CRYPTOSPORIDIUM CONTROVERSY: WHEN DO YOU CONSIDER A REPTILE CRYPTO-FREE?

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Abstract: Management of *Cryptosporidium*-positive reptiles can include isolation and fecal screening for a 1 yr period. Animals that do not shed detectable levels of oocysts can undergo an immunosuppression test. Animals that do not shed detectable levels of oocysts following immunosuppresion can be considered clear of *Cryptosporidium serpentes* and placed back within the collection.

Key words: Cryptosporidium serpentes, parasites, snakes, acid fast staining

INTRODUCTION

The occurrence of *Cryptosporidium serpentes* and its pathogenecity in reptiles is subject to debate. There are several reports of fatal gastric cryptosporidiosis from squamate reptiles, including colubrid snakes and chameleons. This is unusual since other coccidia are generally host-species specific. Various other species have had *Cryptosporidium*-like organisms detected in fecal parasite examinations, revealed either by detection of the characteristic acid fast stained oocyst consistent with *Cryptosporidium* spp. or other methods (Graczyk, et al., 1995). However, many of these reptiles may never develop gastric pathology associated with these organisms, so the pathogenecity of *Cryptosporidium* spp. in reptiles, at least in some species, is not absolute. Furthermore, inactivation of the oocysts is difficult. Ammonia (5-10%), formalized saline, and steam heat are the three suggested efficacious disinfectants, and each has its disadvantages for use in the home or institutional collection. This creates problems for the management of captive reptiles as a specimen that is known to shed *Cryptosporidium serpentes* oocysts could contaminate the rest of the collection. The morbidity and mortality associated with this contamination may vary from collection to collection, and from species to species within a collection, making the decision about the fate of a *Cryptosporidium*-shedding reptile problematic. (See Cranfield & Graczyk, 1996, for a current review of cryptosporidiosis.) After several deaths attributed to cryptosporidiosis prior to 1993, an effort was made to manage the amphibian and reptile collection at the Philadelphia Zoo to minimize the threat of this disease.

METHODS

All incoming amphibians and reptiles were subjected to a quarantine period during which an animal had to produce three consecutive negative fecal samples produced no closer than 3-5 d apart. The fecal samples were evaluated by acid fast staining of the direct wet smear of the feces to detect *Cryptosporidium* spp. and other acid fast organisms, a wet mount examination of feces to detect other protozoa and helminths, and by fecal flotation solution for oocysts and ova. Quarantine time was a minimum of 90 d for snakes, and 60 d for all other reptiles. All collection amphibians and

reptiles received routine fecal parasite examinations at a frequency varying from quarterly to annually. If any *Cryptosporidium*-like oocysts were detected by the acid fast stain screening of a direct wet smear of the feces, that animal was considered to be a Crypto-Quarantine specimen, and was put into a restricted situation.

A Crypto-Quarantine specimens were housed on specific shelves apart from other collection animals. These cages were clearly and distinctly labeled to distinguish them from those cages housing clean animals. Crypto-Quarantine cages were worked at the end of the day after all other collection animals had been serviced, with tools used for no other purpose, and the keeper wore disposable gloves and a disposable gown when working these enclosures. Full strength ammonia (5-10%) was used as a disinfectant for any items contacting the cage or the animal. Contact time was set at a minimum fifteen minutes, and was recommended to exceed thirty minutes.

Crypto-Quarantine specimens were considered so for one year's worth of negative fecals. If a reptile continued to produce positive fecals, it was removed from the collection. Most of these continued shedders were sent to an active research program at the Baltimore Zoo Veterinary Hospital. If a reptile produced consistently negative fecals over the course of 1 yr, it was subjected to a presumed immunosuppressive dose of dexamethasone sodium phosphate (2-4 mg/kg i.m.), and the next three fecal samples, no closer than 5 d apart, were evaluated by acid fast stain for *Cryptosporidium*-like oocysts. If these samples were negative, the reptile was released from quarantine.

RESULTS

At least 27 specimens were classified as Crypto-Quarantine between 1 Jan 1993 and 1 Jan 1996. (See Tables 1 and 2.) Of these, 17 were donated to the Baltimore Zoo's research program, 2 either died or were euthanized and necropsied at the Philadelphia Zoo, 1 was donated to a zoo employee who owned no other reptiles, and 7 passed the screening program.

At least 11 snakes, 4 chameleons (*Chamaeleo montium)*, one bog turtle (*Clemmys muhlenbergi)* and 1 Indian star tortoise (*Geochelone elegans*) were sent to the Baltimore Zoo's research program and were confirmed to be shedding *Cryptosporidium* spp. via a monoclonal antibody technique (MERI*FLUOR Cryptosporidium/Giardia* Monoclonal Antibody Fluorescent Test, Meridian Diagnostic, Cincinnati, OH, USA, 45244) (See Table 1.) Six snakes died or were euthanized before February 1997. Five snakes were still alive at this time (0.0.1 Puerto Rican boa (*Epicrates inornata)*, 0.0.1 Brazilian rainbow boa (*Epicrates cenchria cenchria)*, 2.0 Conant's milksnake *(Lampropeltis conanti)*, and 0.0.1 Northern pinesnake *(Pituophis melanoleucus melanoleucus*)) but were euthanized after February 1997. All chameleons died or were euthanized. All chelonians are still alive.

At least 1.0 timber rattlesnake (*Crotalus horridus)*, 1.1 northern pinesnake (*Pituophis melanoleucus melanoleucus)*, 0.0.2 cornsnake (*Elaphe guttata)*, and 0.0.2 Brazilian rainbow boa (*Epicrates cenchria cenchria)* successfully cleared the screening program and were released to the general collection of the Philadelphia Zoo. (See Table 2.)

DISCUSSION

Cryptosporidiosis is a disease of reptiles for which there are currently no definitive guidelines for risk management. The method outlined in this paper provides an avenue for minimizing the spread of cryptosporidiosis through a collection, however this program is based solely on the detection of *Cryptosporidium*-like oocysts on fecal parasite examinations without regard to clinical signs or confirmation of species-specific identity of the organism. The sensitivity of the acid fast stain of a direct smear or a concentration flotation of feces is far less than methodologies described by Graczyk, et al., 1995, (up to 16 times less sensitive than the MERI*FLUOR* test), but is in wider use in clinical practice at present due to the familiarity of the test and the ready availability of the stains. Screening of multiple fecal samples is recommended with either test to reduce the likelihood of false negatives. A shift toward the more sensitive screening process is encouraged, however the clinician should be aware that monoclonal antibody fluorescent test recognizes oocysts of *Cryptosporidium parvum* as well as *C. serpentis*.

The immunosuppressive part of the protocol is not without additional risk to the patient. The possibility certainly exists for the presumed immunosuppressive dose of dexamethasone to contribute to other health issues including an increased shedding of pathogens such as *Salmonella*, the transformation of subclinical infections to clinically relevant infections, and the enhancement of colonization of the reptile by novel pathogens. These are valid concerns, however immunosuppression tests are routinely performed in domestic dogs and cats with little regard for subsequent problems. The client should be informed of these possible adverse effects of the procedure, and all standard precautions for minimizing the risk of zoonosis, such as proper hand washing, emphasized prior to suggesting this protocol. This protocol is oriented for large collections of reptiles that contain species, such as the cornsnake (*Elaphe guttata*) which are known to be especially susceptible to fatal gastric cryptosporidiosis, and is unnecessary for the pet snake that has no contact with other reptiles. This protocol does not guarantee that the snake or other reptile clearing the quarantine is not a carrier, rather it is an attempt to reduce the risk of maintaining carrier animals within a collection. Ultimately the decision on the disposition of any reptile is a consensus between the veterinarian's advice and the client's wishes. It is hoped that this protocol stimulates further research and thought on the topic of management of reptilian cryptosporidiosis.

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TABLE 1

Fate of Crypto-Quarantine Reptiles

Species	Disposition
Serpentes	
Candoia asper/300847	14 Nov 94 to Baltimore
Crotalus horridus/300193	27 Oct 95 to Baltimore
Crotalus vegrandis/300517	14 Nov 94 to Baltimore
Elaphe obsoleta obsoleta/300445	27 Oct 95 to Baltimore
Elaphe obsoleta obsoleta/300689	27 Oct 95 to Baltimore
Elaphe obsoleta williamsi/300634	Euthanized 08 Apr 93
Epicrates cenchria cenchria/300648	14 Nov 94 to Baltimore
Epicrates inornatus/300385	14 Nov 94 to Baltimore
Lampropeltis t. conanti	*** to Baltimore
Lampropeltis t. conanti	*** to Baltimore
Pituophis melanoleucus melanoleucus/300177	14 Nov 94 to Baltimore
Pituophis melanoleucus melanoleucus/300800	14 Nov 94 to Baltimore
Pituophis melanoleucus sayi/300580	24 Dec 94 donated to zoo employee
Sauria	
Chamaeleo montium/301158	03 Jul 96 to Baltimore
Chamaeleo montium/301161	03 Jul 96 to Baltimore
Chamaeleo montium/301162	03 Jul 96 to Baltimore
Chamaeleo montium/301163	03 Jul 96 to Baltimore
Chelonia	
Geochelone elegans/300961	27 Oct 95 to Baltimore; still alive
Clemmys muhlenbergi/300906	27 Oct 95 to Baltimore; still alive

TABLE 2

Crypto-Quarantine Reptiles That Passed Screening

Species	Diagnosis Date	Release Date	Other Comments
Crotalus horridus 300513	13 Dec 94	20 Feb 96	
Elaphe guttata guttata 300182	11 Dec 94	21 Feb 96	
Elaphe guttata guttata 300442	11 Dec 94	21 Feb 96	
Elaphe guttata guttata 300440	11 Dec 94		Died 04 Nov 95 due to a tumor
Pituophis m. melanoleucus 300801	11 Dec 94	21 Feb 96	
Pituophis m. melanoleucus 300802	11 Dec 94	21 Feb 96	
Epicrates c. cenchria 300379		19 Sep 94	
Epicrates c. cenchria 300649		19 Sep 94	