CRYPTOSPORIDIUM INFECTION IN A COLONY OF LEOPARD GECKOS, *Eublepharis macularius*

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Abstract: During the winter of 1997, a winter storm caused a power outage, which resulted in a dramatic decrease in ambient temperature to a colony of leopard geckos, *Eublepharis macularius.* The breeder resisted veterinary intervention during the initial stages of the disease outbreak. After several hundred deaths and over a thousand dollars in losses, the breeder finally consented to a colony inspection, examination, and diagnostics. The initial presentation of the colony was anorexia, severe weight loss, dehydration, diarrhea, and death. Initial diagnostics provided evidence of an *E. coli* enteritis and coccidiosis. The *E. coli* was resistant to *many* of the antibiotics tested. Treatment with the sensitive antibiotics provided only a temporary cessation of clinical signs. Further diagnostics revealed the evidence of *Cryptosporidium* oocysts on acid fast fecal smear cytology. The diagnosis was confirmed with histopathology and using the *C. serpentis* immunoflourescent antibody (IFA) offered by Animal Diagnostic Laboratory LLC (AniLab, Baltimore, MD). Treatment of the few remaining geckos with paromomycin (Humatin, Parke-Davis, Morris Plains, NJ) orally at 50-800 mg/kg s.i.d. provided some cessation of clinical signs. If treatment was temporarily stopped clinical signs and oocyst shedding returned.

Key words: Cryptosporidium serpentis, leopard gecko, *Eublepharis macularius,* reptile, parasite, *E. coli,* paromomycin

INTRODUCTION

The rising popularity of reptiles in captivity has led to an explosion of new husbandry and medical information. The increased numbers of animals in collections have provided sources of new diseases or new presentation of diseases. Leopard geckos, *Eublepharis macularius,* are one species that has made a large impact on herpetoculture. They are found with various colors and patterns and are bred by the thousands. The leopard gecko is relatively easy to maintain and breed. They have a good temperament, which makes them easy to hold and interact (de Vosjoli, 1990; Tremper, 1997). This case describes the susceptibility of captive reptiles to weather related power outages. The stress that accompanies the environmental changes may predispose the animals to opportunistic infectious and parasitic diseases.

CASE REPORT

Reptile Collection History

The colony of leopard geckos belonged to a local reptile breeder. His collection, at that time, consisted of over 200 reptiles covering several species of geckos, monitors, chameleons, tortoises, chuckwallas, and *Uromastyx*. The majority of the animals live in a converted garage. During November of 1996, the breeder acquired a group of over 300 geckos from a nationally well-known leopard gecko breeder to add to his existing colony of 50 adult breeders. The new group consisted of about four dozen adults and the rest were juveniles of variable "designer" patterns. These geckos were placed in plastic bins within a shelving system with thermostatically controlled heat tape (Flexwatt, Flexwatt Corporation, West Wareham, MA) placed along one end of the bins. The substrate was play sand with plastic water bowls, feeding dishes, and hiding/moisture boxes. The diet was composed with daily rations of crickets and/or mealworms. The food items were dusted with Rep-cal (Rep-Cal Research Laboratories, Los Gatos, CA), a calcium supplement with vitamin D_3 and no phosphorus.

Clinical Presentation and Diagnostics

In February 1997, a severe thunderstorm with high winds created a local power outage. The reptile room heaters and cage heating pads did not have a backup power source and the reptile room temperature dropped to 0-3°C (32-37°F) for 24-48 hr. A gas generator was purchased and the ambient temperatures were raised to 22-24°C (71.6-75.2°F). Several individuals died during those and the next few days.

The activity and appetite of the geckos decreased over the next 2 wk, while the mortality rate increased from a few deaths a day to over a dozen a day. Early March 1997, the geckos became completely anorexic and started having diarrhea, the breeder initiated treatment, without veterinary consultation, with injectable 5% enrofloxacin (Baytril, Bayer de Mexico, Mexico, DF). The breeder employed a treatment regime recommended by another breeder. Food and water were withheld for 7 d. Each gecko was given a drop of the injectable enrofloxacin by mouth every day. After two rounds of this therapy, the mortality rate was still high, with as many as a dozen dying per day. The breeder brought a batch sample of fecal material for fecal examination to the veterinary clinic. The sample was positive for coccidia, (*Isospora* spp) with a zinc sulfate fecal flotation. On direct examination with physiologic saline, a slight increase in flagellates (possible *Tritrichomonas* spp) was noted. After veterinary consultation, the breeder initiated a treatment of sulfadimethoxine (Albon, SmithKline Beecham, West Chester, PA) at an initial dose of 90 mg/kg once by p.o., then a dose of 45 mg/kg p.o. s.i.d.. The sulfadimethoxine syrup was diluted with water due to the small (< 5 g) size of some individuals.

A group of six sick leopard geckos were sacrificed in early April of 1997 for examinations and the limited diagnostics was as follows. Zinc sulfate fecal flotation and direct saline fecal exams were performed on the geckos and were negative. Gram stain fecal exams contained 85-95% gram negative rods. Acid fast fecal stains were negative for any *Mycobacteria* spp or *Cryptosporidia* spp. Fecal culture and sensitivity of the intestinal contents revealed a heavy growth of *E. coli.* The sensitivity pattern was resistant to almost all antibiotics, especially enrofloxacin (Table A). The trimethoprimsulfamethoxazole (Sulfatrim, Barre-National, Baltimore, MD) was selected based on the sensitivity results at a dose of 30 mg/kg p.o. s.i.d. The syrup had to be diluted with water 1:9 to facilitate dosing. The breeder initially instituted a wide spread application to the leopard gecko colony.

During May of 1997, up to a dozen geckos were still dying almost each day. He had stopped treating the geckos due to the lack of available time to catch, restrain, and medicate. He did continue to medicate the more valuable geckos. Another group of variably sick geckos was donated for further testing and treatment. One of the sickest was euthanized and another culture and sensitivity performed. Moderate growth of *E. coli* and a light growth of a *Bacillus* sp were cultured with a different sensitivity pattern resistant to trimethoprim sulfa (Table 1).

Over the next several months, the breeder donated another eight dozen sick leopard geckos for a widespread drug trial of different antibiotics. These animals were divided among several groups of individuals each employing a different antibiotic protocol (Table 2). Many of these geckos had not been treated for weeks. They were very dehydrated (>12%) and emaciated (adult weight less then 50% normal or less than 15 g). The gecko's tails were fat deficient with only a thin, spinal tail remaining. The different groups of geckos were placed with different veterinary technicians and assistants to house, hydrate, and medicate. The geckos were housed in plastic boxes with perforated holes for ventilation. The cages were lined with paper towels with a hiding enclosure and a water bowl. The cages were placed on heating pads or heat tape (Flexwatt, Flexwatt Corporation, West Wareham, MA) to provide their proper preferred optimal temperature zone (POTZ) of 27.78-31.11°C (82-88°F) (de Vosjoli, 1990, Tremper, 1997).

During the next 5 mo, most of the geckos died (Table 2). Some of them responded initially to the antibiotic regimes by increasing in body weight and appetite. Unfortunately, most geckos recrudesced, becoming anorexic and emaciated as before the treatment. Three of the remaining few geckos were euthanized with necropsies performed. A batched zinc sulfate fecal flotation was performed on the colonic contents and was negative for intestinal parasites. Direct saline fecal exams were performed on the intestinal contents with no intestinal parasites detected but numerous hypermotile bacteria present. Gram stain of impression smears of the intestinal lining of the geckos revealed much cellular debris and numerous gram negative bacteria. A culture and sensitivity of the intestinal contents of one gecko was performed. This culture grew an abundant growth of *Escherichia fergusonii* and *Proteus mirabilis* with a different set of antibiotic sensitivity patterns than before (Table 1).

Another set of acid fast stains were performed on impression smears of the intestinal viscera. All three smears contained acid fast positive oocysts consistent with *Cryptosporidium serpentis.* The diagnosis was confirmed with histopathology (Shubot Exotic Bird Health Center, Texas A&M University, College Station, TX). The histopath showed that *Cryptosporidia* organisms were present in moderate numbers on the surface epithelium and in occasional gland crypts. Diagnosis was also confirmed using the *C. serpentis* immunoflourescent antibody (IFA) offered by Animal Diagnostic Laboratory LLC (AniLab, Baltimore, MD). Serum from two more geckos that were acid fast positive for *Cryptosporidia* oocysts were sent to AniLab as well. Unfortunately, the *C. serpentis* enzyme-linked immunosorbent assay (ELISA) offered is somewhat specific. Leopard geckos have not been recognized and tested for *Cryptosporidia* before this point, so a specific ELISA test has not been created (Cranfield, *et al*, 1996; Upton, *et al*, 1989).

An apparently healthy leopard gecko from another breeding facility tested acid fast negative for any cryptosporidium organisms. This leopard gecko was housed with three leopard geckos that were fecal acid fast positive for *Cryptosporidia* oocysts. That gecko over the course of a month, had a decrease in appetite, decreased in weight (to less than 20 g), and produced soft, runny stools. This new gecko with two of the other geckos was euthanized and sent for histopathology. The new gecko was acid fast positive for *Cryptosporidia* in the alimentary tract. A Merifluor™ was performed on the sample and appeared positive, the organisms did not stain but could be detected as black organisms on the IFA. This gecko had no evidence of histopathologic infection by cryptosporidium in the sections examined. The first of the other two geckos had histopathologic evidence of *Cryptosporidia* in the anterior small intestine. The second gecko also had histopathologic evidence of *Cryptosporidia* in the anterior stomach and the anterior small intestine. These geckos were also acid fast positive and *C. parvum* IFA positive as was the above gecko.

At this point, only four geckos from the original group of geckos were still alive. Three of these four geckos underwent treatment with paromomycin (Humatin, Parke-Davis: Morris Plains, NJ) orally at a dose of 1 mg of a compounded 10 mg/ml suspension (BCP Veterinary Pharmacy, Houston, TX) s.i.d. The actual paromomycin dose varied between 50-125 mg/kg depending on exact bodyweight. Another apparently healthy leopard gecko from a third breeding facility was housed with the three geckos and treated with the same paromomycin protocol. Clinical signs decreased in these three sick geckos. They gained some weight and returned to eating. After 2 mo of therapy, two of the geckos had started eating and regained over 50% of normal body weight. The third gecko appeared normal and regained 85% of normal body weight. After a month of discontinuing paromomycin, all three geckos showed signs of decreased appetite and weight. A batched fecal sample of the three geckos showed acid fast positive *Cryptosporidia* oocysts. The fourth gecko that was added appeared normal and was acid fast negative.

Paromomycin was reintroduced to these geckos at an increased p.o. dose of 10 mg of a compounded 100 mg/ml suspension (BCP Veterinary Pharmacy, Houston, TX) s.i.d. The actual dose varied between 300-800 mg/kg depending on exact bodyweight. The one improved gecko continued to improve on the new paromomycin dose. The body weight increased to a normal size (38 g). The gecko outwardly appeared normal. The fourth gecko that was added to the group also continued to appear normal. These two geckos were removed and housed together in a new aquarium. After three months of treatment, the paromomycin on these two geckos was discontinued. These geckos have mated and produced over a half dozen eggs. One mo later, fecal acid fast smears were negative for *Cryptosporidia* oocysts. The two marginal geckos responded minimally to the 10-fold increase in paromomycin. One gecko died after 14 wk of therapy. The gastrointestinal histopathology is pending. The remaining tissues were accidentally destroyed. The other gecko, after 4 mo of therapy with the 100 mg/kg paromomycin, is acid fast positive on fecal smear. A *Cryptosporidia* IFA on all four leopard geckos is positive for shedding of *Cryptosporidia* oocysts.

DISCUSSION

There are numerous modalities currently being employed for the detection of *Cryptosporidia* spp in reptiles (Cranfield, *et al*, 1996; Graczyk and Cranfield, 1996; Graczyk and Cranfield, 1996). The most popularly used diagnostic test is the modified acid fast stain, but other tests are being used with increasing frequency. Other diagnostics include direct IFA (Merifluor Cryptosporidium/Giardia monoclonal antibody fluorescent test, Meridian Diagnostic, Cincinnati, OH), indirect IFA (Hydroflur Combo Cryptosporidium/Giardia, Ensys, Inc., Research Triangle Park, NC), EIA (Prospect Rapid Assay, Alexon Inc., Sunnyvale, CA), modified Sheather sugar flotation, and histopathology. The detection problem is the inconsistent shedding of the oocysts in feces, so repeat fecal exams must be done (Mader, 1993). For snakes, a more accurate diagnosis can be made by using a stomach lavage 3 d after feeding (Graczyk and Cranfield, 1996; Graczyk and Cranfield, 1996). The same should be true for lizards. Regurgitated prey may also be used for detection of *Cryptosporidia* oocyst (Cranfield, *et al*, 1996; Cranfield and Graczyk 1995). Stomach biopsies and barium series have been used, but the size of the patient may also be a factor, as it was with the leopard geckos. Currently the Merifluor antibody test is the best diagnostic tool available, with a sensitivity 16 times greater than the modified acid fast stain for detection in snakes (Cranfield and Graczyk, 1995, Graczyk and Cranfield, 1996, Graczyk and Cranfield, 1996). There were two lizard species infected with *Cryptosporidia* spp that had positive reactions to the Merifluor antibody test in a study done by Graczyk and Cranfield. The same study showed the indirect IFA (Hydroflur) had no difference in sensitivity and specificity from the direct IFA (Merifluor) (Graczyk and Cranfield, 1996). The EIA test (Prospect) was also evaluated in the same study, and some value for detection of *C. serpentis*, but not enough lizards were tested to determine its reliability in squamata. Taking all of the above into account, the direct IFA (Merifluor) and the modified acid fast stain are the most commonly used in current practice.

Sixteen lizard species have been diagnosed with *Cryptosporidia* (O'Donoghue, 1995). The question is which species of *Cryptosporidia* affects which species of reptiles. According to studies done by Upton and Graczyk, more than one species of *Cryptosporidia* may occur in reptiles (Graczyk and Cranfield, 1996; Upton, *et al*, 1989). The Merifluor tested eight naturally infected lizards of two different species and only two lizards showed up positive (Graczyk and Cranfield, 1996; Graczyk and Cranfield, 1996). In the literature, other lizard species found to be infected were diagnosed by modified Sheather sugar flotation, modified acid fast stain, or histopathology of the gastrointestinal tract.

The leopard geckos in this study colony were originally diagnosed by modified acid fast stain. Later, the Merifluor test was used, and did not show the expected lime green coloration. Instead, small, circular 4-8μm unstained oocysts were seen. This was found on repeated samples from the same colony. Histopathology was then performed on four of the remaining geckos, from the cardiac region of the stomach, through the large colon. Three of the four geckos had evidence of *Cryptosporidia* infection. Two had *Cryptosporidia* in the proximal small intestine and the third had *Cryptosporidia* in the cardiac region of the stomach and the proximal small intestine. The fourth original "healthy" gecko, that did not have histopathologic evidence for *Cryptosporidia*, was positive by acid fast so it is possible the oocysts had not infiltrated the gut mucous yet or the histopath sectioning missed the infected lesions. The reason for the oocysts not taking up the stain is still under investigation.

Paromomycin (Humatin) is an aminoglycoside used in human medicine as a treatment for intestinal amebiasis and cestodiasis. The pharmacokinetic activity closely resembles neomycin. The antibacterial activity is effective *in vivo* against *Salmonella* and *Shigella*. Adverse reactions to extremely large doses include nausea, abdominal cramping, and diarrhea. Gastrointestinal absorption is very poor with almost 100% of the drug recovered in the feces. In cases of colonic ulceration or mucosal damage, partial absorption of the aminogycoside may occur with potential ototoxicity and nephrotoxicity (Olin, 1990). The use in reptiles is in the early stages with scattered cases. Some results have been seen with increased doses (Cranfield, *et al*, 1996; Cranfield and Graczyk, 1996; Para, *et al*, 1997). More work is needed to determine the efficacy and therapeutic doses.

CONCLUSION

This case series is an example of a chain of events that began as one diagnosis and ended with another. The *E. coli* in this colony initially began as an opportunistic pathogen, which took advantage of the lowered immune system status of the leopard geckos after the power outage. If the reptile breeder had already owned a generator or provided a quicker response to the lack of heating, this whole colony might still be thriving and producing offspring today. The breeder's delay in seeking veterinary assistance could have also exacerbated the severity of the disease. The coccidiosis seen in the early cases was not observed in subsequent fecal examinations. This was probably an incidental finding rather than a significant factor in the overall disease process. The bacterial enteritis was a difficult factor, which was extremely variable. The variety of antibiotic trials seemed to be without effect. Was this due to presence of *Cryptosporidia* in the beginning of the disease or was the *Cryptosporidia* the result of the chronic disease? Unfortunately, many of the individual breeders who were involved declined background diagnostics to discover the source of the cryptosporidial infection due to the nature of herpetoculture and rumors that could create financial losses. The paromomycin treatment had some success in only a few cases but in the leopard gecko does not seem to be an effective means of eradicating the disease (Upton, *et al*, 1989).

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Table 1: Antibiotic Sensitivity Patterns for Cultured (1) *E. coli*, (2) *Bacillus* spp, (3) *Proteus mirabilis*, and (4) *E. fergusonii*

Dates:	4/97	5/23		10/31	
Antibiotics:	(1)	(1)	(2)	(3)	(4)
Amikacin	S	S	S	S	S
Ampicillin	R				
Carbenicillin	S				
Cefotaxime	S	S	R	S	S
Cefixime		S	R	S	S
Cephalexin	R	S	R	S	S
Chloramphenicol	S	S	S	S	S
Ciprofloxacin		R	S	S	R
Doxycycline	R	S	S	R	R
Enrofloxacin	R	R	S	S	R
Gentamycin	S	R	S	S	S
Oxacillin		R	R	R	R
Penicillin		R	R	R	R
Piperacillin	S	R	S	S	S
Tmp/Sulfa	S	R	R	S	S
Spectinomycin	S				
Tetracycline	R				
Ticarcillin	S	S	S	S	S

Table 2: Antibiotic Drug Trial

Group 1
- 18 Leopard Geckos
- Doxycycline (Vibramycin, Pfizer, New York, NY)
- 2 geckos died by 4 wk
- 20 geckos died by 4 mo

Group 2
- 12 Leopard Geckos
- Doxycycline (Vibramycin, Pfizer, New York, NY)
- NutriBAC df (Midwest Zoological Research, Warsaw, IN)
- 8 geckos died by 4 wk
- 22 geckos died by 4 mo

Group 3
- 6 Leopard Geckos
- Doxycycline (Vibramycin, Pfizer, New York, NY)
- Metronidazole (Flagyl, Rhone-Poulenc Rorer, Mexico, D.F.)
- 1 gecko died by 4 wk
- All geckos died by 2 mo

Group 4
- 6 Leopard Geckos
- Chloramphenicol (Fujisawa USA, Inc, Deerfield, IL)
- 10 geckos died by 4 wk
- All geckos died by 2 mo

Group 5
- 6 Leopard Geckos
- Chloramphenicol (Fujisawa USA, Inc., Deerfield, IL)
- Metronidazole (Flagyl, Rhone-Poulenc Rorer: Mexico, D.F.)
- 4 geckos died by 4 wk
- All geckos died by 2 mo

Group 6
- 4 Leopard Geckos
- NutriBAC df (Midwest Zoological Research, Warsaw, IN)
- All geckos died by 4 wk

Group 7
- 6 Leopard Geckos
- Cephalexin (Goldline Labs, Ft. Lauderdale, FL)
- 2 geckos died by 4 wk
- All geckos died by 2 mo