Canine Protothecosis

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ABSTRACT:
Canine protothecosis is a rare disease that has the ability to cause severe gastrointestinal (GI) pathology as well as ocular, cutaneous, and disseminated disease. Successful therapy for protothecosis has not been previously reported. The case study included in this article involves a dog diagnosed with GI protothecosis in which a novel therapeutic approach was used. This treatment was successful in halting progression and dissemination of the disease. Unfortunately, the long-term benefits of this therapy could not be determined in this patient. Dissemination of disease was not observed in this patient for 12 months following the onset of clinical signs.

Prototheca spp are colorless algae related to the green algae of the genus Chlorella.¹⁻⁴
There are three recognized species of Prototheca organisms: Prototheca zopfii, Prototheca wickerhamii, and Prototheca stagnora.¹⁻³,⁵⁻⁶ A fourth species has also been proposed: Prototheca salmonis.⁷ Of these species, only P. zopfii and P. wickerhamii have been found to be pathogenic.²⁻³,⁵⁻⁶ Prototheca organisms can be found in abundance in nature in sources such as raw or treated sewage, the slime flux of trees, animal waste, soil, food, and flowing or standing water.¹⁻³,⁶⁻⁹ These organisms have a nearly worldwide distribution, having been reported on five of the seven continents: North America,¹,¹⁰⁻⁻²⁶ Europe,²,²⁷⁻⁻³² Asia,³,³³⁻⁻³⁵ Africa,²,³,³⁸⁻⁻₄² and Australia.³,³⁶⁻⁻³⁷ Because Prototheca infections are rare in domestic animals, it is difficult to establish breed, age, and sex predilections. In the canine cases previously reported, the collie and spayed females appear to be overrepresented.¹⁻³ The age range of infected animals is wide: 1.5 to 10 years of age.¹

CLINICAL SIGNS
Despite their abundance in nature, Prototheca organisms very rarely cause disease in domestic animals.¹⁻²,⁴⁻⁻⁴³ The most commonly affected domestic animals are dogs, cats, and cows.³ In cows, manifestation of the disease is usually in the form of mastitis caused by P. zopfii.²,³,²⁷,₃⁵,₄₁,₄²,₄₄⁻⁻₄⁵ Cats are most commonly affected with the cutaneous form of protothecosis and present with large, firm nodules on the limbs and feet.¹,₁⁴⁻⁻₁₈,₃₃,₃₈,₄₀⁻⁻₄⁶⁻⁻₄⁹ Other areas that may be affected are the nose, pinnae, forehead, and tailbase.¹ P. wickerhamii is most commonly isolated from these cutaneous lesions in cats.³,₁⁴⁻⁻₄₀

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Dogs often show a wide range of clinical signs with protothecal infections. The most common clinical presentation is protracted hemorrhagic enteritis. The colon is the most commonly affected portion of the gastrointestinal (GI) tract.\(^1,2,4\) Lesions within the colon include diffuse reddening and multiple raised white nodules as well as ulcerations and may be accompanied by hemorrhage. Necrosis is rarely observed.\(^1,3\) Disseminated disease involving the eyes, ears, skin, skeletal muscles, kidneys, liver, heart, spinal cord, and brain has been reported.\(^1,2,4,50\) It is believed that infection occurs via ingestion, where organisms grow within the GI tract and eventually disseminate via lymphatics and blood.\(^1–4,6,13,25\)

Ocular involvement is one of the most common manifestations of dissemination.\(^1,2,35\) A recent review revealed that of the 26 reported cases of protothecosis, 20 cases showed ocular involvement (77%).\(^2,3,10,11,13,15,16,19–22,24,26,37,50\)

These retinal lesions are most commonly described as white to tan nodules identical to those seen in the colon or on the retina were discovered.\(^1,4\) The kidneys tend to show larger lesions because these clumps of organisms can grow to several centimeters.\(^3\)

The differential diagnosis for dogs infected with protothecosis depends greatly on which organ systems are affected. Protothecosis is not a common disease in domestic animals and should be considered only after diagnostic testing has ruled out more common conditions. However, one presentation that should arouse clinical suspicion of protothecosis is a dog that presents with hemorrhagic enteritis concurrent with acute blindness.\(^1,3,4\)

**DIAGNOSIS**

Diagnosis of *Prototheca* infections can be confirmed with cytology of rectal scrapings when the GI tract is involved.\(^1–3\) Positive cytology results include typical *Prototheca* cells (i.e., 3 to 20 µm in diameter with a thin, refractile cell wall).\(^1,2\) The cells have a granular, basophilic cytoplasm with a small, central nucleus.\(^1,2\) Some cells can be seen undergoing endosporulation, with as many as 20 daughter cells being released from the parent cell\(^1–4,8,9,15\) (see figure on p. 98). *P. zopfii* and *P. wickerhamii* cannot be differentiated via microscopy; an alcohol or sugar assimilation test is accurate in determining the species, but indirect fluorescent antibody tests are much more rapid.\(^1,3,9,15,20,22,51,52\) Culture and sensitivity tests can also be conducted on Sabouraud’s glucose media with clotrimazole-impregnated disks for differentiating between the two species.\(^1,2,53\) *P. wickerhamii* is inhibited by clotrimazole, but *P. zopfii* is not.\(^1,2,53\) Laboratory evaluation, including a complete blood cell count (CBC), chemistry panel, and urinalysis, is often unremarkable.\(^1,3\)

Other diagnostic methods include biopsy specimens of the colon or regional lymph nodes, but any other affected tissue may be sampled.\(^1,3\) Urine can also reveal the presence of these organisms if dissemination to the kidneys has occurred. Microscopic examination of urine sediment can be an excellent means of detecting dissemination to the kidneys.\(^2,4,21\) Cerebrospinal fluid tap or vitreous aspiration may also yield the presence of disseminated organisms when neurologic or ocular infection is present.\(^14,10,19,20,22,26\)
Canine Protothecosis

Signalment and History
A 2-year-old neutered male cocker spaniel was referred to the clinic with an owner complaint of chronic, intermittent, bloody diarrhea for 4 to 5 months. The referring veterinarian had treated the patient with pyrantel pamoate (Strongid T, Pfizer), metronidazole (Flagyl, Searle), and sulfamethoxazole–trimethoprim (Tribrissen, Schering-Plough), none of which reduced the clinical signs. In addition, fecal flotation test results were repeatedly negative. Therefore, the patient was referred for further evaluation and diagnostics.

Physical Examination and Diagnostics
Physical examination findings revealed no significant abnormalities. The patient was overweight at 36 lb (16.4 kg), with an adequate hydration status and no pain on rectal palpation. The stool was dark brown and liquid. Tenesmus and frank blood were present throughout defecation. A complete ocular examination revealed no evidence of lesions. CBC, chemistry panel, and urinalysis results as well as chest and abdominal radiographs were normal. A cytology sample from a rectal scraping revealed the presence of many spherical to ovoid cells identified as *Prototheca* spp (see figure at right). A subsequent colonoscopy revealed markedly hyperemic mucosa in the colon and duodenum. One focal area of necrosis in the intestinal mucosa was also observed in the proximal colon. Biopsy samples were taken and again revealed the presence of *Prototheca* spp. Immunoglobulin levels were checked to ascertain the patient’s immune status. IgG and IgA levels were elevated. The IgM level was within the normal range, as would be expected for the chronicity of the condition.

Treatment
Following diagnosis, the patient was started on a treatment regimen of amphotericin B lipid-based (Abelcet, Liposome Co.; 1 mg/kg IV every other day) and clotrimazole (Monistat 1% ointment enema twice weekly). Amphotericin B was scheduled to be given three times a week, preceded and followed by 2 L of 0.9% sodium chloride (saline) IV to decrease the nephrotoxicity of amphotericin B. Renal function was closely monitored by blood urea nitrogen level, creatinine level, and urinalysis on the mornings of amphotericin B treatments. Amphotericin B treatments were scheduled to continue until a cumulative dose of 12 mg/kg was reached. After the amphotericin B therapy, the patient was to receive itraconazole (Sporanox, Ortho Biotech) therapy (100 mg PO bid) at home for 2 months beyond the resolution of clinical signs.

Rather than three amphotericin B treatments per week for 4 weeks (12 doses total) as planned, the patient received two doses per week for 5 weeks (10 doses total) because of renal toxicity. A low urine specific gravity or elevation of serum blood urea nitrogen and creatinine were cause for canceling the scheduled therapy. Repeat rectal scrapings for colonic cytologic samples were taken at 2 weeks and 4 weeks postinitiation of therapy. Decreasing numbers of *Prototheca* organisms were noted with each progressive sample. Although this could have been reflective of the sample taken and not definitive for success of therapy, the marked decrease in numbers seen combined with improvement of clinical signs were considered positive prognostic factors. The rectal scrapings were always taken by the same person and were evaluated by one pathologist.

A repeat colonoscopy was conducted at 6 weeks, revealing that the mucosa was still hyperemic, but no areas of necrosis were visible. Biopsy samples were also taken at this time, and *Prototheca* organisms were still found within the colonic mucosa. An amphotericin B enema (3% amphotericin B cream [Fungizone, Apothecon]) was administered. It was believed that direct contact with the intestinal mucosa would bring the drug into contact with organisms that had been unreachable via the bloodstream because of damaged or necrotic areas of epithelium. To our knowledge, this procedure had not been performed previously. Ideally, amphotericin B blood levels could have been checked to ensure the safety of this procedure. Chemistry panels and urinalyses were continuously conducted to monitor for signs of renal toxicity. Clinically, the patient showed gradual improvement. Following initiation of treatment with amphotericin B, the patient’s stools progressed from being thin and liquid to becoming more formed but still containing small amounts of frank blood. The frequency of defecation decreased, and tenesmus was no longer observed. Although the stools became progressively
Case Study (Continued)

firmed after the first week of therapy, small amounts of frank blood were often observed. Mild clinical improvement continued; at discharge, the patient’s stools were well formed, with only a small amount of frank blood at the end of defecation.

Repeat cytology results from this time also revealed a marked decrease in the number of *Prototheca* cells compared with the first cytology sample submitted. These biopsy samples were unlikely to be from the same areas as those previously obtained; however, these findings were consistent with cytology of the rectal scrapings and the clinical improvement seen in this patient. The patient was discharged with itraconazole and the owner instructed to return with the patient for a recheck in 30 days. At that time, the patient would be given another 30 days of itraconazole, which would be administered until at least 2 months beyond clinical resolution.

Follow-Up

Follow-up telephone conversations with the owner indicated that the patient was doing very well and had no blood in its stool. The patient did not return for a recheck until 2 months after discharge. The itraconazole prescription had not been refilled after the first 30-day supply because the dog was showing no clinical signs. However, the patient presented after 2 months because of reemergence of bloody diarrhea. The physical examination indicated that the patient’s condition and demeanor were good, but blood was noted on rectal palpation, and feces were again very loose with frank blood. A repeat rectal scrap ing revealed a large number of protothecal cells within the colonic sample. A repeat colonoscopy was conducted. The appearance of the mucosa was a mixture of healthy tissue and patches of inflamed, hyperemic mucosa. No areas of necrosis were noted. An amphotericin B enema was performed after the colonoscopy. Repeat radiography, ultrasonography, and ocular examination showed no evidence of dissemination.

The owners were unwilling to pursue another IV amphotericin B protocol. A total of six amphotericin B enemas were given by the referring veterinarian over the next 2 weeks followed by oral ketoconazole (Nizoral, Janssen) therapy. However, the owners did not heed the recommended advice of continuing the oral medications for 2 months beyond the resolution of clinical signs and did not refill the prescription once the patient was free of signs. The dog presented again 6 weeks after therapy and died the next day. A necropsy was not performed.

Conclusion

This case demonstrates the frustration in treating protothecosis. Despite initial improvement, the clinical signs returned when medications were discontinued. It is unknown whether long-term resolution of the disease could have been achieved if the owners had continued therapy as directed. This dog did survive for approximately 12 months without evidence of dissemination. The combination of IV amphotericin B, oral itraconazole, and amphotericin enemas may hold promise as an effective treatment for protothecosis if early diagnosis precedes widespread dissemination.

It is also important to consider how the infection was acquired. *Prototheca* spp are abundant in the environment but very rarely cause disease in domestic animals. This suggests that animals are immunocompromised at the time of infection. Immunoglobulin levels should be checked to determine whether an animal has a sufficient immune response to warrant treatment. The IgG level should be elevated to indicate an immune response by the host. The IgM level is generally normal because the acute phase of infection will have passed when these patients present for clinical evaluation.

Despite advances in the ability to analyze the functional capacity of leukocytes, such tests are of limited value in forming a diagnosis or prognosis associated with protothecosis. Protothecal organisms decrease the functional capacity of neutrophils, either by deactivating them or by decreasing their chemotactic abilities. One of the features of protothecal infections is that the organisms themselves are surrounded by a very mild inflammatory response. Therefore, leukocyte function tests cannot be used because it is impossible to distinguish between decreased function caused by an underlying immune problem or that caused by the infection itself.

**TREATMENT**

Twenty-six cases have been reported in dogs from 1969 to 2000. Successful treatment regimens have not yet been discovered for disseminated protothecal infections. All dogs were reported to have died within a short period of time after diagnosis. Some treatments have been able to slow progression of the disease, but because all of these cases involved disseminated forms of the disease, there were no survivors. The longest survival time of any dog in this study was 11 months. Cutaneous infections with *P. wickerhamii* have been successfully treated, but therapy for *P. zopfii* has thus far been unsuccessful.

In treating human protothecosis, many drugs (e.g.,...
griseofulvin, potassium iodide, pentamidine isethionate, copper sulfate, gen- 
tian violet, brilliant green, chlorinated lime) have been tried. Because of 
their in-vitro efficacy, aminoglycosides have also been tried but have been 
found to have little effect clinically. Amphotericin B has been used in 
combination with tetracycline, ketoconazole, fluconazole, or clotrimazole to 
successfully combat *P. wickerhamii*. However, treatment is more difficult in 
dogs because *P. zopfii* is the most common species. Amphotericin B has 
been successful in slowing progression of the disease, but it is not curative. 
One dog with cutaneous and systemic infection survived nearly 1 year after 
treatment with amphotericin B alone. However, amphotericin B alone was 
not effective in another case with ocular lesions. Ketoconazole has been 
used successfully twice but only in cutaneous *P. wickerhamii* infections.

Two canine cases had some short-term treatment success. In one patient, 
protothecal chorioretinitis abated during treatment with IV amphotericin 
B. However, the treatment was discontinued because of nephrotoxicity, 
and clinical signs of chorioretinitis returned 12 days later. In the other 
case, a dog presented with ophthalmic lesions only and was treated with 
itraconazole (5 mg/kg bid for 1 month, then once daily for 10 months). The 
lesions in this dog were not reversed, but progression of the disease was 
slowed, and the dog survived for 11 months.

Although the success of these two therapies was limited, they became the 
basis for our treatment protocol. Amphotericin B (1 mg/kg IV three times 
weekly until attaining a cumulative dose of 12 mg/kg) used in combination 
with ketoconazole, fluconazole, or itraconazole (5 to 10 mg/kg/day PO) 
appeared to be the best option for long-term use against *P. zopfii*. Using 
amphotericin B for a short time followed by an azole drug can minimize 
nephrotoxicity without compromising the strength of treatment needed to 
kill the organism. Amphotericin B enemas (3% amphotericin B cream) were 
also introduced to place the drug into direct contact with organisms that 
may not have been reachable via the bloodstream. Surgical resection of any 
involved tissue augments medical therapy, but this is not always possible, 
especially in disseminated cases.

Cost must also be considered in these cases. Antifungal therapies 
(amphotericin B and azole drugs) are very expensive, and a lengthy hospital 
stay is likely. Based on previous reports, owners must be given a grave prog-
nosis for their pets and an expensive estimate.

**SUMMARY**

Twenty-six cases of canine protothecosis have been reported since 1969. 
Successful therapy has yet to be discovered for this disease.

In the case study presented in this article, a 2-year-old castrated male 
cocker spaniel was referred for evaluation of chronic diarrhea that had been 
unresponsive to antibiotics or deworming. An unusual pathogen, *Prototheca 
zopfii*, was discovered from a colonic scrape and subsequent colonoscopy. A 
novel therapeutic regimen was attempted: IV amphotericin B and oral itra-
conazole were used as has been traditionally reported, but amphotericin B 
enemas were also given. These enemas allow the drug to directly contact the 
colonic mucosa and pathogenic organism. Poor owner compliance made 
complete evaluation of this therapy impossible. However, a good clinical
response and no dissemination of disease were seen. Numerous additional cases will be needed to determine whether this therapy offers a better prognosis than what has been previously reported for canine protothecosis.

ACKNOWLEDGMENT

The authors thank the referring veterinarian, Dr. T. C. Branch of Oporto Animal Clinic in Birmingham, Alabama.

REFERENCES

2. Which of the following is not a species of *Prototheca*?
   a. *zopfii*  
   b. *mirabilis*  
   c. *wickerhamii*  
   d. *stagnora*

3. Which breed appears to be overrepresented in protothecosis?
   a. collie  
   b. boxer  
   c. Labrador retriever  
   d. German shepherd

4. Which canine body system is most commonly affected by protothecosis?
   a. ocular  
   b. central nervous  
   c. GI  
   d. cutaneous

5. Lesions of the ocular system do not include
   a. raised, white clusters on the retina.  
   b. hyperpigmentation of the retina.  
   c. granulomatous chorioretinitis.  
   d. retinal detachment.

6. Which of the following most accurately describes the GI lesions associated with protothecosis?
   a. widespread necrosis throughout the small intestine  
   b. widespread necrosis throughout the colon  
   c. congestion of the mucosa and the presence of raised, white foci in the small intestine  
   d. congestion of the mucosa and the presence of raised, white foci in the colon

7. Clinical presentation of a dog with GI protothecosis includes all of the following except
   a. vomiting.  
   b. diarrhea.  
   c. frank blood in the stool.  
   d. tenesmus.

8. Diagnosis of *Prototheca* infections can be confirmed with which of the following tests?
   a. CBC/chemistry panel  
   b. cytology  
   c. cerebrospinal fluid tap  
   d. culture and sensitivity

9. Which of the following has shown no clinical effectiveness against *Prototheca* spp?
   a. aminoglycosides  
   b. amphotericin B  
   c. itraconazole  
   d. ketoconazole

10. ________________ signs should immediately put protothecosis at the top of the differential diagnosis.
   a. GI  
   b. Ocular  
   c. Cutaneous  
   d. Concurrent GI and ocular