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]

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