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The Reptile Survival Guide Online 'Mini Series'

Session 2:
Reptile Diagnostics and Common Conditions

Kevin Eatwell
BVSc (Hons) DZooMed (Reptilian) Dip ECZM (Herp) MRCVS
RCVS & ECZM Diplomate in Zoological Medicine
RCVS & ECZM Recognised Specialist in Zoo & Wildlife Medicine

REPTILE DIAGNOSTICS

Faecal examination

A faecal screen should be performed on every reptile presented to the clinician. Many parasites we see have a direct life cycle. Lizards and chelonians are messy feeders and inhabit the same vivarium, box or run and parasite levels in the reptile and its environment can build up over time. Some can be heavily infested at the time of purchase.

Faecal samples collected for parasitology should be as fresh as possible. Specimens that cannot be examined immediately should be fridged and a portion added to equal part of 10% Formalin. (Histology fixative strength). A fresh sample is needed due to the fact that eggs, oocysts and other life cycle variation may alter due to development.

Small Animal Faecal Collection

1) The owner may collect the faecal sample immediately after defecation and place into a plastic bag, wrap it in cling film, use a clean jar or other suitable container such as a 30ml/60ml container. This is important as the sample needs to be moist. The sample container should not be contaminated.

2) Pooled samples may be used if a number of animals are housed together. These samples are used to give a general idea of the degree of parasitic load.

3) Collection directly from the animal can be performed using a gloved finger or using a faecal loop, although this only gives a tiny sample. Many animals may void during examination and this should be collected if available.

Invertebrates fed to insectivorous species generally have free range throughout the tank and can quickly contaminate themselves with reptile faecal material if not ingested quickly. They can therefore be a source of autoinfection. Any uneaten livefood should be removed to prevent damage to the lizard but also destroyed such that they do not contaminate other livefood by being replaced in the tub and fed out again another day.

Parasitic infections can be reduced by using a substrate that can be easily and completely removed. All uneaten food should be removed promptly. Any livefood that is uneaten should be removed and destroyed. Running regular faecal screens is useful to identify if any treatment is warranted. Ensuring reptiles have a negative faecal sample prior to going onto a soil based substrate or run is vital to limit pathogen build up over time.

Many tortoises will be presented twice a year for deworming. I do not recommend this. By all means test for faecal parasites twice a year instead. Fenbendazole toxicity is now well recognised and has been reported in Feas vipers. It causes radiomimetic lesions (gastrointestinal ulceration, bleeding, immune suppression and secondary infections). The biochemical changes after treatment have been published in Hermanns' Tortoises. The effective kill is increased by using a lower dose over a few days, eliminating the need for high one off doses.

Initially a fresh wet preparation examined under the microscope will give an indication of parasite numbers and any motility can be noted. Only a tiny amount of faecal material is needed and this can be diluted with warmed saline and examined immediately. A common mistake is to have a thick

preparation. A floatation is the next useful preparation to make. A saturated saline solution is fine and a coverslip is used to collect any parasites or ova that float. A common mistake is to be impatient and not give sufficient time for adequate floatation! A concentration technique can be performed using 10% formalin overnight (to encourage cyst formation) followed by filtration then the addition of ethyl acetate (to dissolve fat). This mixture is centrifuged and the deposit examined. This allows identification of even lower numbers of parasites. These three techniques should be used for all samples. Performing an egg count by the McMaster's technique is unnecessary as we really only need to know a rough idea of numbers (is it bad enough to treat?) in most cases I would advise the treatment of any worm burden in reptiles given the likelihood of autoinfection and sever burdens developing. A compromise is to add 1 gram of faeces to 15mls of saturated saline and fill the chambers of a McMaster's slide. Iodine staining for parasitic cysts is not required as concentration techniques encourage any protozoa to cyst up and be readily identified.

FAECAL ANALYSIS

Faecal Examination

1) Faecal samples must be handled with care. The faeces you are handling may contain parasites, bacteria or viruses that are zoonotic (i.e. hazardous to people). Appropriate clothing (plastic aprons) and gloves are available for use. If you choose not to wear gloves, hands should be frequently washed with the antibacterial soap provided and washed prior to departure from the laboratory.

Gross Examination of Faeces

It is helpful if several characteristics of the faeces can be noted:

1) Consistency: Fresh normal faeces should be somewhat formed depending on the species of animal. Diarrhoea or constipation may be an indicator of parasitic infection.

2) Colour: Colour may be affected by food eaten. Malabsorption from the intestinal tract or parasites.

3) Blood: Can be reddish brown/and or bright red streaks or digested blood which has a dark tarry appearance. In any case blood of any description may indicate severe parasite infection or a serious intestinal disease.

4) Mucus: Can be the result of parasitic infection or digestive disorder.

5) Parasites: Adult parasites or tapeworm segments may be found. Please note tapeworm segments must be kept moist by the addition of normal saline.

Methods

Direct Smear

Materials required: Microscope slide and cover slip.
Applicator stick or loop/sterile saline or water.

Optional: Lugols iodine (Gram stain strength) or new methylene blue.

Procedure

1) Place a drop of warmed saline or water on the slide with an equal amount of faeces. A drop of stain may be added at this time.

2) Mix thoroughly with the applicator stick to form a homogeneous solution.

3) Remove any large pieces of faecal material.

4) Note: Smear should be thin enough to read print through add more diluent if required.

5) Cover with cover slip.

6) Examine under x10 objective for eggs and larvae and then under x40 for motile protozoa and cysts.

Top tips: If you feel something should be motile and it's not moving, heat a penny or any coin through a flame and rest the slide on it to warm, alternatively heat gently with a lighter or match flame. A common mistake is to make a preparation that is too thick.

Flotation Method

Salt Flotation

Most commonly used but in fact the least desirable. It corrodes laboratory equipment, forms crystals and severely distorts parasite eggs. The maximum specific gravity obtainable is only 1:200, which allows heavier eggs to remain submerged. It also needs TIME for the parasites to rise to the surface.

Materials required: Flotation solution (saturated sodium chloride)
Waxed paper cups
Tea strainer – Metal small pores/or cheesecloth/gauze or muslin
Tongue depressor
Glass test tube/universal

Procedure

1) Take about 2 grams faeces (1/2 teaspoon) and place in a paper cup.

2) Add approximately 20mls of flotation solutions and gently mix thoroughly to form a thin 'soup'.

3) Pour this mixture through the tea strainer or alternative and discard large faecal lumps.

4) Pour filtered liquid into a test tube or a universal to the top to form a meniscus. If there is not enough fluid, top up with flotation solution.

5) Gently place a cover slip on top of the tube.

6) Allow the tube to remain UNDISTURBED for a minimum of 20/30 minutes but not longer than 1 hour. If the preparation is not allowed to sit this long, some eggs will not have time to float to the surface and if left longer than 1 hour, eggs may become waterlogged and sink and are also prone to distortion.

7) Carefully remove the cover slip by picking it STRAIGHT UP (don't slide it off) and place on a slide with the wet side adjacent to the slide.

8) Examine under the microscope at x10 and x40 objectives.

Top tip: At least ten minutes is required for eggs to float. Do not be impatient and it may be best to set a timer as ten minutes is a long time while you are waiting.

'Cold' ZN for Cryptosporidium

Materials required: Pro lab Cryptosporidium staining kit which contains:

(1) Fixative
(2) ZN Carbol Fuchsin
(3 & 4) Differentiator 1 & 2
(5) Malachite Green

Microscope slides
Swabs
Immersion Oil
Timer
Microscope

Procedure

1) Using a swab make a paper thin smear of faeces on to a slide. Smears can also be made from swabs taken from the outside of regurgitated items. Smears should be almost transparent.

2) Cover with fixative (1) and leave until evaporated (about 10 – 15 minutes).

3) Flood the slide with ZN carbol fuchsin (2) and leave for 10 minutes.

4) Wash off with tap water and flood slide with differentiation one (3), leave until no more pink colour appears to come out.

5) Rinse under the tap again, wipe the back of the slide with tissue. Flood the slide with differentiation two (4) and leave until no more pink colour appears to come out.

6) Rinse under the tap again and flood the slide with malachite green (5) and leave for 30 seconds.

7) Rinse off under the tap and leave to dry.

8) Examine the slide under a x100 oil immersion. Oocysts appear as BRIGHT red ovals, whereas bacteria and yeasts stain pale green.

Note: Do not use tap water when using this method for a hot ZN or looking for acid fast bacilli. It is possible for false positives to be seen as AFB can occasionally be found in tap water.

In some animals obtaining a faecal sample can be difficult. Snakes in particular void infrequently. A chronically anorexic snake may not have a sample to give! In these cases a cloacal flush should be performed using a tube. 1% of the animals bodyweight in warmed saline can be infused into the cloaca/colon. A large tube must be used as the aim is to collect faecal material. In many animals the insertion of the tube can induce voiding prior to infusion. This is preferred as the faeces are more concentrated. However if infusion is required, concentrating techniques can be used. The back end of the reptile is massaged and then the watery contents aspirated. Caution is to be used as it is possible to obtain urates via any method which can lead to non diagnostic results. Many owners and vets will also present urate concretions for faecal analysis.

There are a vast number of parasites seen in reptiles. Ascarids are commonly identified in chelonia and in a recent study had a prevalence of 13%. The adults can be up to 10cm long and can lead to intestinal impaction, vomiting and death. They have direct and indirect life cycles. The most common species identified is *Angusticaecum sp*. The eggs are thick walled with scalloped edges. Oxyurids can be found in about half of faecal samples form tortoises and lizards and large burdens can develop due to their direct life cycle. They are small worms up to 1cm in length. Clinical signs reported include anorexia, obstruction and rectal prolapse. The eggs are D shaped with thin walls.

Ciliates are also commonly identified in chelonian faecal smears and many of these can be evident in high numbers in sick chelonia. The difficulty is attaching any significance to their presence. They may be secondary overgrowth with digestive disturbances. Many are commensals such as *Balantidium sp* or *Nytcotherus sp*. It is difficult to identify individual species without special training and stains. I am of the opinion if the chelonian is sick and high numbers are identified then treatment should be given.

Coccidia, Amoeba, Flagellates and Cryptosporidium can be identified in chelonia. They do not normally present as a clinical problem. Coccidiosis is a common parasite, particularly in bearded dragons. In one American study 23% of beardies were positive. In this species *Isopsora amphibolouri* is found. These cysts have two sporocysts, each containing four sporozoites. Cryptosporidium is a common pathogen in squamates and two species are recognised. *Cryptosporidium serpentis* in snakes and *C. saurophilum* in lizards. In snakes it infests the stomach leading to hyperplasia of the mucous glands and hypertrophy of the stomach itself. This causes a mid body swelling and regurgitation and emaciation. The parasite life cycle is speeded up after feeding. In lizards is causes small intestinal tract disease and leads to a wasting condition. It is particularly common in leopard geckos. The diagnosis is made by a ZN stain of the faeces or a regurgitated meal. Ultimately a biopsy of the intestinal tract or stomach sent for histopathology will lead to a diagnosis. 80% of the cysts reinfect the host without being shed. The remaining 20% are immediately infective upon being passed and are extremely resistant to most disinfectants. Heat (60^0C for 5 minutes), formalin (10% contact time 18 hours) and ammonia (5% contact time 18 hours) were effective a neutralising the oocyst in the environment.

Fungal overgrowth is common after antibiotic therapy and can be fatal and can be easily detected.

Standard treatments used include fenbendazole for nematodes and metronidzaolre for protozoa. Toltrazuril and TMPS are frequently used for coccidiosis. Ivermectins should not be used in chelonians as it can enter the central nervous system and cause paralysis by activation of GABA receptors. Ivermectins can be used in squamates but have led to mortality in chameleons.

Microbiology

Culture and sensitivity is indicated from all lesions where infection is suspected. Samples can be taken from any site (eye, tracheal wash, choanal swab, nasal discharges, mouth, skin lesion, faecal sample, abscess capsule biopsy etc). It is important to obtain a suitable sample. Thus where there is obvious purulent material sampling from the edge of lesions is more likely to lead to a positive culture result. The thick consistency of pus in reptiles means that in many cases surgery is the treatment of choice with the physical removal of all infected tissue including the abscess capsule is of paramount importance. Antibacterial therapy based on culture is used to prevent a recurrence from contamination of the surgical wound during surgery or to 'mop up' residual infection where complete excision is not possible (for example osteomyelitis). The centre of many abscesses is biologically sterile. Freshly voided faeces should be used for culture. Swabs taken from the choana, nasal passages or from tracheal washes should be minimally contaminated with oral flora as this may confuse potential treatment and guarded swabs may be required to avoid touching the oral mucosa. Starving the animal prior to sampling will reduce the level of bacterial flora and food in the oral cavity.

If possible the material collected should be placed immediately onto culture media to maximise the growth of fastidious organisms. This is only possible if a laboratory is within walking distance of the practice or the practice has their own. This usually means that samples will need to be placed into a medium prior to transport. Charcoal medium is ideal and this should be held at room temperature for one hour (to allow the bacteria to colonise the medium) and then be kept cool or posted immediately (to reduce the chances of overgrowing the medium). This is fine for aerobic bacteria. Cooling swabs will kill off the more fastidious anaerobes. Samples should be submitted for aerobic, anaerobic and fungal culture. Anaerobic infections can be significant in all species and are typically cultured from deep infections. In one reptile study 54% of samples yielded anaerobes. More recently anaerobes have been considered significant pathogens in periodontal disease in lizards. Reptilian cultures will typically grow gram negative bacteria with multiple resistance patterns. Agents commonly identified include *Actinobacter, Aeromonas, Campylobacter, Citrobacter, Klebsiella, Moraxella, Pastuerella*, *Prevotella, Proteus, Pseudomonas, Salmonella, Serratia* and *Yersinia*. Some of these produce specific clinical syndromes such as Septicaemic cutaneous ulcerative disease (SCUD) in aquatic chelonians *(Citrobacter* and *Serratia).* Although there has been a lot of concern regarding positive *Salmonella* and *Campylobacter* cultures in reptiles particularly, many reptiles shedding these organisms show no clinical signs at all and treatment is likely to be contraindicated. Many isolates are likely to be pathogenic, but they may not be the primary cause or agent or have any significance whatsoever. Their growth in a culture should always be questioned, as all bacteria isolated from reptiles have been cultured from healthy individuals.

Supportive evidence of an inflammatory response or suitable clinical signs is required to confirm pathogenicity or any cultured organism. There has been debate regarding the temperature cultures should be incubated at. What determines the appropriate temperature is the organisms being cultured, not what the host species was (unless the bacteria is host adapted). Thus most of the pathogens we find in reptiles will grow at 37^0C as they are not host specific (i.e. many are zoonotic and most are found as pathogens in mammals as well). However the fact they have grown is irrelevant without supportive evidence of an inflammatory response or suitable clinical signs. Thus other testing is required to confirm their significance.

It should be noted that other atypical bacterial pathogens may be involved such as *Mycoplasma, Chlamydophilla* and *Mycobacteria* and antibiotic cover for these may need to be included and this

may alter the selection of antimicrobial agents considered. *Mycoplasma* are common in respiratory problems in reptiles. *Chlamydophila* is in snakes and chelonians.

Cytology

Cytology is an inexpensive yet powerful tool in clinical practice and samples can be taken from any lesions, nares, tongue, eyes, colon, stomach or trachea, either on swabs or as a wash. These samples can be used for culture but the cytology is not to be underestimated. Cytology can confirm the presence of an organism but also an inflammatory response to the organism confirming its pathogenicity. Presumptive therapy can be started while waiting for culture results or in the event of the culture failing to grow organisms. Cytology can also identify organisms that may be difficult to culture. Slides should be made by gently rolling or dabbing material onto the slide. In-house cytology can be performed, but it is wise to get samples verified by a suitable external laboratory until you are confident at reading them.

Nasal and tracheal washes

PCR analysis is now available for two common pathogens in chelonia (Herpesvirus and Mycoplasma) and ocular, nasal or oral swabs and flushes are acceptable samples to send. Starving the tortoise and using saline moistened swabs will help to improve isolation of the DNA. Pressing under the jaw can lead to discharges being produced from the nares. For nasal flushing a syringe with a needle hub on the end can be used to blast material form the nares out the mouth. Occasionally discharges will be so thick that a nasal discharge is absent despite having upper respiratory disease.

Tracheal washes are commonly performed as pneumonia is common. 1% of the bodyweight of warmed saline is infused down an appropriately sized catheter. The snake is rolled and the fluid aspirated. Cytology and culture should be performed. PCR is also available for PMV. The catheter used can be bent to attempt to obtain a wash from a single side (lizards and chelonians) and radiography can be used to confirm the location. Lung worm larvae (*Rhabdius* or *Entomelas*) can be seen on cytology in snakes and lizards. Samples of the respiratory tract can also be taken during endoscopic examination of birds and reptiles and samples submitted for cytology, culture or histopathology and are preferred to tracheal washes as direct visualisation and selection of the site to be sampled is possible.

Oral cytology

In reptiles with a stomatitis a sample for cytology should be submitted and if there is a lot of fluid production an aspirate should be kept moist in a small collection tube. *Rhabdias* larvae and flagellates can be found on wet preparations.

Skin cytology

Cytology of skin lesions can be performed in all species. In snakes and the shed exuvium is very useful in identifying external parasites such as *Ophionyssus natricus*. Other external parasites are rare in reptiles, but ticks and other mites can be seen). In many cases skin biopsy is indicated for a detailed assessment of skin lesions.

Urine analysis

Urine analysis is generally not considered helpful in reptiles. However urine specific gravity and pH are a useful measure of hydration status in chelonians. Herbivorous chelonia have a urine pH of 7.5 and a specific gravity of 1.003 - 1.012. Carnivorous reptiles should have a pH of 6 – 7. Reptiles lack loops of henle so urine should consistently have a specific gravity of 1.005 – 1.010. In individuals with elevated osmolarity the urine specific gravity may elevate to 1.034. Glucoseuria is abnormal. The colour of the urates voided can also give an indication of health status. Pink stained urates are common in chelonians and are of no clinical significance. However green staining can suggest increased biliverdinuria and be correlated with liver pathology. Parasites should not be found and hexamita are rarely seen in the urine of tortoises and can lead to significant renal pathology.

Antibacterial therapy

Selection of an antimicrobial requires careful thought and I would suggest that clinical parameters and likely pathogens are far more important in the selection process than the cascade. A good exotic animal formulary should be utilised to identify the most suitable regime based ideally on pharmacokinetic studies in the same or similar species. These can be highly variable between species and a blanket dose for all reptiles is inappropriate.

Aminoglycosides are bactericidal and act on bacterial ribosomes preventing protein production. They are much less active in purulent material. Side effects include nephrotoxicity and ototoxicity. They are effective against gram negative aerobic infections only and are not absorbed from the gastrointestinal tract. The risk of nephrotoxicity, in humans, is based on the antibiotic remaining above a certain threshold in the serum. This level may well be below the MIC for many pathogens. Less frequent dosing may need to be considered where pharmacokinetic studies have not been performed to reduce the risk of nephrotoxicity. The use of these agents with cephalosporins or penicillins leads to synergism due to increased cell permeability to the aminoglycosides and may help to maintain effectiveness in this situation. To improve effectiveness further a concentration of 3 – 5 times the MIC of the organism at the site of infection is recommended for the aminoglycosides. I only utilise them where a culture result shows resistance to all other therapies. Amikacin is safer than gentamicin and is a second generation aminoglycoside. Generally amikacin has greater activity than gentamicin.

Ceftazidime is a third generation cephalosporin with excellent anti-pseudomonal activity. Ceftiofur has also been used in exotic animal medicine. Anaerobic bacterial resistance is low. It is useful as a primary agent or alongside aminoglycosides or fluoroquinolones to extend the spectrum of cover. They are widely distributed both into bone and the CNS if meningitis is present. It is excreted by renal tubular secretion and glomerular filtration. This is available as an injection only. Once re constituted it only maintains potency for 24 hours at room temperature. Refrigerated injections last for 7 days. Frozen injections remain potent for 3 – 4 months.

Synergism has been noted between bactericidal antibiotics such as gentimicin and ceftazidime. This is because antibiotics that act on the cell wall (such as the penicillins and cephalosporins) increase the cell permeability to other antibiotics that act within the bacterial cells. Interactions between gentamicin and ceftazidime at peak serum levels have led to the recommendation that they be injected on separate days. No such action occurs between the fluoroquinolones and cephalosporins and it is acceptable to inject these drugs in combination.

Enrofloxacin is the only licensed antibiotic for use in exotic species. This is bactericidal and effective against aerobic bacteria (particularly *Pseudomonas)* and *Mycoplasma* only. It acts by inhibiting

DNA gyrase and resistance is currently less likely compared to other antibiotic classes and occurs via mutation of the bacteria. Fluoroquinolones are metabolised by hepatic biotransformation and oral administration leads to a first pass effect. Thankfully the hepatic extraction ratio is low. Side effects include cartilage damage in growing animals. Excitation and diarrhoea has been reported in Galapagos tortoises. Muscle damage due to the alkaline pH (11) of the injectable enrofloxacin is well known. Dilution of samples in saline or administration intracoelomically has been suggested to avoid these local tissue effects.

Sadly abuse of this agent is leading to increasing resistance. Enrofloxacin has been shown to be effective at the elimination of *Salmonella* from iguanas. Ciprofloxacin has greater anti-pseudomonal activity and many isolates resistant to enrofloxacin prove sensitive to ciprofloxacin. This is available as an oral preparation and as eye drops (which can be given intranasally). Sadly the bioavailability of ciprofloxacin is less than enrofloxacin when given orally. Marbofloxacin is increasingly used in preference and resistance is lower. The injection has an acidic pH.

Pulse therapy works well and efficacy is determined by obtaining a plasma level eight times or more than the MIC. There is a significant post antibiotic effect (PAE) that acts to inhibit bacteria even when levels drop below the MIC. This effect can last up to 8 hours and depends on bacterial species, drug concentration and effective time above the MIC. Essentially the PAE allows for more infrequent dosing compared to the regime calculated from pharmacokinetic studies.

Penicillins act by inhibiting bacterial cell wall synthesis, but beta lactamase resistance is possible. These require steady state pharmacokinetics and maintaining the serum level above the MIC for an extended time period is important in their effectiveness. Broad spectrum penillcillins in combination with clavulanic acid, such as ticarcillin is commonly used as it has activity against *Pseudomonas*. It can be frozen in a similar fashion to ceftazidime.

Blood sampling

Tortoise veins are hard to find. The first site to consider is the jugular vein. This runs on either side of the neck (the right is larger) from the tympanum coursing dorsally. The carotid artery can also be sampled and runs more ventrally. They are both superficial vessels. Care has to be taken as haematomas are common particularly if the carotid vessels are inadvertently punctured. I have seen a tortoise bleed out from a carotid vessel into its oesophagus. Skin disinfection should be thorough prior to venipuncture. 25 gauge needles will be required in most cases.

Other sites include the subcarapacial sinus which is my preferred second option. This sinus is a confluence of the common intercostals veins and the dorsal branch of the jugular veins. Lymph dilution is possible at this site but usually has minimal effect on results with careful technique. The lymph vessels lie directly over the sinus site and so some contamination is inevitable. In one study the PCV of tortoises was reduced by only a small percentage compared to jugular vessels. This can be accessed with the head in or out and a longer 1” or 1.5” needle required in larger tortoises. Spinal damage has been reported.

The dorsal tail vein leads to marked lymph dilution and should not be used for blood sampling. It is however useful in aggressive or strong specimens, but in an ideal world should be used to administer a sedative to allow for jugular sampling more safely! Tortoises with hinges may also need sedation. 10mg/kg propofol into the tail vein or subcarapacial sinus can greatly facilitate sampling (or other diagnostics). If finding a vein is impossible then intra-osseous propofol or intra muscular agents such as medetomidine/ketamine or tiletamine/zolazepam can be used.

In lizards and snakes the ventral tail vein is the first site to consider. This runs in the ventral midline of the tail. Care has to be taken to avoid the hemipenes in males and the musk sacs in females. The ideal site is beyond 12 ventral scales down the tail. Jugular sampling can be performed in lizards and the vessel courses from the point of the shoulder to the angle of the jaw. In snakes it is only accessable by a surgical procedure. It lies just underneath the end of the ribs. An ideal site to make your incision is 12 ventral scales cranial to the apex beat of the heart. The ventral abdominal vein has been used in lizards but it is impossible to control and haemorrhage from this site. Cardiac puncture is used commonly in snakes. The apex beat is identified 22 – 33% of length from the snout to the vent. The heart is stabilised and a small gauge needle is inserted into the ventricle. Blood is aspirated with each beat. The palatine vessels have been used in anaesthetised snakes. Skin disinfection should be thorough prior to venipuncture. 25 gauge needles will be required in most cases.

In general 0.5 - 1% of the body weight may be collected for diagnostic testing. Smaller samples should be collected in critical animals.
Heparin is the tube of choice for reptiles. This is because the sample can be used for haematology and biochemical analysis. Careful mixing is important to reduce white cell and platelet aggregation. Plasma can be separated and used for biochemical analysis. Heparin gel tubes are also available but these prevent the sample from being used for haematology but they do reduce the risk of red cell leakage. However generally the artefacts created by this are well recognised (elevated potassium) and the altered biochemical parameters ignored. For measurement of ionised calcium keeping the lithium heparin concentration below 15IU/ml is ideal.

Haematology

Reptilian haematology is a specialised field and films can be evaluated in house if the clinician has suitable experience, otherwise films should be examined by a haematologist with a special knowledge of reptiles. Recently studies in America have concluded that EDTA is preferable for the assessment of cellular morphology in the iguana and in those species of chelonians where haemolysis does not occur. However given the small sample sizes in many cases it is far more prudent to fill one tube from which both biochemical and haematological analysis can be performed and as a result heparin samples are preferred.

Reptile red cells are larger than mammalian cells and are nucleated. They are able to regenerate from pleuripotential thrombocytes within the circulation. They last for 800 days and regenerative responses may not be obviously marked on films. Polychromasia and mitoses can indicate a regenerative response. This may be seen after hibernation. A low level of polychromasia is expected on all films. A particular feature of chelonian samples is that red cell vaculolation can occur in anorexic individuals. This is a non specific response and the precise reason is unknown. Basophilic stippling can be seen as a result of heavy metal toxicity, anaemia or iron deficiency. Viral inclusions have also been reported in reptiles (herpesviruses in iguanas for example). Illness due to these inclusions seldom occurs. For many years *Pirhaemocyton* was reported as a protozoal disease and caused acidophilic intracytoplasmic inclusions. More recently an iridiovirus has been implicated in some species.Reptiles can cope with blood loss well due to their ability to tolerate anaerobic metabolism. The PCV varies depending on hydration status.

Reptile PCVs vary depending on hydration status but is generally much lower than values in mammals. Comparing the PCV to the protein values (and considering the venipuncture site) can be invaluable in determining if there is dehydration, whole blood loss or anaemia.

Red cell counts must be performed manually due to the nucleated corpuscles. Automated machines are particulate counters and will record red cells with white blood cells. As a result many laboratories avoid a total red cell count for simplicity.

Anaemia is defined as a low blood Hb estimation. It is possible to be anaemic with a PCV within reference ranges. A number of types of anaemia are possible based on the indicies. In reptiles a regenerative response is indicated by increased polychromasia, binucleated cells and mitoses in the circulation. Pleuripotential thrombocytes can lead to erythropoiesis in the circulation and many juvenile red cells can be identified. Typically these cells are more rounded and basophilic with a prominent nucleus. Reptiles cope with acute blood loss much better than mammals. Non regenerative anaemia occurs when there is a lack of appropriate red cell regeneration. In reptiles this can occur if they are hypothermic. Monitoring the PCV is a simple way to assess the regenerative response.

White cell counts must be performed manually in reptiles. As a result some laboratories use indirect methods for speed. Some authors have advocated the use of the buffy coat percentage to predict the total white cell count. This may give a suggestion for an elevated count but has been shown to be inaccurate. Another commonly used method is an estimated count based on the blood film. This also leads to inaccurate results. It is far preferable to perform a direct count as outlined below. It should be noted that direct counting in mammals is subject to a 30% variance compared to automated results.

White cell morphology

Reptiles have both granulocytes and agranulocytes. Their morphology on a smear is more important than their numbers. Numbers can be low even in the presence of severe infections. The main granulocytes are the heterophil. The reptilian heterophil has similar functions to the mammalian neutrophil. It is an acute inflammatory cell that responds to infection and inflammation.

Heterophils represent about 40% of the white cells. Signs of toxic activity include degranulation, cytoplasmic vacuolation, darkening granules (increasing basophilia), nuclear hypersegmentation and bacteria may be seen within the cytoplasm. The granules are rod shaped and usually pink to orange in colouration. The cells can have variable shape with uneven staining cytoplasm. High heterophil counts have been seen in response to bacterial and fungal infections. A left shift can be seen and suggests a significant response to inflammatory disease. Lower heterophil counts are recorded post hibernation and can be due to viral disease or overwhelming infections.

In reptiles lymphocytes can commonly be misidentified as thrombocytes. Thrombocytes have cytoplasmic pseudopodia and tend to aggregate on films. Lymphocytes are small round to oval basophilic cells with no granules. Reactive lymphocytes can occur in response to viral infections. This is indicated by darker blue cytoplasm with a paler region (golgi region) close the nucleus and nuclear changes. Elevations or reductions can occur in viral disease. In reptiles numbers can elevate during the summer months. They can compose up to 80% of circulating white cells in some reptile species. Lymphopaenia can reflect chronic immunosuppression, stress and malnutrition in reptiles.

Monocytes perform phagocytic functions. They are the largest leukocyte. These agranulocytes respond in chronic granulomatous diseases. In reptiles they can be elevated with severe bacterial infections and granulomatous disease. Leukaemia can lead to marked elevations in monocytes.

Snakes and iguanas have another group of monocytes that stain differently and are called azurophils. These have the same function as normal monocytes. Up to 20% of the leucocytes can be azurophils in these species. Other authors disagree placing all monocytes together. From a clinical point of view the interpretation of altered levels is the same.

Reptiles usually have low numbers of basophils. Fresh water chelonians can have higher levels (up to 50% of the white cells). The nucleus can be obscured from view due the number of intracytoplasmic granules evident. Trauma, viral, parasitic and chronic disease can lead to elevations.

Eosinophils have round granules that stain bluish compared to heterophils. They typically have a clear differentiation between nucleus and cytoplasm. Nuclei are single or bilobed and eccentrically placed. Reptile eosinophils can be up to 20% of the cells and can be influenced by seasonality (lower in summer). Many snakes can have no circulating eosinophils at all. Eosinophils are much less common in birds. The nucleus can be obscured from view due the number of intracytoplasmic granules evident. In reptiles it is assumed they respond to parasitic infestations. However caution is to be advised should you diagnose an eosinophilia as it is likely that a heterophilia has been misidentified.

Reptiles have thrombocytes and these are the second most numerous cells after the erythrocytes. These usually aggregate on the smear. Decreased thrombocytes can be associated with coagulopathies. Pseudopodia or cytoplasmic enlargement characterises reactive thrombocytes. This can be for phagocytic defence. They can be misidentified as lymphocytes. They typically have pale cytoplasm.

Intracellular parasites are usually in the red blood cells. In reptiles common intracellular haemoparasites seen include *Plasmodium, Haemogregarina, Hepatozoon* and *Haemoproteus*. *Saurocytozoon* can be found in the leucocytes. It is impossible to distinguish between Haemogregarine species on a blood film, however, snakes typically are infected with *Hepatozoon* and *Haemogregarina* in chelonians. These all require intermediate hosts and are usually self limiting in captive reptiles. In addition microfilaria of filarial nematodes and trypanosomes can be seen free in the plasma. It is best to look for these on the smear edges.

Biiochemistry

Selecting the correct biochemical profile is important. both supportive care and possible diagnostics you would like to perform next. Sadly biochemical analysis is not an exact science and many animals can be quite severely ill but yet have a biochemical analysis within reference ranges. This does not mean that the screen should be discarded but assessment of the results should be compared to the status of the patient.

Protein excretion varies depending on the habitat of the species we are considering and the season. Typically terrestrial species excrete high levels of Uric Acid. Sadly this only is elevated after 70% of functioning nephrons have been destroyed. Mild elevations can reflect folliculogenesis or high protein meals in omnivores or carnivores (it can be elevated two fold the day after feeding). It is also possible for an animal to sustain a uric acid spike which the settles back down to reference ranges but yet still be predisposed to the development of gouty tophi. Thus animals with visceral or articular gout may not always have elevated uric acid. In reptiles a value of 1457 umol/L is considered the point at which tophi form and animals with values over 2000 umol/L will be highly unlikely to survive.

Urea also is a useful parameter to measure as terrestrial tortoises increase production of urea in order to raise blood osmolarity. Fresh water chelonian species excrete more urea. Uric acid is elevated only when two thirds of renal function has been compromised. Mild elevations can reflect folliculogenesis or high protein meals in omnivores (can be elevated two fold the day after feeding). Urea is useful in terrestrial chelonians to evaluate dehydration. Creatinine is of no use in reptiles.

Sodium is useful to quantify the hydration status. Low levels can be due to gastrointestinal infections or over perfusion. Potassium should be analysed immediately as it leaks from erthrocytes in aged samples. Potassium can elevate in dehydrated reptiles due to reduced renal excretion.

Total protein and albumin can elevate in dehydration and in reproductive activity. Reduced values can be due to anorexia, gastrointestinal tract disease, liver disease or blood loss.

Total calcium consists of protein bound calcium, complexed calcium and free or ionised calcium. Ionised calcium consists of 18 – 67% of the total calcium. There is no physiological control of total calcium levels and values are primarily influenced by protein binding. Hypocalcaemia and lymph dilution decrease levels and ionised calcium should be measured. It is decreased when there is an acute demand leading to flaccid paralysis. This is most common in reproductively active females laying eggs. Many juveniles can have normal levels despite having marked NSHP.

Elevated phosphorus is the hallmark of renal secondary hyperparathyroidism. Mild elevations can occur in reproductive activity where it is liberated from the bone matrix. Haemolysis also leads to elevated values. Reductions can be due to starvation, nutritional deficiency or lymph dilution. Many reptile texts compare phosphorus to total calcium to produce a ratio and a solubility index. An inverted total calcium to phosphorus ratio (less than one) can suggest renal pathology. In green iguanas the solubility index is calculated by the product of the total calcium and phosphorus values. If this exceeds 9 then metastatic calcification in predisposed tissues such as the cardiac muscle and renal tissue becomes possible. If the value exceeds 12 then calcification in all tissues is possible. Mineralisation of the tissues depends on a number of factors other than serum values and the importance of the solubility index has been overstated. That said attention to calcium and phosphorus values should be made when deciding on the appropriate treatment for calcium deficient reptiles.

Glucose can be performed on a heparin sample or a glucometer. Glucose estimation from glucometers designed for human patients lead to variable results. There is marked physiological variation. High levels can reflect diabetes or pancreatic disease but have been poorly quantified in reptiles. Hypoglycaemia can occur in liver disease, starvation, malnutrition and septicaemia.

AST and CK are useful in combination. AST is found in liver and muscle tissue. CK is only present in muscles. Thus an AST elevation alone can reflect hepatic damage. If in concert with CK it can be disregarded. Bile acids are highly species specific throughout the animal kingdom. Assays developed are for mammalian bile acids and the usefulness of this test has not been conclusively confirmed in reptiles. Many clinicians consider values lower than 60µmol/L to be acceptable. Total cholesterol has also been measured in reptiles but is yet to be quantified. Measuring high density lipoproteins and triglycerides enables the low density lipoproteins to be calculated. This may be useful in assessing hepatic lipidosis but there has been no work undertaken in reptiles. The formula for this calculation is: -

LDL = Total Cholesterol – HDL – 0.45(Triglycerides)

The HDL is good cholesterol the LDL has been associated with cardiac disease in humans.

Betahydroxybutyrate is the major ketone produced in chelonian blood and is elevated in post hibernation or in drought.

Bone marrow aspiration

In reptiles bone marrow can be obtained from the tibia in lizards and chelonians. In snakes it is generally best to surgically remove a fraction of a rib and send this for histological analysis.

Reptile radiography

There are two standard views for squamates. Dorso-ventral vertical beam. This is useful for gastrointestinal tract disease, bladder stones, eggs and bone abnormalities. Anaesthesia may be needed to examine snakes so that they can be pulled into a straight line! Marking the snake along its length with radio-opaque markers at set points helps to localise a lesion. Lizards may need to be anaesthetised for limb or head radiography.

Lateral horizontal beam radiographs are required as squamates do not have a diaphragm and coelomic contents can compress the chest. These are useful for assessing the lung fields. Once again a snake may need to be marked along its length.

Barium studies are useful to assess gastrointestinal transit time and 1% of bodyweight can be given by stomach tube. Transit time can be increased by using iodinated compounds in KY jelly.
There are three standard views for chelonia. Dorso-ventral vertical beam. This is useful for gastrointestinal tract disease, bladder stones, eggs and bone abnormalities. Anaesthesia may be needed to examine the extremities so that they can be pulled beyond the margin of the carapace and kept still!

Horizontal beam radiographs are required as tortoises do not have a diaphragm and coelomic contents can compress the chest. Lateral and crainio-caudal views should be taken. These are useful for assessing the lung fields.

Barium studies are useful to assess gastrointestinal transit time and 1% of bodyweight can be given by stomach tube. Transit time can be increased by using iodinated compounds in KY jelly. Pro-kinetic agents had no effect on transit time in a study in gopher tortoises. Heat and fibre have greater influences on transit time.

Reptile ultrasound

Probes with a small footprint are required. 7.5MHZ scanners are best for most situations. Snakes can be scanned along their entire length from the ventral aspect. Lizards can be scanned from the ventral aspect or from the thoracic inlet. Ultrasound examination is a useful tool should the practice possess a unit with probes that have a small footprint. 7.5mHz probes are ideal for most individuals and can be used via the prefemoral fossae or between the neck and front legs to view the internal organs. They are most useful to assess the reproductive status of female chelonians, renal disease, hepatic disease and liver disease.

Reptile CT/MRI

CT and MRI are also options increasingly becoming available to clients. CT is extremely useful as it does not require anaesthesia and allows a thorough evaluation of the skeletal elements of a tortoise

looking for minor fractures and evaluation of soft tissue structures such as the liver (for fatty liver detection) and the gonads. Fine detail of the respiratory tract of snakes is also possible.

Reptile endoscopy

Rod lens telescopes (produced by Storz®) are regularly used in exotic animal medicine. These telescopes allow far greater light transmission and so smaller diameter instruments can be used. They also can have an angled end. Most usually a 30^0 angle is utilised and this allows a greater field of view by rotation of the telescope. The telescope is used with an examination sheath in place for insufflation. Lizards come in a variety of shapes and sizes and the approach for each species may well vary based on their anatomy. Thankfully their anatomy allows for a larger variety of entrance sites.

The lizard coelomic cavity is accessible from a single entry point. The most common entry site used is the paralumbar fossa on the left side. The region is bounded by the lumbar vertebrate and musculature dorsally, the pelvis and hindlimb caudally and the rib cage cranially. The aim is to enter the coelomic cavity just behind the last rib. As mentioned above there is a degree of flexibility in the approach and it can be more cranial if desired (if pneumonoscopy is required). The skin is incised using a fine pair of round ended scissors halfway up the animal. I prefer to then tent up the area with a pair of fine rat toothed forceps prior to inserting a fine pair of artery forceps in a cranial direction under the last rib. Tenting the body wall reduces the risk of trauma to coelomic contents. Caution is required in other species of lizards such as the chameleons as it is possible for their air sacs to extend this far back and damage to the hollow tentacular divertiulae is possible. Care has to be taken when insufflating as there is no diaphragm and there is a risk of lung compression. Intermittent positive pressure ventilation is recommended (and of course is standard for a reptilian anaesthesia).

For this entry site a vast array of internal organs can be visualised. Cranially the lung fields, heart and liver can be assessed. Caudally the coelomic fat pads are evident and need to be by passed in order to evaluate the kidneys, gonads and intestinal tract. If pneumonoscopy is performed the lung must be brought out of the skin incision and anchored with stay sutures. The lung must be closed airtight post operatively otherwise there is a likelihood of pneumocoelom.

The skin is incised and tented as before and a pair of artery forceps inserted. In this situation the liver is directly visualised and available for biopsy. This is a useful technique as liver pathology is common in lizards and all too often there is little indication from biochemical parameters. Wound closure can be performed with a single horizontal mattress suture that incorporates the coelomic musculature and the skin incision. A single horizontal mattress suture can be used to close the surgical wound. Endoscopic examination of the oesophagus, trachea and cloaca are possible.

Snake endoscopy is increasing in popularity. Entry points can be made on either side of the snake at any point down the length of the coelomic cavity between the ribs. The surgical approach used is identical for that in lizards. Initially a decision has to be made about what organs you wish to visualise. This may be based on clinical signs (for example the site of a swelling), clinical history (for example respiratory signs) or based on radiography or biochemical results. It is also indicated for biopsy of tissues for histopathology to screen for inclusion body disease. Snake organs have a set order in which they lie in the cavity of the snake. This means there are fairly predictable in their location. This is assessed using the snout vent length. Below is a quick guide to the organ location based on this. There have been differences reported between snake families and this is just a guide to the location based on colubrid snakes.

Heart	20 – 24% SVL (arboreal species may have a more cranial heart)
Right Lung	22 – 32% SVL
Liver	32 – 48% SVL
Stomach	48 – 58% SVL
Left Gonad	64 – 72% SVL (males have shorter gonads)
Right Gonad	72 – 82% SVL
Left Kidney	84 – 94% SVL
Right Kidney	88 – 96% SVL

It is practically simple to split the snake into quadrants. The first quadrant contains the heart and lung, the second the liver and stomach, the third the gonads and the final quarter the kidneys.

One of the more useful techniques developed is the use of the respiratory system to visualise internal organs. As snakes have a well developed right lung the entry site is the on the right side of the snake. The aim is to enter the air sac just caudal to the lung. This occurs at approximately 50% of the SVL. The surgical approach is comparable to other sites. Entry is gained between the first two rows of scales. However it is important that the lung is fixed to the body wall to prevent pneumocoelom and that the lung is sutured separately once the procedure is completed. There is minimal haemorrhage at this site and the endoscope can be passed cranially to examine the lung field and caudally to examine the air sac. Transillumination through the air sac allows a number of internal organs to be visualised. Both flexible and rigid endoscopes have been used in this way. In snakes that are large enough flexible endoscopy is possible via the trachea and the air sac can be entered and examined this way. However length can be a problem and the image quality is less than that afforded by the rod lens telescope.
Once again oesophagoscopy and cloacoscopy are possible techniques depending on the clinical indications.

The chelonian shell prevents access to the body cavity without major trauma. Endoscopic examination via the prefemoral fossa allows an assessment of the coelomic cavity using a soft tissue approach. The site of surgical entry is bounded dorsally by the lung field and caudally by the kidney and hindlimb musculature. Thus an entry point mid fossa is ideal. Chelonians have a divided body cavity by an oblique post pulmonary septum that runs from the pericardium to the carapace. It is this divided in two.

The skin is incised and curved artery forceps can be used to puncture into the coelomic cavity. Gentle pressure is applied in a craniomedial direction. The force required is greater than in birds. Once punctured the artery forceps are opened and withdrawn. The left sided approach is technically easier for right handed individuals as the shell requires negotiation. I prefer to use the left side despite being left handed as it is the same approach as that used in birds and lizards. Having the tortoise on its side should encourage the bladder to fall away from the entry point limiting any complications.

This entry point has been used for examination of the coelomic cavity. One of the first organs encountered will be the liver. This is large in chelonians and may be pigmented with melanin and can also be quite pale due to fatty infiltration. Advancement further forward can allow inspection of the heart. Rotation of the endoscope caudally allows visualisation of the left kidney and gonad. The intestines lie ventral to this. Biopsy is possible from this site and liver and kidney biopsies are the most likely organs requiring biopsy. Once again assessment of the right side is not possible without a right sided approach.

One of the most common complications is entry into the lung fields by mistake due to the incision being too dorsal. I actually consider this a welcome complication as it allows a thorough assessment of the lung fields. You do need to be careful of creating a pneumocoelom and the post pulmonary septum must be closed if it has been breached. Usually entry is gained via the body wall and the coelomic cavity has to be entered by a second incision in the body wall. With careful placement of the incision it is possible to gain entry into the lung fields and the coelomic cavity via a single skin incision. A single horizontal mattress suture can be used to close the surgical wound. I prefer this technique for the assessment of the lung fields unless there is a specific indication for gaining entry via the carapace. Carapacial entry is possible over the site of a lesion identified radiographically. A hole is drilled through the carapace over the site of the lesion. The midline must be avoided to prevent spinal damage. The size of the hole determines the extent to which the endoscope can be angled and hence the field of view. A 30^0 angled endoscope allows a larger field of view if the endoscope is rotated. The hole created can be used as a port for administration of therapeutics or alternatively sealed after use with technovit. Endoscopic examination of the cloaca, oesophagus, ear, choana and trachea are also of value in many cases. Tortoises have a habit of ingesting inappropriate items and these can be retrieved endoscopically. Air or saline infusion can be used for better visualisation in a similar method to that used in lizards.

Common conditions of reptiles

Snake mites (*Ophionyssus natricus*)

Snake mites are the most common ectoparasite of snakes and lizards seen in practice. The initial source of infection is usually from a newly acquired animal. These are motile parasites that produce eggs which are laid in the environment. These hatch to produce nymphs which then feed on the host animal prior to developing into adults. The whole life cycle takes 13 – 19 days, which allows numbers to increase dramatically. The mites like to hide in skin folds and a close inspection around the eyes, mouth, gular fold and cloaca are good places to find them. They are photophobic and examination of the environment with torchlight can give an indication of the level of infestation. Reptiles with mites are pruritic and spend a lot of time soaking in water bowls and can do self trauma. Dysecdysis is a common secondary complication in snakes. Anaemia can develop in severe infestations. Treatment involves environmental control and topical treatment of the reptiles. Ivermectin made into a spray formulation works well. 10ml of cattle pour on can be added to 500ml water and shaken up to form a spray for immediate use. Injectable ivermectin given at 0.2mg/kg every two weeks can be given depending on the species. Adverse reactions in chameleons, skinks and indigo snakes have been reported. An alternative is to use fipronil as a wipe and a spray in the environment. Treatment every few days is recommended until there are no mites. Bathing the snake regularly also helps. All disposable items should be burned and the vivarium simplified. Products such as dichlorvos (Vapona) should not be exposed to reptiles. Any enclosure should be thoroughly washed prior to reintroduction. OP toxicity and pyrethroid toxicity have been reported. Pterygosomid mites are being increasingly found on lizards and treatment is identical to that used for snake mites.

Dysecdysis

Thermal burns

Stomatitis

This is a commonly presenting condition in snakes and many can be due to inappropriate husbandry like bite wounds, or interaction with transparent surfaces. Some are secondary to immunosuppressive viral conditions. Severe cases can develop septicaemia or pneumonia as an extension of the initial condition. Infections with bacterial and fungal agents are common. Cytological examination and if required even biopsy of the tissues are required to identify pathogens. Many snake owners have no money and so presumptive treatment with enrofloxacin or ceftazidime will be required. Debridement under anaesthesia will help lesions to heal. Topical treatment with silver sulphasalazine is possible even in the mouth and this is a useful agent to consider as it provides analgesia and has anti fungal properties.

Periodontal disease

This is probably the most underdiagnosed condition in lizards. Acrodont species such as the tuatara, agamids (beardies and water dragons) and chameleons do not replace their teeth. These sit on top of an exposed bony ridge in the mouth and can be prone to trauma and gingival recession down the bone of the jaw. This leads to infection in the bone of the face and gum and lip recession. Early treatment involves correcting the husbandry (covering transparent surfaces) and diet (giving harder invertebrates). Dental therapy to include scaling and debridement of abscesses and necrotic bone is required alongside systemic analgesics and antibiotics.

Upper respiratory tract disease

Runny nose syndrome (RNS) and stomatitis (mouth rot) are historic diseases of tortoises. These were attributed to poor husbandry. Many pathogens can be involved and will occur in both good and bad husbandry. Clinical signs include conjunctivitis, rhinitis, stomatitis, dyspnoea, respiratory stridor and noise. Caseous discharges can accumulate. Secondary infections with bacterial and fungal agents are common.

Mycoplasma agassizzi has recently been identified as a common pathogen in tortoises. It has been responsible for mass mortality in wild tortoises in America and has been spread by translocation of wildlife casualties and the release of unwanted pets. Thus quarantine is an important factor for limiting exposure and disease outbreaks. It is endemic in the U.K. population. Horsfield tortoises have a higher incidence compared to other Mediterranean species. Transmission is via oculo-nasal discharges. Testing is possible by PCR on conjunctival or choanal swabs. A positive test means an animal is positive for the rest of it's life. Recrudescence of clinical disease is possible despite clinical cure. Topical and systemic therapy using antibiotics effective against *Mycoplasma* should be used. Antibiotics to consider include enrofloxacin, doxycycline, gentamicin or azithromycin. F10 disinfectant has been used as a nasal flush and found to be effective. Supportive therapy with an oesophagostomy tube may be required.

Herpes viruses are also common pathogens and can be found concurrently with *Mycoplasma*. Two types of herpes viruses can be found in terrestrial tortoises. To date the type II herpes has only been found in one species. PCR testing is available and sampling technique is similar to the above. Stomatitis is generally a feature of Herpes viruses. Tongue scrapings can be taken for cytology or histopathology to check for intraneuclear eosinophillic inclusion bodies. Secondary fungal and/or bacterial infections are common. Leopard tortoises and Hermanns tortoises commonly show severe clinical signs. Marginated and Spur thighed tortoises are more resistant. Latency is a common feature and recrudescence after a number of years is possible. Mixing naive individuals is a common predisposing factor. Treatment is supportive and involves oesophagostomy tube

placement for anorexic animals. Covering antibiotic therapy and topical treatment (silver sulphasalazine cream is fine) should be used with topical debridement to reduce the risk of invasion by secondary pathogens. Specific therapy with acyclovir has been used at 200mg/kg given via oesophagostomy tube. Despite the lack of an obvious clinical benefit no side effects were noted. It may be more beneficial to provide the acyclovir as a prophylactic measure for in contact tortoises. Recently a pharmacokinetic study confirmed that 80mg/kg once daily dosing was absorbed, but failed to achieve therapeutic levels in tortoises. Thus more frequent dosing and a higher daily dose is required. Iridioviruses are another differential for similar clinical signs as the herpes viruses. Currently virus isolation or TEM are the only diagnostic tests available.

Pneumonia

Lower respiratory tract disease is a common finding and can be an extension of stomatitis. Viral, bacterial and fungal agents have been identified. Horizontal beam radiography, haematology and tracheal washing should be considered in the initial foray of diagnostic testing to consider. It is difficult for reptiles to clear respiratory secretions and manipulation secretions out of the snakes' glottis by handing it upside down and milking it has been suggested. Ultimately a lung biopsy via endoscopic examination can be performed. Histopathology, cytology or culture of endoscopic biopsy samples can be performed to confirm diagnoses required. Treatment can include nebulisation with F10 disinfectant at 1:250 dilution. Antibiotic therapy is indicated. Treatment may be required for 4 – 6 weeks. Chlamydia and mycoplasma have been found as a cause of pneumonia. Fungal and viral disease is also possible. Repeat testing to confirm that the infection has resolved is recommended.

Septicaemia

This is a common presenting problem in snakes. These may have immunosuppressive viruses or poor husbandry. Many present as anorexic and have a flushed reddening on their ventral scales. Blood culture can be performed and this should be assessed alongside haematology. Many septic snakes can seed infection into other sites around the body. This can lead to spinal osteoarthritis. This has previously been known as 'pagets disease' or spinal osteopathy. Recent studies on rattlesnakes have confirmed there to be a progression of cases from acute osteomyelitis through to chronic osteomyelitis and ultimately osteoarthritis of the spinal joints. Proliferative bone production occurs and this can lead to a reduced flexibility of the snake and obvious bony swellings over the site. The long term prognosis for these cases is poor. Salmonella and other gram negative bacteria have been cultured from some snakes. A similar condition is also increasingly being recognised in aged lizards.

Paramyxovirus

Ophidian PMV has been around since 1972. Initially venomous snakes were infected but the disease now includes the boas, pythons and is becoming increasingly a problem in colubrid snakes. There are three presentations possible. Acute infection presents as sudden death. Minimal signs are noted by the owner. Respiratory signs or neurological signs are possible. The incubation period can be up to 10 weeks. Some snakes can become chronically infected leading to immune suppression. Respiratory signs, regurgitation and other gastrointestinal signs are possible. Some animals can remain healthy for up to 10 months after exposure. There is a serological test for OPMV which can be performed on a heparin sample. It is best to take two titres. If an animal is in quarantine then sample it upon admission and before release. Chronically infected animals have higher titres; acutely presenting animals (generally with clinical signs) have lower titres. Post mortem examination shows pneumonia which can be markedly haemorrhagic. Histopathology

shows fairly typical proliferation of type II pneumocytes within the lung and occasionally an eosinophillic intracytoplasmic inclusion bodies can be seen. Other tissues such as the CNS, pancreas, liver etc should be submitted. There is no treatment beyond supportive care for the presenting clinical signs. Quarantine and test all new animals in a collection. PMV viruses have been isolated from lizards causing similar signs.

Inclusion body disease

Inclusion body disease (IBD) is a common condition affecting boas and pythons. It has recently been identified in colubrid snakes and vipers as well. It produces eosinophillic intracytoplasmic inclusion bodies in the snakes and it is believed to be a retrovirus but no complete transmission study has been performed. Boas are more resistant than pythons and it is best never to mix these two groups in a collection for this reason. Clinical signs can vary from neurological disease, regurgitation or weight loss. Neurological disease is more common in pythons. Boas suffer from secondary conditions such as pneumonia. The diagnosis depends on demonstration of the typical inclusion bodies. The kidneys, liver, CNS, pancreas are good sites to find them. In pythons the CNS is the best site. Diagnosis in the live animal is difficult. Liver or oesophageal tonsillar biopsies are the current methods used. This is sadly invasive and costly. Quarantine should be performed for a minimum of six months. There is no treatment and affected snakes should be isolated. Transmission is unknown but these have been links with the snake mite (*Ophionyssus natricus*) and mite control should be performed as detailed below.

Nutritional secondary hyperparathyroidism

NSHP or metabolic bone disease is a common finding in juvenile lizards and chelonians. Problems are caused by dietary calcium deficiency, imbalances in the calcium to phosphorus ratio, or hypovitaminosis D. A lack of vitamin D (cholecalciferol) can be due to either dietary deficiency or failure to provide adequate UV-b radiation (naturally or artificially). A critical review of heating, lighting and diet is therefore mandatory in every case that is seen. Clinical signs commonly seen include a soft compressible jaw and a lack of truncal lifting. In males hemipenile prolapse is a common finding. Fractures of the limbs are common and there can be marked deviations of the limbs leading to difficultly ambulating. Tetany is possible if the blood ionised calcium drops and is most common in juveniles. In chelonians clinical signs include a soft compressible carapace and a lack of truncal lifting. In males hemipenile prolapse is a common finding. Thickening of the plastrocarapcial bridge and ventral deviation of the carapace often occur. Deviation and fractures of the limbs are possible in severe cases. Flaccid paralysis is possible if there is a decreased blood ionised calcium.

Diagnosis is fairly self evident based on clinical history and examination. Radiography can demonstrate gastrointestinal stasis, poor bone mineralization and in cases with renal disease articular gout. A blood profile should be taken to assist with the long term prognosis. Treatment includes placement of an oesophagostomy tube, UV-b light, phosphate binders such as aluminium hydroxide and oral or systemic calcium therapy. Calcium gluconate at 100 – 200 mg/kg every six hours is recommended if the ionised calcium is reduced.

Pyramiding

Pyramidal carapace growth is a common finding in captive bred tortoises. Over feeding high protein sources and a low humidity environment are predisposing factors. Existing pyramiding will never go away but with correction of husbandry can instigate more normal growth. A higher humidity

environment can be created by providing a box with a small entrance hole. Foam stuck into the roof can be sprayed repeatedly with water to keep the relative humidity high. We recommend using F10 at a 1:250 dilution to stop fungal proliferation.

Reproductive disease

This is common in female lizards and chelonians and less so in snakes. Many reptiles need to be exposed to a male to induce ovulation. Naturally female reptiles go off food when gravid. This is due to the fill of the coelomic cavity with developing follicles. This places a huge demand on their reserves and metabolic problems with hypocalcaemia and condition loss can be seen leading to collapse. Nutrition is important, producing eggs requires high levels of protein and calcium and marginal husbandry can have a greater effect on reproductively active females.

Infections and abscessation of the follicles is possible and they can rupture leading to an egg coelomitis. Stasis of the follicles is also possible. Egg stasis is also possible due to a lack of appropriate environmental stimuli such as a suitable nesting site, temperature, heat, privacy etc. Isolated females may require social cues from males (pheromones or physical interaction) to induce ovulation. This can lead to the eggs being retained. These conditions are known as pre ovulatory ova stasis (POOS) or post ovulation egg stasis (POES).

Diagnosis requires a good clinical history and radiography/ultrasound. It can be difficult differentiating between normal gravidity and egg retention. If shelled eggs are present and there is no obvious reason for the stasis (i.e. its behavioural not pathological) then providing the correct environment for oviposition at home is an option. Medical induction is the next step. Calcium gluconate 100mg/kg by injection every six hours (ideally based on blood ionised calcium levels) is given followed by oxytocin at 2- 10 IU/kg can lead to oviposition. In tortoises atenolol at 7mg/kg PO can be given prior to induction.If there are no shelled ova present then surgery is required.

Hepatic disease

Hepatic disease is very common in reptiles. The most common problem is fatty infiltration. This can be physiological in the case of breeding females or after hibernation. However if the level of fat becomes excessive then anorexia, lethargy, increased weight, green stained urates or no clinical signs at all can be seen. The diagnosis rests on ultrasound, endoscopic or CT examination with endoscopic biopsy confirming the diagnosis. Treatment rests on supportive care with the addition of anabolic steroids, carnitine, methionine and possibly thyroid supplementation. Typically reproductively active females are affected.