (and the procedure can be repeated for the next egg) or the snake can be allowed to try to pass the deflated egg on its own.

Cloacoscopy

Cloacoscopy can also be utilized in an attempt to manipulate the egg out through the cloaca.[3,5-7] The snake is anesthetized as described above for egg manipulation technique. A 2.7-mm rigid endoscope with a 5.0-mm Taylor sheath with ports is used to evaluate the cloaca. A continuous warmed saline drip is used through the port system of the sheath to keep the cloaca dilated. An intravenous fluid bag is hung above the surgery table to facilitate the fluid running through the sheath system.

The endoscope is gently placed into the cloaca and the fluid drip initiated to allow distention of the cloaca. Once the cloaca is dilated a thorough investigation of the cloacal anatomy can be performed. The openings into the oviducts are located dorsally in the urodeum. In some cases the oviducts may be entered and ova or retained fetus identified. Gentle manipulation of the ova or fetus with the endoscope or grasping forceps (along with the infusion of the warm saline) will allow all or portions of the ova or fetus to be removed. The technique is not always successful. In some cases entry into the oviduct via the endoscope is not possible as the opening appears to be "sealed closed". This is often the oviduct with retained reproductive products and often the other oviduct can easily be entered. Sometimes entry into the oviduct is possible but adhesions are unable to be safely

broken down and the dystocia is not resolved. However the endoscopy allows visualization of the oviduct and the associated obstruction. If severe thickening, hyperemia, obvious infection or abnormal looking ova/fetus is evident then it allows appropriate decisions to be made about the next steps. Treatment with antimicrobials and non-steroidal anti-inflammatory drugs such as meloxicam (Metacam; Boehringer Ingelheim) can be utilized to help with active salpingitis. After medical treatment the endoscopist can repeat the procedure in 10-14 days often with increased success in manipulating the retained ova/fetus.

Percutaneous aspiration

If the egg cannot be manipulated to the cloaca or endoscopy and or surgery is not an option percutaneous aspiration can be performed. This procedure does have the inherent risk of leakage of egg contents into the coelom and/or oviduct possibly inducing a ceolomitis. These potential complications should be discussed with owners. The author prefers endoscopy and surgical management as a better option to ensure safe reduction of the dystocia and to attempt to ensure future reproductive potential. However, sometimes these options are not available and percutaneous aspiration must be considered. The snake is preferably anesthetized as for egg manipulation above. The egg is isolated against the lateral body wall. The area is sterilely prepped and a 20-gauge needle is inserted between the first and second row of lateral scales and into the egg. The contents of the egg are aspirated into the syringe using caution to avoid any leakage of egg material into the coelomic cavity. The snake will usually pass the egg within 12-24 hours of aspiration. Subsequent eggs behind the first may also have to be aspirated in turn or they may pass on their own after the first egg is removed. Eggs retained more than two weeks may not be successfully aspirated as the egg contents may solidify. These eggs will have to be surgically removed. Ultrasound can be utilized to determine if the ova have solidified. This procedure cannot be utilized in ovoviviparous snakes.

Surgery

Surgery may be necessary if medical therapy, egg manipulation, cloacoscopy or ovocentesis have failed or are not the best options as discussed above.[1-3,8,9] After anesthetizing with propofol and/or isoflurane as described above, an incision is made between the first and second row of lateral scales over the retained egg or fetuses. The oviduct is isolated and incised to remove the egg or fetuses. If there is more than one egg or fetus they may be able to be removed from the same incision. However, if they are adhered higher up in the oviduct or in the opposite oviduct multiple incisions may have to be made. The oviduct is closed with a simple continuous pattern using a fine absorbable suture (ie, 4.0-5.0 PDS). The coelom is closed with an absorbable suture and the skin with a non-absorbable suture in an everting pattern.

References

1. DeNardo D. Dystocias. In: Mader DR, ed. *Reptile Medicine and Surgery.* Philadelphia, PA: Saunders;1996:370-374.

2. Stahl SJ. Reptile Obstetrics. *Proc 13th Annu N Am Vet Conf.* 2000;971-974.

3. Stahl SJ. Veterinary management of snake reproduction. *Vet Clin N Am Exot Anim Pract.* 2002;5: 615-636.

4. Lloyd ML. Reptilian dystocias review-causes, prevention, management and comments on the synthetic hormone vasotocin. *Proc Annu Conf Am Assoc Zoo Vet.* 1990;290-296.

5. Coppoolse KJ, Zwart P. Cloacoscopy in reptiles. *Vet Quart.* 1985;7:243-245.

6. Stahl SJ. Reptile cloacoscopy. *Proc Int Conf Exot.* 2003;57-60.

7. Stahl SJ. Cloacal endoscopy and associated endosurgical techniques in snakes. *Proc Assoc Rept Amph Vet.* 2005;44-46.

8. Divers SJ. Medical and surgical treatment of reptile dystocias. *Proc 21st Waltham OSU Symp.* 1997;75-81.

9. Lock BA. Reproductive surgery in reptiles. *Vet Clin N Am Exot Anim Pract.* 2000;3:733-752.s