

Cutaneous Hyalohyphomycosis Caused by *Geotrichum candidum* in a Red-Eared Slider Turtle (*Trachemys scripta elegans*)

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ABSTRACT

Background: Mycoses in Chelonians have different features; they can be affected by fungi causing shell, tegumental, and/or systemic disease, which can be considered as conventional agents. *Geotrichum* species are colonies similar to yeast, producing arthroconidia without blastoconidia. The diseases of the shell are one important disorder that might affect the survival of chelonians. They are more often found in aquatic species kept in captivity and are usually caused by poor environmental conditions. Also, bites and accidents that hurt the shell are factors that allow pathogenic organisms, such as bacteria, fungi, or even algae, to colonize and penetrate the shell. This can lead to conditions called septicemic cutaneous ulcerative disease, which causes deep ulcerations. This report describes a case of cutaneous hyalohyphomycosis caused by *Geotrichum candidum* in a turtle.

Case: A 3-year-old female Red-Eared Slider Turtle weighing 0.3 kg attacked by a dog 2 weeks earlier and presenting many deep wounds with necrotic areas in the carapace as the one relevant clinical feature, was assisted at the Service of Dermatology, Hospital de Clínicas Veterinárias, UFRGS, Porto Alegre, Brazil. Samples were obtained from the shell scrapings and the wounded necrotic areas of shell tissues were removed for routine microbiological and histological diagnosis. Direct microscopic examination with mount in 10% KOH revealed the presence septate hyaline hyphae. Bacteriological cultures done on Blood agar and yielded few colonies of *Citrobacter freundii*. For mycological diagnosis, the material was inoculated onto Sabourad's Dextrose Agar supplemented with chloramphenicol and Mycosel Agar. Smooth, one-celled, subglobose to cylindrical arthroconidia quite variable in size, consistent with description of *Geotrichum candidum*. Histology findings of biopsied material stained with Hematoxylin and Eosin (HE) and Periodic Acid-Schiff (PAS), revealed the presence of numerous hyaline septate hyphae among necrotic tissues. Immediately after the clinical examination, in view of the extensive injuries, a subcutaneous single dose of 10,000 UI of benzathine penicillin G, a long therm-acting antibiotic, was administered and a daily topical application of 10% iodine was recommended. Supportive therapy was not necessary due to the animal general good health. The turtle was reexamined only 45 days later showing a little improvement of the lesions and new collected material yielded no bacteria but only pure colonies of *G. candidum* and still the presence of hyaline septate hyphae on the histopathologic assessment. Then, a treatment with topical nystatin was prescribed and 6 months later, the success of the therapy, was confirmed by the complete resolution of the wounds and the negative results of the third fungal and histological evaluations.

Discussion: Geotrichosis has already been reported in animals, including lizards and snakes and reports about *Geotrichum* species as etiological agents among chelonians have been increasing in recent years. Although the conditions that enable opportunistic infections are similar for all saprobic fungi, clinical cases by *Geotrichum* species are rare in hosts. We believe that misreporting or misdiagnosis with other species, like *Candida*, could be the reason it is rarely reported. In this report, and in the other reviewed cases of animal geotrichosis, no predisposing factors are mentioned and the bite probably caused the wound in the shell and allowed the growth of the pathogenic fungus. The appropriate medium and the cultivation of *Geotrichum candidum*, as well as the use of molecular analysis, can contribute to the successful isolation of these species and make a correct final diagnosis.

Keywords: geotrichosis, *Geotrichum* sp., turtle, Chelonians, emerging mycoses, shell mycoses.

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INTRODUCTION

Geotrichosis are mycotic infections caused by *Geotrichum* species that live in soil, water, vegetables, and the digestive tract of animals. *Geotrichum candidum* is the most common type, a cosmopolitan mushroom that can parasitize different families of vegetables and also cause cutaneous or systemic infections in humans and animals [2,6,7].

The diseases of the shell are one important disorder that might affect the survival of chelonians. They are more often found in aquatic species kept in captivity and are usually caused by poor environmental conditions, like bad quality water, low temperatures, low UV light, overpopulation or a diet that is not balanced. Also, bites and accidents that hurt the shell are factors that allow pathogenic organisms, such as bacteria, fungi, or even algae, to colonize and penetrate the shell. This can lead to conditions called septicemic cutaneous ulcerative disease, which causes deep ulcerations [15,19].

The adoption of exotic animals, such as turtles, has been particularly widespread in a world-wide basis. In Brazil, the Red-Eared slider Turtle (*Trachemys scripta elegans*) is an aquatic turtle belonging to the suborder Cryptodira and to the family Emydidae native to North America. It is distributed geographically from the eastern United States to northeastern Mexico [6,19] and it is now a very popular pet. This report presents a case of cutaneous hyalohyphomycosis caused by *Geotrichum candidum* in a Red-Eared Slider Turtle (*Trachemys scripta elegans*).

CASE

A 3-year-old female American Red Eared turtle weighing 0.3 kg attacked by a dog 2 weeks earlier and presenting many deep wounds with necrotic areas in the carapace (Figure 1) as the unique relevant clinical feature, was referred to the Service of Dermatology of the Hospital de Clínicas Veterinárias (HCV) - UFRGS, Porto Alegre, Brazil. Samples were obtained from the shell scrapings and the wounded necrotic areas of shell tissues were removed for routine microbiological and histological diagnosis.

Direct microscopic examination with mount in 10% KOH on slide revealed the presence of few fragmented septate hyaline hyphae. Bacteriological cultures done on blood agar and MacConkey agar¹ yielded few colonies of *Citrobacter freundii*.

For mycological diagnosis, the material was inoculated onto Sabourad's dextrose Agar² (supplemented with chloramphenicol) and Mycosel Agar³. In both media, the germinated colonies were fast growing, flat, creamy, finely suede-like without pigmentation. Microscopic features showed hyphae branched and broken up into chains of hyaline, smooth, one-celled, subglobose to cylindrical arthroconidia quite variable in size, consistent with description of *Geotrichum candidum* (Figure 2). On SDAC 2 colonies of *Penicillium* sp. were also isolated, but without lesion involvement.

Histology findings of biopsied material stained with Hematoxylin and Eosin (HE)⁴ and Periodic Acid-Schiff (PAS)⁴, revealed the presence of numerous hyaline septate hyphae (Figure 3) among necrotic tissues.

Immediately after the clinical examination, in view of the extensive injuries, a subcutaneous single dose of 10,000 UI of benzathine penicillin G⁵, a long therm-acting antibiotic, was administered and a daily topical application of 10% iodine was recommended. Supportive therapy was not necessary due to the animal general good health. The turtle was reexamined only 45 days later showing a little improvement of the lesions and new collected material yielded no bacteria but only pure colonies of *G. candidum* and still the presence of hyaline septate hyphae on the histopathologic assessment. Then, a treatment with topical nystatin⁶ was prescribed and 6 months later, and the success of the therapy was confirmed by the complete resolution of the wounds (Figure 4) and the negative results of the 3rd fungal and histological evaluations.

DISCUSSION

The *Trachemys scripta elegans* (red-eared slider turtle), a North American semi-aquatic species that has been introduced in various water ecosystems around the world and in Brazil, is an exotic species whose illegal trade has led to the abandonment of specimens in water bodies [7].

Geotrichosis has already been reported in animals [4,5,13], including lizards and snakes [8,15]. Despite the scarcity of reports about mycosis diseases on turtles, the species *Fusarium solani*, *Paecilomyces* sp., *Aphanomyces* sp., *Purpureocillium lilacinum* and *Trichosporon jirovecii* are also described as etiological agents of cutaneous disorders [3,9,10,12,17].



Figure 1. Deep wounds necrotic areas on the left side of carapace of one Red-Eared Slider Turtle (*Trachemys scripta elegans*).

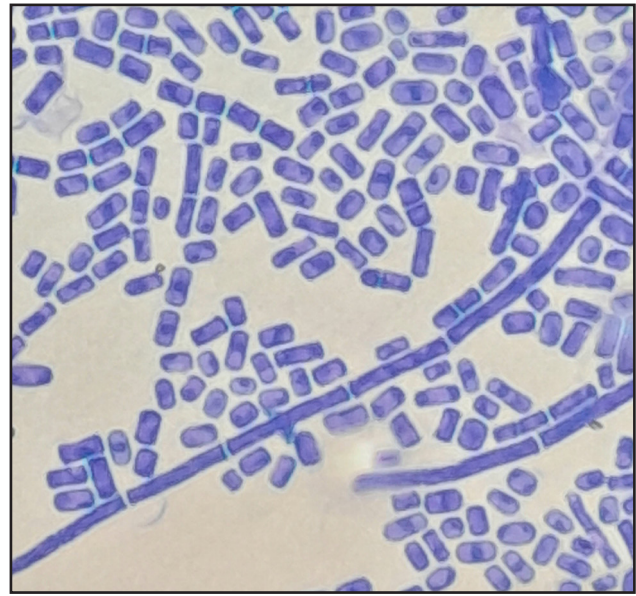


Figure 2. Microscopic appearance of *Geotrichum candidum* showing barrel shaped unicellular arthroconidia arranged in chains and branching hyphae.

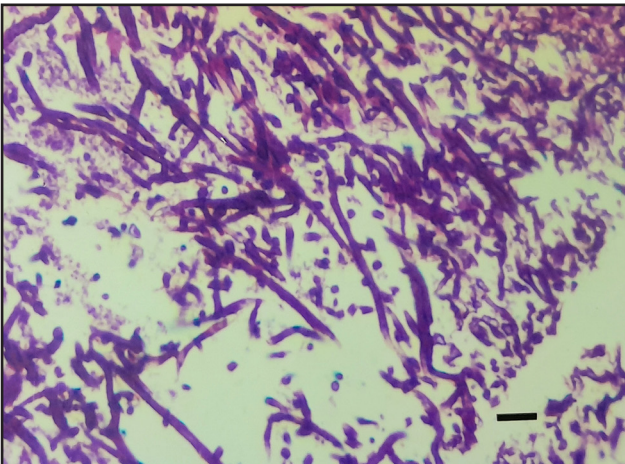


Figure 3. Stratum corneum showing numerous hyaline septate hyphae on PAS-stained histological section. [Bar= 25 µm].



Figure 4. View of the complete healing of the wounds after 6 months of treatment of one Red-Eared Slider Turtle.

The 1st description of *G. candidum* in animals was about an experimental infection with arthroconidia suspensions of plant and animal isolates being inoculated in 2 species of turtles. All isolates were found in at least 2 organs, with led to the conclusion that isolates from both sources can parasitize turtle tissues and establish *G. candidum* as a potential biopathogen [16].

Even though it's considered a rare situation, the mycotic cutaneous infection of chelonians has already been described as being able to spread to 1 organ, cause granuloma formation in several others and

even lead to osteomyelitis with a fatal outcome [9,14]. *Trichosporon cutaneum* is reported as causing agent of a nuchal haematoma of a Carolina anole lizard (*Anolis carolinensis*), subsequent to a bite from conspecific [11]. The involvement of shells alone rarely in turtles and the occurrence of yeasts like *Candida albicans* and non-*albicans* *Candida* species, *Cryptococcus* sp. and *Rhodotorula* sp., together with molds hyalohyphomycetes and feohyphomycetes has been recorded [2,12].

The initial therapy was to prevent internal disseminations that could be a life-threatening for

the turtle. After that, nystatin was chosen based upon the success achieved on treating animals and human geotrichosis, even though topical drugs such as Topical iodine, terbinafine, ketoconazole, miconazole, lugol's solution, 10% solution of iodine in alcohol good results cutaneous mycoses of chelonians [1,3,18].

Although the conditions that enable opportunistic infections are similar for all saprobic fungi, clinical cases by *Geotrichum* species are rare in hosts. In this report, and in the other reviewed cases of animal geotrichosis, no predisposing factors are mentioned. However, in one report, it is reasonable to suppose that interaction of a concurrent underlying disease caused the death of 2 dogs from the same house, since they died in 2 weeks after onset of an acute illness leading to the development of nephritis, hepatitis and pneumonia [16], or to admit at least, to a particular powerful pathogenicity of the involved *Geotrichum* isolates.

In conclusion, reports about *Geotrichum* species as etiological agents among chelonians have increased in recent years. We believe that misreporting

or misdiagnosing with other species, like *Candida*, could be the reason it is rarely reported. The appropriate medium and the cultivation of *Geotrichum*, as well as the use of molecular analysis, can contribute to the successful isolation of these species and make a correct final diagnosis.

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