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Eumycetoma caused by *Madurella mycetomatis* in a mare

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A case of equine eumycotic mycetoma caused by *Madurella mycetomatis* is described. This is the first report of *M. mycetomatis* as the etiologic agent of human or animal infections in Israel. The definitive identification of the isolate was established by molecular methods. In addition the mold was cultured on various media through which we found that its growth rate was significantly augmented on incubation on trypticase soy agar, more so if horse serum was added to this medium.

Sixteen previously published cases defined by the authors as mycetoma in horses are briefly reviewed. Among these, only one indicated that the infection was caused by *M. mycetomatis*.

Keywords Eumycetoma, horse, *Madurella mycetomatis*, Trypticase Soy Agar

Introduction and literature review

Eumycetoma is a mycotic infection that may be visceral or subcutaneous. If the latter, sinuses and fistulae exuding purulent material containing fungal aggregates in a matrix, i.e., 'granules', are formed. The infection results from the traumatic implantation of the etiologic agent [1]. Human cases are usually restricted to what has been defined as the 'mycetoma belt', between 15° south and 30° north [1]. In Israel, situated just north of this 'belt', there have been no human cases and only one involving a dog [2].

We found 16 publications [3–18] reporting cases defined by their authors as eumycetoma in horses, with the etiological agent identified by culture or immunological techniques in 11 (Table 1). Among these, three cases [3,6,8] were caused by *Bipolaris spicifera* (*Helminthosporium spiciferum* or *Curvularia spicifera* at the time of publication). However, the illustrations in these three papers, as well as in another in which the etiology was not established [7], seem to indicate that the cases represented chromoblastomycosis or phaeohyphomycosis rather than eumycetoma. The isolates from the remaining cases were identified as *Scedosporium apiospermum* (*Monosporium apiospermum* at the time of some publications) on four occasions and one each of *Aspergillus versicolor*, *Curvularia verruculosa*, *Phialophora oxyspora* and *Madurella mycetomatis*.

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Case report

In April 2007 a 3-year-old Haflinger mare was presented with a wound, measuring about 3 cm, located on the left hind foot at the fetlock which had been refractory to treatment for the last 6 months. No limping was observed. The mare was used for riding on a rocky terrain, abundant with thorny bushes, about 15 km west of Jerusalem. The lesions consisted of one main lesion and several smaller ones where fistulae were formed (Fig. 1a). Black 'granules' measuring up to 0.5 cm were seen in the wound and on bandages (Fig. 1b). Additional, smaller, wounds were seen between the fetlock and the hoof. These wounds were interconnected by sinuses. Initial treatment consisted of debridement and bandaging with an ointment containing triamcinolone, nystatin, neomycin, and gramicidin. Systemic antibacterial and antiparasitic treatment was initiated (sulfa-trimethoprim combination and Ivermectin, respectively). About 10 cc Polidine was injected daily into the lesion through the fistulae (Fig. 1c).

A biopsy was submitted for histopathology and examination of hematoxylin-eosin stained slides revelaed a granulomatous reaction containing pigmented fungal aggregates. Granules and swabs from the lesion were taken and submitted for bacteriological and mycological culture, as detailed below. Due to economic constrains and the very low probability of therapeutic success the lesion was not treated. The tumefaction continued to increase and new sinuses appeared (Fig. 1d). Currently, 3 years after the appearance of the lesion, the tumefaction has increased to about one and a half its initial size but there is still no limping, indicating that ligaments, joints and bones have

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Table 1 Publications reporting cases defined by their authors as eumycetomata, in chronological order. Current nomenclature used for isolates

Country [Ref.]	Anatomic location	Etiology	Diagnosis/Identification	Remarks
USA [4]	Subcutaneous	NE	Histopathology	Pigmented granules present
Germany [5]	Subcutaneous	*Scedosporium apiospermum*	Histopathology, Culture	Granules present
Canada [9]	Retrobulbar	*Scedosporium apiospermum*	Histopathology, Culture	Granules present
USA [10]	Uterus	*Scedosporium apiospermum*	Culture	Culture only. Granules present but no histological information given
South Africa [11]	Subcutaneous	NE	Histopathology	Two cases. Granules present
Australia [12]	Subcutaneous	NE	Histopathology	Two cases. Pigmented granules present
Czech Rep. [13]	Subcutaneous	See remarks	Histopathology	Granules present. Assumed to be *Pseudallescheria boydii* based on histopathological characteristics
South Africa [14]	Subcutaneous	*Madurella mycetomatis*	Histopathology, Culture	Granules present
USA [15]	Subcutaneous	See remarks	Histopathology, Immunological	Granules present. Identification methods do not allow differentiation between *Pseudalescheria boydii* and *Scedosporium apiospermum*
Argentina [16]	Subcutaneous	*Curvularia verruculosa*	Histopathology, Culture	Granules present. Three cases
USA [17]	Subcutaneous	*Aspergillus versicolor*	Histopathology, Culture	Granules absent. Classified as 'mycetoma like'
USA [18]	Mouth	*Phialophora oxyspora*	Histopathology, Molecular	Granules present

NE, not established; WA, Water agar.

not been affected. The mare continues to take part in riding trips. An update on the final outcome of the case will be published as a letter to the editor.

Microbiological examination

All media, unless otherwise specified were produced by Becton Dickinson, USA. The swabs from the lestions were inoculated onto 5% sheep blood agar, nutrient agar and McConkey agar for bacteriologic examination. In addition, swabs and granules were inoculated onto Sabouraud's dextrose agar (SDA) for mycologic examination. The bacteriologic media were incubated at 37ºC under aerobic (all) and anaerobic (5% sheep blood agar) conditions resulting in the growth of a mixed flora of contaminants. Cultures on SDA were incubated at 30ºC. Three weeks after

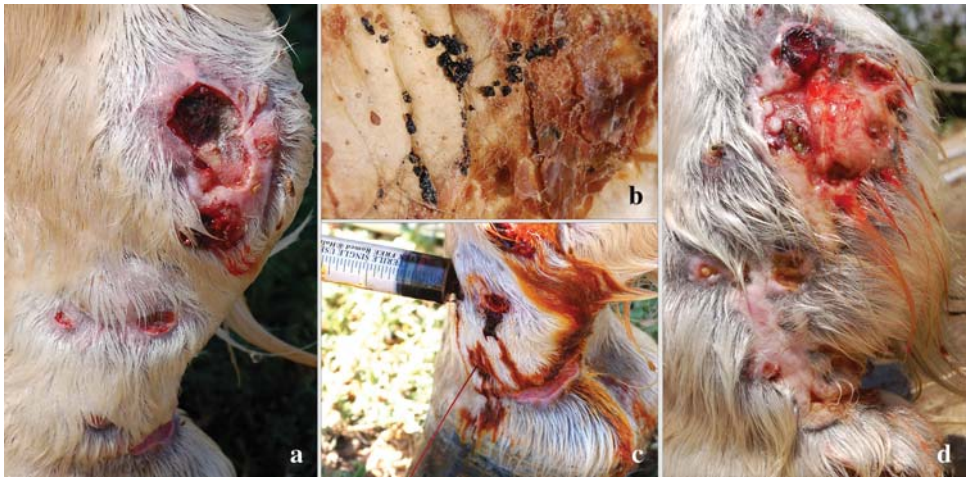

Fig. 1 Clinical aspects. (a) Lesions upon presentation, May 2007. (b) Bandage containing secreted granules. (c) Sinuses and fistulae: Polidine injected through one fistula exiting through another. (d) Lesions a year later, May 2008.

inoculation, colonies of a pigmented mold, having a diameter of about 5 mm, were detected in the cultures inoculated with granules. Hyphae were sterile and a tentative identification of *Madurella* spp. was made. Since SDA and 30ºC are unfavorable for the growth of this fungus, it was subcultured to 2% water agar (WA), potato dextrose agar (PDA), corn meal agar (CMA) and CMA with 1% Tween80 (CMA-TW; Sigma-Aldrich, USA) and potato carrot agar (PCA) (prepared according to the ATCC recipe – www.atcc.org/Attachments/3654.pdf) to induce conidial formation. In addition, it was inoculated onto trypticase soy agar (TSA) and TSA with 5% (vol/vol) horse serum (TSA-HS), in the hope that the latter will boost the growth rate of the fungus. All media were incubated at 37ºC for two weeks. The *M. mycetomatis* isolate recovered in this case grew faster on TSA than on the other media, more so if horse serum was added. The slowest growth was observed on PDA. On TSA, TSA-HS and PDA colonies were downy brown and produced diffusible pigment (Fig. 2). Colonies on PCA and WA were lacey, white and sclerotia were produced. On CMA and CMA-TW colonies were lacey, white and growth was submerged in the agar. The fungus did not produce conidia on any of the media. *Candida albicans*, as identified by the ID32C kit (bioMerieux, France), was recovered in the SDA cultures inoculated with swabs. Since the clinical and histological aspects of the lesions did not support candidasis, this was deemed a secondary contaminant.

The definitive identification of the fungus was achieved by molecular methods. Fungal development and DNA extraction were performed as previously described [19] except that the fungus from which DNA was to be recovered was grown at 37ºC rather than 30ºC. Fungal universal primers [20], synthesized by Hy Laboratories (Rehovoth, Israel), were used to amplify the spacer ITS1-5.8S-ITS2 region fragment of the genomic rRNA gene: GGAAGTAAAAGTCGTAACAAGG for ITS5 and TCCTCCGCTTATTGATATGC for ITS4. For the PCR reaction a solution containing 5 units of taq DNA polymerase (AmpliTaq, Applied Biosystems, USA), 300 mM Tris-HCL pH = 8.5, 75 mM $(NH_4)SO_4$, $MgCl_2$, 1 µl template DNA and 100 µM Dxtp (deoxynucleotide triphosphate) was prepared and 100 ng of each primer were suspended in 25 µl of this solution. For the amplification, a PTC 200 Peltier Thermal Cycler (MJ Research, USA) was used. DNA was denatured at 95°C for 10 min. The subsequent steps included 30 cycles of 30 sec at 94°C, 50°C and 72°C and was concluded with an extension step consisting of 10 min at 72°C. Results were visualized on an 1.5% agarose gel stained with ethidium bromide. A 564 bp product was obtained that was purified (GenElute agarose spin columns, Sigma, USA) and sequenced in an Automatic Sequencer 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA) with ITS4 and ITS5 primers.

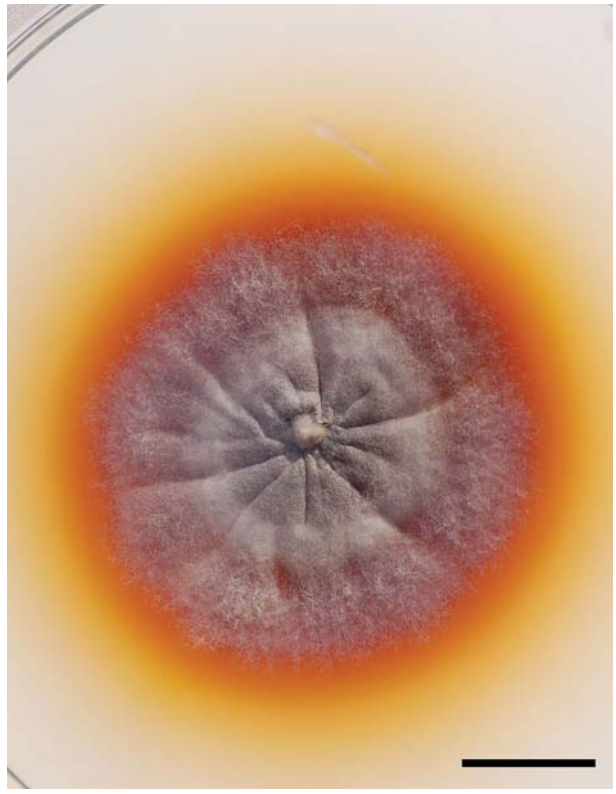

Fig. 2 Colony on trypticase soy agar after 8 days of incubation at 37°C. Bar = 1 cm.

A BLAST search (www.ncbi.nlm.nih.gov/blast/blast.cgi) of the GENBANK and EMBL data bases (accession number GQ415325) revealed a perfect (100%) homology between the 564 bp PCR fragment and *Madurella mycetomatis* sequences (accession number DQ 836767, DQ836768, DQ836769, DQ836770, DQ836771, DQ836772, DQ836773, DQ836774) [19].

This is the first case of an infection caused by *M. mycetomatis* in Israel and only the second involving a horse. However, the mycological information regarding the isolate in the other case is limited [14]. The source of our isolate most likely is the thorny area in which the animal was used for riding. However, since mycetomas have not been reported in other horses that routinely pass through the same area, there must be additional risk factors affecting this specific mare. TSA-HS has been found to significantly boost the growth of *M. mycetomatis*. Whether this activity is species specific, i.e., horse serum acts on equine isolates and human serum on human isolates, is still to be determined.

Declaration of interest: No conflict of interest exists for any of the authors.

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