Introduction to Amphibian medicine

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Amphibian medicine is an emerging field, an emerging field close to “emergency” field. The amphibian patient is often presented late in the disease process and most frequently apparent clinical signs are non pathognomonic. However skills, medications and protocols used in mammalian medicine and surgery are applicable to ill amphibians.

General presentation
The term Amphibian created by Linné before, included the Reptiles. It was then restrained to Anura (frogs and toads), Caudata (salamanders, newts, and sirens) Latreille in 1825. Afterwards, Gymnophiona (caecelians) were finally added. Anurans represent by far the greatest diversity of amphibians with more than 4100 species divided among 21 families. Caudatans comprise 450 species. Urodeles have long tails, with toothed larval forms often being similar in appearances to the adults. Neotony is common among the salamander families, with the axolotl (Ambystoma mexicanum) being the most common example. Gymnophiona are approximately 165 know species of caecilians. They are sporadically seen in zoological collection.

In spite of visible heterogeneity in these 3 orders of Actual Amphibians. Today all the amphibians have at least 2 permanent and common anatomical characteristics.
- pedicel teeth : the crown is loosely attached to the base or pedicel , of the tooth that is in turn attached to the jaw.
- they have a second ossicle in the inner ear

There no chance statistically to have these 2 characters appearing independently of one another in the three orders, we must recognize a monophyletic origin to the amphibian class.

The class “Amphibians” name refers to the dual life stages: aquatic and terrestrial. All are poikilotherms (ectotherms). Physiology, behaviour, pathology and therapies are all influenced by temperature; therefore it is important to keep the animals within the Preferred Optimal temperature zone (POTZ). Amphibians that are kept above their POTZ may show signs of inappetence, weight loss, agitation changes in skion color and immunosuppression. Those kept below the POTZ may become inappetent, lethargic, develop abdominal bloating associated with bacterial overgrowth from poor digestion, have poor growth rates or become immunocompromised. Enclosures that contain a mosaic of thermal zones are ideal to allow the amphibian to thermoregulate normally;

Quick clinical datas
General cavity is not divided. Eyes are voluminous, in terrestrial forms, especially in anurans where the vision plays a great role in the nutritional behaviour. Pray movement triggers the feeding response. Anurans in particular are voracious feeders and tend to eat anything that fits their mouth.

Gastric overload and impaction, as well as ingestion of non-food items, such substrate gravel or moss are fairly common.

Although many larval amphibians are herbivorous, all amphibians’ adults are carnivorous, with a wide variety of invertebrates constituting a large part of the diet. The intestinal tract is relatively short and follows the normal vertebrate plan. Absorption of water from the gastrointestinal tract is negligible in most species, thus oral fluids are little benefit in rehydrating an amphibian. For most terrestrial species, shallow water soaks and subcutaneous or intracoelomic dilute fluid administrations are most effective in combating dehydration.

Cutaneous respiration is important in both larval and adult forms. Lungs are simple saclike structures that lack true alveoli.

Amphibians are presented for:
- Expertise
- High mortality in a breeding farm and facilities
- Non pathognomonic symptoms: anorexia, weight loss, loss of pigmentation
Physical examination

Because amphibians are very close depend on their environmental conditions, husbandry records are very important for the clinician. Reviewing information on apparently healthy animals and the sick individual is part of the diagnostic process. Like with any other animal, the physical examination includes evaluation of the locomotion, responsiveness to stimulation and a novel environmental factors, behavior, respiratory rate, body condition, etc. The clinician should always wear gloves on palpation.

Fresh faeces should be collected for a fecal exam and a fecal culture. Direct and fecal floats should be done on all ill amphibians, because parasitic disease is common. In anorexic animals, force-feeding may be necessary before obtaining a fecal. The presence of organism or ova in a fecal sample is not a sufficient indicator of disease. Many healthy amphibians have a low parasite burdens and some organism are non pathogenic.

Blood sampling and blood work. Blood can be obtained from most species, though sedation may be needed if animal are struggling. The blood volume of many aquatic species is relatively high ranging from 13.4 % of body mass (Xenopus laevis) to 25 % in aquatic caecilians. Terrestrial amphibians are similar to terrestrial vertebrates where the bloodvolume ranges from 7.4 % to 9.5 % of bodyweight. 10 % of the total blood volume may be withdrawn safely from a healthy frog and 5 % in case of an ill frog (Wright, 2001). In the author experience, cardiocentesis is the easiest way in the majority of amphibians. Even if a small amount of blood is taken, evaluation of a blood smear can help the clinician appreciate changes associated with sepsis (such as bacteria) or neoplasia. This may be run in-house or sent to a laboratory experienced with amphibians. A fresh blood smear should be used for the assessment of the differential white cell count, cellular morphology, and incidence of toxic changes, inclusion bodies, blood parasites, bacteraemia, and so on. Blood pathology can only be made manually (because of nucleated erythrocytes). Amphibian leukocytes are similar to those of Birds and Reptiles, consisting of eosinophiles, basophils, monocytes, heterophils and lymphocytes, but few works have been done on granulocytic leukocytes that are found in Amphibian blood.

Diagnostic imaging

Radiography, endoscopy and laparoscopy can be used to explore intracoelomic masses. Radiographs are most useful to look for bone fractures or malformations, evidence of osteomyelitis and gastrointestinal changes consistent with foreign body ingested. But there is limited radiographic contrast in the normal amphibian abdomen and generally, no sharp distinction between the liver, intestines, fat pads, and reproductive organs. Coelomic or gastric endoscopy can be performed on amphibians as in other animal. Rigid endoscopes are mostly used. Exploratory surgery may be used for diagnostics and treatment.

Cytology, histology and culture

If abnormalities are observed on the integument, skin scraping, impression smears and/or a biopsy should be conducted for histological examination and bacterial culture with concurrent antibiogram. Samplings of course are best performed on the live subject. Dead amphibians decompose very rapidly, confusing the diagnostic picture in terms of bacteriology, virology and histopathology. For histology, acid-fast stains should be asked routinely on all samples, because of the frequency of environmental mycobacterial infection. One may also run in-house acid-fast staining. All samples should be prepared and/or kept in an appropriate medium to be used for in-house analysis or for send outs to another diagnostic lab. In general, histopathology allows for a definitive diagnosis or, at the very least, contributes valuable information for the individual and the colony. Even if a specific skin disease is not identified histologically, non specific findings such as epidermal hyperplasia and hyperkeratosis can suggest unsuspected environmental problems.

Necropsy

Post-mortem examination should systematically be immediately followed by histological evaluation and bacterial cultures. A complete necropsy that includes submission of samples for histopathology and bacteriology is an invaluable aid both in diagnosing single cases involving pets and in detecting health problems in an entire collection.
Some clinical cases
Metabolic bone disease, obesity and corneal lipidosis are common recognized nutritionally related disorder of captive amphibians. Traumas like abrasions, skeletal fractures are mostly due to inadequate husbandry. Like in any other animal, neoplasia may be seen in every organ. Bacterial diseases have a high prevalence in amphibian facilities. Most of bacterial environmental agents become pathogens in stressed amphibians. Mycobacteriosis is very common in amphibians. Clinical signs, when observed, are lethargy, weight loss, emaciation, skin abnormalities and masses. The diagnosis of mycobacteriosis is based on histologic appearance and Ziehl-Neelsen tissue staining. The identification of Mycobacterium species is based on comparison of the 16S rRNA gene sequence with several GenBank databases. If mycobacteriosis is confirmed in a stock, given the fact that there is no known efficacious treatment for any form of mycobacteriosis, the whole group of infected animal should be promptly euthanized to avoid a rapid spread of infection to the colony. Red leg syndrome in amphibians is so named due to hyperemia of the ventral skin of the thighs and abdomen of septicemic anurans, and is now synonymous with any generalized bacterial infection in amphibians. A more appropriate term for the condition is bacterial dermosepticemia. Historically this syndrome is associated with *Aeromonas hydrophila*, but many other infectious agents produce similar integumentary signs.

Many viruses have been isolated from amphibians, but few with a primary disease. The Lucke herpesvirus responsible of renal adenocarcinoma is well known. The viruses of the indoviniidae family have gained interest since they are said to contribute to the world decline of the Amphibians by infecting wild amphibians. Fungal disease is common in amphibians. *Batrachochytrium dendrobatidis* is a pathogenic fungal organism that causes mortalities in captive and wild amphibians. The fungus disrupts the skin, leading to severe dehydration and death. Chytridiomycosis may be diagnosed using cytology and histology. Infection with *Batrachochytrium dendrobatidis* occurs through waterborne zoospores that invade the superficial keratinized epidermal layers of amphibian skin, causing hyperkeratosis sloughing and erosions of the epidermis and occasional ulcerations. Diagnosis of chytridiomycosis relies in identification of the agent in skin scraping or other tissue samples. Protozoan and metazoan infections are commonly encountered but often, without remarkable clinical disease. Disseminated microsporidiosis is a quite interesting disease. Clinical presentation may range from progressive ulcerative dermatitis to sudden death with no clinical signs. The disease is insidious as it mimics several other amphibian pathogens, particularly of bacterial origins and antibiotics have no effect on this intracellular protozoan parasite

General considerations
Amphibians have a low metabolic rate but a fort turn over of their body fluids. So if we search for a posology for a drug, we should empirically take a posology “between the Mammals and the Reptiles”. The weight is very variable depending on the state of hydration. One should not hesitate to reweigh the animal. The sick Amphibians have a metabolic rate higher than of the healthy subjects.

Injection sites and medicating routes
Bath are most common and effective and can be used for hydration. We use isotonic solutions.
IM: should be in front limbs… in theory.
Subcutaneous : dorsal area over shoulders
Intraperitoneal : ventrolateral quadrant

Surgery
Amphibians are generally good candidates for surgery. They are quite resistant to blood loss. For general anaesthesia tricaine methanesulfonate (MS-222) or inhalant isoflurane are commonly used for anesthesia procedures. Anaesthetic solution is made by mixing the MS-222 (that comes as a powder) with non-chlorinated water. Sodium bicarbonate powder at equal weight to the MS-222 powder should be added to help buffer the solution. The solution can typically be dosed at 500 mg/L of bath. Induction with isoflurane is at 5 % in oxygen delivered by way of face mask or chamber induction. Many animals attempt to breath-hold: gentle stimulation encourages continued respiration. We can also use the combination ketamine (50 mg/kg) – medetomidin (0.2 mg/kg) IM and the combinaison ketamine (50 mg/kg) – dexdetomidin (0.1 mg/kg) IM. Animals can be reversed with atipamezole hydrochloride at equal volume to medetomidine IM.

Biopsies and skin surgery may not need anesthesia. These techniques follow the same as seen in other vertebrates. One would be care to take only small surface of skin as it is not very extensible.
When the surgery is too extensive (neoplasia or abscess), we can make a chemical cauterisation with Lotagen.

Major surgeries need surgical anesthesia with an adequate analgesia treatment (meloxicam, 0.3 mg/kg, p.o., i.m.).

Laparotomy. Presurgical preparation includes soaking in a shallow water bath (to ensure hydration) and prophylactic antimicrobial therapy (either by bath or injection). For incision, it's better to make a one bold stroke leaving a clean incision. One must take care of macroscopic glands, lymph hearts and blood vessels, especially the mid-ventral vein. The abdominal membrane is punctured and dissected smoothly. Everting-type suture patterns with simple interrupted suture using an absorbable material are recommended for the skin closure.

Endoscopy. Insufflation is needed to improve the visibility of all the organs. General cavity is not divided, with one way entry, all the organs may be seen.

The management of a cloacal prolaps may sometime need a cloacal suture. When relaps are too often, in parallele to an etiologic treatment, we can make a colopexy. After coeliotomy, the lungs are isolated for a better view of the intestinal track. The colon is sutured to the peritonuem. Abdominal cavity and the skin are sutured in one layer. One will take care to prevent dehydration of the lungs and skin.

Reproductive surgery
Some veterinarians are sometime asked to withdraw surgically eggs (for research purpose). The technique of laparotomy is the same as described before. Eggs are withdrawn by smooth dissection. No coagulation is needed.

Other therapeutics
The adult Amphibians are carnivorous, a treatment of support can be provided by force feeding with food for cats. The A/D from Hill’s for cat, mixed with 1/1 with water, can be used as nutritional support. Hydration can be accomplished while placing the animal in clean water. Optimize temperature and hydgrometry will contribute to convalescence.

Literature


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