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Entamoeba invadens: The Chelonian Connection

By Sandy Barnett, MATTS President

Like many MATTS members, I keep a variety of reptiles at my house–snakes, lizards, and turtles. All my turtles are "boxies" (*Terrapene carolina carolina*), although I occasionally foster other species on a short-term basis. During a recent conversation with a fellow MATTS member, I mentioned that I am always careful to tend my snakes and lizards before dealing with any chelonians in my house, and I always take care of tortoises before turtles. When asked why, I said it was out of concern for possible transmission of *Entamoeba invadens* (an amoebic protozoan) from my turtles to more susceptible animals. (I don't know that my boxies are carrying *Entamoeba*, but I want to play it safe.)

My friend asked where he could read more about this organism and the illness (amoebiasis) that it causes. I didn't have any material on hand to loan him but said I would look into it. That was the inspiration for this article. In the following paragraphs, I describe the life history of *E. invadens*, the symptoms and pathology of *E. invadens* infections in chelonians, and procedures for diagnosing, treating, and preventing such infections.

Who is affected?

E. invadens is considered the most significant pathogenic (disease-causing) amoebic protozoan of captive lizards and snakes worldwide[1,2,3]. Most sources suggest that *E. invadens* is usually nonpathogenic to crocodilians[4], turtles and *some* tortoises[2,5], and lives in the gut of these animals without harming them (a living arrangement called "commensal symbiosis"). A notable exception is the giant tortoises, which are highly susceptible to fatal infection[6].

While the incidence of *E. invadens*-associated amoebiasis is relatively small, it has been documented in a number of species of turtles and tortoises, including species generally considered to be relatively resistant (see table right).

It appears that tortoises, as a group, are prone to more serious illness by *E. invadens* infection than other chelonians[7]. However, some species of the genera *Geochelone* and *Testudo* are reported to be natural carriers that do not normally develop active infections.[2]

Box turtles (*Terrapene* spp.)[6] and semi-aquatic and aquatic turtles (*Chelodina*, *Chelydra*, *Chrysemys*, *Cuora*, *Emydura*, *Podocnemis*, *Pseudemys* and *Trionyx)*[2] are also reported to be carriers and do not normally develop active infections. Resistance may be highest in purely aquatic turtles[7].

Although there may be differences in species susceptibility, all chelonians are probably vulnerable if they are sufficiently immune-compromised or underdeveloped (as in the case of very young, very old, and debilitated animals) and if presented with a sufficiently high load of the parasite. Prime candidates for active infections would be newly imported, wild-caught animals that normally serve as carriers for *E. invadens*. These animals are often severely immune suppressed (and ill) due to the atrocious way in which they are handled, transported and held before sale.

Life History

E. invadens has a direct life cycle (there is no intermediate host). An animal is infected by ingesting cyst-laden feces, or material contaminated by such feces (e.g. bedding, food)[2]. When a susceptible animal ingests cysts, they develop into motile trophozoites in the lumen (cavity) of the animal's small intestine. After multiplying, the tro-

**with minor modifications by the author. (Contact: www.matts-turtles.org)*

phozoites pass into the colon where they mature. Many invade the mucosa (wall) of the gastrointestinal tract where they may or may not initiate pathology; others are passed in the stool, while still others are transformed into cysts before being passed in the stool.

The excreted cysts are ready to infect yet another animal when ingested. Any trophozoites that are passed rapidly desiccate once outside the body, most likely rendering them non-infective.

Survival data on *E. invadens* cysts in the environment are not available. However, such information has been reported for *E. histolytica,* a closely related protozoan. The infective cyst stage of *E. histolytica* can survive in the environment for more than 14 days at a temperature of 8°C (46.4°F), up to eight days at 28-34°C (82.4-93.2°F) and 1-2 days at 37°C (98.6°F)[11]. It is possible that *E. invadens* is equally sensitive. It has been found that *E. invadens* survives but will not multiply at a temperatures of 12.5°C (54.5°F)[12].

Symptoms

Clinical signs can vary widely and often are the same vague signs seen with many other chelonian illnesses. In some cases, symptoms, if present at all, may be limited to mild weight loss (or failure to grow at a normal rate) and poor appetite right up to the time of death[9].

The most common signs are diarrhea (possibly accompanied by blood and excessive mucus) and irregular appetite. Other clinical signs include vomiting, listlessness, weakness, low weight and signs of dehydration (e.g. sunken eyes, thick oral mucus). If the lungs are infected (a condition generally associated with more chronic infections), labored breathing, mouth/nasal discharge and wheezing may be noted.

Mild symptoms may continue with little change for months, or progress rapidly towards death. Unfortunately, in some cases *no* clinical signs are obvious until a

Species of chelonians in which E. invadens *associated amoebiasis has been reported* [a]

Common Name	Scientific Name
• Gulf coast box turtle	*Terrapene carolina major*
• Three-toed box turtle	*T. c. triungulis*
• Malayan box turtle	*Cuora amboinensis*
• Yellow-margined box turtle	*C. flavomarginata*
• Indochinese box turtle	*C. galbinifrons*
• Serrate box turtle	*C. serrata*
• Giant Asian pond turtle	*Heosemys grandis*
• Spiny turtle	*H. spinosa*
• Malayan flat-shelled turtle	*Notochelys platynota*
• Black-breasted leaf turtle	*Geoemyda spengleri*
• Sulawesi forest turtle	*Heosemys* (*Geoemyda*) *yuwonoi*
• Black marsh turtle	*Siebenrockiella crassicollis*
• Mata mata	*Chelus fimbriatus*
• African mud turtle	*Pelusios subniger*
• Loggerhead musk turtle	*Sternotherus minor. minor*
• Loggerhead turtle	*Caretta caretta* [b]
• Green sea turtle	*Chelonia mydas* [b]
• Gopher tortoise	*Gopherus polymerus*
• Travancore tortoise	*Indotestudo forsteni*
• Bell's hinge-back tortoise	*Kinixys belliana*
• Serrated hinge-back tortoise	*K. erosa*
• Home's hinge-back tortoise	*K. homeana*
• Impressed tortoise	*Manouria impressa*
• Red-footed tortoise	*Geochelone carbonaria*
• Yellow-footed tortoise	*G. denticulata*
• Indian star tortoise	*G. elegans*
• Leopard tortoise	*G. pardalis*
• West African spurred tortoise	*G. sulcatta*
• Elongated tortoise	*Graptemys nigrinoda*

Table Notes

[a]This table is compiled from cases brought to a small number of veterinarians[2,7,8,9,10], and should not be interpreted as an all-inclusive list of species in which amoebiasis has occurred.

[b]This infection was only possible because the animal was maintained in freshwater. *E. invadens* cannot survive in saltwater where the species naturally occurs[2]

day or so before death, eliminating any opportunity for treatment[7,9].

There have been no studies published on the duration of illness in chelonians experimentally inoculated with *E. invadens*. However, deaths have been reported to occur in experimental infections in snakes as early as 13 days and up to 77 days after inoculation[2]. The length of the illness and time to death is undoubtedly dependent upon a number of factors, including the size and possibly the strain of the infectious dose, the health status of the animal at the time of inoculation, the level of maturity of the animal's immune system, and probably the species of animal involved.

"Unfortunately, in some cases no *clinical signs are obvious until a day or so before death, eliminating any opportunity for treatment."*

Pathology

E. invadens most commonly affects the host's gastrointestinal tract, especially the colon, invading the intestinal mucosa and destroying cells. The extent of damage is variable, but may result in intestinal inflammation, erosion and often ulceration and perforation. Perforation can can lead to acute inflammation of the coelom (the membrane that lines the wall of the abdomen and covers the abdominal organs) and life-threatening septicemia (blood poisoning). There may be thickening of the gut wall, even obstruction of the gastrointestinal tract. Secondary bacterial infection is usually present, and may contribute significantly to the deteriorating health of the animal.

E. invadens trophozoites may spread through the circulatory system from the gut to other organs, most notably to the liver, spleen and lung. At these extraintestinal sites, damage may vary from none to focal to diffuse areas of necrosis or abscesses. Often there is secondary bacterial infection. Blood vessels often contain thrombi (clots) resulting in necrosis of the vessel wall and hemorrhaging. An excellent review of the pathology of *E. invadens* amoebiasis is available in Frye[5].

Diagnosis

If your animal has any of the symptoms described above, I would recommend taking it to a vet. Undoubtedly, your vet will want to do a fecal screening, as part of his or her evaluation, to check for internal parasites.

Unfortunately, a standard fecal exam may not give reliable results with regard to *E. invadens*. Such screening rarely detects *E. invadens* unless there is a very large number of trophozoites present and the sample is very fresh (trophozoites breakdown rapidly once excreted from the body, thereby evading detection, and identification based solely upon the cyst stage is difficult)[7].

It may be possible to obtain a stool fixative (e.g., polyvinyl alcohol) from your vet that prevents the breakdown of the trophozoites. However, even if a stool sample is very fresh or properly preserved, it is often a case of luck that an animal is shedding the organism at the time that the stool sample is collected since shedding is often intermittent. Even animals with severe cases of amoebiasis may have repeated negative fecal screenings[9].

A more accurate means of diagnosing an active *E. invadens* infection involves a cloacal flush in which fluid is gently flushed into the cloaca via a catheter-tipped syringe, and then suctioned back out[13]. The sample is then centrifuged to separate the solids from the liquid, and the retrieved material is examined under a microscope or cultured for identification.

If you follow this route, you should insist that the flush sample be cultured. In an extensive study[13], it was determined that microscopic examination of in vitro cultures is more efficient in detecting *E. invadens* cysts and trophozoites than microscopic examination of fresh cloacal flushes. Once again, however, repeated sampling may be necessary since animals often shed the parasite intermittently.

C. BARNETT

The Eastern Box Turtle (*Terrapene carolina carolina)* is generally considered to be a carrier of *Entamoeba invadens*

It is possible but expensive to make a diagnosis histologically (involving microscopic examination) from small samples of colon, intestine or liver, the organs most likely affected by the pathogen. The tissue may be harvested using an endoscope (for intestinal sample) or laparoscope (for sampling other organs)[7].

Treatment

Patient

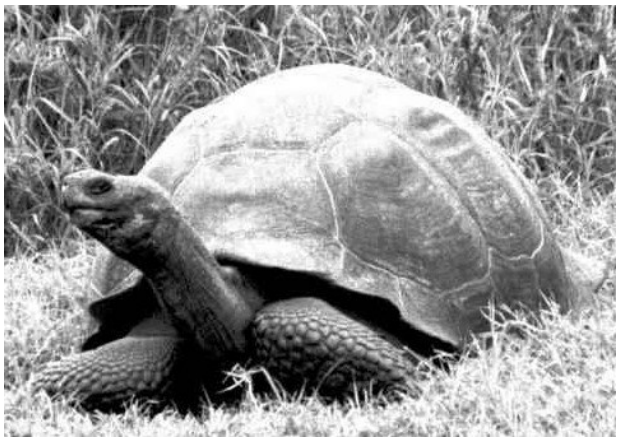
Metronidazole (Flagyl) is the drug most commonly used to treat reptile amoebiasis[5]. While it is effective in killing trophozoites and can reach a wide variety of body tissues, it generally is not effective at eliminating cysts from the gut lumen.

Increasingly more veterinarians are using metronidazole concurrently with or immediately followed by lumen active agents such as diiodohydroxyquin (Iodoquinal) or paromomycin (Humatin)[3,6,7]. The treatment regime depends on the species being treated. Where pathology extends beyond the GI tract, metronidazole may be used in combination with chloroquine phosphate (Aralen Phosphate)[3]. Unfortunately, no single drug or combination of drugs has been demonstrated to completely eliminate *Entamoeba* organisms from an infected animal[8]. The disease can at best be managed so that an animal shows few or no clinical signs of illness.

Bacterial infections often accompany amoebiasis and may contribute significantly to the disease problem. Broad-spectrum antibiotics such as gentamycin (Gentocin) or chloramphenicol (Chloromycetin) are among the drugs used to treat these infections[6,14].

Temperature has a significant effect on amoebiasis in snakes and presumably in other reptiles too. In one experiment, snakes inoculated with *Entamoeba* did not develop an active infection when maintained at a temperature above 33°C (91.4°F)[14]. In other experiments[12,15] inoculated snakes did not develop active amoebiasis when maintained at 34-37°C (93.2-98.6°F) but some did develop active infections when maintained at a *constant* 35°C (95°F). Those infections were never as pathogenic as those at yet lower room temperatures. It has been determined[16] that *E. invadens* loses its pathogenicity in snakes maintained at 13ºC (55.4ºF). At two higher temperatures—25ºC (77ºF) and 30ºC (86.8ºF)—the inoculated snakes developed active infections.

You must be very careful in using high temperature to control amoebiasis. It is very easy to cause life-threatening dehydration in an animal already having difficulty with its water balance due to diarrhea and poor fluid intake. Also, a compromised animal, even one normally adapted to a warm climate, may have difficulty coping with an elevated temperature from which it cannot escape.



J. OSWALT

The Galapagos Tortoise (*Geochelone elephantopus)* is highly susceptible to fatal infection from *Entamoeba invadens.*

Contaminated Enclosure/Equipment

It is likely that you could properly disinfect surfaces contaminated with *E. invadens* by washing them with very hot water or steam (small, relatively inexpensive steam units can be found on the Internet.) This is based on a study[7] of *E. histolytica*, a closely related protozoan, which is killed in less than a minute when exposed to temperatures over 52ºC (126ºF).

You might also be able to use high room temperatures to disinfect an indoor enclosure (after removing sensitive animals). I have already mentioned that *E. histolytica* is killed in a period of days at high temperatures (see Life History); it is likely that *E. invadens* would be similarly affected. Presumably, outdoor enclosures subject to high summer temperatures would be sanitized naturally if not subject to re-contamination.

Two other effective treatments for *E. invadens* contamination are UV sterilization of the water supply and application of 0.002% mercuric chloride ($HgCl_2$) for at least 24 hours to contaminated surfaces[7]. Neither of these is practical for the hobbyist.

Prevention

Prevention and control of amoebiasis must include a

good husbandry and sanitation program, and strict isolation of new animals and their supplies and cleaning equipment from the existing collection. Ideally, new animals should be quarantined in a different room, as far away as possible from the rest of the collection. This reduces the likelihood that you will cross contaminate enclosures, and that insect vectors will spread disease. (Flies and cockroaches can carry the infective cyst stage of *E. invadens* mechanically on their bodies from enclosure to enclosure[2].)

It also seems prudent to permanently isolate aquatic and semi-aquatic species of chelonians from terrestrial ones, or even better, house each species in its own enclosure, each with its own set of supplies and cleaning equipment.

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