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Veterinary Dermatology

CASE REPORT

# Intralesional amphotericin B in a cat with cutaneous protothecosis

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**Abstract**

A domestic cat was presented with nodular lesions on the nose/muzzle and pinnae. Protothecosis was diagnosed through cytological and histopathological examination, and culture. Molecular identification confirmed *Prototheca wickerhamii* infection. Intralesional application of amphotericin B in conjunction with oral terbinafine resulted in a significant reduction of the nasal lesion and complete resolution of the pinnal lesion, without adverse effects.

**KEYWORDS**

amphotericin B, cat, prototheca, protothecosis

## INTRODUCTION

The genus *Prototheca* consists of achlorophyllic microalgae that are ubiquitous in the environment and animal intestines.[1] *Prototheca wickerhamii* is associated with cutaneous disease and has been isolated from many affected cats,[2–6] although there also are reports of cats with *P. cutis*[7] and *P. bovis (*formerly *P. zopfii* genotype 2)[8] infections. Cats acquire *Prototheca* spp. after ingestion or following penetrating cutaneous injury,[1] and most of them exhibit only skin involvement.[2–9] Diagnosis of protothecosis is based on cytological and/or histopathological examination and microbiological culture. PCR and mass spectrometry enable the identification of *Prototheca* species.[1]

Intralesional use of amphotericin B has been reported in cats for the treatment of feline sporotrichosis.[10] Oral terbinafine was used in a case of disseminated protothecosis in a dog, stalling the progression of disease with the improvement of cutaneous lesions, despite the fatal outcome expected in most of the disseminated infections,[11] and also has been mentioned by some authors as a possible option against *Prototheca* spp.[1] Combination therapy is likely to be superior to monotherapy with a single azole in most cases of Protothecosis.[1] Although amphotericin B is known to be a therapeutic option for protothecosis in humans and animals, to the best of the authors' knowledge, this is the first published report of its intralesional application for the treatment of cutaneous protothecosis in a cat.

## CASE REPORT

A seven-year-old, 4 kg, intact female, domestic longhair cat was presented with a history of nodular lesions affecting the nasal planum and right pinnal margin. No other clinical signs were reported. The owner reported that the mass had grown for three years and had been diagnosed as cryptococcosis. Before referral to the dermatology service, previous oral treatment with itraconazole, 100 mg once daily for five months, ketoconazole, 10 mg/kg once daily for one month, dexamethasone (unknown dose and duration), and ampicillin (unknown dose and duration) had failed to produce clinical improvement. Before adoption, the animal had free access to the outdoors in a humid environment, and with access to an open sewer system. Other animals with which the cat had contact, including one other cat and two dogs, exhibited no clinical signs of skin disease.

  

Upon physical examination, a nodular lesion involving the philtrum, nasal planum, and bridge of the muzzle, measuring approximately 5 cm in diameter was observed (Figure 1a). The lesion was partially ulcerated, and the nostrils were moderately occluded producing a perceptible respiratory stertor. A smaller nonulcerated nodule, measuring approximately 1 cm in diameter, was evident on the edge of the right pinna (Figure 1b). The cat was mildly underweight yet otherwise appeared to be in good health.

Analysis of a complete blood count and serum biochemical profile revealed mild neutrophilia (15.705/mm$^3$), mild lymphopenia (1.396/mm$^3$), hyperproteinemia (8.2 g/dL), hyperglobulinemia (5.21 g/dL) and a slight increase in alanine aminotransferase (65.4 UI/l). Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) tests were both negative. Abdominal ultrasound and thoracic radiographs also were unremarkable. Cytological examination of a fine needle aspirate from the nasal lesion, stained with a modified Wright–Giemsa stain, revealed a pyogranulomatous inflammatory reaction with moderate numbers of macrophages and degenerating neutrophils. Numerous round micro-organisms with slightly basophilic cytoplasm and a thick clear halo were evident, mostly extracellularly. These organisms also showed internal septations, giving them a morula-like appearance (Figure 2a), morphologically consistent with *Prototheca* algae.

Four punch biopsies (6 mm) were collected from the large nodule. Fragments were processed for histopathological examination and microbiological culture. Histopathological analysis showed an inflammatory infiltrate of the dermis and subcutis with an abundance of neutrophils, macrophages and giant cells, as well as lymphocytes and plasma cells, and numerous PAS-positive encapsulated round organisms, 20–30 μm in diameter, exhibiting internal endospores and a morula shape (Figure 2b). Organisms were located within the cytoplasm of macrophages and extracellularly within the tissue, compatible with *Prototheca* species.

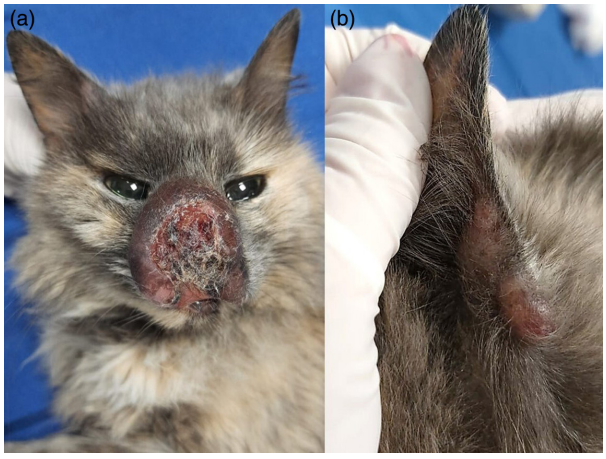


**FIGURE 1** (a) Nodular lesion affecting the nasal planum, philtrum and muzzle, measuring approximately 5 cm in diameter, exhibiting surface ulceration; (b) smaller nonulcerated nodule, measuring approximately 1 cm in diameter, on the edge of the right pinna.

Tissue fragments were plated to defibrinated sheep blood agar medium (5%) and MacConkey agar medium and incubated under aerobic conditions for three days at 37°C. Simultaneously, fragments were inoculated onto Sabouraud agar medium, and incubated aerobically at 37°C. Yeast-like colonies, 1–2 mm in diameter, were observed by Day 15, and microscopically showed single and endosporulating cells suggestive of *Prototheca* algae. Further analysis of the isolate using a PCR based on the mitochondrially-encoded cytochrome b (*Cytb*) gene marker confirmed the agent as *P. wickerhamii*.[12]

Treatment with oral terbinafine, 20 mg/kg once daily, plus intralesional administration of amphotericin B was initiated. A flask containing 50 mg of amphotericin B was diluted in 10 mL of distilled water to obtain a final concentration of 5 mg/mL. The cat was anaesthetised with dexmedetomidine 10 μg/kg intramuscularly, ketamine 4 mg/kg i.m. and methadone 0.3 mg/kg i.m., and was maintained on intravenous fluid therapy with 0.9% NaCl and oxygen mask throughout the procedure. Parameters such as blood pressure and oxygen saturation were monitored continuously, without exhibiting abnormal changes. Amphotericin B was directly infiltrated into the lesions with a needle and syringe until swelling was achieved. The needle was moved in different directions to guarantee infiltration of the whole lesion (Figure 3). The final volumes applied were 1.5 and 0.5 mL, into the nasal and pinnae nodules, respectively. The cat received three applications at two weeks intervals.

After applications, the cat exhibited a reduction of about 50% in the size of the nasal lesion with a resolution of ulceration, and a complete reduction of the pinnal lesion. (Figure 4a,b). No adverse effects were noted, apart from mild lethargy and hyporexia during the day of the procedure. Repeated blood analyses

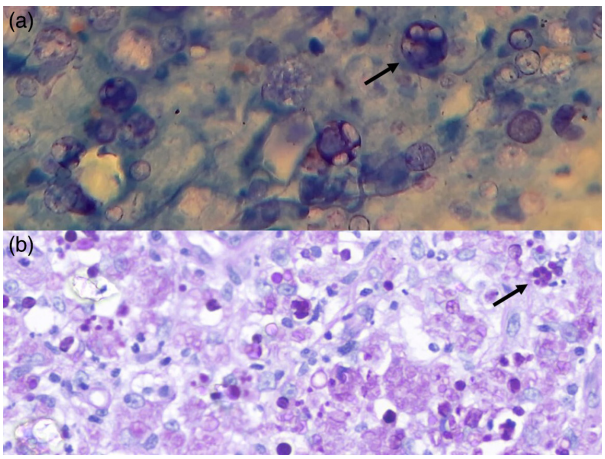


**FIGURE 2** (a) Cytological features of a fine–needle aspirate obtained from the nasal lesion. Unicellular round- to oval-shaped structures of different sizes with a deeply basophilic granular cytoplasm surrounded by a thin cell wall. Some structures display internal septation with a variable number of endospores (black arrow). Modified Wright–Giemsa stain, ×100. (b) Histopathological features of the nasal nodule. There is an inflammatory infiltrate of the dermis and subcutis with an abundance of neutrophils, macrophages and giant cells, as well as lymphocytes and plasma cells, and numerous periodic acid Schiff–positive staining encapsulated round–shaped organisms (20–30 μm), some of which exhibit a morula shape (black arrow).

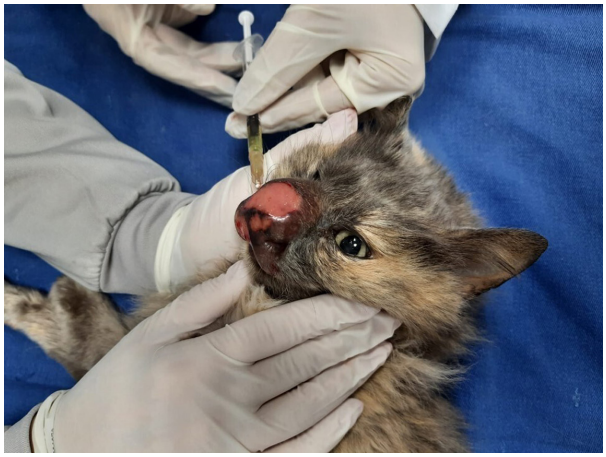

**FIGURE 3** Administration of intralesional amphotericin B into the large nodular lesion on the muzzle.

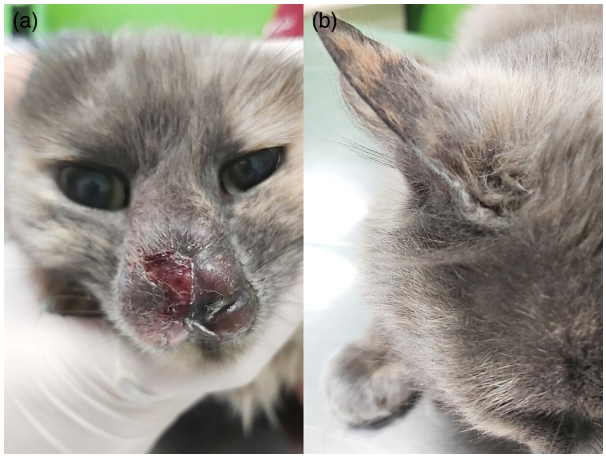


**FIGURE 4** (a) Reduction of lesion volume visually approximates 50% after three intralesional injections of amphotericin B. (b) Complete resolution of the pinnal nodule.

showed no changes. Despite the recommendation to continue amphotericin B applications until complete resolution of the nasal lesion was achieved, the owner declined additional injections as a result of the need for general anaesthesia. The cat was maintained on daily oral terbinafine monotherapy and at each of the subsequent seven monthly rechecks, the nasal lesion remained static and there was no recurrence of the pinnal lesion (Figure 5a,b), which was acceptable to the owner.

## DISCUSSION

To the best of the authors´ knowledge, this is the first report describing the use of intralesional amphotericin B combined with oral terbinafine in the treatment of a cat with cutaneous protothecosis, which was refractory to azole monotherapies. This novel therapy produced partial clinical improvement of the lesions without adverse effects.

Previously reported treatments for feline cutaneous protothecosis consist of surgical excision of the lesions,[9] oral ketoconazole combined with topical terbinafine[8] and oral itraconazole with surgical resection.[5,6]


**FIGURE 5** (a) Following six months of daily maintenance therapy with oral terbinafine, the nasal lesions remained unchanged. (b) The pinnal nodule had not recurred.

Surgical excision was not considered an option for our case owing to the size and location of the nasal lesion, and the inability to achieve clean borders. Our patient did not demonstrate any improvement after several months of oral itraconazole therapy, nor to a shorter course of ketoconazole. This lack of response to azoles is consistent with similar cases reported in cats and could be explained by the fact that ergosterol is less relevant for the algal cell wall than for the fungal cell wall.[6]

Amphotericin B is a useful agent for the treatment of protothecosis. In dogs, it has been used intravenously or subcutaneously as a monotherapy or combined with azoles,[1] and intrathecally in a case affecting the nervous system.[13] In cats, it has been applied intravenously or subcutaneously in serious mycoses, such as disseminated forms of sporotrichosis.[10] The duration of intravenous amphotericin B therapy can be limited by cephalic or jugular thrombosis and nephrotoxicity.[10] Intralesional amphotericin B has been administered in cats with sporotrichosis refractory to oral medications,[10] and has not been reported in the treatment of cutaneous protothecosis.

The intralesional route of administration is associated with high tissue concentrations, tissue conservation and few adverse systemic consequences.[10] In the present case, the decision to administer intralesional amphotericin B with oral terbinafine was made as a consequence of reports in other species which suggest better efficacy of the combination versus amphotericin B alone.[1] Terbinafine, an allylamine antifungal, was selected based on the cat's poor response to azoles. In addition, oral terbinafine has been reported to stall the systemic progression of protothecosis in a dog, while improving cutaneous lesions.[11]

In spite of a recommendation to dilute amphotericin B in local anaesthetics,[10] we used the standard concentration of 5 mg/mL of amphotericin B in distilled water, applying a final volume of 1.5 mL into the largest nodule and 0.5 mL into the smallest one, as described in other studies for deep mycosis, which recommend a drug volume of 0.5–1.5 mL per application.[10] Adverse effects reported previously include oedema and sterile abscessation,[10] complications that were not observed

in our case. Furthermore, no significant haematological changes were noticed. The cat received three applications at two weeks intervals, demonstrating a reduction of about 50% of the nasal lesion, and exhibiting improvement in ulceration and a complete resolution of the pinnae lesion without recurrence.

Our observations suggest that intralesional amphotericin B in conjunction with oral terbinafine could be an interesting option in cases of feline cutaneous protothecosis caused by *P. wickerhamii* refractory to other (oral) therapies. It is important to highlight that the positive clinical outcome observed in this cat was the result of a combined therapy; thus it cannot be known if amphotericin B would have been an effective monotherapy. Prospective clinical trials are needed to establish the most reliable dose and frequency regimen.

## AUTHOR CONTRIBUTIONS

**Wendie Roldán Villalobos:** conceptualisation; investigation; writing—original draft; methodology; validation; writing—review and editing; supervision. **Tássia Ferreira:** investigation; methodology; writing—review and editing. **Vanessa Gmyterco:** investigation; methodology. **Renata Bastos:** methodology. **Louise Bacher:** methodology. **Tomasz Jagielski:** investigation; methodology. **Márcio Ribeiro:** investigation; methodology; writing—review and editing. **Marconi Farias:** conceptualisation; investigation; methodology; validation; writing—review and editing; supervision.

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## CONFLICT OF INTEREST STATEMENT

None declared.

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## REFERENCES

1. Masuda M, Jagielski T, Danesi P, Falcaro C, Bertola M, Krockenberger M, et al. Protothecosis in dogs and cats—new research directions. Mycopathologia. 2021;186:143–52.
2. Kaplan W, Chandler FW, Holzinger EA, Plue RE, Dickinson RO 3rd. Protothecosis in a cat: first recorded case. Sabouraudia. 1976;14:281–6.
3. Coloe PJ, Allison JF. Protothecosis in a cat. J Am Vet Med Assoc. 1982;180:78–9.
4. Dillberger JE, Homer B, Daubert D, Altman NH. Protothecosis in two cats. J Am Vet Med Assoc. 1988;192:1557–9.
5. Endo S, Sekiguchi M, Kishimoto Y, Kano R, Aoki S, Sichinohe T, et al. The first case of feline *Prototheca wickerhamii* infection in Japan. J Vet Med Sci. 2010;72:1351–3.
6. Kessell AE, McNair D, Munday JS, Savory R, Halliday C, Malik R. Successful treatment of multifocal pedal *Prototheca wickerhamii* infection in a feline immunodeficiency virus–positive cat with multiple Bowenoid in situ carcinomas containing papillomaviral DNA sequences. JFMS Open Rep. 2017;3:2055116916688590.
7. Maboni G, Elbert JA, Stilwell JM, Sanchez S. Genomic and pathologic findings for *Prototheca cutis* infection in cat. Emerg Infect Dis. 2021;27:979–82.
8. Huth N, Wenkel RF, Roschanski N, Rösler U, Plagge L, Schöniger S. *Prototheca zopfii* genotype 2–induced nasal dermatitis in a cat. J Comp Pathol. 2015;152:287–90.
9. Finnie JW, Coloe PJ. Cutaneous protothecosis in a cat. Aust Vet J. 1981;57:307–8.
10. Gremião I, Schubach T, Pereira S, Rodrigues A, Honse C, Barros M. Treatment of refractory feline sporotrichosis with a combination of intralesional amphotericin B and oral itraconazole. Aust Vet J. 2011;89:346–51.
11. Milley CD, Labelle P, Tolar E, Griffin J, Reeder C. *Prototheca wickerhamii* in a dog with cutaneous, ocular and systemic manifestations and treatment with terbinafine. Vet Rec Case Rep. 2015;3:e000225.
12. Jagielski T, Gawor J, Bakuła Z, Decewicz P, Maciszewski K, Karnkowska A. *Cytb* as a new genetic marker for differentiation of *Prototheca* species. J Clin Microbiol. 2018;56:e00584-18.
13. Young M, Bush W, Sanchez M, Gavin P, Williams M. Serial MRI and CSF analysis in a dog treated with intrathecal amphotericin B for protothecosis. J Am Anim Hosp Assoc. 2012;48:125–31.

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**摘要**

一只家猫的鼻子/口周和耳廓出现结节性病变。通过细胞学、组织病理学检查和培养，诊断出原藻病。分子鉴定证实了魏氏原壁菌感染。应用两性霉素B联合特比萘芬口服，病灶内可见鼻腔病变显著减少，并完全消除耳廓病变，无不良药物反应。

**Résumé**

Un chat domestique est présenté avec des lésions nodulaires sur le nez/museau et le pavillon auriculaire. La protothécose est diagnostiquée par un examen cytologique et histopathologique, ainsi que par une culture. L'identification moléculaire confirme l'infection par *Prototheca wickerhamii*. L'application intralésionnelle d'amphotéricine B, associée à la terbinafine orale, permet une réduction significative de la lésion nasale et une résolution complète de la lésion du pavillon auriculaire, sans effets indésirables.

**Zusammenfassung**
Eine Hauskatze wurde mit knotigen Läsionen auf der Nase/dem Maul und an den Pinnae vorgestellt. Mittels zytologischer und histopathologischer Untersuchung und einer Kultur wurde eine Protothekose diagnostiziert. Mittels molekularer Identifizierung wurde eine Infektion mit *Prototheca wickerhamii* bestätigt. Eine intraläsionale Verabreichung von Amphotericin B in Kombination mit Terbinafin *per os* resultierte in einer signifikanten Verbesserung der Nasenläsionen und einer völligen Abheilung der Läsionen an den Pinnae, ohne dass Nebenwirkungen auftraten.

**要約**
**鼻/マズルおよび耳介に結節性病変を有する家猫が受診した。細胞学的検査、病理組織学的検査および培養検査によりProtothecosisと診断した。分子生物学的同定により*Prototheca wickerhamii*感染が確認された。アムホテリシンBの鼻腔内投与およびテルビナフィンの経口投与を併用した結果、鼻病変は有意に縮小し、耳介病変は副作用なく完全に消失した。**

**Resumo**
Um gato doméstico foi apresentado com lesões nodulares no nariz/focinho e pavilhões auriculares. Prototecose foi diagnosticada por exame citológico e histopatológico, e cultura. A identificação molecular confirmou a infecção por *Prototheca wickerhamii*. Aplicação intralesional de anfotericina B associada à terbinafina por via oral resultou em redução significativa da lesão nasal e resolução total da lesão na orelha, sem efeitos adversos.

**RESUMEN**
Un gato doméstico se presentó con lesiones nodulares en la nariz/hocico y orejas. Se diagnosticó prototecosis mediante examen citológico, histopatológico y cultivo. La identificación molecular confirmó la infección por *Prototheca wickerhamii*. La aplicación intralesional de anfotericina B junto con terbinafina oral dio como resultado una reducción significativa de la lesión nasal y una resolución completa de la lesión auricular, sin efectos adversos.