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Cutaneous histoplasmosis in a roadkill European badger (*Meles meles*), Czech Republic

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Abstract

Histoplasmosis is caused by a thermally dimorphic ascomycete fungus, *Histoplasma capsulatum*. Europe has long been considered a non-endemic area for histoplasmosis; however, in recent decades, sporadic cases of histoplasmosis have been confirmed in the European badger (*Meles meles*) in Austria, Denmark, Germany and Switzerland. In 2014, an adult male badger carcass was found on a highway on the outskirts of the city of Brno (Southern Moravia, Czech Republic). A gross pathological examination revealed a good overall body condition prior to receiving multiple traumatic injuries from a car impact. However, multiple irregular nodular lesions measuring up to 5 cm in diameter were observed on the skin of the ventral part of the body and the thoracic limbs. Histopathology revealed a granulomatous inflammatory reaction in the lesions, with abundant macrophages multifocally forming large multinucleated giant cells, lymphocytes, plasma cells and scarce granulocytes. The macrophages contained oval to spherical 2.5–3.0 µm yeast-like structures in the cytoplasm. These stained mildly to moderately with haematoxylin and eosin and well with periodic acid Schiff, revealing a small central nucleus surrounded by a clear zone. Other organs showed no macroscopic or microscopic pathology. The internal transcribed spacer rDNA sequence from the skin was identical to a sequence previously detected in a *H. capsulatum*-infected badger in Germany. This case report detailing cutaneous histoplasmosis in a European badger marks the first documentation of *H. capsulatum* in the Czech Republic and emphasises the potential for early detection of new and emerging pathogens through wildlife disease monitoring.

Endemic mycosis, European wildlife, Histoplasma capsulatum

The causative agent of histoplasmosis, *Histoplasma capsulatum*, a thermally dimorphic ascomycete fungus, is known to switch from filamentous saprophytic growth in natural habitats or in culture at 25 to 28 °C to budding yeasts when parasitic in warm-blooded hosts or in culture at 37 °C (Gauthier 2015). The sexual stage of the fungus is termed *Ajellomyces capsulatus*. *Histoplasma* thrives in specific habitats, particularly in soil mixed with faeces of birds or bats in caves (Guillot et al. 2018). Mammals, including humans, typically contract the infection by inhaling airborne propagules (conidia), often after disturbing contaminated material. Consequently, people undertaking activities such as archaeology, speleology, gardening, farming or landscaping may be at increased risk of infection. While individuals with compromised immune systems or those exposed to large doses are at greater risk of developing pneumonia and disseminated infection (Scully and Baddley 2018), there is no evidence that histoplasmosis spreads between infected animals or people.

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Subspecies of *H. capsulatum* show variable geographical distribution and clinical signs in different species (Guillot et al. 2018). For example, *H. capsulatum* var. *capsulatum*, found in many regions around the world, causes pulmonary and systemic infections with small-sized yeast-like cells in humans and animals. In contrast, *H. capsulatum* var. *duboisii*, reported from Western and Central Africa, develops as large-sized yeast cells with lymphadenopathy and dissemination to the skin and bones in primates (Develoux et al. 2021), whereas *H. capsulatum* var. *farciminosum*, developing in the skin and subcutaneous lymphatic system, is recognised as 'epizootic lymphangitis' in horses and other equids (Weeks et al. 1985).

Histoplasma capsulatum is a complex of at least nine phylogenetic clades differing in geographical range (Arunmozhi Balajee et al. 2013; Kasuga et al. 2003). Multiple typing methods have been developed for the identification and study of *H. capsulatum* epidemiology, with PCR fingerprinting, sequencing of ITS1 and ITS2 internal transcribed spacers (ITS rDNA region) and sequencing of four protein-coding genes most often applied (Muniz et al. 2010). Though all methods have comparable usability, multi-copy ITS rDNA is easily accessible and is routinely used for identification and genetic studies (Tamura et al. 2002; Murata et al. 2007; Muniz et al. 2010; Landaburu et al. 2014).

While *H. capsulatum* is widely distributed in tropical and subtropical areas of the world where it causes systemic mycosis in numerous mammalian hosts including humans, Europe has long been considered a non-endemic area (Taylor et al. 2022). Nevertheless, wild mammals infected with *H. capsulatum* have been reported in Europe, including European badgers (*Meles meles*; Akdesir et al. 2018; Bauder et al. 2000; Eisenberg et al. 2013; Wilmes et al. 2022), a European hedgehog (*Erinaceus europaeus*; Jacobsen et al. 2011), and the common noctule bat (*Nyctalus noctula*; González-González et al. 2013). Interestingly, case reports also include an outdoor domestic cat (*Felis catus*; Mavropoulou et al. 2010) and a Dorcas gazelle at a Spanish zoo (*Gazella dorcas neglecta*; Fariñas et al. 2009). Human histoplasmosis in Europe is mostly attributed to travel to and migration from endemic regions outside Europe; however, increasing numbers of cases in Italy, Germany, Türkiye, Switzerland, Spain and France appear to be autochthonous (Antinori et al. 2021; Ashbee et al. 2008). This suggests that the geographic distribution and/or epidemiology of this fungus may be evolving (Ashraf et al. 2020), highlighting the need for broader surveillance of new emergent pathogens (Moreira et al. 2022).

Here, we report the first case of cutaneous histoplasmosis in a badger in the Czech Republic, confirming the presence of this fungal agent in the Central European region.

Materials and Methods

An adult male badger, found dead on a highway on the outskirts of the city of Brno (Southern Moravia, Czech Republic), was submitted for necropsy on the day of finding in August 2014. Following a macroscopic examination, tissue specimens were collected for histopathological examination (skin lesions, lungs, liver, kidneys). The tissues were fixed in buffered 10% neutral formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 4 µm, and routinely stained with haematoxylin and eosin (H&E) and periodic acid Schiff (PAS) for the detection of fungal elements in the tissues. Total DNA from the skin lesions was isolated using the ArchivePure DNA yeast and Gram2+ kit (5PRIME Inc., Gaithersburg, Maryland). PCR amplification and sequencing of the ITS rDNA region (ITS1-5.8S-ITS2 rDNA) was conducted using the protocols of Hubka et al. (2014), alongside the fungal-specific primers ITS1F-KYO2 (Toju et al. 2012) and ITS4S (Kretzer et al. 1996). The complete ITS rDNA sequence (529 bp) was deposited under GenBank accession code PX237214 and compared with published data deposited in NCBI GenBank. Fungal cultures of samples collected from skin lesions were performed on Sabouraud dextrose and brain heart infusion agars with antibiotics (chloramphenicol and gentamicin) at 25 and 37 °C for eight weeks (Guemas et al. 2020). The results were compared with those for eight other roadkill badger specimens (three males, five females) collected along the same stretch of highway between 2015 and 2024 and also tested for histoplasmosis.

Results and Discussion

Gross pathology

The badger was in good overall body condition; however, gross pathological examination showed multiple traumatic injuries from a car impact, including fractures and severe bleeding in the thoracic and abdominal cavities. Skin examination revealed multiple irregular nodular lesions in the ventral, predominantly abdominal, part of the body and in the skin of the thoracic limbs in the ulnar region. The nodules were prominent, appeared as solid, grey-coloured masses, firm on palpation, displaceable with the skin, and measured up to 5 cm in diameter, often with the presence of superficial ulcerations and haemorrhages (Plate I, Fig. 1). Other organs were without any macroscopic pathology.

Histopathology

The multiple nodular skin lesions detected macroscopically were differently sized foci, often coalescing, of granulomatous inflammatory reaction that spread throughout the dermis (Plate I, Fig. 2). The granulomas consisted of abundant macrophages multifocally forming large multinucleated giant cells, lymphocytes, plasma cells and scarce granulocytes within the site of the inflammatory reaction. Focally in the skin, there was moderate to marked proliferation of fibrous tissue. Most granulomas were superficially exulcerated. The macrophage cytoplasm contained oval to spherical yeast-like structures measuring 2.5–3.0 µm. Numerous fungal elements were found intracellularly and diffusely scattered throughout the proliferating fibrovascular tissue in the dermis due to cell rupture. The fungal cells stained well with PAS and mildly to moderately with H&E, and showed a small central nucleus surrounded by a clear zone (Plate II, Figs. 3 and 4). Tissue sections of the lungs, liver and kidneys did not show the presence of fungal elements, signs of inflammatory reaction or any other pathological features.

Gross and microscopic pathology findings were consistent with cutaneous histoplasmosis, suggesting a primary skin infection without dissemination (Grosse et al. 1997; Bauder et al. 2000; Eisenberg et al. 2013; Wilmes et al. 2022). The fungal culture failed to grow the agent in this case, which is not uncommon (Eisenberg et al. 2013; Wilmes et al. 2022).

Molecular detection

The ITS rDNA sequence obtained from the tissue sample showed 100–96% homology with published *H. capsulatum* (*A. capsulatus*) sequences, and a 90% closest match to the next taxon, *Emmonsia parva* (AF038329). The sequence proved identical to that obtained from an infected badger in Germany (HE806328; Eisenberg et al. 2013), and to other published sequences of comparable length (coverage ≥ 86%), differing by two or more substitutions. As both the present specimen and that from Germany belong to the same ITS haplotype, not known from other studied material so far, this would suggest that it belongs to the same, or a very closely related, fungal clone with geographic- and host-limited distribution. See Eisenberg et al. (2013) providing a detailed discussion about genetic relatedness and epidemiology. The German specimen, which was characterised using multiple protein-coding genes, was identified as belonging to the Eurasian clade (Eisenberg et al. 2013), which originated from within one of the Latin American phylogenetic species of the *H. capsulatum* species complex (Kasuga et al. 2003). The Eurasian clade, which comprises isolates from Britain, India, Thailand, China and Japan, is typically found in horses, badgers and dogs but rarely causes clinical histoplasmosis (Kasuga et al. 2003; Murata et al. 2007). Some evidence suggests that Eurasian clade isolates originating from animals could affect humans; however, their veterinary or zoonotic potential is little known and, based on the number of reported cases, is assumed to be very low (Kasuga et al. 2003; Murata et al. 2007; Eisenberg et al. 2013).

The present finding represents the eleventh report of histoplasmosis in a European badger, which, considering the fungus is rarely found in other hosts in Central Europe, would suggest the species is a typical host of the fungus (Eisenberg et al. 2013). Large-scale monitoring of carnivore road mortality data indicates that the European badger is one of the most common species killed on roads in the Czech Republic (Červinka et al. 2015). Interestingly, many histoplasmosis-positive badgers have been diagnosed through the examination of such road casualties (Eisenberg et al. 2013), suggesting that the disease may increase the animals' risk of dying on the road. Evidence shows that *H. capsulatum* is present in the habitat of the European badger, and its immunity to this fungal pathogen appears limited because badger macrophages fail to produce nitric oxide, a key effector of cellular innate immunity (Bilham et al. 2017). Host defences may also be suppressed due to exposure to environmental pollutants (Bandouchova et al. 2011).

Having confirmed histoplasmosis in the present specimen, we undertook follow-up testing of other road casualty badgers collected from the same region over the past decade, but found no other histoplasmosis-positive specimens, indicating the disease's sporadic nature. Neither the reservoir nor any possible source of histoplasma infection has been identified in the Czech Republic. In warmer areas, the fungus is typically found in habitats with nitrogen-rich soils, especially caves; however, it appears to be absent in caves of Central Europe (Nováková et al. 2010; Nováková et al. 2012; Vanderwolf et al. 2013). We can only hypothesise, therefore, that the tunnelling activities of badgers bring them into contact with soil containing the fungus, and that the confined spaces of excavated badger setts provide favourable microclimatic conditions for *H. capsulatum*. Theoretically, earthdogs and hunters may be exposed to soil contaminated with this fungus. The public health importance and geographic distribution within the Czech Republic are much lower than those of other zoonoses (Pikula et al. 2003; Pikula et al. 2004).

In conclusion, this study confirmed presence of *H. capsulatum* infection in the Czech Republic for the first time. Further studies will be needed to fully understand the disease's ecology and epidemiology, including the apparent sentinel role and susceptibility of the European badger.

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References

Akdesir E, Origgi FC, Wimmershoff J, Frey J, Frey CF, Ryser-Degiorgis MP 2018: Causes of mortality and morbidity in free-ranging mustelids in Switzerland: necropsy data from over 50 years of general health surveillance. BMC Vet Res **14**: 195

Antinori S, Giacomelli A, Corbellino M, Torre A, Schiuma M, Casalini G, Parravicini C, Milazzo L, Gervasoni C, Ridolfo AL 2021: Histoplasmosis diagnosed in Europe and Israel: a case report and systematic review of the literature from 2005 to 2020. J Fungi **7**: 481

Arunmozhi Balajee S, Hurst SF, Chang LS, Miles M, Beeler E, Hale C, Kasuga T, Benedict K, Chiller T, Lindsley MD 2013: Multilocus sequence typing of *Histoplasma capsulatum* in formalin-fixed paraffin-embedded tissues from cats living in non-endemic regions reveals a new phylogenetic clade. Med Mycol **51**: 345-351

Ashbee HR, Evans EGV, Viviani MA, Dupont B, Chryssanthou E, Surmont I, Tomsikova A, Vachkov P, Ener B, Zala J, Tintelnot K, The ECMM Working Group on Histoplasmosis 2008: Histoplasmosis in Europe: report on an epidemiological survey from the European Confederation of Medical Mycology Working Group. Med Mycol **46**: 57-65

Ashraf N, Kubat RC, Poplin V, Adenis AA, Denning DW, Wright L, McCotter O, Schwartz IS, Jackson BR, Chiller T, Bahr NC 2020: Re-drawing the maps for endemic mycoses. Mycopathologia **185**: 843-865

Bandouchova H, Pohanka M, Kral J, Ondracek K, Osickova J, Damkova V, Vitula F, Treml F, Pikula J 2011: Effects of sublethal exposure of European brown hares to paraoxon on the course of tularemia. Neuroendocrinol Lett **32** (Suppl 1): 77-83

Bauder B, Kübber-Heiss A, Steineck T, Kuttin E.S., Kaufman L 2000: Granulomatous skin lesions due to histoplasmosis in a badger (*Meles meles*) in Austria. Med Mycol **38**: 249-253

Bilham K, Boyd AC, Preston SG, Buesching CD, Newman C, Macdonald DW, Smith AL 2017: Badger macrophages fail to produce nitric oxide, a key anti-mycobacterial effector molecule. Sci Rep **7**: 45470

Červinka J, Riegert J, Grill S, Šálek M 2015: Large-scale evaluation of carnivore road mortality: the effect of landscape and local scale characteristics. Mamm Res **60**: 233-243

Develoux M, Amona FM, Hennequin C 2021: Histoplasmosis caused by *Histoplasma capsulatum* var. *duboisii*: a comprehensive review of cases from 1993 to 2019. Clin Infect Dis **73**: e543-e549

Eisenberg T, Seeger H, Kasuga T, Eskens U, Sauerwald C, Kaim U 2013: Detection and characterization of *Histoplasma capsulatum* in a German badger (*Meles meles*) by ITS sequencing and multilocus sequencing analysis. Med Mycol **51**: 337-344

Fariñas F, Flores L, Rodríguez P, Sabalete T, Quevedo MA 2009: Disseminated histoplasmosis in a dorcas gazelle (*Gazella dorcas neglecta*) kept in captivity conditions in Spain. Rev Iberoam Micol **26**: 152-154

Gauthier GM 2015: Dimorphism in fungal pathogens of mammals, plants, and insects. PLoS Pathog **11**: e1004608

González-González AE, Ramírez JA, Aliouat-Denis CM, Demanche C, Aliouat EM, Dei-Cas E, Chabé M, Taylor ML 2013: Molecular detection of *Histoplasma capsulatum* in the lung of a free-ranging common noctule (*Nyctalus noctula*) from France using the *Hcp100* gene. J Zoo Wildlife Med **44**: 15-20

Grosse G, Staib F, Rapp J, Rang H, Heise W, Kaufma L 1997: Pathological and epidemiological aspects of skin lesions in histoplasmosis: observations in an AIDS patient and badgers outside endemic areas of histoplasmosis. Zentralblatt Bakteriol **285**: 531-539

Guemas E, Sobanska L, Demar M 2020: *Histoplasma capsulatum* and histoplasmosis: current concept for the diagnosis. In: Bongomin F (Ed.): *Histoplasma* and histoplasmosis. IntechOpen, London, pp. 1-19

Guillot J, Guérin C, Chermette R 2018: Histoplasmosis in animals. Emerging and epizootic fungal infections in animals. In: Seyedmousavi S, De Hoog GS, Guillot J, Verweij PE (Eds): Emerging and epizootic fungal infections in animals. Springer International Publishing, Switzerland, pp. 115-128

Hubka V, Dobiášová S, Dobiáš R, Kolařík M 2014. *Microsporum aenigmaticum* sp. nov. from *M. gypseum* complex, isolated as a cause of tinea corporis. Med Mycol **52**: 387-396

Jacobsen B, Baumgartner W, Bialek R 2011: Disseminated histoplasmosis in a European hedgehog (*Erinaceus europaeus*) in Northern Germany. Mycoses **54**: 538-541

Kasuga T, White TJ, Koenig G, McEwen J, Restrepo A, Castañeda E, Da Silva Lacaz C, Heins-Vaccari EM, De Freitas RS, Zancopé-Oliveira RM, Qin Z, Negroni R, Carter DA, Mikami Y, Tamura M, Taylor ML, Miller GF, Poonwan N, Taylor JW 2003: Phylogeography of the fungal pathogen *Histoplasma capsulatum*. Mol Ecol **12**: 3383-3401

Kretzer A, Li Y, Szaro T, Bruns TD 1996: Internal transcribed spacer sequences from 38 recognized species of *Suillus* sensu lato: phylogenetic and taxonomic implications. Mycologia **88**: 776-785

Landaburu F, Cuestas ML, Rubio A, Elías NA, Daneri GL, Vecino C, Iovannitti CA, Mujica MT 2014: Genetic diversity of *Histoplasma capsulatum* strains isolated from Argentina based on nucleotide sequence variations in the internal transcribed spacer regions of rDNA. Mycoses **57**: 299-306

Mavropoulou A, Grandi G, Calvi L, Passeri B, Volta A, Kramer LH Quintavalla C 2010: Disseminated histoplasmosis in a cat in Europe. J Small Anim Pract **51**: 176-180

Moreira LM, Meyer W, Chame M, Brandão ML, Vivoni AM, Portugal J, Wanke B, Trilles L 2022: Molecular detection of *Histoplasma capsulatum* in Antarctica. Emerg Infect Dis **28**: 2100-2104

Muniz MDM, Morais e Silva Tavares P, Meyer W, Nosanchuk JD, Zancope-Oliveira RM 2010: Comparison of different DNA-based methods for molecular typing of *Histoplasma capsulatum*. Appl Environ Microbiol **76**: 4438-4447

Murata Y, Sano A, Ueda Y, Inomata T, Takayama A, Poonwan N, Nanthawan M, Mikami Y, Miyaji M, Nishimura K, Kamei K 2007: Molecular epidemiology of canine histoplasmosis in Japan. Med Mycol **45**: 233-247

Nováková A, Kolařík M 2010: *Chrysosporium speluncarum*, a new species resembling *Ajellomyces capsulatus*, obtained from bat guano in caves of temperate Europe. Mycol Prog **9**: 253-260

Nováková A, Šimonovičová A, Kubátová A 2012: List of cultivable microfungi recorded from soils, soil related substrates and underground environment of the Czech and Slovak Republics. Mycotaxon **119**: 189

Pikula J, Treml F, Beklová M, Holešovská Z, Pikulová J 2003: Ecological conditions of natural foci of tularaemia in the Czech Republic. Eur J Epidemiol **18**: 1091-1095

Pikula J, Beklova M, Holesovska Z, Treml F 2004: Ecology of European brown hare and distribution of natural foci of tularaemia in the Czech Republic. Acta Vet Brno **73**: 267-273

Scully MC, Baddley JW 2018: Epidemiology of histoplasmosis. Curr Fungal Infect Rep **12**: 51-58

Tamura M, Kasuga T, Watanabe K, Katsu M, Mikami Y, Nishimura K 2002: Phylogenetic characterization of *Histoplasma capsulatum* strains based on ITS region sequences, including two new strains from Thai and Chinese patients in Japan. Jpn J Med Mycol **43**: 11-19

Taylor ML, Reyes-Montes MDR, Estrada-Bárcenas DA, Zancopé-Oliveira RM, Rodríguez-Arellanes G, Ramírez JA 2022: Considerations about the geographic distribution of *Histoplasma* species. Appl Environ Microbiol **88**: e02010-21

Toju H, Tanabe AS, Yamamoto S, Sato H 2012: High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. Plos One **7**: e40863

Vanderwolf KJ, Malloch D, McAlpine DF, Forbes GJ 2013: A world review of fungi, yeasts, and slime molds in caves. Int J Speleol **42**: 9

Weeks RJ, Padhye AA, Ajello L 1985: *Histoplasma capsulatum* variety *farciminosum*: a new combination for *Histoplasma farciminosum*. Mycologia **77**: 964-970

Wilmes D, Mayer U, Wohlsein P, Suntz M, Gerkrath J, Schulze C, Holst I, von Bomhard W, Rickerts V 2022: Animal histoplasmosis in Europe: review of the literature and molecular typing of the etiological agents. J Fungi **8**: 833

Fig. 1. Multiple ulcerated nodules with superficial haemorrhages in the skin of the ventral abdomen.

Fig. 2. Large area of granulomatous inflammatory reaction in the superficial dermis (left). H&E stain, × 40 magnification.

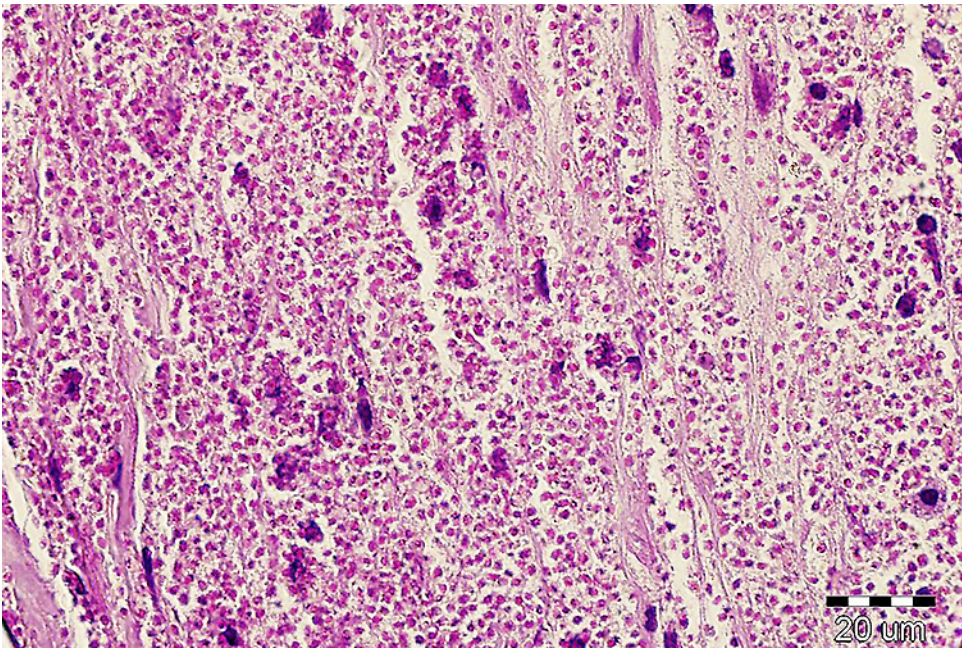

Fig. 3. Fungal cell masses scattered diffusely over the dermis, with multifocal presence of giant cells. PAS stain, × 1000 magnification.

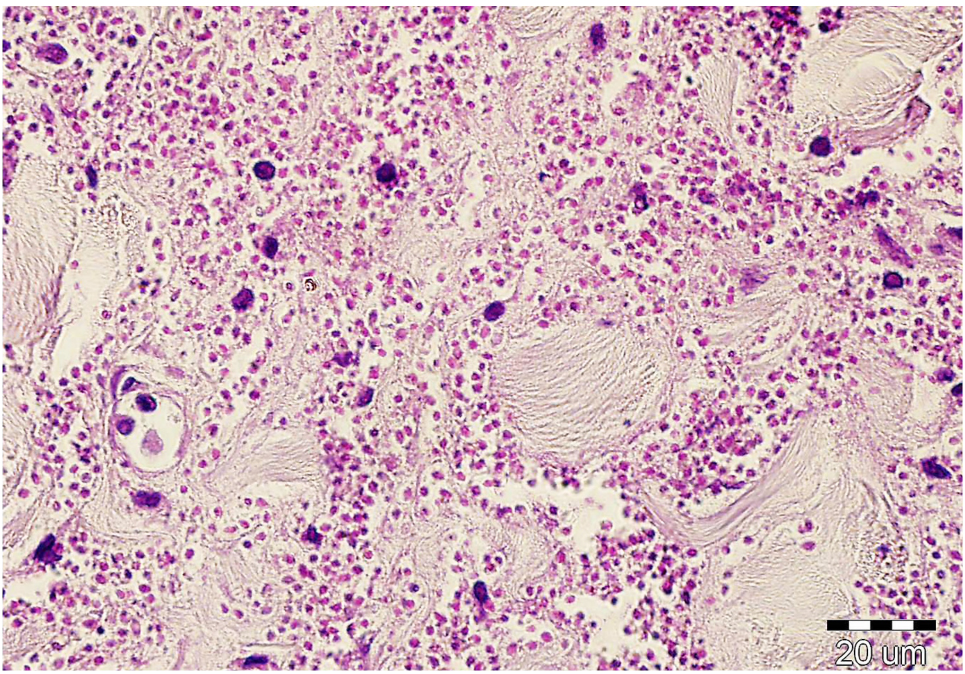

Fig. 4. Numerous fungal cells in the dermis surrounded by clear zones. PAS stain, × 1000 magnification.