Three isolations of Chaetomium globosum from erythematous epilation of canine skin

KAZUTOSHI SUGIYAMA*,†, AYAKO SANO‡, MASARU MURAKAMI‡, TAKASHI OGAWA§, HIROYUKI MISHIMA§, HIROYUKI OTAKE#, KATSUHIKO KAMEI† & SHUNITI SUGIYAMA*

* Sugiyama Veterinary Clinic, Shizuoka, † Medical Mycology Research Center (formerly: Research Center for Pathogenic Fungi and Microbial Toxicosis), Chiba University, ‡ Faculty of Veterinary Medicine, Azabu University, Kanagawa, § Ogawa Animal Hospital, Shizuoka, and # Shikatani Animal Hospital, Shizuoka, Japan

Chaetomium globosum is commonly found in natural environments worldwide [1] and is known to be a causative agent for emerging fungal infections. The present study describes a case of erythematous epilation of a dog caused by C. globosum. A mixed-breed young dog, a 4-months-old male, weighing 7.25 kg, showed depilation, scales, and dermatitis with slightly itchiness on his skin. The main symptom was an erythematous epilation on the left subocular skin 7.5 cm in diameter, accompanied by elephantiasis-like hyperplasia and scales. Similar lesions were observed on the skin on both sides of the ear lobes, the heels, tail, and left angulus oris. The scales from the crusted lesion were cultured on chrolamphenicol-added potato dextrose agar plates at the first visit, as well as followed by ambulatory practices. The isolates at the first visit, 1 and 3 weeks after treatment, were identified as C. globosum by mycological study and the D1/D2 domain of the large subunit rRNA gene sequence. The patient dog was treated by ketoconazole both orally and externally. The lesions were cured, showing new hair growth 9 weeks later. In addition, the susceptibilities to antifungal agents for the present C. globosum isolate were as follows: amphotericin B, 4.0 μg/ml; 5-FC 64.0 μg/ml; itraconazole, 0.5 μg/ml; miconazole, 1.0 μg/ml; fulconazole, 16.0 μg/ml; ketoconazole, 0.25 μg/ml; and micafungin, 16.0 μg/ml.

Keywords Chaetomium globosum, dog, erythematous epilation

Introduction

Chaetomium species are commonly found in natural environments worldwide [1] and are known to be mycotoxin-producing fungal species [2–4]; they are also, however, recognized as industrially important fungal species because of their ability to induce cellulose degeneration [5] and to be used in the production of medically useful compounds [6,7]. On the other hand, the fungal species are known to be causative agents for emerging fungal infections [8]. More than 20 human cases of Chaetomium spp. caused by C. globosum, C. atrobrunneum, C. strumarium, C. perlucidum, C. funiculum, and C. murorum [9] have been found to cause onychomycosis, superficial and deep mycoses, sinusitis, pneumonia, and fatal disseminated infection [8–19]. Furthermore, C. globosum has been recorded as one of the cutaneous fungal flora of dogs [20], though there are no documented cases of Chaetomium spp. infection in animals. The present study describes a cutaneous infection of C. globosum in a young dog showing erythematous epilation having repeated isolations of the fungal species from the cutaneous lesions.

Case report

A 4-month-old male mixed-breed dog from Shizuoka prefecture, the middle part of Japan, weighing 7.25 kg,
received vaccinations for rabies, canine distemper, parvovirus, adenovirus type 2, parainfluenza virus, and leptospirosis on a routine schedule, and was fed commercial dry chow. The dog had a very intimate relationship with the owners, being kept indoors and sharing a bedroom with the family.

The main symptom was an erythematous epilation on the left subocular skin, 7.5 cm in diameter, accompanied by elephantiasis-like hyperplasia and scales (Fig. 1a). According to the owner, scratching behavior was slightly increased and the lesion was expanding 10 days before the first visit. Similar lesions were observed on the skin on both sides of the ear lobes, the heels, tail, and left angularis oris. The dog’s appetite was normal and physiological and biochemical data for the blood were within normal values. Direct microscopic observation of skin scales in 20% potassium hydroxide (KOH) showed mycelial components. The wood-light test showed a change to a slightly pinkish color, though the results were indeterminate. In order to avoid contamination, the scales from the lesions were collected by a sterilized brush after the surface was scrubbed with a cotton mass immersed in 70% ethanol. White cottony colonies with slight yellowish reverse pigment were sprouted from the scales cultured on Sabouraud’s glucose agar (SDA) supplemented with antibiotics at 25°C for 3 days. We identified the fungal sprouts as *Mycrosporum canis* and began to treat the skin lesions by topical application of antifungal and antiseptic agents such as ketoconazole cream and chlorhexidine, once to 3 times daily.

The redness and hypertrophy of the skin at the left subocular lesion showed remission after 3 weeks, but the diameter was expanded and other lesions appeared at the right subocular, the oral angular, inside the ears, on both tarsal joints, and the root of the tail. The dog continued to scratch intensely. Direct microscopic observation of scales mounted by 20% KOH also showed fungal elements. The wood-light test was slightly positive. The culture on SDA plates also produced a white floccose colony having a slightly yellowish color on the reverse, the same as the appearance of the first isolate.

We then began systemic administration of 50 mg *semel in die* (SID: single administration per a day) of ketoconazole followed by reference datum of minimum inhibitory concentration (MIC) ketoconazole [18] in parallel with topical administration of antifungal and antiseptic agents. The lesions entered remission and showed signs of hair growth after 9 weeks (Fig. 1b). The skin lesions had no fungal elements, as observed by microscopy. The wood-lighting test and culture on SDA became negative.

The systemic and topical administration of ketoconazole was continued for two weeks, at which time the lesions were completely healed; no relapse of infection has since been observed.

Colonies on SDA and potato dextrose agar (PDA, Difco, Detroit, MO, USA) plates at 25°C, 37°C, and 42°C were as follows. The colony on SDA after 4 weeks at 25°C was floccose and grey at the center and yellowish-grey at the margin; colonies at 37°C and 42°C after 4 weeks on SDA were white velvety. The diameters at 25, 37, and 42°C after 4 weeks on SDA were 9.0, 6.7, and 3.7 cm, respectively. That on PDA after 4 weeks at 25°C was deep green, having a granulated texture and a white floccose margin on the surface, and dark brown on the reverse. The textures of colonies on PDA at 37°C were light brown floccose at the center and white lobate cottony at the margin without ascomata, and at 42°C were ashy green and wrinkled. The diameters at 25, 37, and 42°C after 4 weeks on PDA were 9.0, 7.3, and 2.3 cm, respectively (Fig. 2a, 2b). The morphology of fungal colonies isolated at 3 and 6 weeks after treatment were equivalent to the first isolate.

**Fig. 1** The skin lesion showing erythematous epilation with scales at the first visit (a), and 9 weeks after treatment (b).
Structures of ascomata on the PDA plate and slants at 25°C after 4 weeks under stereoscopy and light microscopy were as follows. Spherical ascomata with coiled hair were observed on the PDA plate under a stereomicroscope. The diameters of the ascomata were approximately 200 μm. The perithecia were filled with asci, including 8 ascospores. The matured ascospores were flat, brown, and lemon-shaped, with both sides being 10–12 μm in diameter, and had germination pores (Fig. 3a–3f).

The maximum growth temperature of the isolate was 42°C on the PDA slants using a series of different temperatures; 37, 42, 45, 48, and 50°C.

A partial sequence of the large subunit ribosomal DNA gene (LSU rDNA) sequence was processed by a standard method described by Kurtzman and Robnet [21] using DNA extracted from a culture on a PDA slant at 25°C after 2 weeks. The sequence was compared through BLAST search to the GenBank database [http://www.ncbi.nlm.nih.gov/BLAST/]. A partial sequence of the large subunit ribosomal DNA gene (LSU rDNA) sequence of the isolate had more than 99% identity in the GenBank database, located at a cluster of C. globosum by a distance tree of results, and was deposited as AB292591.

Antifungal susceptibility tests on conidia of the present isolate cultured on a PDA slant at 25°C at 4 weeks were performed according to the broth microdilution modified method of the CLSI M38-A [22] approved standard using RPMI 1640 medium (Sigma, Poole, UK) buffered to pH 7.0 with 3-(N-Morpholino) propanesulfonic acid (MOPS) (Sigma) using a kit (Dryplate, Eiken, Tokyo Japan) containing antifungals: amphotericin B, fluconazole, itraconazole, miconazole, and micafungin. Isolates of Candida albicans IFM 40213 equal to ATCC 90028 were included as quality-control strains for susceptibility testing. The microdilution plates were incubated in air. Readings were made after 48 h of incubation at 37°C (the Candida control strain was examined after 24 h) in RPMI medium. The minimal inhibitory concentration (MIC) endpoints for amphotericin B and itraconazole were read visually as the lowest drug concentration that prevented any discernible growth. The MIC endpoints for other antifungal drugs were read visually and taken as that which reduced growth by 80% compared with the drug-free control. The susceptibilities to antifungal agents for the present C. globosum isolate were as follows: amphotericin B, 4.0 μg/ml; 5-FC, 64.0 μg/ml; itraconazole ITC, 0.5 μg/ml; miconazole, 1.0 μg/ml; fluconazole, 16.0 μg/ml; ketoconazole, 0.25 μg/ml; and micafungin, 16.0 μg/ml. The values were not changed after 72 hours.

The owner of the patient dog agreed to publish this paper.

Discussion

The skin disease of the dog was diagnosed as dermatitis caused by C. globosum identified as Chaetomium globosum based on mycological [23] and molecular biological techniques. The systemic administration of 50 mg SID of ketoconazole with a combination of topical treatment of ketoconazole cream for 12 weeks was effective. The present case was the first documented canine case of C. globosum infection in Japan, although Chaetomium spp. have been reported as species of a normal fungal flora in a foreign country [20].

C. globosum is one of the environmental saprophytic fungal species worldwide, and has been isolated from soil, woods, and wet walls [1]. Systemic infection of the fungal species has been thought to occur by inhalation of airborne spores [15]. The infection route of the
present case might be direct contact with the soil or other environments.

*C. globosum* is listed as one of the causative agents for emerging fungal infection in immunocompromised hosts [16–18]. Human cases in immunocompromised patients after transplantations have been reported [17,18], though the present case had no immunodeficient profile except for the dog’s age of 4 months, indicating an under-developed immune system.

Morphological identification of the present *C. globosum* isolate on SDA plates was difficult. The colony on the SDA plate at room temperature within 4 days closely resembled *Microsporum canis* and lacked ascomata, even on the colony cultured at 25°C after 4 weeks. In contrast, PDA plates of slants were excellent media for observation of ascoma of *C. globosum* cultured at 25°C after 4 weeks, aiding in morphological identification. We supposed that considerable numbers of white flucosse mycelial colonies resembling *M. canis* might be misidentified as *M. canis*, and many cases of dermatitis diagnosed as *M. canis* infection in pet animals might involve *C. globosum* infection. In

![Fig. 3 Microscopic aspects of the isolate cultured on PDA plates or slants at 25°C for 4 weeks. Spherical ascomata with coiled hair on the plate (a) and on the slant (b), as seen by the stereomicroscope; peritheium (c), immature ascospores in a peritheium (d), maturing ascospores (e), and mature ascospores (f), as seen by light microscopy.](image-url)
C. globosum has been effective in the present case. In addition, other triazoles such as voriconazole, miconazole, and itraconazole, as reported in human medical treatment. The present isolate is susceptible to ketoconazole and shows equivalent susceptibilities to amphotericin B. It is also noteworthy that ketoconazole might be a further consideration when the drugs become commercially available in Japan.

Fungal species cause systemic infection depending on the host immune condition [17,18]. Therefore, hygiene control for owners holding infected animals with C. globosum seems to be important, especially for infants, the elderly and immunodeficient persons. The owners in the present case were advised to avoid intimate contact with the dog, to wash hands after touching, and to indefinitely keep the dog’s environment very clean.

Recently, the number of emerging fungal infections has been increasing [25–27]. C. globosum infections in the veterinary field might not be an exception to this trend, and likely their incidence will increase in the near future, not only in cutaneous infections but also systemic ones such as in human cases.

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