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Case report

Keratomycosis in a Percheron cross horse caused by *Cladorrhinum bulbillosum*

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This report describes an infection of a horse's cornea caused by *Cladorrhinum bulbillosum*. Minor surgery and treatment with antibiotics successfully resolved the infection. The only previous reported case involving this fungus was an Argentinian boy who was infected while working with horses.

Keywords *Cladorrhinum bulbillosum*, cornea, horse, keratomycosis

Introduction

More than 60 species of fungi have been identified as aetiological agents of keratomycosis, with species in the genera *Fusarium, Aspergillus, Candida* and *Curvularia* among the commonest pathogens [1]. The horse seems to have a predisposition to keratomycosis [2,3], and the disease is being diagnosed more frequently. A wide variety of fungi have been involved, the most common being species of *Aspergillus*, *Fusarium*, *Cladosporium* and *Alternaria* [4–7].

In Argentina a case of keratomycosis was reported in a boy whose eye had been scratched by straw while he worked in a horse transport van [8]. The fungus isolated was identified as *Cladorrhinum* sp. Further study of the isolate from that case (CBS 604.75) resulted in it being described as the new species *Cladorrhinum bulbillosum* Gams et Mouchacca [9]. This report is of a second case of keratomycosis caused by the same species; the disease occurred in a Percheron cross horse.

Case history

An 11-year-old Percheron cross gelding weighing 650 kg was presented to the University of Queensland Teaching Hospital for evaluation of an eye problem. The left eye had suffered a penetrating wound of unknown cause 14 days earlier and was treated by the referring veterinarian with topical cloxacillin daily and 1 g phenylbutazone *per os* daily.

The wound initially healed but the horse was referred as the cornea became cloudy with lacrimation and blepharospasm. On examination the superficial layers of the cornea were necrotic and infiltrated with inflammatory cells. The deeper cornea was oedematous and had increased vascularization. The anterior chamber could not be visualized.

A guarded prognosis was given and surgical keratectomy was carried out under general anaesthesia. The necrotic cornea was debrided, a subpalpebral lavage system installed [10] and a temporary tarsorraphy performed. A sample of debrided cornea was submitted for cytology and culture; fungal hyphae were seen on cytology. Treatment was initiated with miconazole topically three times daily; ciprofloxacin topically three times daily; atropine topically four times daily until pupil dilatation, then daily; phenylbutazone 2 g orally twice daily for six days followed by 1 g twice daily until the cornea was healed.

The horse dislodged the subpalpebral lavage system within 24 h, but accepted medication directly onto the eye, so the lavage system was not replaced. The horse was discharged to the owner with continuing medication six days after surgery. The antibiotics were discontinued 10 days after surgery but the miconazole was administered

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until the lesion healed. The horse was examined 37 days after surgery. The cornea was clear except for a ventral area of granulation tissue and vision appeared normal. Blood vessels with pigment had migrated from both lateral and ventral limbi to the area of granulation tissue. There appeared to be no pain present and no other abnormalities were found. Miconazole treatment was continued for another four weeks. After a further 26 weeks the owner advised that a small amount of corneal scarring was present with apparent normal vision, and that the horse was being used for his normal work load.

Mycology

The sample of debrided cornea was cultured on plates of 10% sheep's blood agar (Oxoid) and MacConkey agar (Oxoid) which were incubated at 37 °C, and on Sabouraud glucose agar (Oxoid) and inhibitory mould agar (Difco) which were incubated at 30 °C. After 24 h the sheep's blood agar was observed to have a heavy growth of *Branhamella catarrhalis* [11] which was not considered to be of pathological significance. The fungal plates were examined after 48 h and a heavy growth of a single fungal species was seen.

The fungus was not identified to species and was studied further at the University of Alberta where the isolate was grown on a variety of culture media including potato glucose agar (PDA; Difco), phytone yeast extract agar (PYE; BBL), Pablum cereal agar [12], oatmeal-salts agar [12] and V-8 juice agar [12] at 25 and 37 °C. Tolerance to antifungal compounds was tested by growing the isolate on mycosel agar (BBL) containing cycloheximide at a concentration of 400 μg ml^{-1} and on PDA with and without benomyl at a concentration of 2 μg ml^{-1} [13]. The isolate from this case has been deposited in the University of Alberta Microfungus Collection and Herbarium as UAMH 8095.

The fungus was first recognized as *Cladorrhinum bulbillosum* by its growth characteristics on V-8 agar. This medium and growth at 37 °C promoted optimum sporulation. Colonies are fast growing on all media reaching a diameter of 65–80 mm within four days at 25 °C and 80–100 mm at 37 °C, and at seven days had reached the edge of the petri dish. On V-8 agar, initial growth consists of tufts of whitish-grey aerial hyphae and by seven days, colonies are fasciculate with greyish-brown velvety patches of sporulation, with numerous black microsclerotia embedded in the medium (Fig. 1). The reverse is greyish black. Colonies on PDA and PYE appear brownish grey, dense, and felty with radial folds; microsclerotia form among strands of aerial mycelium by 14 days. Numerous droplets of yellowish-brown exudate formed on PYE but not on PDA. Greyish-black diffusing pigment occurred on both media by seven days. The isolate was sensitive to cycloheximide and to benomyl.

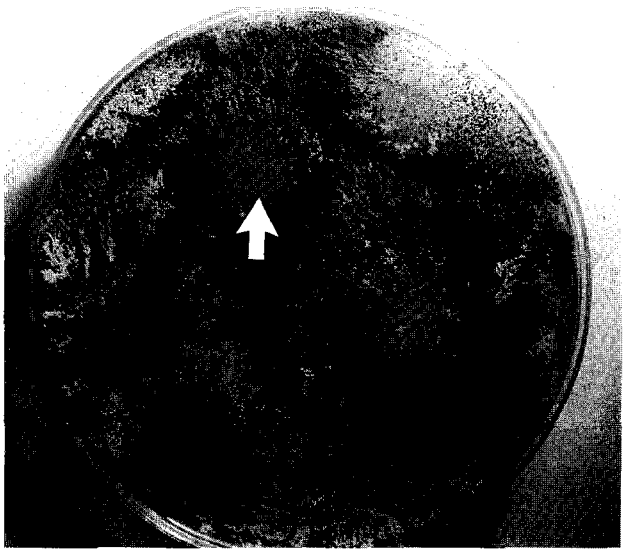

Fig. 1 Colony of *Cladorrhinum bulbillosum*, UAMH 8095, on V-8 agar showing hyphal tufts and microsclerotia (arrow) embedded in the agar.

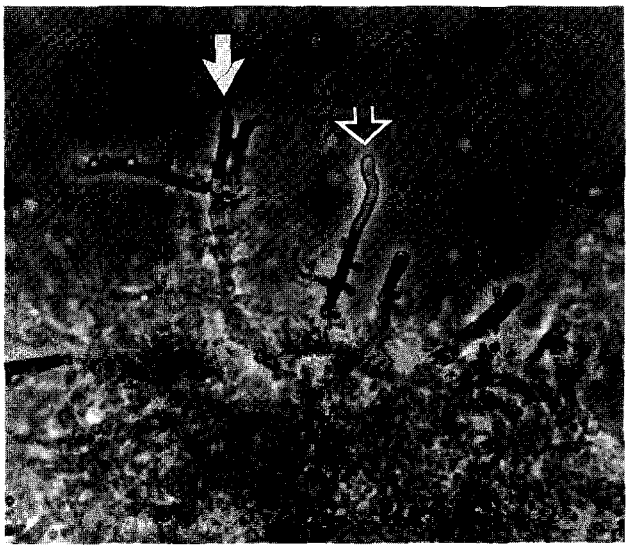

Fig. 2 Fertile hyphae showing terminal and lateral phialidic apertures. Note that terminal cells may be fertile (arrow) or non-fertile (arrowhead). (Phase contrast, × 580.)

Sporulation in *C. bulbillosum* occurs in greyish-brown clusters of branched, septate, fertile hyphae, 2·5–3·5 μm in width, in which individual cells form conidia in slimy masses from a series of integrated phialidic cells, and in which the terminal cell is either fertile or non-fertile (Fig. 2). Conidia are produced from lateral phialidic apertures which are 1–1·5 μm wide and long, and terminate in a

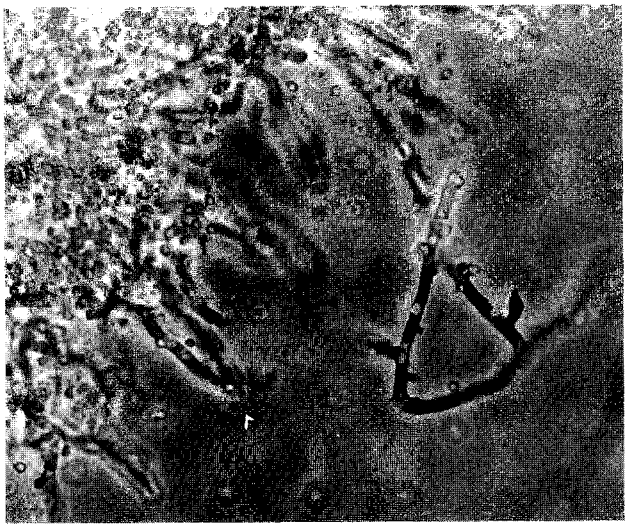

Fig. 3 Integrated phialidic cells and larger, lateral phialide. (Phase contrast, × 580.)

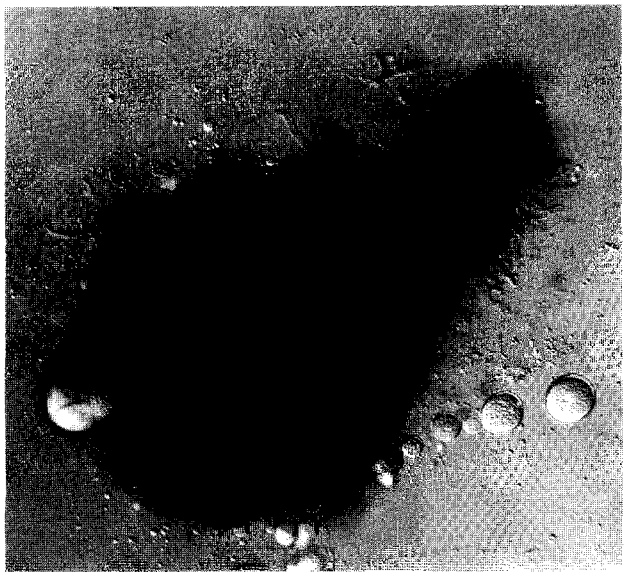

Fig. 4 Microsclerotium of *C. bulbillosum*. (Nomarski optics, × 175.)

short, distinct collarette (Fig. 2). Terminal cells bear phialidic openings either terminally or subterminally or consist of non-fertile clavate vesicles. Rarely, lateral conidiogenous cells are discrete, flask-shaped and measure up to 10 μm long (Fig. 3). Conidia are single-celled, dacryoid, 2·5–3 × 1·8–2·2 μm and appear pale greyish-brown in mass. Vegetative hyphae show great variation in width, ranging from 1·5 to 10 μm wide; some wider hyphae show evidence of narrow, intrahyphal hyphae. Microsclerotia measuring up to 150 μm form either in the aerial hyphae or submerged in the agar and consist of loose or compact aggregations of hyphae composed of swollen, pigmented cells (Fig. 4). Individual cells measure from seven to 20 μm in width and show varying intensity of pigmentation from subhyaline to dark brown.

From the excellent description and illustrations of the original authors [9] and from the thermotolerance of *C. bulbillosum*, there can be no doubt that the fungus isolated from our case of keratomycosis is identical to the isolate previously reported from the Argentinian boy [8,9]. The patient in that case was infected following abrasion of the cornea by straw, suggesting a possible similar route of infection in the case described here. Other isolates of *C. bulbillosum* have come from the soil in the dry desert areas of Egypt and from the root of *Saccharum officinarum* in Taiwan. *Cladorrhinum* anamorphs are known for several species of *Apiosordaria* and *Fimetariella* classified in the Lasiosphaeriaceae, but several strictly anamorphic species are known [9,14]. None of the isolates of *C. bulbillosum*, including the one described here, has been connected to a teleomorph.

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