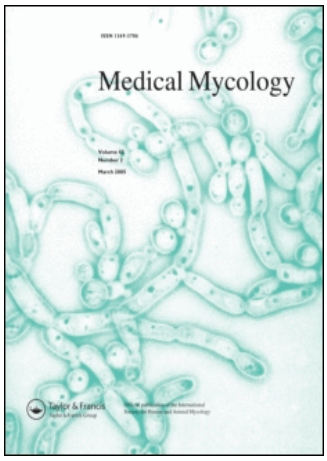

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Case Report

Deep fungal dermatitis in three inland bearded dragons (*Pogona vitticeps*) caused by the *Chrysosporium* anamorph of *Nannizziopsis vriesii*

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The *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV), a keratinophilic fungus that naturally and experimentally causes severe and often fatal dermatitis in multiple reptile species, was isolated in pure culture from skin samples of three inland bearded dragons (*Pogona vitticeps*) with deep granulomatous dermatomycosis. The first animal presented with a focal maxillary swelling involving the skin and gingiva. This lizard died while undergoing itraconazole and topical miconazole therapy. The second presented with focally extensive discoloration and thickening of the skin of the ventrum and was euthanized after 10 weeks of itraconazole therapy. A third lizard presented with hyperkeratotic exudative dermatitis on a markedly swollen forelimb. Amputation and itraconazole therapy resulted in a clinical cure. Histopathology of tissue biopsies in all cases demonstrated granulomatous dermatitis with intralesional hyphae morphologically consistent with those produced by the CANV. The second lizard also had granulomatous hepatitis with intralesional hyphae. Evidence in this report suggests that the CANV is the etiologic agent of an emerging condition in captive bearded dragons that has been called 'yellow fungus disease'.

Keywords *Chrysosporium* anamorph of *Nannizziopsis vriesii*, inland bearded dragons, dermatitis, yellow fungus disease

Introduction

The *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV) is a keratinophilic ascomycetous fungus that has been implicated in several cases of mycotic dermatitis in lizards [1–3], snakes [4,5] and crocodilians [2,6]. Lizard species from which this fungus has been recovered include day geckoes (*Phelsuma* sp.), a Parson's chameleon (*Chamaeleo parsonii*), a jewel chameleon (*Chamaeleo lateralis*), a Jackson's chameleon (*Chamaeleo jacksoni*), and ameivas (*Ameiva* spp.) [1–3]. Recent studies indicate that the CANV is uncommon on the normal microflora of reptilian skin [7]. The CANV reliably induced dermatomycosis in veiled chameleons (*Chamaeleo calyptratus*) under different modes of experimental exposure [8]. The source of this fungus in naturally occurring disease, its prevalence in the environment (e.g., soil, cage substrates), and the factors regulating its pathogenicity for captive reptiles are not well

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understood. Although improper diet, substandard husbandry, and environmental stressors may predispose animals to infection, the CANV appears to be capable of behaving as a primary pathogen in some species of reptiles [8]. This report describes three cases of deep granulomatous dermatitis in inland bearded dragons (*Pogona vitticeps*) caused by the CANV and provides evidence to suggest that this fungus is the etiologic agent of a sometimes fatal bearded dragon dermatological condition known in the pet trade as 'yellow fungus disease' [9].

Case reports

A 2-year-old, 425 g, pet female inland bearded dragon (Case 1) was presented to the University of Wisconsin, Veterinary Medical Teaching Hospital (UW-VMTH) with a focal swelling of the right maxilla. The lizard, acquired two days earlier from an online breeder in Mississippi and shipped by mail with a male bearded dragon, had been anorexic since arrival but was active, alert, responsive, and in good body condition. The lizards were housed together in a 285-liter aquarium with a screen lid, broad-spectrum ultraviolet lighting, and calciferous sand for substrate. Daytime temperature approximated 30–32°C (24–27°C at night). The lizards were offered a diet of dark leafy greens, mixed vegetables, and gut-loaded crickets. The crickets were coated with a vitamin/mineral powder prior to feeding. At the Mississippi breeding facility, the lizards were housed in pairs in $120 \times 60 \times 60$ cm enclosures with sand or red clay as substrate. Animals were housed indoors from October through March, and then placed outdoors from April through September. Indoor enclosures had broad-spectrum ultraviolet lighting and were kept at an ambient temperature of 30–32°C with a basking site of 46°C. The lizards were fed collard greens, gut-loaded superworms (*Zoophobus* sp.), and pelleted cockatiel food. No skin lesions were noted on any of the other bearded dragons at this facility, including the male cagemate.

Abnormal physical examination findings at presentation included a 5-mm diameter raised crusty skin lesion ventral to the right eye and an associated 1-cm diameter maxillary gingival swelling, which elicited a pain response upon palpation (Fig. 1). Caseous or purulent material could not be expressed from the swelling. Mucous membrane color at the site of the oral lesion was normal. Differential diagnoses included an infectious stomatitis/ dermatitis (bacterial or fungal), foreign body reaction, and trauma. Diagnostic testing was declined initially, and empirical treatment with oral enrofloxacin (5 mg/kg, PO, q 24 h for 21 days) and topical chlorhexidine solution (0.125% daily oral rinse) was initiated. Further diagnostic procedures were recommended if the oral lesion did not resolve.

Fig. 1 Adult female inland bearded dragon (Case 1) with maxillary cutaneous lesion and gingival swelling. (See colour online)

The lizard was re-evaluated 16 days later when the oral lesion was larger. Scrapings and impression smears of the external skin lesion revealed numerous heterophils and few bacterial cocci. The animal was anesthetized with isoflurane and biopsies were obtained of the external lesion and the subjacent gingival tissue using disposable 2-mm biopsy punches. Histologic examination revealed a chronic, severe, pyogranulomatous dermatitis and stomatitis with extensive discrete granuloma formation. Grocott's methenamine silver (GMS) stain disclosed fragments of hyphae within the skin and oral granulomas (Figs. 2, 3). Bacteria and acid-fast organisms were not

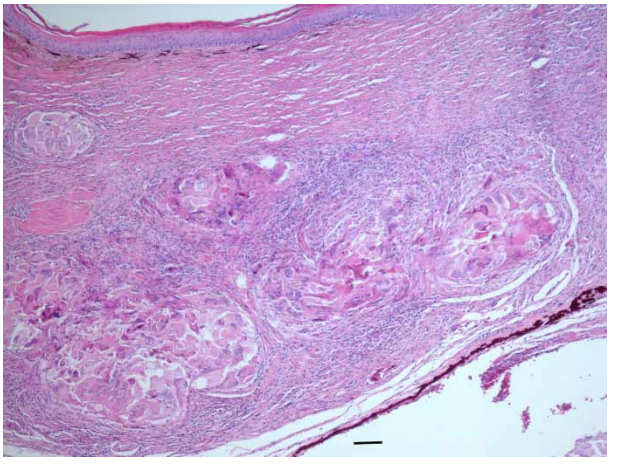

Fig. 2 Histopathology of the maxillary skin of the Case 1 lizard showing multiple, coalescing granulomas in the dermis. H & E stain; Bar = 100 μm. (See colour online)

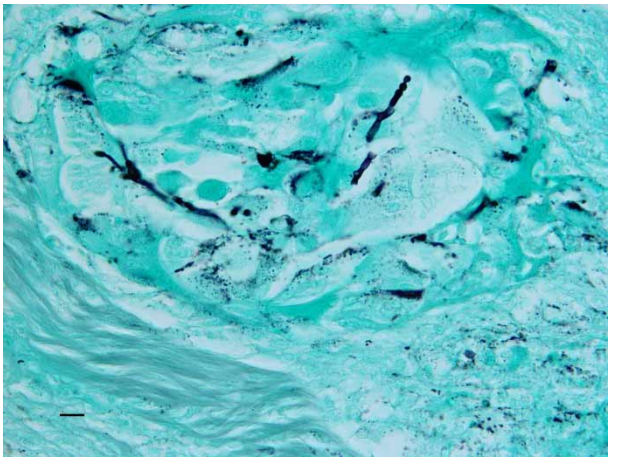

Fig. 3 Histopathology of the maxillary skin of the Case 1 lizard showing hyphae within the oral granulomas. GMS stain; Bar = 10 μm. (See colour online)

observed with additional staining. A white powdery fungus was cultured from the biopsy tissue at the UW-VMTH and identified as the CANV based on growth characteristics on potato dextrose agar at 30°C and 35°C, the microscopic appearance of the conidia (aleurioconidia and arthroconidia) in slide culture preparations, and on physiological characteristics on bromcresol-purple milk-solids-glucose agar (available commercially as dermatophyte milk agar; Hardy Diagnostics, Santa Maria, CA) and Christensen's urea broth (Difco Laboratories, Detroit, MI) as described previously [1,6]. The isolate was deposited in the University of Alberta Microfungus Collection and Herbarium (UAMH) in Edmonton, Alberta, Canada as UAMH 10171.

Blood was collected for a serum biochemistry profile to establish baseline values prior to initiating antifungal therapy. All values were within reference limits [10]. The lizard was treated with an oral suspension of itraconazole (10 mg/kg, PO, q 24 h for 6 weeks) along with continued topical treatment with a 0.125% chlorhexidine solution. After three weeks of antifungal therapy, the maxillary lesion had decreased in size by approximately 50%, and the biopsy sites had healed. Follow-up biochemistry profile values remained within reference limits. The lizard was active and alert, but a slight decrease in appetite was noted which was reflected in a mild weight loss of 20 grams. Subcutaneous fluids were administered and supplemental hand-feeding was recommended. After an additional three weeks of treatment, the maxillary lesion had nearly resolved, and the lizard's weight had improved. An additional three weeks of itraconazole therapy was recommended, but the animal changed owners and was temporarily lost to follow-up.

The lizard returned to the UW-VMTH 6 months after discontinuation of antifungal treatment. The right maxillary lesion had recurred and extended slightly beyond the margins of the original lesion. Punch biopsies were repeated and submitted for histopathology and fungal culture. Histologic examination confirmed a recurrence of mycotic dermatitis and stomatitis, and the CANV was again cultured from the affected tissues. Oral itraconazole therapy was reinstituted, along with daily topical treatment with 2% miconazole cream. Twenty-two days after therapy was initiated, the lizard presented for anorexia and weight loss. It was alert and responsive, but examination revealed moderate dehydration, a weight loss of 70 grams, and only mild improvement of the maxillary lesion. Blood was collected for a complete blood count and serum biochemistry profile. Hematologic abnormalities included a leukocytosis (36.57×10^3 cells/μl; reference range, $6.736–19.946 \times 10^3$ cells/μl), a lymphocytosis (18.65×10^3 cells/μl; reference range, $4.012–12.033 \times 10^3$ cells/μl), a monocytosis (8.78×10^3 cells/μl; reference range, $0–0.499 \times 10^3$ cells/μl) and an azurophilia (2.19×10^3 cells/μl; reference range, $0–1.085 \times 10^3$ cells/μl) [10]. Biochemical abnormalities included hyperglycemia (1088 mg/dl; reference range, 139–291 mg/dl), hyperproteinemia (10.3 g/dl; reference range, 4.5–9.5 mg/dl), and hyperuricemia (16.5 mg/dl; reference range, 1.6–11.4 mg/dl) [10]. Oral antifungal treatment was discontinued and supportive care with subcutaneous fluids and supplemental hand-feeding was initiated. The lizard expired 48 h later. Postmortem examination revealed a diffuse hepatopathy in addition to the dermatitis of the right maxilla. Histologically, there was a granulomatous, ulcerative and crusting fungal dermatitis of the right maxilla, severe and diffuse vacuolar degeneration of the liver with minimal, multifocal, lymphocytic hepatitis, severe arterial mineralization of the great vessels with multifocal mononuclear arteritis, and a moderate, subacute, multifocal, heterophilic and mononuclear colitis. There was no histological evidence of fungal dissemination.

A 2.5-year-old, 250 g, female inland bearded dragon (Case 2) was examined at the Avian and Animal Hospital of Bardmoor in Largo, Florida. The lizard had been rescued from improper care and donated to the clinic. This animal's cagemate had reportedly died 6 months previously of a fungal-like disease. Upon presentation, the lizard was active and alert, but there was a region of discolored and necrotic skin along the left caudoventral abdomen, extending to the perineum

and tail base. Fecal flotation was positive for oxyurid ova. No other diagnostic tests were performed initially due to financial constraints. The lizard was empirically treated with subcutaneous fluids, enrofloxacin (9 mg/kg, IM, q 24 h for 3 days), and fenbendazole (80 mg/kg, PO, once). On day 3, antibiotic treatment was changed to ceftazidime (20 mg/kg, IM, q 72 h for 13 days) and amikacin (2.5 mg/kg, IM, q 72 h for 13 days). After 16 days of treatment, the lizard's appetite was good and its weight had increased to 300 g. Therapy was changed to oral enrofloxacin (9 mg/kg, PO, q 24 h for 13 days) and topical silver sulfadiazine cream on affected skin once daily. Since the skin lesions worsened during oral antibiotic treatment, therapy with parenteral ceftazidime and amikacin was reinitiated. One month after presentation, the lizard was anesthetized with propofol and isoflurane for wound debridement. Histologic examination of debrided tissue revealed severe, diffuse, granulomatous dermatitis with intralesional hyphae. A portion submitted to the Athens Diagnostic Laboratory, Athens, GA for culture grew *Enterococcus* sp., *Brevibacterium* sp., and coagulase-negative *Staphylococcus* sp. on aerobic bacterial culture, and *Trichosporon* sp. on fungal culture.

Tissue from a second debridement performed 3 weeks later was submitted to the UW-VMTH for fungal culture. A *Chrysosporium* species was cultured and identified as the CANV. The isolate was deposited as UAMH 10211. Antibiotic therapy was discontinued and systemic antifungal therapy with itraconazole (10 mg/kg, PO, q 24 h for 10 weeks) was initiated in addition to daily baths in dilute povidone-iodine solution. The dermatitis improved during the first 8 weeks of antifungal therapy, but the animal became progressively anorexic with significant weight loss, and was euthanized 2 weeks later. Postmortem examination revealed a marked, locally extensive, ulcerative dermatitis of the ventral abdomen (Fig. 4) and a focal, hepatic granuloma. Histologic findings included an ulcerative, multinodular, granulomatous dermatitis with gram-negative rods, gram-positive cocci, and GMS-positive hyphae. Bacterial rods and hyphae were also observed within the hepatic granuloma. Hyphae were linear, septate, and occasionally branched. Acid fast staining was negative for mycobacteria.

A 2-year-old, 130 g, male inland bearded dragon (Case 3) presented to the UW-VMTH for evaluation of a non-weight bearing lameness of the right front limb. The lizard was bright, alert, and in good body condition. The right forelimb was markedly swollen distal to the elbow (Fig. 5) The skin was thickened and creviced, and exuded a serosanguinous exudate. Complete blood count, serum biochemistry profile, and whole body radiographs were performed. Hematologic abnormalities included a low normal hematocrit (19%; reference range, 17–50%) and a basophilia (3.88×10^3 cells/μl; reference range, $0.2–3.19 \times 10^3$ cells/μl) [10]. Serum biochemistry values were within reference limits [10]. Radiographs revealed a marked soft tissue swelling culminating around the carpus, and marked osteolysis of the right distal radius-ulna and carpus. The following day the lizard was anesthetized with propofol and isoflurane, and the affected limb was amputated at the level of the proximal humerus. Surgery was uneventful and treatment with ceftazidime (20 mg/kg, SC, q 72 h for 12 days) and itraconazole (5 mg/kg, PO, q 48 h for 14 days) was initiated. Histological examination of the decalcified limb revealed granulomatous fungal dermatitis, myositis, and osteomyelitis through the full thickness of the limb. GMS staining disclosed numerous mostly parallel-walled, branching, sparsely septate hyphae throughout the forelimb and within granulomas. Bacteria and acid-fast organisms were not observed with additional staining. Fungal culture of

Fig. 4 Ventrum of the Case 2 bearded dragon with chronic fungal dermatitis. (See colour online)

Fig. 5 Swollen right forelimb of the Case 3 bearded dragon affected with deep fungal granulomatous dermatitis and osteomyelitis. (See colour online)

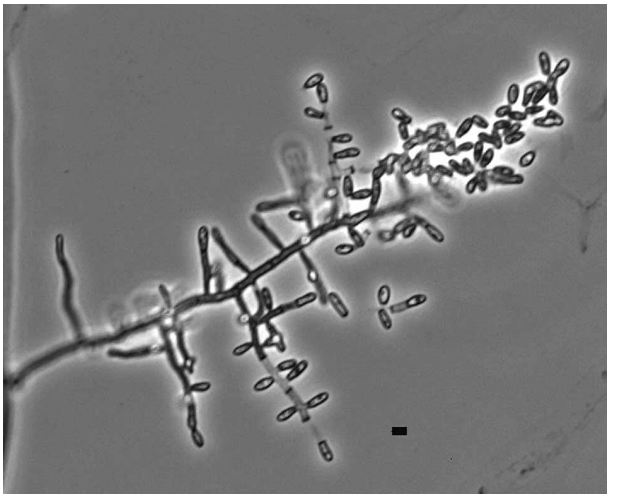

Fig. 6 Microscopic appearance of the isolate from Case 3 (UAMH 10351) showing aleurioconidia borne laterally on branched hyphae. Bar = 5 μm.

affected tissue sections yielded the CANV in pure culture (Fig. 6). This isolate was deposited as UAMH 10351. Two weeks later, the surgical site was healed and the lizard had gained weight. Six months after presentation, the lizard remained free of lesions.

Discussion

These are the first reported cases of mycoses caused by the CANV in a *Pogona* species. The isolation of the fungus in pure culture along with the presence of morphologically consistent hyphae within histological sections of affected tissues firmly establishes this fungus as the etiologic agent of infection in these bearded dragons [2]. The CANV etiology is further supported by isolation of the fungus from repeat tissue biopsies in Case 1. The source of infection in these cases is not known. Husbandry and diet in two cases appeared adequate, but the improper diet of the Case 2 lizard may have been a predisposing factor contributing to systemic fungal infection. Although feeding practices were not ideal for this animal, its overall physical condition was good at the time of initial presentation. Breaches in cutaneous integrity have been experimentally shown to increase the risk of infection with the CANV in veiled chameleons, and presumably in other lizard species [8]. Although there were no reports of previous skin trauma in these three cases, secondary fungal infection from the environment or from a conspecific is possible.

A condition termed 'yellow fungus disease' has been identified in the pet trade as a leading cause of mortality in captive bearded dragons and in commercial bearded dragon breeding operations [2,9]. Gross lesions, clinical presentations, and disease progression in the three cases presented here are consistent with those described for yellow fungus disease and the isolation of the CANV from all infected animals is strongly indicative that this fungus is the etiological agent of this emerging disease. Isolation and identification of the fungus in the majority of cases of yellow fungus disease has rarely been pursued. A few laboratories have reported a *Trichophyton* sp. from infected bearded dragons [9] but the CANV has been misidentified previously as a *Trichophyton*, or as other fungal species including *Geotrichum* or *Trichosporon* [1,2]. This may have been the situation in Case 2 when a fungus cultured originally was identified as *Trichosporon* sp. There is strong anecdotal evidence to suggest that infection with the agent of yellow fungus disease is contagious, and that disease does spread among animals within breeding operations, but there is no evidence, thus far, that infection may be transferred to human handlers. An environmental origin is probable, but has not been confirmed. It is also suspected that some bearded dragons are asymptomatic carriers of the disease, but testing has not yet been performed to confirm this hypothesis.

The characteristics of the bearded dragon isolates agreed with those described for the CANV in recent reports of reptile infection [1,2,4–6]. These included pyriform to cylindrical aleurioconidia borne sessile on branched hyphae (Fig. 6), presence of undulating lateral hyphae, production of urease, no pH change but some clearing of the medium on bromcresol-purple milk-solids-glucose agar, and hair digestion with perforating bodies. However, the isolates from bearded dragons differed in growing well at 35°C, in contrast to isolates from other reptile species which do not grow at this temperature. Reptile isolates of the CANV have been considered to belong to a single species based on morphological similarities, but preliminary molecular phylogenetic analysis based on comparison of DNA sequences from the internal transcribed spacer regions reveals the existence of several subgroups that are allied to specific reptile hosts (L. Sigler and S. Hambleton, unpublished results). Descriptions of several new fungal species are in progress. As species within the CANV complex become more precisely defined it will be possible to gain a better understanding of the relationship between the pathogens and the disease.

The use of itraconazole in lizards has not been well documented, and few pharmacokinetic studies with this drug have been performed in reptiles [11]. Itraconazole has been shown to have a broad spectrum of activity including many filamentous fungi, a relatively high

efficacy, and lower toxicity than other antifungals in the azole family [12]. CANV isolates from bearded dragons were found to be sensitive to itraconazole *in vitro*, although MICs were slightly higher then those for CANV isolated from other reptiles [13]. The dosage of 10 mg/kg used in the first two cases was based on a previous case report of the CANV in chameleons [1]. Anorexia and weight loss were the only reported side effects in this report [1]. Since hepatotoxicity is the main adverse effect of this medication in other species [12], hepatic enzymes were monitored closely during the first bearded dragon's treatment. Elevations in hepatic enzymes were not observed after three weeks of antifungal treatment, but partial anorexia and weight loss did occur. Similar clinical signs were noted after a second course of itraconazole in this lizard, as well as after an initial course of antifungal therapy in the second bearded dragon. Postmortem findings in the first two cases were not supportive of drug toxicity. The hepatic vacuolar degeneration observed in the first lizard, as well as the biochemical abnormalities, are consistent with anorexia. A lower dose of itraconazole was used for the third animal to avoid the anorexia observed in the two previous lizards. The third lizard recovered clinically and appeared to tolerate the medication well, but limb amputation alone may have been curative in this animal. Pulse therapy with itraconazole is now advocated for dermatomycoses in human medicine and should be considered in reptile patients [14,15].

Fungi are commonly present within the environment and on the skins of captive reptiles [7]. The circumstances under which mycotic diseases occur in reptiles are unknown, although inadequate diet and husbandry, environmental stresses, trauma, and existing dermatitis likely predispose these animals to opportunistic infections [2]. Since fungal species are routinely isolated from the skins of healthy reptiles, histopathology of affected tissues is necessary to confirm a causal relationship with the fungus isolated in culture [2]. Recently, yellow fungus disease has been associated with significant morbidity and mortality among bearded dragons. The gross pathology, histopathology, and culture evidence presented in these three cases of bearded dragon mycotic dermatitis strongly suggest that the CANV is the causal agent of yellow fungus disease, and that this fungal agent is emerging as a significant cause of dermatomycosis in bearded dragons. The prevalence of the CANV in the captive environment and the factors governing its pathogenicity in reptile populations remain incompletely understood. Further studies are warranted to fully understand the origin and nature of the CANV as a reptile pathogen.

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