TREATMENT OF MYCOTIC RHINOSINUSITIS IN A BENNETT'S WALLABY (*MACROPUS RUFOGRISEUS*) USING TOPICAL VORICONAZOLE SUSPENDED IN A REVERSE THERMODYNAMIC PLURONIC GEL

Author(s): Josephine Bryk Rose, D.V.M., Sarah Davies, B.V.Sc., M.S., Dipl. A.C.V.R., Kadie M. Anderson, D.V.M., Graeme S. Allan, B.V.Sc., Dipl. A.C.V.R., F.A.C.V.Sc., Patricia M. Dennis, D.V.M., Ph.D., Dipl. A.C.Z.M., and Richard Malik, D.V.Sc., Ph.D., F.A.C.V.Sc.
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TREATMENT OF MYCOTIC RHINOSINUSITIS IN A BENNETT'S WALLABY (*MACROPUS RUFOGRISEUS*) USING TOPICAL VORICONAZOLE SUSPENDED IN A REVERSE THERMODYNAMIC PLURONIC GEL

Josephine Bryk Rose, D.V.M., Sarah Davies, B.V.Sc., M.S., Dipl. A.C.V.R., Kadie M. Anderson, D.V.M., Graeme S. Allan, B.V.Sc., Dipl. A.C.V.R., F.A.C.V.Sc., Patricia M. Dennis, D.V.M., Ph.D., Dipl. A.C.Z.M., and Richard Malik, D.V.Sc., Ph.D., F.A.C.V.Sc.

Abstract: An approximately 4-yr-old female Bennett's wallaby (*Macropus rufogriseus*) was evaluated for chronic left-sided facial swelling and nasal discharge. Computed tomography, endoscopy, biopsy, mycologic culture, and panfungal polymerase chain reaction were consistent with destructive mycotic rhinosinusitis. The patient's infection was treated with a long-term injectable antibiotic, oral antifungal therapy, and multiple intranasal infusions of voriconazole suspended in a reverse thermodynamic pluronic gel. This case represents the first documented case of mycotic rhinosinusitis in a macropod and underlines the importance of advanced cross-sectional imaging in the diagnosis, monitoring, and management of nasal cavity disease in zoo animals.

Key words: Bennett's wallaby, computed tomography, *Macropus rufogriseus*, mycotic rhinosinusitis, pluronic.

BRIEF COMMUNICATION

A female Bennett's wallaby (*Macropus rufogriseus*; approximately 4 yr) presented with a left-sided facial swelling in December 2015. One year prior, the wallaby had blepharospasm and suppurating facial swelling that resolved with treatment (Table 1). A purulent discharge occluded the left naris. Skull radiographs showed no dental abnormalities. The swelling resolved with anti-inflammatory and antibiotic treatment (Table 1).

The swelling recurred in January 2016 (Table 1). Computed tomography (CT) of the head demonstrated bilateral soft-tissue opacity throughout both frontal sinuses and loss of turbinates on the left side of the nasal cavity (Fig. 1). The fluctuant swelling was incised. A segment of frontal bone (1-cm diameter) at this location was eroded. The nasal turbinates were debrided and flushed. The lumen of this debrided region was then infused with a pluronic polymer (Pluronic F127, 30%; compounded by GEM Edwards Pharmacy, Hudson, Ohio 44236, USA) instilled with ceftazidime, as described by McBride,[11] and the incision was closed. *Cryptococcus* antigen by latex agglutination was negative, but *Aspergillus* antibody testing by radial immunodiffusion was positive. Antibiotic and anti-inflammatory therapies were initiated (Table 1).

Three weeks later, a firm yellow plaque was identified in the nasal cavity using an otoscope. CT demonstrated increased air space (Fig. 1). Endoscopy of the left nasal cavity demonstrated tiny mucosal nodules rostral to the lytic region. Rare yellow plaques were visualized, but friable tissue precluded plaque biopsy. Several blind biopsies were collected and submitted for histopathology. Mucosal surface samples were collected for culture and susceptibility testing for fungi and aerobic and anaerobic bacteria.

Histologically, there was severe lymphoplasmacytic and eosinophilic sinusitis with seromucous glandular hyperplasia, follicular lymphocytic hyperplasia, mild bony remodeling, and plant foreign body fragments. Methenamine silver staining revealed a focal clump of fungal hyphae more bulbous and less parallel than is typical for *Aspergillus* species. Polymerase chain reaction (PCR) testing for the presence of 5.8S rDNA and intergenic spacer regions 1 and 2 was positive,

From the Cleveland Metroparks Zoo, 3900 Wildlife Way, Cleveland, Ohio 44109, USA (Rose, Anderson, Dennis); Veterinary Imaging Associates, P.O. Box 300, Saint Leonards, New South Wales, Australia, 1590 (Davies, Allan); and The University of Sydney, B22 Veterinary Science Conference Centre, New South Wales, Australia 2006 (Malik). Present addresses (Rose): Indianapolis Zoo, 1200 West Washington Street, P.O. Box 22309, Indianapolis, Indiana 46222, USA; (Anderson): Point Defiance Zoo and Aquarium, 5400 North Pearl Street, Tacoma, Washington 98407, USA. Correspondence should be directed to Dr. Rose (jrose@indyzoo.com).

Table 1. Treatment timeline of 4-yr-old female Bennett's wallaby (*Macropus rufogriseus*) with fungal rhinitis.

Date	Clinical findings	Procedure	Antibiotic	Anti-inflammatory	Oral antifungal	Clinical outcome
22 Nov 2014	Left-sided facial swelling Monocytosis 910 cells/μl (37–519 cells/μl)[14]	Lanced and placed drain	Ceftiofur CFA[a] 16 days			Swelling resolved
01 Dec 2015	Swelling recurred Monocytosis 1,950 cells/μl Culture negative	Ceftiofur CFA 14 days	Meloxicam[b] sq			Swelling resolved
22 Jan 2016	Swelling recurred Monocytosis 1,680 cells/μl	Lanced and placed drain	Ceftiofur CFA once	Meloxicam sq		
27 Jan 2016	CT: Frontal bone destruction *Aspergillus* AB positive	Debridement Ceftazidime[d] pluronic infusion	Ceftiofur[c] start	Meloxicam im sid 3 days		Swelling resolved
16 Feb 2016	CT: improved sinusitis Plaques visible *Penicillium* sp. cultured PCR fungal DNA Hyphae on biopsy					Static
03 Mar 2016	CT: unchanged Monocytosis 1,254 cells/μl Galactomannan index negative	Voriconazole[e]-pluronic treatment (no. 1)		Meloxicam im		Static
04 Mar 2016					Itraconazole started	Static
29 Mar 2016		Ceftiofur completed		Terbinafine[g] started		Static
18 Apr 2016	CT: turbinate regrowth Few nasal plaques No monocytosis Negative fungal culture Biopsy: reduced inflammation, no hyphae	Voriconazole-pluronic treatment (no. 2)		Meloxicam im		Static
11 Jun 2016					Terbinafine stopped	Static
26 Sep 2016	CT: turbinate improvement	Voriconazole-pluronic treatment (no. 3)	Ceftiofur	Meloxicam im		Static
14 Dec 2016	No plaques or nodules	Voriconazole-pluronic treatment (no. 4)			Itraconazole stopped	Firm fibrous callus over frontal bone defect

[a] Ceftiofur crystalline-free acid (CFA), Excede®, Zoetis, Parsippany, New Jersey 07054, USA; 6.6 mg/kg im q72 hr.
[b] Loxicom®, Norbrook Inc., Overland Park, Kansas 66210, USA; 0.2 mg/kg.
[c] Naxcel®, Zoetis; 2.2 mg/kg im sid.
[d] Tazicef™, Hospira, Lake Forest, Illinois 60045, USA.
[e] VFend®, Pfizer, New York City, New York 10017, USA.
[f] Itraconazole, 100-mg capsules, Sporanox®, Titusville, New Jersey 08560, USA; 7.3 mg/kg po sid.
[g] Terbinafine, 250-mg tablets, Lamisil®, Novartis Pharmaceuticals Corporation, East Hanover, New Jersey 07936, USA; 8.3 mg/kg po sid.

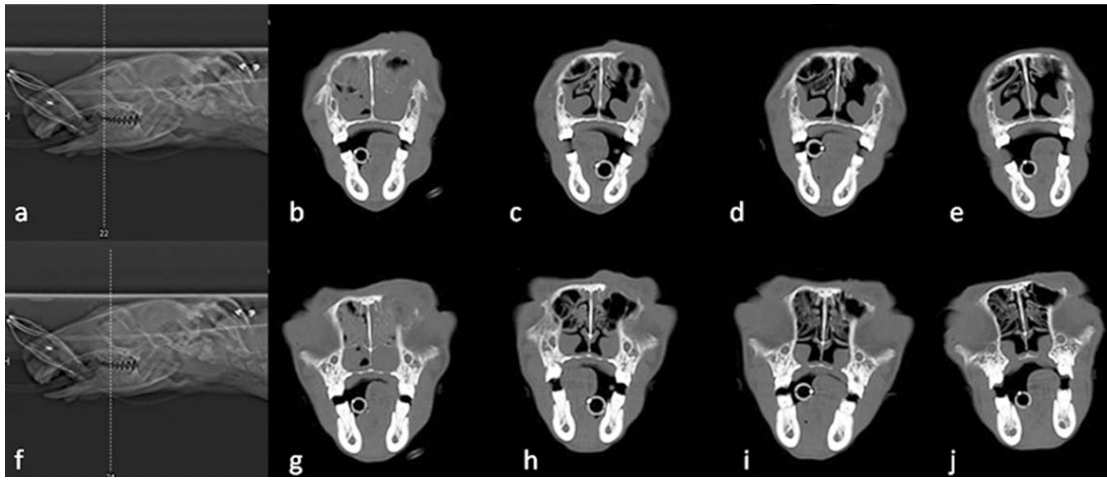

Figure 1. CT images of approximately 4-yr-old female Bennett wallaby (*Macropus rufogriseus*) at initial diagnosis and at treatment revisits. **A.** and **F.** are CT scout images each showing a hashed localizer line that indicates slice location of axial images presented to the right. Level **F.** is very slightly caudal to level **A.** From left to right, CT images were obtained in January (**B.** and **G.**), February (**C.** and **H.**), April (**D.** and **I.**), and September (**E.** and **J.**) of 2016. Initial CT imaging (**B.** and **G.**) shows increased soft tissue and fluid density within the left and right sides of the nasal cavity and within the frontal sinuses. The left choana is obstructed. There is expansile lysis of the dorsal aspect of the left maxilla and left frontal bone, and there is soft-tissue swelling dorsal to the left maxilla and frontal bone within subcutaneous soft tissues. Progressively, there is reduced soft tissue and fluid-dense material in the nasal cavity and frontal sinuses. Nasal turbinate density is reduced in the left side of the nasal cavity, with some progression of turbinate lysis and atrophy between February and September. There is remodeling of the dorsal aspect of the left maxilla and left frontal bone, with a more normal contour developing between April and September. Subcutaneous soft-tissue swelling dorsal to the left side of the nasal cavity and left frontal sinus improves and resolves over time.

indicating ascomycetes (fungal) DNA was present. PCR amplification for *Aspergillus* DNA was negative. Both a culturette and a section of tissue produced two colonies of *Penicillium* spp.

On 3 March 2016, the patient was anesthetized for topical intranasal antifungal therapy. The debridement site was reopened aseptically. The frontal sinus was flushed with saline and reaspirated for cytology (Table 1). To prepare the infusion, half of a 200-mg voriconazole tablet was pulverized and mixed with 2 ml of propylene glycol (Table 1). Immediately prior to the infusion, the propylene glycol-voriconazole solution was suspended in 7 ml of pluronic reverse-thermodynamic polymer by flushing between two connected syringes situated on an ice pack. The left maxillary sinus was instilled with the suspension through the incision over the frontal sinus by using a 14-ga red rubber catheter; the incision was then sutured. The right maxillary sinus was infused via the right naris with a separate 10 ml of suspension in a similar manner. Oral itraconazole was started the following day. Four weeks later, oral terbinafine was added to the regimen (Table 1). On 8 March 2016, cryopreserved serum from 16 February 2016 (prior to any antifungal administration) was analyzed for *Aspergillus* galactomannan, and the level was 0.3; an index value considered negative for disseminated disease.[5,15]

On 18 April 2016, the patient was reevaluated. Monocytosis was no longer present (Table 1). CT demonstrated better aeration of the nasal sinuses. Rhinoscopy demonstrated smooth, pink nonfriable mucosa with some yellow plaques in the left nasal cavity. Biopsies and specimens for fungal and aerobic and anaerobic bacterial culture were collected. Following biopsy collection, both sinonasal cavities were infused with a voriconazole-pluronic suspension via the nares. Hemorrhage developed postbiopsy and infusion. Bleeding was controlled with digital pressure and cotton-tipped applicators soaked with 0.1% epinephrine. Hemorrhage recurred upon recovery, and the patient became dyspneic. A complete recovery was made with assisted ventilation via a resuscitator bag. Histologic

examination demonstrated less severe inflammation than in previous biopsies. Turbinates showed mild remodeling. Methenamine silver staining was negative for fungi. Culture produced three colonies of an *Aspergillus* spp. (not *Aspergillus niger* or *Aspergillus fumigatus*) and light growth of mixed upper respiratory flora (Table 1). Terbinafine was discontinued on 11 June 2016.

The patient was reevaluated on 26 September 2016 (Table 1). CT showed decreased soft-tissue opacity in the frontal sinuses, with some bony regrowth of turbinates (Fig. 1). A small, nonadhered yellow plaque was detected in the rostral left nasal cavity with an otoscope. This plaque was swabbed and submitted for cytology and culture. Both nasal passages were infused with a voriconazole-pluronic suspension for a third treatment. Cytology of the plaque demonstrated slender beaded filamentous organisms that resembled *Actinomyces* spp., but the culture produced moderate mixed upper respiratory flora with no fungi or *Actinomyces* spp.

The patient was anesthetized for final evaluation on 14 December 2016. Keepers reported chronic mucoid nasal discharge since the previous infusion in September. Nasal examination demonstrated no plaques or nodules. The mucosa was no longer friable on probing. Both nasal passages were infused with a final voriconazole-pluronic treatment. Blood was collected for a trough plasma itraconazole level (24 hr after the last dose). Neither itraconazole nor its hydroxyl metabolite was detectable. Itraconazole was discontinued. It has been 11 mo since the last antifungal infusion, and the wallaby has had no recurrence of facial swelling.

To the authors' knowledge, this is the first reported case of fungal rhinosinusitis in a macropod. Mycotic rhinosinusitis is well documented in dogs (*Canis familiaris*)[4] but less commonly in cats (*Felis catus*).[3] The most common upper respiratory tract infection in marsupials is cryptococcosis (*Cryptococcus gattii*), with koalas (*Phascolarctos cinereus*) as the most affected species.[1,7]

Aspergillus species (*A. fumigatus*, *A. niger*, and *A. flavus*) are the most common causes of fungal rhinosinusitis in dogs, while *Penicillium* spp. and *Scedosporium* spp. are implicated less frequently.[2,4] Cryptococcosis is also an important cause of sinonasal disease, especially in cats.[8] All these agents are ubiquitous environmental saprophytes.[4] Affected animals are typically systemically healthy. Risk factors for infection in dogs include dolichocephalic head conformation, young age, large breed, and possibly a history of nasal trauma or foreign body inhalation.[4,9] The wound observed on this wallaby's face and the plant foreign material found on biopsy suggest that such factors may have played an etiologic role in this patient.

Definitive diagnosis was challenging. There is no single clinical or laboratory test to conclusively diagnose nasal aspergillosis in dogs or cats,[12] and no serologic test has been established for macropods. Though the *Aspergillus* antibody assay by radial immunodiffusion was positive, fungal elements were visualized microscopically in biopsy material, and the mucosal surface cultured *Aspergillus* spp., none of the cultures, cytology, or biopsies confirmed the presence of *A. niger* or *A. fumigatus*. However, any species of *Aspergillus* is potentially pathogenic.[4] The radial immunodiffusion assay for *Aspergillus* antibodies has a specificity of 98% and a positive predictive value of 93% in dogs.[12] The positive antibody result was almost certainly valid, as the fungal plaques observed were consistent with an *Aspergillus* or *Penicillium* species infection. The negative galactomannan suggests either the infection was not sufficiently extensive or invasive,[5] galactomannan was not released at that stage in the fungal growth cycle of *Aspergillus*,[4] or the cause of the sinusitis was another fungus that did not produce galactomannan.[5,15] *Penicillium* spp. was cultured from a blind biopsy sample, suggesting it may have been the etiologic agent. However, this culture may have represented contaminant spores trapped in the mucus overlying the nasal mucosa. Despite these reservations, the unequivocal visualization of fungal plaques with anterior rhinoscopy, microscopic demonstration of fungal elements in biopsy material, positive PCR amplicon, and the positive response to antifungal therapy (based on serial CT and endoscopic examinations), strongly support a diagnosis of fungal rhinosinusitis. The favorable long-term response would not have occurred with neoplastic or untreated dental disease.

The pluronic-voriconazole instillation overcame several challenges presented by intranasal liquid azole infusions, the treatment of choice for dogs and cats with sinonasal aspergillosis.[4,9] Poloxamer pluronic is a reverse thermodynamic gel polymer that is a liquid when refrigerated but hardens to a gelatinlike consistency that maintains its form at room and body temperature. Infusing the pluronic gel containing the potent triazole

voriconazole permitted all surfaces of the nasal sinuses to be treated with a product that slowly exudes active antifungal therapy for a period likely in excess of 2 wk. Previous studies with a poloxamer infused with clotrimazole in healthy beagles demonstrated that it was absorbed over the course of 2 wk, producing minimal inflammatory response histologically.[10] Voriconazole was chosen as it is has demonstrated efficacy against *Aspergillus* species, including cryptic *Aspergillus* spp. and other hyphomycetes, such as *Penicillium* spp.

Systemic treatment is often used as an adjunct to topical antifungal therapy. Systemic treatment alone can require months to years to clear an infection.[4,9] In this wallaby, the undetectable trough level at 24-hr postadministration suggests either that the drug had poor bioavailability or that itraconazole was degraded to pharmacologically inactive metabolites by rapid hepatic biotransformation.[6] It is likely that either a higher dose of itraconazole or a shorter dosing interval is required in macropods. Close observation and serial monitoring of this animal is warranted. Delayed recurrence has been reported 2 mo to 3 yr after resolution of infection has occurred in dogs.[13]

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