Prevalence of *Cryptosporidium* Infection and Characteristics of Oocyst Shedding in a Breeding Colony of Leopard Geckos (*Eublepharis macularius*)

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PREVALENCE OF *CRYPTOSPORIDIUM* INFECTION AND CHARACTERISTICS OF OOCYST SHEDDING IN A BREEDING COLONY OF LEOPARD GECKOS (*EUBLEPHARIS MACULARIUS*)

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Abstract: Cryptosporidiosis is an emerging problem in reptile medicine and has been associated with a wasting syndrome in leopard geckos (*Eublepharis macularius*). This study determined the prevalence of infection in a breeding colony of leopard geckos to be 9.8%. Two groups of 20 geckos, one that was fecal positive for oocysts of *Cryptosporidium* sp., and one, whose individuals were fecal negative at the inception of the study, were followed for 2 mo. Fecal samples were tested for oocysts every 2 wk, body weights were measured, and a body condition score was assigned for each gecko. Selected geckos from these two groups were euthanized and necropsied. There were statistically significant differences ($P < 0.05$) between the two groups for mean body weight, mean body condition score, and prevalence of infection. *Cryptosporidium* sp. infection is endemic in this breeding colony, and there were a large number of geckos with a subclinical or carrier state of infection. These animals continued to be infected with *Cryptosporidium* sp. but gained weight and remained in good body condition. Only one gecko in the entire group of 40 was confirmed to be negative for oocysts or developmental stages by repeated fecal exams and histopathology. An additional 37 severely emaciated geckos from the breeding colony were euthanized, and all were positive for *Cryptosporidium* sp. on histopathologic examination of the gastrointestinal tract. The results of this study indicate that although some animals can recover from a clinical infection, if a gecko is severely wasted, it should be euthanized because of the poor prognosis and possible source of infection to other geckos.

Key words: Cryptosporidiosis, *Cryptosporidium* sp., leopard gecko, *Eublepharis macularius*, prevalence, wasting syndrome.

INTRODUCTION

Cryptosporidiosis has been recognized as a cause of diarrheal disease in several mammalian species since 1907.[9,15] It has recently been seen as an emerging problem in reptile medicine, causing serious disease in snakes, lizards, and chelonians.[3,6] In 1977, the parasite was reported for the first time in a reptilian species, in association with a severe, chronic, hypertrophic gastritis in snakes.[1] This organism was found to be different from that causing disease in mammals and was named *Cryptosporidium serpentis*.[8] More recently, cryptosporidiosis has been seen in association with a wasting syndrome or ''going light'' in leopard geckos (*Eublepharis macularius*), characterized by chronic weight loss, diarrhea, lethargy, a normal appetite initially, followed by anorexia, and death.[2,11] One study described juvenile geckos that were experimentally infected with a species of *Cryptosporidium* found in skinks.[7] It was proposed that this was a distinct species of *Cryptosporidium* and it was named *C. saurophilus*.[7] However, recent sequence analysis of both the 18S rRNA and actin genes indicates *C. saurophilus* is identical to *C. varanii*, and it has been proposed that the older name of *C. varanii* should take preference over *C. saurophilus*.[11] Furthermore, studies using polymerase chain reaction (PCR)-restriction length polymorphism and rRNA sequence analysis indicate there is considerable genetic diversity of *Cryptosporidium* spp. isolated from captive reptiles.[17]

Cryptosporidiosis in reptiles has been studied most extensively in snakes and has been reported to be present in both subclinical and clinical forms.[3,5] Once the clinical form of the disease is apparent, it is invariably fatal in snakes. The subclinical form may not be a true infection with a pathogenic reptilian form of cryptosporidiosis, since most snakes are fed rodents, which may be infected with *C. parvum* or *C. muris*. The oocysts from these species pass directly through the gastrointestinal tracts of several different species of snakes and lizards and are not thought to cause disease in reptiles.[4,14] However, they may be easily confused with the reptilian *Cryptosporidium* species on fecal flotation, leading to misdiagnosis of a subclinical infection.[14,16] On necropsy, pathologic changes were found in the stomach in snakes, including mucosal edema, thickening, petechiae, fo-

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cal necrosis, increased stomach diameter, and decreased lumenal diameter.[15]

In geckos, *Cryptosporidium* sp. infection is more common in the small intestine than in the stomach. Organisms were restricted to the anterior intestine in a case of cryptosporidiosis in a free-ranging house gecko (*Hemidactylus turcicus*) from Israel.[10] In infected leopard geckos, the gastrointestinal tract is filled with a clear, mucinous fluid; organisms may or may not be present in the stomach and are occasionally associated with atrophic gastritis.[2] *Cryptosporidium* sp. infection in leopard geckos has also been observed to be localized mainly within the small intestine, and associated with hyperplastic, lymphoplasmacytic enteritis.[13] Clinically, cryptosporidiosis in leopard geckos resulted in progressive weight loss and a mortality rate of approximately 50%.[12] However, the geckos in this study were also found to be infected with large numbers of flagellate protozoa, which may also have contributed to the clinical signs.[12]

Leopard geckos are a popular reptile pet and are found in many pet stores, zoological parks, and breeding facilities. In these types of environments, the geckos may be predisposed to cryptosporidiosis, secondary to stress from overcrowding. This can be exacerbated if there are other stresses in the environment, such as other infectious diseases, reproductive stress, or shipping to a new environment. At this point, the information on cryptosporidiosis in geckos is insufficient to guide treatment and control measures in these commercial facilities.

The goals of this study were 1) to determine the prevalence of cryptosporidiosis in a productive breeding colony of leopard geckos, 2) to characterize the frequency and persistence of oocyst shedding in two groups of selected geckos, and 3) to further characterize the association of *Cryptosporidium* sp. infection with the ''wasting syndrome'' in leopard geckos.

MATERIALS AND METHODS

Animals

The leopard geckos used in this study were from a colony of approximately 3,500 animals housed at a commercial breeding facility. Geckos are housed together in breeding groups composed of five females, with one male that rotates between two groups of females. They were housed in 54 × 39 × 14 cm Rubbermaid containers (Rubbermaid Co., Wooster, Ohio 44691, USA) at an ambient temperature of 85°F with heat tape placed underneath the back of each container to provide a hot spot, and newspaper lining the bottom of the containers. Each breeding group was provided with a 32 × 19 × 8 cm Rubbermaid (Rubbermaid Co.) nest box filled with a mixture of vermiculite and bottled water. Fresh water and mealworms (*Tenebrio* sp.) dusted with a vitamin supplement (Vionate Vitamin Mineral Powder, Gimborn, Atlanta, Georgia 30340, USA) were provided in 8-oz plastic cups. Crickets (*Acheta domestica*) were also dusted with the vitamin supplement and fed three times a week. During the breeding season, the nest boxes were checked for eggs three times a week.

Prevalence study

Each breeding group was numbered (1–678) and 203 breeding groups were randomly selected to evaluate the prevalence of *Cryptosporidium* sp. infection. It was assumed that since transmission of cryptosporidiosis is fecal–oral, if one gecko in a breeding group tested positive, then all geckos in that group were positive. In addition, all of the geckos within a breeding group defecate in a specific location in their cage. This may concentrate the oocysts in one area and possibly increase the likelihood of fecal–oral transmission. A group fecal was collected from each of the selected breeding groups and evaluated by fecal flotation in Sheather's sugar for *Cryptosporidium* sp. The prevalence of infection was calculated from the results of the fecal flotation tests.

Frequency and persistence of shedding

Two groups of 20 geckos each were selected from the breeding groups tested in the prevalence study. One group consisted of animals from groups that tested negative and were in good body condition (Negative group). The second group consisted of animals from groups that tested positive and were in poor body condition (Positive group). All selected animals were female. These animals were separated from the breeding colony and housed individually in 32 × 19 × 8 cm Rubbermaid containers (Rubbermaid Co.). They were provided mealworms and crickets dusted with the vitamin supplement, and a nesting area was provided in 8-oz plastic cups, similar to the setup for the larger breeding groups. Fecal samples were collected from these geckos every 2 wk on weeks one, three, five, and seven. Additional samples were collected at week 13. Body weights were measured using a gram scale and a body condition score (BCS) of one–five was assigned each time a fecal sample was collected.

Animals with severe wasting syndrome

Thirty-seven geckos that were not part of the selected groups were euthanized due to severe ema-

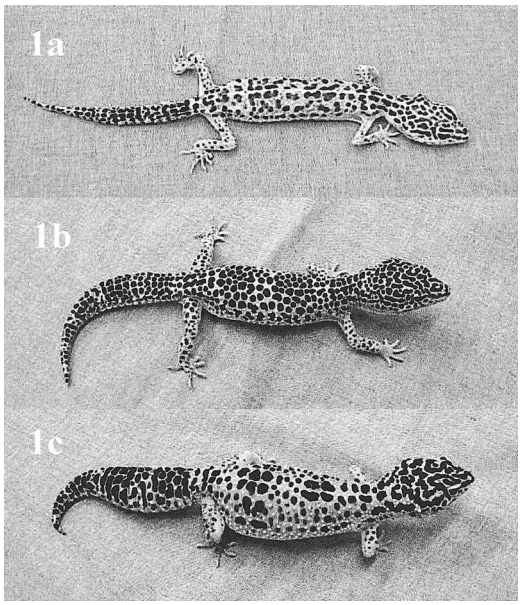

Figure 1. Leopard geckos with different body condition scores (BCS). **a.** An example of a gecko with the lowest BCS (one). **b.** Gecko with a BCS of three. **c.** Gecko with a BCS of five, which was the highest BCS score given.

ciation (BCS = one), anorexia, and at the request of the breeding facility. Following euthanasia, they were necropsied as described below.

Fecal samples

Fecal samples were examined using Sheather's sugar flotation with centrifugation. Each sample was first placed in a 15-ml glass or plastic tube. The tube was partially filled with Sheather's sugar solution and mixed using a wooden applicator stick and vortexed for approximately 5 sec. The remainder of the tube was filled with Sheather's sugar solution and a coverslip was placed at the top. Each tube was placed in the centrifuge and spun at 1,500 rpm for 10 min. The coverslips were then placed on clean, labeled glass slides. Slides were examined for the presence of *Cryptosporidium* sp. oocysts by light microscopy.

Body condition scoring

A scoring system for the body condition of the geckos was created, and examples are shown in Figure 1. The scoring system was defined as: one = emaciated. No fat deposits visible anywhere in the body, vertebral column easily visible, eyes appear sunken; two = thin. Minimal fat deposits throughout the body, tail is straight and thin, vertebral column is visible, but not prominent; three = average. Tail is slightly plump, vertebral column is not easily seen, abdomen is slightly rounded; four = fat. Fat deposits have noticeably thickened the tail, abdomen is wide in proportion to the body; and five = obese. Tail is very rounded and thick; abdomen is very wide in proportion to body size.

Histopathology

At any time during the study, if an animal tested positive for *Cryptosporidium* sp. and had lost 20% of its original body weight, it was euthanized by intracoelemic injection of an overdose of sodium pentobarbital (Veterinary Laboratories, Lenexa, Kansas 66215, USA). A necropsy was performed, and all visceral organs were removed and preserved in 10% neutral buffered formalin. Several representative sections from the stomach, small intestine, and large intestine were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). At the conclusion of the study, selected *Cryptosporidium* sp. negative animals and *Cryptosporidium* sp. positive animals were euthanized and prepared for histopathology as described above.

Each section was examined by light microscopy, and the presence or absence of characteristic *Cryptosporidium* sp. organisms was noted (Fig. 2). The extent of *Cryptosporidium* sp. infection was scored on a scale of one to five, with one signifying the least intense infections seen, and five indicating an extremely high concentration of parasites with the presence of hyperplastic villi or inflammatory infiltrates.

Statistical analysis

Prevalence was calculated as the percentage of positive groups out of the total groups sampled. For the Positive and Negative groups, the mean and standard error were calculated for body weight and BCS each week samples were collected. The prevalence of infection for each group was also calculated for each week fecal samples were tested. A paired *t*-test was used to compare the body weights, BCS, and prevalence between the two groups for each sampling week. For the severely emaciated group of geckos, the prevalence of *Cryptosporidium* sp. infection was also determined as described above. The mean and standard error were calculated for the degree of infection determined microscopically. Mean values between groups were compared by one-way analysis of variance (ANOVA) using a computer-based statistical program (SigmaStat, Jandel Corp., Point Richmond, California 94804, USA). Differences between individual

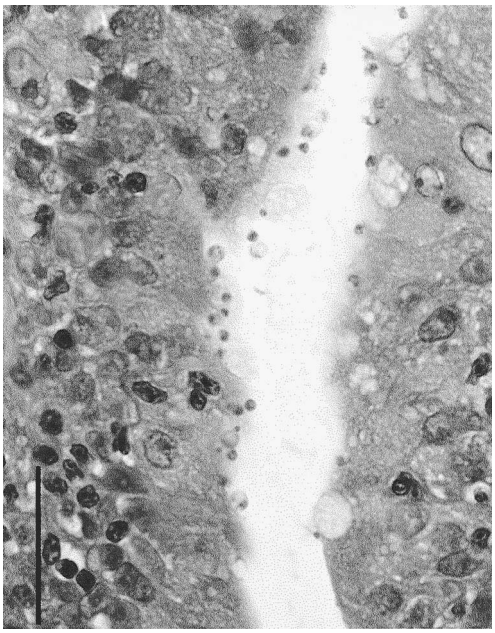

Figure 2. Leopard gecko small intestine with a light to moderate (score of 2) infection. Characteristic *Cryptosporidium* sp. stages are visible along the lumenal border of the enterocytes. Other common pathologic changes noted in infected geckos included hyperplasia of the intestinal epithelium and a mild to moderate infiltrate of lymphocytes. H&E, bar = 30 μm.

means were assessed by Bonferroni's method of all pairwise multiple comparison.

RESULTS

Overall prevalence study

Of the 203 breeding groups sampled, 20 tested positive for *Cryptosporidium* sp. by fecal sugar flotation. The group prevalence in the colony was 9.8%.

Frequency and persistence of shedding

The mean body weights (Fig. 3) and BCS (Fig. 4) between the Negative and Positive groups were statistically different ($P < 0.05$). The prevalence of *Cryptosporidium* sp. was significantly higher in the Positive group than in the Negative group ($P < 0.05$) at all weeks sampled (Fig. 5). There were two geckos in the Negative group that died of unknown causes during the course of the study. One severely emaciated gecko in the Positive group died before it had lost 20% of its body weight. These three geckos were not available for necropsy. After week seven, six emaciated geckos from the Positive group that had lost over 20% of their body weight were euthanized and necropsied.

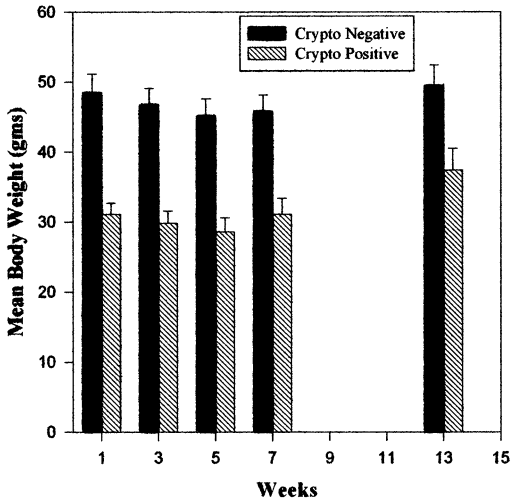

Figure 3. Mean body weights of the initially *Cryptosporidium* sp. Negative and Positive groups of geckos. *Cryptosporidium* sp. positive geckos had consistently lower body weights at all time points examined compared with *Cryptosporidium* sp. negative geckos ($P < 0.05$).

There was a trend from weeks 3–13, in which the mean body weights increased while the prevalence of *Cryptosporidium* sp. infection decreased (Fig. 5). However, the euthanasia of the most severely emaciated animals, which were positive, prior to week 13 may have affected this trend.

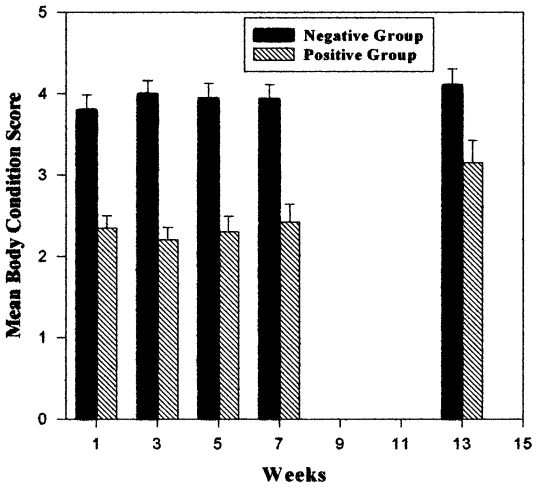

Figure 4. Mean body condition scores (BCS) of the initially *Cryptosporidium* sp. Negative and Positive groups of geckos. *Cryptosporidium* sp. positive geckos had consistently lower BCS at all time points examined compared with *Cryptosporidium* sp. negative geckos ($P < 0.05$).

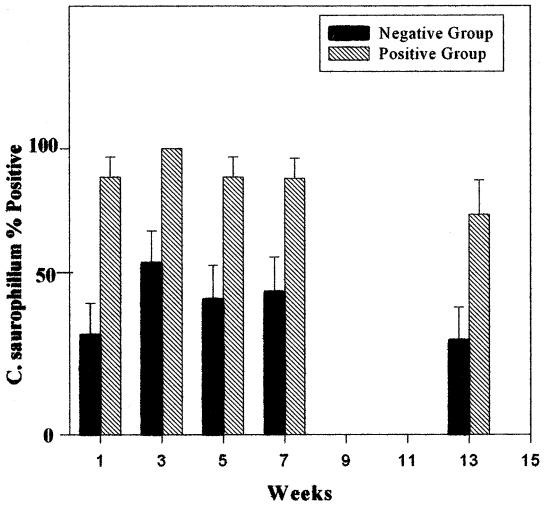

Figure 5. Prevalence of *Cryptosporidium* sp. infections in the initially *Cryptosporidium* sp. Negative and Positive groups of geckos. Although geckos in the initially *Cryptosporidium* sp. Negative group did not remain negative, the prevalence of *Cryptosporidium* sp. positive animals was generally less than 50%. In contrast, the prevalence of *Cryptosporidium* sp. in the initially positive group was well over 50% at all time points investigated.

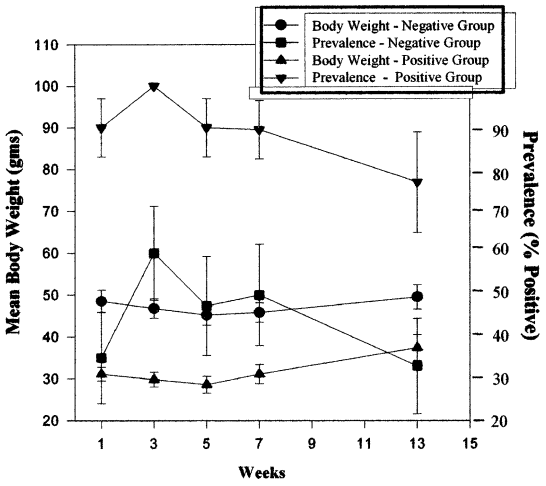

Figure 6. Prevalence of *Cryptosporidium* sp. infection and mean body weights over time from the initially Negative and Positive groups of geckos. The prevalence and mean body weights appear to be inversely proportional in both groups of geckos.

Animals with severe wasting syndrome

All 37 emaciated geckoes were infected. The numbers of animals at each level of infection are shown in Figure 6. The mean (± standard error) histologic level of infection in this group was 2.6 ± 0.2.

Histopathology

Six geckos were euthanized after testing positive for *Cryptosporidium* sp. and losing at least 20% of their original body weight. All six were in the Positive group and were assigned a body condition score of one or two at the time of euthanasia. On histopathologic examination, all six geckos had *Cryptosporidium* sp. organisms visible on the mucosa of the small intestine.

At the conclusion of the study, four additional geckos were euthanized and necropsied. Two were from the Negative group and had been consistently negative for *Cryptosporidium* sp. on every fecal sample. An additional fecal sample was examined prior to euthanasia and was negative for both animals. The other two geckos were euthanized because they tested positive for *Cryptosporidium* sp. infection two and three times, respectively, and were then negative on all consecutive fecals. One of these two was from the Negative group and one was from the Positive group. An additional fecal sample was examined prior to euthanasia and was negative for both animals. All four geckos had all gained weight since the last fecal sample taken 7 wk previously (Table 1). On histopathologic examination, three of the four had *Cryptosporidium* sp. organisms visible within the small intestine. However, the degree of infection was light (score of one) and there were no signs of hyperplastic villi

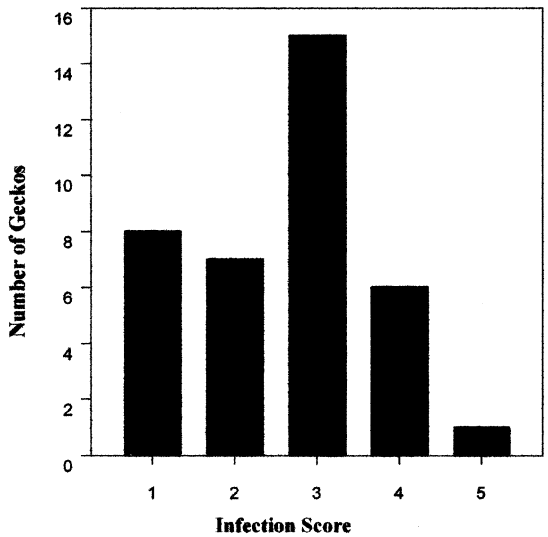

Figure 7. Level of histopathologic *Cryptosporidium* sp. infection in thirty-seven geckos euthanized for severe wasting disease. The mean (± standard error) histologic infection score was 2.6 ± 0.2.

Table 1. Euthanized gecko body weights (g) and fecal results (+/−) of euthanized geckos.[a]

Gecko No.	Group	Week 1	Week 3	Week 5	Week 7	Week 13	Week 20	Histo+
571	Neg.	29.0 (−)	32.0 (−)	30.0 (−)	33.0 (−)	35.0 (−)	39.0 (−)	No
41	Neg.	63.0 (−)	58.0 (−)	66.0 (−)	63.0 (−)	72.0 (−)	76.0 (−)	Yes
289	Neg.	32.0 (+)	30.0 (+)	29.0 (−)	32.0 (−)	32.0 (−)	37.0 (−)	Yes
217	Pos.	44.0 (+)	45.0 (+)	45.0 (+)	50.0 (−)	56.0 (−)	63.0 (−)	Yes

[a] Histo+, intestinal epithelial infection; Neg., negative; Pos., positive.

or enteritis. One of the two consistently negative geckos had no evidence of *Cryptosporidium* sp. infection on histopathology.

DISCUSSION

A previous study examined twenty-three geckos from two large breeding facilities and one zoological collection with the characteristic wasting syndrome.[2] All showed histologic evidence of *Cryptosporidium* sp. infection, and it was estimated that 5% of the geckos at those facilities were affected with the wasting syndrome.[2] The calculated prevalence of about 10% for the current study is based on oocyst shedding observed in fecal samples collected from over 200 breeding groups and represents the overall prevalence within this colony. It would be expected that the prevalence of clinical disease in the breeding colony should be lower than the 9.8% prevalence calculated. When the geckos were tested individually for *Cryptosporidium* sp., even the prevalence found in the initially Negative group was much higher (Fig. 5) than that of the entire colony. One possible explanation for this is that the feces examined from the breeding groups were collected as a group fecal sample, which may not be as accurate as an individual fecal sample in detecting the presence of *Cryptosporidium* sp. oocysts. At the time when the breeding groups were being tested, the breeding season of the geckos had just begun. The fecal sample obtained from individual animals were first tested for *Cryptosporidium* sp. 3 wk after the last sampling of the breeding groups. It may be possible that the geckos became more stressed as the breeding season progressed, resulting in a higher prevalence of oocyst shedding.

The mean body weights and body condition scores of geckos in the Positive group were significantly lower than those in the Negative group. The prevalence of *Cryptosporidium* sp. infection remained significantly higher in the Positive group than in the Negative group over the entire time period sampled, and a high prevalence of infection was associated with low BCS. The prevalence of infection began to drop around 3 wk of isolation, which suggests isolation might decrease infection. The mean body weights then gradually increased as the prevalence decreased. Initially, the two groups should have been significantly different because they were selected on the basis of infection status and body condition. However, this difference persisted throughout the length of the study. The most severely emaciated geckos were consistently associated with a higher prevalence of infection, and the geckos in better condition were consistently associated with a lower prevalence of infection. This finding reinforces the observation that this unique wasting syndrome is associated with *Cryptosporidium* sp. infection in leopard geckos. In addition, no other pathogens were identified on histopathologic examination of wasted geckos, and every gecko of body condition score one was positive for *Cryptosporidium* sp. The trend in the data could represent some type of stressful insult to both groups that resulted in weight loss and increased oocyst shedding over several weeks. Removal of the geckos from their breeding groups, and housing in a new environment may have been a significant stress to these animals. At the time when these geckos were removed from the breeding groups, they were producing eggs regularly. When housed individually, some of the reproductive stress may have decreased, resulting in weight gain and a decrease in the prevalence of infection after several weeks.

In the geckos that were severely wasted, the majority of the animals had a moderate (score of three) histologic level of infection in their small intestines. There were fewer animals with a moderately high (score of four) or high (score of five) degree of infection. It is possible that geckos infected with high numbers of parasites succumb more rapidly to the disease. These geckos may have some underlying immunodeficiency that could predispose them to a higher parasite burden. Such animals would be expected to die acutely and thus would not have survived long enough to be identified and included in this study. In the geckos with a moderate (score of three) level of infection, the disease may have a more chronic course, and the geckos may be able

to compensate for a period of time. In the Negative and Positive groups, all geckos of BCS one became progressively more emaciated and were euthanized. However, there were geckos of BCS two that progressively lost weight and then regained weight after a period of time. The lightest gecko to regain weight after testing positive for *Cryptosporidium* sp. weighed 20 g at week five and had a BCS of two. By week 13, she weighed 27 g and had a BCS of three. These observations suggest that infected geckos reach a critical threshold of weight loss, and additional factors, such as reproductive stress, introduction to a new environment, competition for food, or overcrowding, may determine whether the gecko continues to waste or is capable of a clinical recovery.

Of all 40 geckos from both groups of individual geckos, only one animal could be confirmed as negative for *Cryptosporidium* sp. infection, based on repeated fecal exams and histopathologic examination of the gastrointestinal tract. The other gecko that was consistently negative on fecal flotation did have a light (score of one) infection histologically. In addition, two geckos that initially were positive for *Cryptosporidium* sp. and later tested negative on several fecal exams were also confirmed to have a light (score of one) infection histologically. However, since these three animals had progressively gained weight and were generally healthy, it seems that the presence of *Cryptosporidium* sp. is not always associated with clinical disease. The value of a single negative fecal exam is questionable based on the findings in these three geckos and should not be used as an absolute screening test for infection. These animals may represent a population of geckos with subclinical infection or a carrier state for *Cryptosporidium* sp., and it is possible that a stressful incident may induce clinical disease in these geckos at a later time. It is also possible that geckos with subclinical infections eventually clear the infection. The one gecko that appeared to be truly negative for *Cryptosporidium* sp. infection could represent an animal that has never been exposed, an extremely light infection not detectable on the histologic sections examined, or a gecko that has cleared a previous infection. Based on the husbandry situation in the breeding colony, it is unlikely that this gecko was never exposed.

This breeding colony occasionally receives geckos and other reptiles from outside sources, which could be a source of infection for the breeding colony. Once eggs are laid by the breeders, they are removed from the nest boxes and kept separately in an incubation room until they hatch. The newborn geckos are housed separately from the breeding colony and should be less likely to become infected with *Cryptosporidium* sp. The majority of the newborn geckos are shipped out, but a small number are kept as replacement breeders. These geckos likely become infected once they are introduced to a breeding group. It is likely that *Cryptosporidium* sp. oocysts are also spread through the colony by fomites. Equipment used in feeding, egg collection, and cleaning of cages is sprayed with a dilute bleach solution before being used. However, *Cryptosporidium* sp. oocysts are not killed by bleach, so this is not an effective method of preventing transmission. Through fomite transmission, the newborn geckos could become infected without direct contact with the rest of the breeding colony. Hyperimmune bovine colostrum has been used to treat moribund leopard geckos with cryptosporidiosis. However, it took treatments over several weeks to have an effect, and the feasibility of this type of treatment in a large breeding operation is yet to be determined.[4]

CONCLUSIONS

Cryptosporidium sp. is endemic in a breeding colony of leopard geckos and is associated with the wasting syndrome previously reported in geckos. The prevalence of infection is higher than the prevalence of the clinical wasting disease. All severely wasted geckos in this study (BCS = one) were infected with *Cryptosporidium* sp. Once a gecko reaches a BCS of one, it will not clinically recover. In a management situation, wasted geckos should be isolated and euthanized once they reach a BCS of one due to the poor prognosis and potential source of infection to other geckos. It may be possible to screen a population of geckos for *Cryptosporidium* sp. infection through serial fecal samples, but some lightly infected animals will likely be missed. All geckos with a positive fecal result can be considered infected. There appears to also be a subclinical or carrier form of the disease that is not associated with the clinical wasting disease. It is unknown at this time what factors may play a role in triggering clinical disease, or recovery from clinical disease.

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