

Diagnosis and treatment of truncal cutaneous pythiosis in a dog

Kelley M. Thieman, DVM, MS; Kristin A. Kirkby, DVM, MS, DACVS; Alison Flynn-Lurie, DVM, DACVD; Amy M. Grooters, DVM, DACVIM; Nicholas J. Bacon, MA, VetMB, DACVS

Case Description—A 4-year-old spayed female Boxer was evaluated for a cutaneous mass located on the dorsum. The mass had been present for 6 weeks and was increasing in size.

Clinical Findings—A mass of approximately 10 cm in diameter was detected on the dorsum cranial to the right ilial wing. Histologic examination of a tissue sample from the mass led to the diagnosis of cutaneous pythiosis. Computed tomography of the abdomen and the mass were performed and revealed a contrast-enhancing soft tissue mass of the dorsum and enlarged intra-abdominal lymph nodes.

Treatment and Outcome—The dog underwent surgical excision of the cutaneous mass, including 5-cm skin margins and deep margins of 2 fascial planes. The mass was completely excised on the basis of results of histologic examination of surgical margins. The dog received itraconazole and terbinafine by mouth for 3 months following surgery. Recheck examination at 20 months postoperatively showed no signs of recurrence of pythiosis at the surgical site.

Clinical Relevance—Aggressive surgical excision in combination with medical treatment resulted in a favorable long-term (> 1 year) outcome in this dog. Thorough workup including diagnostic imaging and lymph node evaluation is recommended. If surgery is to be performed, skin margins of 5 cm and deep margins of 2 fascial planes are recommended. (*J Am Vet Med Assoc* 2011;239:1232–1235)

A 4-year-old 22.7-kg (50-lb) spayed female Boxer was referred to the University of Florida Veterinary Medical Center for evaluation of a cutaneous mass on the dorsum. The mass had been present for approximately 6 weeks and was increasing in size despite treatment with cefpodoxime (4.4 mg/kg [2 mg/lb], PO, once daily) and topical ointment^a containing nystatin, neomycin sulfate, thiostrepton, and triamcinolone acetonide. Incisional biopsy of the mass had been performed prior to referral and revealed severe necrotizing and fibrosing granulomatous and eosinophilic dermatitis and cellulitis. Concurrent with the appearance of the mass, the dog had mild intermittent lethargy and a decreased appetite. Two additional cutaneous masses developed approximately 10 days prior to referral: 1 in the interdigital space of the right thoracic limb and 1 on the haired skin of the right upper lip.

On initial examination at the University of Florida Veterinary Medical Center, the dog was bright and alert. Rectal temperature, pulse rate, and respiratory rate were within normal limits. A 10-cm-diameter cutaneous mass with a central tract draining purulent to

From the Department of Small Animal Clinical Sciences, Veterinary Medical Center, College of Veterinary Medicine, University of Florida, Gainesville, FL 32611 (Thieman, Kirkby, Flynn-Lurie, Bacon); and the Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 (Grooters). Dr. Kirkby's present address is Seattle Veterinary Specialists, 11814 115th Ave NE, Kirkland, WA 98034. Dr. Flynn-Lurie's present address is Miami Veterinary Specialists, 8601 Sunset Dr, Miami, FL 33173. Dr. Thieman's present address is Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843.

The authors thank Dr. Ian Hawkins for histologic interpretation. Address correspondence to Dr. Thieman (kthieman@cvm.tamu.edu).

ABBREVIATIONS

CT	Computed tomography
GMS	Gomori methenamine silver

serosanguinous fluid was noted just cranial to the right ilial wing (Figure 1). In addition, there was a 2-cm dermal nodule located between the fourth and fifth digits on the right thoracic limb and a 5-mm ulcerated plaque located on the haired skin of the right upper lip.

An impression smear of the fluid from the draining tract was stained^b and revealed numerous degenerative neutrophils, eosinophils, and activated macrophages. No organisms were seen. Clear acetate tape cytology examination of the interdigital and lip lesions revealed small numbers of yeast organisms and no other abnormal findings. A CBC revealed mild leukocytosis (16,160 WBCs/ μ L; reference interval, 5,000 to 14,500 WBCs/ μ L) with neutrophilia (13,770 neutrophils/ μ L; reference interval, 3,000 to 11,500 neutrophils/ μ L). No clinically important abnormalities were detected on serum biochemical analysis or urinalysis.

Wedge biopsy specimens were obtained from the 3 skin lesions and submitted for dermatopathologic testing as well as for cultures for aerobic and anaerobic bacteria, fungi, mycobacteria, and oomycetes. After 3 days, culture on blood agar of tissue from the mass on the dorsum yielded hyphal growth that was morphologically consistent with *Pythium insidiosum*. Cultures of the interdigital lesion and lip lesion were positive for *Staphylococcus intermedius*. All other cultures were negative.

Tissue samples were routinely processed for dermatopathologic testing by use of neutral-buffered 10% buffered formalin for H&E, GMS, periodic acid–Schiff, and



Figure 1—Preoperative photograph of a cutaneous mass on the dorsum of a 4-year-old spayed female Boxer with a subsequent diagnosis of truncal cutaneous pythiosis. The mass had been present for approximately 6 weeks and was increasing in size despite treatment with cefpodoxime (4.4 mg/kg [2 mg/lb], PO, once daily) and a topical ointment^a containing nystatin, neomycin sulfate, thiostrepton, and triamcinolone acetonide.

Brown-Brenn stains. Histologic examination of sections from the mass on the dorsum revealed pyogranulomatous and eosinophilic dermatitis and panniculitis. Wide (4- to 7- μ m), irregularly branching hyphae with nonparallel walls and rare septa were identified in GMS-stained sections. Biopsies of the lip and interdigital lesions revealed superficial pyoderma with neutrophilic folliculitis. The GMS-stained sections were negative for hyphae.

Abdominal ultrasonography revealed enlarged medial iliac lymph nodes and sediment within the urinary bladder, but no other abnormalities. Cytologic examination of a fine-needle aspirate of the medial iliac lymph nodes revealed reactive lymphoid hyperplasia with mild mastocytic and eosinophilic inflammation. Slides stained with GMS failed to demonstrate the presence of hyphae. A CT study of the abdomen and thorax was performed to detect extension of disease and to facilitate surgical planning. The only abnormalities noted on CT examination were the mass on the dorsum and a single small cystic calculus. The dorsal soft tissue mass was unencapsulated and had ill-defined peripheral and deep margins within the surrounding subcutaneous fat. The mass was heterogeneously contrast enhancing, and the fascial planes of the epaxial musculature did not appear to be affected (Figure 2).

Serum was submitted to the Pythium Laboratory^c at Louisiana State University for ELISA-based evaluation of anti-*P insidiosum* antibody concentrations. Results of this assay are expressed as percent positivity in comparison with a strong positive control sample, with values > 40% positivity having been shown to be 100% sensitive and specific for pythiosis in dogs and values in healthy dogs ranging from 3% to 15%.¹ Results in our patient were consistent with active pythiosis, with a percent positivity of 44%. Medical treatment was started with itraconazole (9 mg/kg [4 mg/lb], PO, q 24 h) and terbinafine (11 mg/kg [5 mg/lb], PO, q 24 h).

Surgical removal of the dorsal skin lesion was recommended because the location was amenable to wide surgical excision and because no definitive evidence of

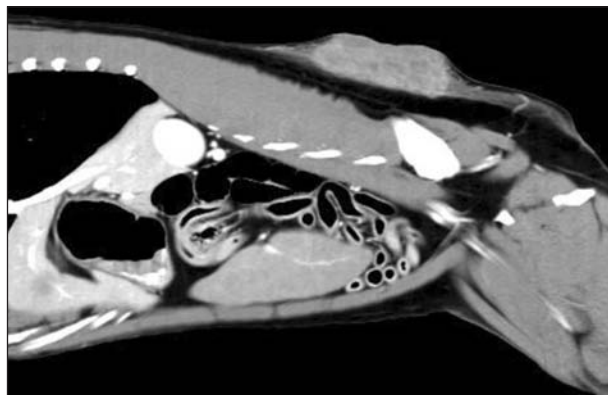


Figure 2—Computed tomographic image of the dog in Figure 1 following IV administration of contrast agent. The cutaneous mass is unencapsulated and has ill-defined peripheral and deep margins. The mass is heterogeneously enhancing with contrast uptake extending up to 3 cm from the mass within the surrounding soft tissues.

regional lymph node infection or distant disease extension had been detected. The dorsal mass was excised with 5-cm skin margins. Two fascial planes were included in the deep margin. This required removal of spinous processes of L5-L7 vertebrae and the wing of the right ilium. The wound was closed with an ipsilateral caudal superficial epigastric axial pattern flap rotated 90° dorsally to lie vertically covering the caudal end of the wound. In addition, a large single pedicle advancement flap was raised from the cranial dorsal thorax and advanced caudally to cover the cranial end of the wound and join the cranial extent of the axial pattern flap. The cut edges of the resected tissue were stained with India ink. Histologically, there was no evidence of infection extending to the surgical margins.

Postoperatively, the patient received an IV constant rate infusion of hydromorphone at 0.005 mg/kg/h (0.002 mg/lb/h) and lidocaine at 25 μ g/kg/min (11.4 μ g/lb/min) for analgesia. The wound was treated with laser therapy^d at 5 J/cm² once daily for 7 days to facilitate wound healing. Seven days after surgery, the wound became infected with *Enterococcus faecium*, which was susceptible to chloramphenicol. Following administration of chloramphenicol (38 mg/kg [17.3 mg/lb], PO, q 8 h for 12 days) and topical silver sulfadiazine, the infection resolved, and the wound healed without further complication. The dog was discharged after 18 days of hospitalization and was maintained on itraconazole (9 mg/kg, PO, q 24 h) and terbinafine (11 mg/kg, PO, q 24 h) for 3 months.

Serum samples were obtained and submitted for ELISA evaluation of anti-*P insidiosum* seroreactivity¹ at 1, 3, and 13 months following surgery, with results of 16%, 7%, and 6% positivity, respectively. Recheck examination at 20 months postoperatively showed no signs of recurrence at the surgical site.

Twenty-four months following surgery, the dog was examined for evaluation of multiple 2- to 3-cm cutaneous nodules that had been present for approximately 2 weeks. The owner reported that the dog had continued to swim frequently in lakes and ponds. Retesting of anti-*P insidiosum* antibody concentrations was performed, and results were negative at 8% positivity. Histologic

evaluation of masses on the left antebrachium, left crus, and ventrum indicated granulomatous inflammation with intralesional hyphae. Culture of tissue from one of these lesions yielded an isolate that was confirmed to be *P insidiosum* on the basis of amplification with a species-specific PCR assay.² In addition, histologic evaluation of a subcutaneous nodule near the base of the tail indicated hemangiosarcoma. As the locations of the lesions precluded aggressive surgical management, the dog was treated with wide excision of the *P insidiosum* lesions and the subcutaneous hemangiosarcoma, followed by administration of itraconazole (9 mg/kg, PO, q 24 h) and terbinafine (11 mg/kg, PO, q 24 h). The dog recovered uneventfully. As of 1 year after the second surgery (3 years after the initial surgery), the dog had had no recurrence of *P insidiosum* or hemangiosarcoma and was no longer permitted to swim in lakes or ponds.

Discussion

Pythium insidiosum is an oomycete that infects animals mainly in temperate, tropical, and subtropical climates.³⁻⁷ The organism requires an aqueous environment to sporulate, and the resultant zoospores are attracted to hair, broken skin, or open wounds.⁷ Therefore, it is not surprising that many animals with pythiosis have a history of exposure to water.^{3,5} Animals with cutaneous pythiosis are often initially examined for nonhealing wounds, masses, or chronic draining tracts.³⁻⁶ Cutaneous lesions are typically characterized as draining, ulcerated masses occurring on the limbs, ventral aspect of the thorax, perineum, tailhead, and abdomen.^{3,4} Medical management of pythiosis is rarely successful, with < 20% of animals responding to treatment with itraconazole and terbinafine.⁶ This poor response rate is likely attributable to the fact that traditional antifungal agents target ergosterol, which is generally lacking in the oomycete cell membrane. Because of the poor response to medical treatment, surgery is considered the treatment of choice for pythiosis when lesions are potentially resectable.

To our knowledge, this is the first report of survival > 2 years for a dog with large, truncal cutaneous pythiosis. Other reports³⁻⁵ suggest a poor prognosis associated with cutaneous pythiosis, often because lesions were considered nonresectable. Long-term (36-month) resolution of the initial lesion in the dog in the present report was most likely the result of aggressive surgical resection. Unfortunately, current guidelines^{3,5,6} for surgical resection of cutaneous pythiosis are vague. Recommendations for surgical management of cutaneous lesions include amputation if the lesion is located on an extremity^{3,6} or wide excision if located on the trunk of the body.⁶ Few specific guidelines are made, and the exact margins required are unknown. In our patient, the mass was treated as for a large, invasive soft tissue malignancy, and 5-cm margins of skin and deep margins of 2 fascial planes were obtained, including the dorsal spinous processes of underlying vertebrae and dorsal surface of the right ilium. Although it is impossible to predict whether a smaller excision would have been successful, we suspect that surgical excision in this patient was successful because of the deliberately aggressive margins obtained. We propose that similar

aggressive surgical management of 5-cm margins and 2 fascial planes be considered for other dogs with large cutaneous lesions of the trunk.

Prior to considering aggressive surgical resection, imaging of the thorax and abdomen (and tissue sampling, if indicated) should be performed to determine the extent of disease. Although disseminated pythiosis has been described in only a single dog,⁵ extension of infection to regional lymph nodes is common. In the dog described in the present report, enlarged medial iliac lymph nodes were detected on both CT and ultrasound examinations. Cytologic examination of aspirates from these lymph nodes was consistent with reactive lymphoid hyperplasia, and hyphae were not detected on GMS-stained slides. Therefore, lymphadenopathy alone may not be a contraindication to proceeding with surgery.

In the present patient, the CT examination was able to identify soft tissue changes that were impossible to detect by palpation. To our knowledge, the CT appearance of cutaneous pythiosis in a dog has not been previously described. Contrast CT images revealed an ill-defined area of contrast enhancement extending up to 3 cm into the surrounding palpably normal tissues (Figure 2). The extension of disease beyond the grossly palpable mass suggested that the local behavior of *P insidiosum* infection is similar to that of a high-grade neoplasm. Computed tomographic scans are often recommended prior to excision of a tumor to determine the extent of disease and detect metastasis. Margins suggested for resection of high-grade soft tissue sarcomas in animals are 3 to 5 cm for skin and 2 fascial planes deep.⁸ Because of the aggressive nature of cutaneous pythiosis and the CT findings of a poorly defined halo of disease extending several centimeters beyond the palpable edge of the lesion, 5-cm skin margins and 2 fascial planes were used as guidelines for resection in this dog.

It is not known to what degree the adjunct antifungal treatment contributed to the successful outcome in this patient. Postoperative medical treatment is often recommended in dogs with pythiosis, especially when the surgeon is not certain that clean margins were obtained. It has been the clinical experience of one of the authors (AMG) that anti-*P insidiosum* antibody concentrations may decrease rapidly in animals that have complete surgical resections, with percent positivity values decreasing by 50% or more within 2 months in most patients that go on to have no long-term (> 1 year) recurrence. In these patients, concentrations are often < 15% within 6 months after surgery. In contrast, values typically decrease more slowly in animals that respond to medical treatment alone, sometimes taking > 12 months to reach similar values. However, these are anecdotal observations that have not yet been published. The rapid decrease in anti-*P insidiosum* seroreactivity in the dog described here (from 44% before surgery to 16% 1 month after surgery) suggested that the surgical resection in this patient was complete and that the postoperative medical treatment may not have played an important role in the outcome. However, because the prognosis associated with recurrent disease is poor, postoperative medical treatment should be recommended for 2 to 3 months until recheck serologic testing results pro-

vide information about whether or not the surgical resection appears to have been complete.

Treatment options for pythiosis in human patients are similar to those available for dogs. Although potassium iodide, amphotericin B, itraconazole combined with terbinafine, and immunotherapy have been used successfully in a small number of isolated human cases, no medical treatment has been shown to have consistent efficacy.⁹⁻¹¹ Aggressive surgical treatment (including amputation) has also been pursued when cutaneous or vascular lesions are limited to a single limb. In previously described human patients with *P insidiosum* arteritis involving the lower extremities, amputation has occasionally been curative,⁹ but disease progression and death despite amputation have been reported with equal frequency.⁹

The dog described in the present report developed new *P insidiosum* lesions approximately 1 year after resection of the first lesion. Unfortunately, it is impossible to definitively determine whether the newer lesions represented recurrence of disease related to the original lesion or reinfection. However, the distant locations of the newer lesions, the duration of disease-free interval, the finding that anti-*P insidiosum* antibody concentrations had returned to the reference range after the initial surgery, and the history of continued exposure to the same aquatic environments all suggested that reinfection was more likely. It is interesting that anti-*P insidiosum* antibody concentrations were not elevated in this dog at the time the newer lesions developed. One possible explanation would be that the dog had not yet mounted a sufficient humoral immune response. However, this seems unlikely given that the lesions had been noted by the owners for 2 weeks prior to the time the sample was obtained for serologic testing. It is also possible that the dog's previous *P insidiosum* infection may have altered the humoral response to the recurrence or

reinfection. Regardless of the reason, it is important to note that serologic testing was not able to confirm the recurrence of pythiosis in this patient.

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- a. Resoroin ointment, Schuyler LLC, Vancouver, Wash.
 - b. Diff-Quik, Dade Diagnostics, Newark, Del.
 - c. Pythium Laboratory, Louisiana State University, Baton Rouge, La.
 - d. Gallium arsenide, 904 nm, 500 mW, Respond Systems Inc, Branford, Conn.
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References

1. Grooters AM, Leise BS, Lopez MK, et al. Development and evaluation of an enzyme-linked immunosorbent assay for the serodiagnosis of pythiosis in dogs. *J Vet Intern Med* 2002;16:142-146.
2. Grooters AM, Gee MK. Development of a nested polymerase chain reaction assay for the detection and identification of *Pythium insidiosum*. *J Vet Intern Med* 2002;16:147-152.
3. Dykstra MJ, Sharp NJH, Olivry T, et al. A description of cutaneous-subcutaneous pythiosis in fifteen dogs. *Med Mycol* 1999;37:427-433.
4. Neto RT, De MG Bosco S, Amorim RL, et al. Cutaneous pythiosis in a dog from Brazil. *Vet Dermatol* 2010;21:202-204.
5. Foil CSO, Short BG, Fadok VA, et al. A report of subcutaneous pythiosis in five dogs and a review of the etiologic agent *Pythium* spp. *J Am Anim Hosp Assoc* 1984;30:959-966.
6. Grooters AM. Pythiosis, lagenidiosis, and zygomycosis in small animals. *Vet Clin North Am Small Anim Pract* 2003;33:695-720.
7. Mendoza L, Hernandez F, Ajello L. Life cycle of the human and animal oomycete pathogen *Pythium insidiosum*. *J Clin Microbiol* 1993;31:2967-2973.
8. Vaccine-Associated Feline Sarcoma Task Force. The current understanding and management of vaccine-associated sarcomas in cats. *J Am Vet Med Assoc* 2005;226:1821-1842.
9. Prasertwitayakij N, Louthrenoo W, Kasitanon N, et al. Human pythiosis, a rare cause of arteritis: case report and literature review. *Semin Arthritis Rheum* 2003;33:204-214.
10. Shenep JL, English BK, Kaufman L, et al. Successful medical therapy for deeply invasive facial infection due to *Pythium insidiosum* in a child. *Clin Infect Dis* 1998;27:1388-1393.
11. Franco DM, Aronson JF, Hawkins HK, et al. Systemic *Pythium insidiosum* in a pediatric burn patient. *Burns* 2010;36:e68-e71.