

Cutaneous lesions due to *Trichosporon jirovecii* in a tortoise (*Testudo hermanni*)



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ABSTRACT

Cutaneous mycoses have been rarely reported in Chelonians. A *Testudo hermanni* adult male showed a thick erosion of the dorsal neck covered by necrotic material. *Trichosporon* sp. was cultivated, while arthrospores and hyphae were observed in histological sections. The causative agent was identified as *Trichosporon jirovecii* by PCR. After a surgical intervention povidone iodine and Iruxol® ointment were daily administered through the drainage for 2 weeks, along with enrofloxacin 5 mg diluted in saline 0.5 ml via the intracelomatic route. After treatment the ulcer healed with residual scars. No relapse was registered after 12 months. *T. jirovecii* is considered as a rare yeast pathogen and the presented case is the first report of a dermatomycosis in tortoises caused by this yeast species.

1. Introduction

Although dermatomycoses among reptilian species are usually reported in lizards and snakes [1], Chelonians can also be prone to superficial mycoses, mostly infecting the shell [2–4]. Moreover cutaneous mycoses by *Fusarium solani* have been reported in injured sea turtles [5,6] and mycoses probably due to *Fusarium* sp. and *Mucor* sp. were reported in European pond turtles (*Emys orbicularis*) from Serbia [7], but to the best of our knowledge the only record of tegument involvement in tortoises is referred to a cutaneous and renal geotrichosis in a giant tortoise (*Geochelone elephantopus*) due to *Geotrichum candidum* [8].

The genus *Trichosporon* appears as yeast-like anamorphic cells belonging to the phylum of the Basidiomycota [9]. They widely occur in the environment, as well as on the skin and in anatomical locations of human beings. *Trichosporon* spp. are known emerging opportunistic agents of invasive fungal infections, mostly in severely immunocompromised patients. The species identification can be achieved by molecular methods only, considered that several fungal species have been recognized within the genus and 17 of them are reported as pathogens [10].

2. Case

A *T. hermanni* adult male had been referred to clinical visit at day 0

for a cutaneous lesion (about 1 cm in diameter) of the dorsal neck, consisting in a thick erosion covered by necrotic material lasting from 20 days (Fig. 1a). The animal was 11 year-old, weighting 505 g, was living outdoor and was in a good general health status. The owners had shaped the carapax believing that the skin lesion was caused by rubbing. The wound was surgically reduced and sutured. The tortoise was given enrofloxacin 5 mg diluted in saline 0.5 ml via the intracelomatic route. At day 20 the animal was readmitted to clinician for a worsening of the lesion, which appeared enlarged in size showing a wide ulcer still covered by fibrinous necrotic material (Fig. 2b). The wound was further deeply excised.

Exudate was collected from the lesion by means of sterile cotton swabs and seeded onto Blood Agar (Blood agar sheep, Biolife, Italy) and Malt Extract Agar (MEA, Conda, Madrid, Spain) added with 0.01% gentamicin, for microbiological analyses. The plates were incubated at 25 °C until a noticeable growth was observed.

Portions of tissue from the lesion were fixed in buffered formalin and routinely processed for histopathology. Four-micrometer serial sections from paraffin-embedded material were submitted to Hematoxylin and Eosin (H-E) and Periodic Acid Schiff (PAS) stains.

Blood agar did not show any bacterial growth, while cerebriform, cream colonies with a membranaceous aspect indicative of yeast were isolated on MEA as pure culture starting from day 4 post incubation. Histopathological investigations revealed the presence of large superficial pustoles covered by a dense necrotic material rich in heterophils,

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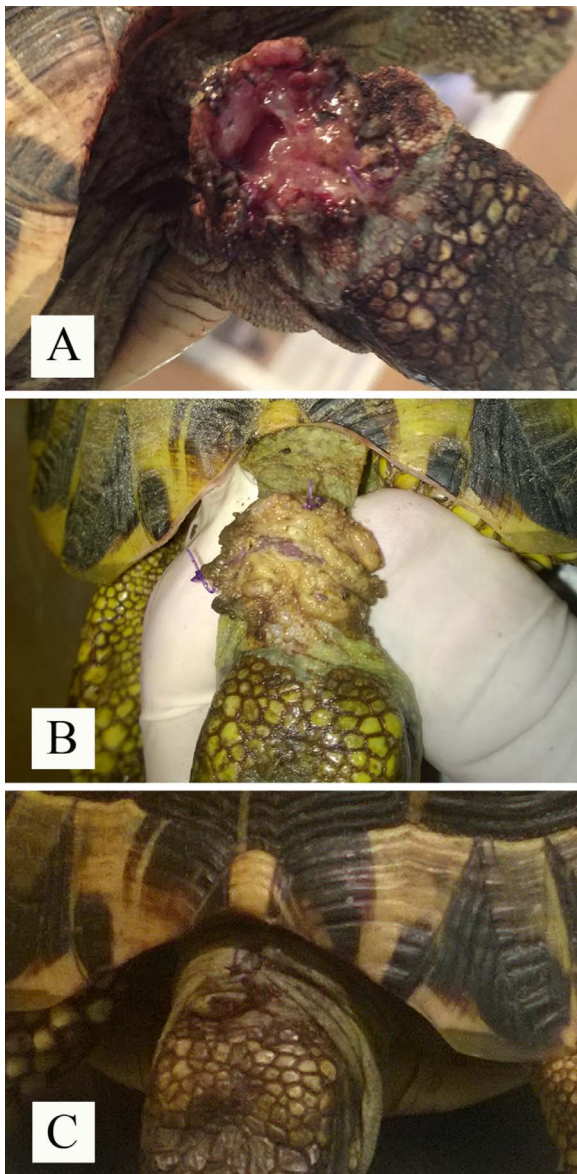


Fig. 1. Clinical signs of *Trichosporon jirovecii* infection in the examined turtle: A) Wide ulcerative focal lesion detected in the dorsal neck at the first clinical visit; B) Twenty days later was still present a wide ulcer covered by necrotic material; C) At the end of the treatment, healing of the ulcer with a residual scar was observed.

macrophages and rare eosinophils (Fig. 2a). Subjacent epidermis showed spongiosis and edema of the superficial dermis. Yeast aggregates and pseudohyphae might be detected in H-E stained sections (Fig. 2a), but were more obvious in PAS stained section (Fig. 2b), which revealed brilliant magenta round yeast measuring 14–16 μm in diameter as well as tangled hyphae suggestive of *Trichosporon* sp. (Fig. 2c). Genus identification was confirmed by auxanographic test (ID32C, BioMérieux, France).

Molecular methods were used to identify the fungal isolate at a species level. Yeast DNA was extracted by Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, CA, U.S.A.) following the manufacturer's instructions and used for PCR sequencing of rDNA genes employing universal primers for ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCGCTTATTGATATGC-3') [11]. PCR products were sent to a commercial sequencing lab (BMR genomics, Padova, Italy). DNA sequence obtained (CTGCGGAAGGATCATTAGTGAATTGC TCTTTGAGCGTTAACTACATCCATCTACACCTGTGAACTGTTGATTGAC TCCGGTCAATTACTTTACAAAACATTGTGTAATGAACGTATGTTATTAT

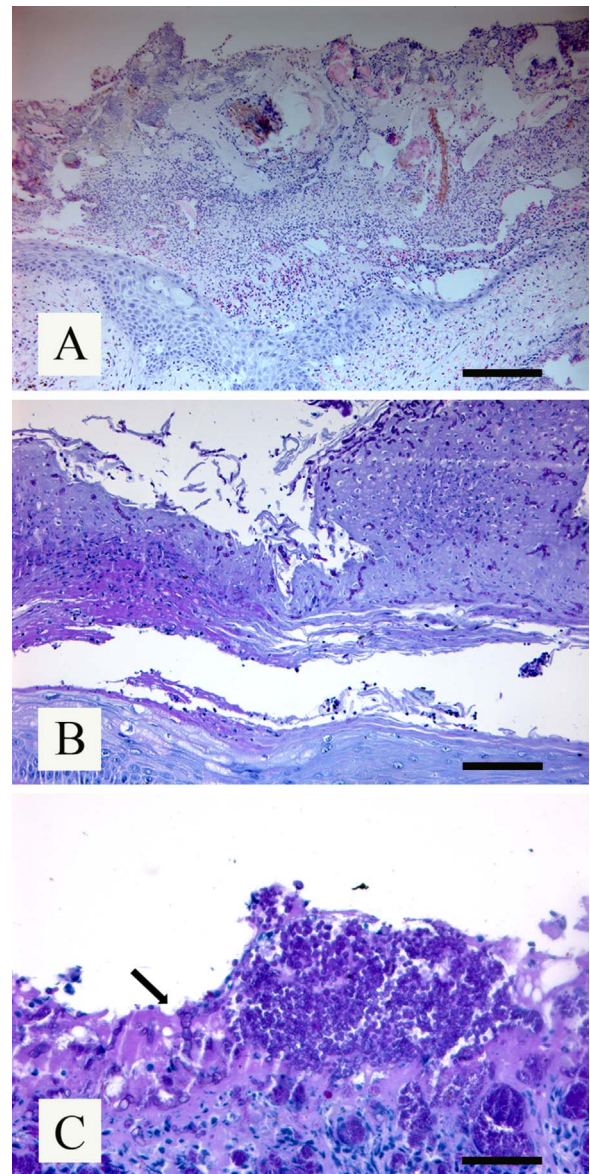


Fig. 2. Microscopic lesions associated with *Trichosporon jirovecii* infection in the examined turtle: A) Superficial ulcer covered by a dense necrotic material infiltrated by heterophils, macrophages and rare eosinophils (H-E stain; bar = 200 μm); B) PAS staining highlighted the presence of yeast aggregates and pseudohyphae (PAS stain; bar = 100 μm); C) Large aggregates of brilliant magenta round yeasts measuring 14–16 μm in diameter and tangles pseudohyphae (PAS stain; bar = 50 μm).

AACAAAAATAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGA AGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG AATCATCGAATCTTTGAACGCAACTTGCGCTCTCTGGTATTCCGGAGA GCATGCCTGTTTGTAGTATCATGAAATCTCAACCATTAGGGTTTCTTAA TGGCTTGGATTTGGGCGCTGCCACTTGCCCTGGCTCGCTTAAAAGAGT TAGCGTGTAACTTGTGCGATCTGGCGTAATAAGTTTCGCTGGTGTAG ACTTGAGAAGTGGCGTTCTAATCGTCTTCGGACAATTCTTGAACCTCTG GTCTCAAATCAGGTAGGGCTACCCGCTGAACCTTAAGCATAT) was submitted to BLAST software (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and showed a 100% identity with *Trichosporon jirovecii*.

A further treatment along with a surgical intervention was started at day 40. Povidone iodine and Irujol® ointment (containing chloramphenicol and collagenase) were daily administered through the drainage for 2 weeks. Enrofloxacin administration was continued. After treatment the ulcer healed with residual scars (Fig. 1c). No relapse was registered at day 365.

3. Discussion

The present report firstly describes a cutaneous trichosporonosis in Chelonians.

Trichosporon spp. seem to occur in specific ecological niches. They have been isolated from the gastrointestinal tract of reptiles [12,13]. Although the ecology is reported as unknown [14] *T. jirovecii* has been isolated from water and sediments of Central Lake in Colombia [15], suggesting an environmental distribution.

The taxonomy of this yeast genus has been object of several investigations. *T. jirovecii* was firstly described by Fragner [16] from a fungal isolate cultured from human nails, later the species was classified as synonym of *Trichosporon cutaneum* [14], then was reconsidered as a proper species on the basis of a phylogenetic analysis [17], describing two isolates. The other type strain was described as *Trichosporon beemeri* sp. nov. [18], and had been isolated from a *Crocodylus niloticus* affected by a mucocutaneous infection.

Moreover *T. cutaneum* is reported as causing agent of a nuchal haematoma of a Carolina anole lizard (*Anolis carolinensis*), subsequent to a bite from conspecific [19], and of several cases of dermatitis and carapace inflammation in lizards and tortoises, and histological evidence showed that this yeast was also present in cases of stomatitis, enteritis, pneumonia and polygranulomatosis [2].

Azoles and in particular voriconazole are first choice antimycotic agents for treating invasive *Trichosporon* spp. infection in human beings. In the present case topical iodine povidone was applied on the wound, due to lack antifungal drugs for veterinary use, to avoid an off label voriconazole administration.

T. jirovecii is considered as rare yeast pathogen by ESCMID and ECMM [20]. While in fact *Trichosporon asahii* and *Trichosporon mucoides* are responsible for deep human infections, other species are considered as causative agents of superficial infections [17], not spreading to underlying tissues. For these reasons the zoonotic risk would be considered negligible.

Ethical form

Please note that this journal requires full disclosure of all sources of funding and potential conflicts of interest. The journal also requires a declaration that the author(s) have obtained written and signed consent to publish the case report from the patient or legal guardian(s).

The statements on funding, conflict of interest and consent need to be submitted via our Ethical Form that can be downloaded from the submission site www.ees.elsevier.com/mmcr.

Conflict of interest

There are none conflict of interests.

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