CLINICAL PARASITOLOGY OF THE CHELONIA

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Internal and external parasites, including ticks, insect larvae, protozoa, and metazoan worms are common in chelonia. The clinician must use judgement to assess the clinical implications of specific parasites. While wild turtles survive with a variety of parasites, turtles in captivity may be prone to superinfection under conditions of immunosuppression, crowding, and poor hygiene.

Ticks may cause localized inflammation, and may be vectors of disease. Ticks removed from imported chelonians should be submitted to the USDA laboratory in Ames, Iowa for identification. In the past, exotic ticks carrying the bacterial agent that causes heartwater disease have been found on tortoises imported into the United States from Africa. Introduction of such disease into the United States could be devastating for livestock. Ticks are most commonly found on the axillary and pre-femoral skin. Ticks may be removed using forceps. A commercially available permethrin spray (Provent-a-mite, Pro Products, Mahopac, NY) has been approved by the FDA for use in reptiles, and safety and efficacy studies against ticks have been conducted in tortoises. Sporadic reports of mite infestation of chelonia suggest that mites are much less of a problem for chelonia than for snakes and lizards.

Fly larvae are often found within the subcutaneous tissues of North American box turtles (*Terrapene* sp.). Large numbers of larvae can be found in some cases. The larvae are detected by examining the soft tissues of the neck and pre-femoral skin. Characteristic 1- to 2-cm soft tissue masses with a central orifice are present. Larvae must be manually removed. Leeches are commonly found on freshwater chelonia, and may be vectors of hemoparasites. They are removed manually.

Hemoparasites of chelonia do not always cause clinical signs. Intraerythrocytic parasites such as *Hemogregarina*, as well as extracellular hemoparasites such as *Plasmodium,* may be encountered during examination of blood smears. Anecdotal reports of anemia that resolved with treatment of hemoparasites suggest that these organisms may be problematic in some cases. Primaquine and chloroquine, at doses extrapolated from the treatment of malaria in birds, have been used in chelonians. Chloroquine is available for both intramuscular injection and oral administration. Previous reports of chloroquine's use in reptiles to treat *Plasmodium* infections have used a dose of 125 mg/kg PO every 48 hours for three doses. In humans a dose of 50 mg/kg per week is generally used to prevent malaria, while a dose of 50 mg/kg/day may be used in treatment of malaria. The author has used chloroquine in three chelonian patients at a dose of 50 mg/kg IM once weekly for three doses. One patient developed moderate ataxia in the limb used for injection after the second dose; however, normal function was regained over several weeks, and it was unclear whether the drug was the cause of the problem. Other adverse effects, such as vision abnormalities seen in humans, were not detected but would be difficult to document in chelonians.

A systemic intranuclear coccidian parasite has been reported in chelonia over the past 15 years. Species affected have included the radiated tortoise (*Geochelone radiata*), impressed tortoise (*Manouria impressa*), leopard tortoise (*Geochelone pardalis*), Bowsprit tortoise (*Chersina angulata*), and Sulawesi tortoise. Organisms have been most commonly found in intestine, pancreas, liver, and kidney, with less frequent involvement of the eustachian tube, splenic macrophages, middle ear, lung, and stomach. Most cases were diagnosed post mortem. Intranuclear coccidia and *Mycoplasma* spp. were identified ante mortem from the nasal cavity of five Sulawesi tortoises *(Indotestudo forsteni)* affected by chronic rhinosinusitis and oronasal fistulae. Recent investigation identified this coccidian as a novel species, likely of a novel genus. Successful treatment of infected tortoises has not been described; however, toltrazuril has been used clinically in several unconfirmed cases. Ponazuril has recently shown promise for the treatment of coccidiosis in bearded dragons, and may have use in chelonia. However, the author is not aware of its use in chelonia at this time.

Entamoeba invadens may cause significant pathology in chelonians, particularly under conditions of immunosuppression. There appears to be a high prevalence of amoebiasis in recently imported Southeast Asian and African chelonians. After a lengthy collection and importation process, these animals are often emaciated, dehydrated, heavily parasitized, and suffering from various bacterial and fungal infections. Clinical experience in rehabilitating these animals has shown that eliminating *Entamoeba* infections appears to greatly increase survivorship. In addition, clearing *Entamoeba* infection from chelonians may be desirable if the chelonians are to be housed with more susceptible lizards or snakes.

The characteristic multinucleated cysts of *Entamoeba* are passed in feces and are directly infectious to a new host. Upon ingestion of the cysts, excystation occurs and motile trophozoites penetrate the wall of the gastrointestinal tract. While some infections remain within the GI tract, *Entamoeba* may spread to the kidneys or liver resulting in abscess formation. Symptoms of infection generally include watery, bloody, or mucoid diarrhea, anorexia, dehydration, and lethargy. Diagnosis of amoebiasis is generally made by detection of cysts or trophozoites on direct fecal wet mount or fecal floatation, although newer diagnostic modalities such as polymerase chain reaction (PCR) will likely be applied in the future.

The choice of medication for treating amoebiasis in humans often depends on the suspected location of the infection and the severity of the infection. Drugs are generally classified by their site of action and their ability to kill trophozoites and/or cysts. If a patient is an asymptomatic cyst shedder, a drug that is active within

the intestine and effective against cysts is chosen. If the patient suffers from extraintestinal infection, combination therapy is generally used to kill migrating trophozoites as well as intestinal cysts.

The most commonly reported therapy for reptilian amoebiasis has been metronidazole given orally at relatively high doses and relatively infrequent intervals. However, recent pharmacokinetic studies in reptiles indicate that more frequent, lower doses may be more appropriate. Three reports of the pharmacokinetics of orally administered metronidazole in reptiles have been published; however, no pharmacokinetic study of oral metronidazole has been published for chelonian species. The pharmacokinetics of subcutaneously administered metronidazole have been recently described for the red-eared slider. The drug was absorbed well, but several turtles became ill during and after the study. Unfortunately the study design did not allow the authors to determine whether the illness was due to metronidazole, or was unrelated to the drug.

Treatment for *Entamoeba* should continue for at least 2 weeks, although much longer courses may be required. Unfortunately, although metronidazole in considered effective against amoebic trophozoites and is an effective extraintestinal amoebicide, it may be only partly effective or ineffective against amoebic cysts. As a result, combination drug therapy may be needed to completely clear an infection.

Iodoquinol or diiodohyroxyquin has been used to treat amoebiasis in humans, and has been used in many chelonian species. Many reptile practitioners may have unknowingly used this drug, as it is a component of Flagenase, a metronidazole suspension from Mexico that has often found its way into the hands of US veterinarians. Iodoquinol may be difficult to obtain. Compounding pharmacies may be able to obtain iodoquinol powder and formulate a palatable suspension. A dose of approximately 50 mg/kg orally once daily for 21 days has resulted in cessation of cyst shedding (not necessarily proving complete eradication) in the majority of cases. Adverse effects documented in humans, including neurologic signs, have not been observed, and necropsy of several treated animals has not shown lesions indicative of drug toxicity. Iodoquinol is thought to act mainly as an intestinal amoebicide, and is effective against both the trophozoite and cyst form.

Chloroquine is also labeled for treatment of extraintestinal amoebiasis and is effective against trophozoites. It is likely that chloroquine may need to be used in conjunction with a drug effective against cysts. Nevertheless, chloroquine is deserving of further investigation, particularly because of its potential convenience in chelonians as it is the only amoebicide that is available for IM injection.

Paromomycin is often used as part of combination drug therapy for treating human amoebiasis. Paromomycin is an aminoglycoside antibiotic that is theoretically not absorbed from the gastrointestinal tract. It is generally used as a lumenal amoebicide to eradicate amoebic cysts. Paromomycin has been used in reptiles for treatment of amoebiasis and cryptosporidiosis. Pare

reported elimination of cryptosporidium shedding in two Gila monsters treated with 300 to 360 mg/kg paromomycin every other day for 14 days. No adverse affects were noted. In addition, Lane and Mader report that Schweinfurth used paromomycin in snakes at a dose of 25 to 100 mg daily for 4 weeks to eliminate amoebiasis. The human pediatric dose of paromomycin is generally 30 mg/kg TID for 7 days. There are reports of renal failure in cats treated with paromomycin.

Use of diloxanide for treatment of amoebiasis has not been widely reported in reptiles, but the drug is often recommended in human cases. In humans, diloxanide may have fewer side effects in children than other amoebicidal drugs. Like paromomycin, diloxanide is a lumenal amoebicide, effective against the cyst stage. It may be used alone for treatment of asymptomatic cyst passers, or in combination with metronidazole for invasive amoebiasis. The human pediatric dose of diloxanide is generally 20 mg/kg SID for 10 days. Unfortunately, diloxanide is not readily available at this time in the US.

In addition to treatment with amoebicidal drugs, infected patients may also need nutritional support, antibiotics, and antifungal medications. A thorough assessment of the patient must be made, and amoebae treated with consideration of other concurrent disorders. Repeated evaluation of fecal samples over a several month quarantine period is required to ensure eradication.

Fatal *Cryptosporidium* infection of the small intestine has been described in several chelonian species. Paromomycin, as described above can be considered for these cases. In addition, there is a recent anecdotal report of the use of ponazuril to treat cryptosporidium infection in snakes. Hyperimmunized bovine colostrum has also been used in the management of *Cryptosporidium* infection in reptiles.

Some flagellated protozoa may be pathogenic to chelonia. In the author's experience, small numbers of flagellates found on fecal examination may not be problematic, particularly for herbivorous tortoises. However, flagellated protozoa such as *Hexamita* and *Myxidium* have been found to cause significant renal infection in some chelonia. Antiprotozoal therapy may be warranted if flagellated protozoa are found in chelonian urine.

Nematodes, such as the ascarid *Angusticaecum*, can cause disease in chelonia. In general, the author will seek to eliminate ascarids and strongyle-type nematodes from chelonia. There are rare reports of illness in chelonia caused by oxyurid (pinworm) nematodes. However, oxyurids are extremely common in herbivorous tortoises, and seem to cause no pathology in most cases. These can be difficult and frustrating to eliminate, and the author does not try to eliminate them if no clinical illness is present. Some authors postulate that pinworms may help to break down plant material in the large intestine of tortoises.

Acanthocephalans are commonly found in North American aquatic turtles, such as the red-eared slider. Their pathogenicity is unclear. Successful treatment of

these parasites in turtles has not been reported, but they have been treated in some primates using albendazole.

Trematodes may be problematic for sea turtles and freshwater turtles. While in many cases these parasites seem to be found only incidentally at necropsy, there are numerous case reports of more severe disease. The most significant trematode infections in chelonia are caused by the cardiovascular trematodes, such as *Spirochis* sp. Much of the pathology associated with these worms is due to embolism caused by the trematode ova. Unfortunately, turtles that are clinically affected are often at an advanced state of disease, and carry a poor prognosis. One may attempt to treat them using principles for the treatment of heartworm disease of dogs, for example. Praziquantel, anti-inflammatories, and supportive care may be useful.

Diagnosis of gastrointestinal parasites may require fecal direct and floatation examination, fecal acid-fast cytology or ELISA testing (eg, *Cryptosporidium*), endoscopic gastrointestinal biopsy, etc. Once a diagnosis of parasitism is made, specific antiparasitic therapy may be selected. There is relatively little pharmacokinetic data available for anthelmintic use in chelonia; and there is even less safety and efficacy data. Oral praziquantel pharmacokinetics have recently been reported for loggerhead sea turtles (*Caretta caretta*). Fenbendazole has been used clinically in chelonia for many years; however, one recent report suggests that fenbendazole may have caused myelosuppression in Herman's tortoises (*Testudo hermanni*). However, an untreated control group was not utilized in this study. The author has had anecdotal success in treating oxyurid infection in several tortoises using fenbendazole administered per rectum. The dose and frequency of fenbendazole use is left to the discretion of the clinician. Some clinicians use it weekly, while others use it daily for 3 to 5 days. The author has used pyrantel (5 mg/kg PO q7d x 3–4 weeks) with apparent safety in efficacy several chelonian species. Ivermectin appears to be toxic to most chelonian species and should not be used.

The ciliated protozoan, *Nyctotherus*, is often seen in fecal examination of herbivorous tortoises. It does not appear to cause disease. The motile, ciliated stage of this organism is easily identified. The cyst stage of the organism is also seen commonly, and is a fairly large, slightly D-shaped, ovoid cyst, often with a single operculum at the tip of the long axis. Due to its resemblance to trematode ova of mammals, clinicians may be misled into treating with praziquantel. Trematodes are exceedingly rare in most tortoises. *Monocystis* is a parasite of earthworms that may be seen in fecal examination of chelonia that have been fed earthworms. It does not cause disease in chelonia. The ova of this organism resemble miniature *Trichuris* or *Capillaria* ova, and are often found in small packets of multiple ova.

Clinicians should thoroughly pursue the identification and characterization of chelonian parasites. Many of the parasitic diseases of chelonia that have been identified and characterized over the past two decades have been discovered due to the diligent effort of motivated clinicians.

References are available from the author upon request.