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Research article

## Fungal diseases of horses

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## ABSTRACT

Among diseases of horses caused by fungi (=mycoses), dermatophytosis, cryptococcosis and aspergillosis are of particular concern, due their worldwide diffusion and, for some of them, zoonotic potential. Conversely, other mycoses such as subcutaneous (i.e., pythiosis and mycetoma) or deep mycoses (i.e., blastomycosis and coccidioidomycosis) are rare, and/or limited to restricted geographical areas. Generally, subcutaneous and deep mycoses are chronic and progressive diseases; clinical signs include extensive, painful lesions (not pathognomonic), which resemble to other microbial infections. In all cases, early diagnosis is crucial in order to achieve a favorable prognosis. Knowledge of the epidemiology, clinical signs, and diagnosis of fungal diseases is essential for the establishment of effective therapeutic strategies. This article reviews the clinical manifestations, diagnosis and therapeutic protocols of equine fungal infections as a support to early diagnosis and application of targeted therapeutic and control strategies.

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### 1. Introduction

Over the last two decades, the number of fungal and fungal-like diseases of plant and animals in both natural and controlled systems has increased, most likely as a consequence of environmental changes (Fisher et al., 2012). Similarly to other mammals, horses may be affected by several fungal diseases, although only some (i.e., dermatophytosis, pythiosis and epizootic lymphangitis, and aspergillosis) are well described (Al-Ani, 1999; Chermette et al., 2008; Gaastra et al., 2010; Cafarchia et al., 2012b). Most reports of fungal diseases are available as sporadic clinical cases, and comprehensive reviews on the topic date back to the 80s (Blackford, 1984). This article aims at summarizing the clinical manifestations, diagnosis and therapies of equine fungal infections, and at supporting clinicians and their efforts to diagnose the infections and set out targeted therapeutic and control strategies.

In the present review, equine fungal diseases are classified into: (i) superficial mycoses, caused by

pathogens confined to the *stratum corneum* except hairs; (ii) cutaneous mycoses, by pathogens invading keratinized tissues (including hairs, horns and skin); (iii) subcutaneous mycoses and; (iv) deep mycoses which affect the upper and/or lower respiratory tracts, as well as internal organs (de Hoog et al., 2000).

### 2. Superficial mycoses

Superficial mycoses are caused by facultative or commensal pathogens, which are responsible for mild inflammatory, usually benign infections, generally associated with underlying immunodepressive conditions in the mammalian host. Yeasts belonging to the genus *Malassezia* are the most frequent agents of superficial mycosis in horses (Nell et al., 2002; White et al., 2006; Kim et al., 2011).

#### 2.1. *Malassezia* infections

*Malassezia* yeasts have recently been under the spotlight in equine dermatology since they cause dermatitis in immunocompromised individuals (Nell et al., 2002; White

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et al., 2006; Kim et al., 2011). These yeasts can be isolated from the axilla, interbulbar region, groin and anus of healthy horses (~60%) (Nell et al., 2002), the intermammary region of mares and the preputial fossa in geldings (White et al., 2006). Out of the 14 species ranked within the genus, only *M. furfur*, *M. slooffiae*, *M. obtusa*, *M. globosa*, *M. restricta*, *M. equina* and *M. pachydermatis* have been isolated from horses (Cabañes et al., 2007), in which they cause benign infections of limited or no significance. However, perineal and ventral abdominal exudates associated with pruritus, and alopecic areas without inflammation, exudates or crusts have been described (White et al., 2006). The diagnosis usually requires cytological and cultural examinations and/or histology. For cytology, samples are usually collected by sticking tape strips on the skin of the infected animal; subsequently, the material collected is stained with May-Grunwald Giemsa (Nell et al., 2002; Kim et al., 2011). Samples are considered positive if large populations of *Malassezia* cells are counted at 40× magnification (cf. Cafarchia et al., 2005). Histological examinations of the skin of *Malassezia*-infected horses have previously led to the observation of superficial hyperplastic dermatitis with a predominance of lymphocytes and macrophages (Kim et al., 2011). Because of the lipophilic properties of these yeasts, fungal media supplemented with a range of lipid sources are suitable for their culture (e.g., Dixon's medium). Treatment protocols of the clinical disease are reported in Table 1.

### 3. Cutaneous infections

Cutaneous mycoses include fungal infections of keratinized tissues including hairs, horns and skin, which cause significant destruction of the keratinized tissues and induce variable (protective) immunological responses. Skin infections might also result from widely disseminated fungal infections; in these cases, a prompt observation of the clinical signs, followed by an appropriate diagnosis, is crucial for positive treatment outcomes.

#### 3.1. *Chromoblastomycoses*

Chromoblastomycosis (Syn: *Chromomycosis*, *Cladosporiosis*, *Fonseca's disease*, *Pedroso's disease*, *Phaeosporotrichosis*, *Verrucous dermatitis*) is a slow-developing chronic granulomatous fungal infection, which results in the formation of pigmented hard yeast cells, known as "muriform cells" or "sclerotic bodies". Clinically, the disease consists in the development of verrucose, dyschromic, scaly plaques, as well as atrophic patches and ulcerative lesions of the skin (Abid et al., 1987; López and Méndez, 2007). This disease occurs sporadically in horses; only a few cases, caused by *Fonsecaea* spp., have been reported in animals from the United States and Canada, localized to areas characterized by heavy rainfalls (Abid et al., 1987; López and Méndez, 2007). The infection is acquired following the accidental inoculation of the etiologic agent into the skin or the subcutaneous tissue. Following penetration of the tissue, the fungus transforms from filamentous to parasitic stages, known as muriform

bodies, which are not destroyable by macrophages and polymorphonuclear phagocytic cells (López and Méndez, 2007).

At first, from one to two months after the infection, nodular granuloma-like lesions with no draining tracts appear (Table 1—Abid et al., 1987); these lesions are clinically indistinguishable from melanomas, foreign body granulomas, squamous cell carcinomas, habronemosis, onchocerciasis, mycetomas, phaeohyphomycosis and sporotrichosis (Abid et al., 1987). The laboratory diagnosis requires a direct cytological examination of the affected skin sample using potassium hydroxide (KOH) and/or histopathological examination using Hematoxylin-Eosin (H&E) or Periodic acid-Schiff (PAS) or Gomori's methenamine silver (GMS) stains, followed by fungal culture of scrapings or biopsy material (Tables 1 and 2—Abid et al., 1987). Culture on Sabouraud dextrose agar (SAB) for 10 days produces velvet colonies, which are initially deep green in colour, and black later. Since spontaneous recovery from the disease is rare, cases of chromoblastomycoses require adequate therapies. In horses, surgery has been proposed as the only definitive solution (Abid et al., 1987; López and Méndez, 2007).

#### 3.2. *Dermatophytoses*

Dermatophytoses are superficial, cutaneous mycoses caused by dermatophytes. These diseases are considered zoonoses, since they can be transmitted from animals to humans. Dermatophytes are filamentous fungi which invade keratinized tissues of humans and animals, causing mild to severe, localized and/or diffuse infections. Zoophilic dermatophytes infect both animals and humans, whereas anthropophilic ones are mainly found on humans. Geophilic dermatophytes can cause disease in both animals and humans. Fungi of the genera *Microsporum* and *Trichophyton* cause animal dermatophytoses; amongst these, *Microsporum canis* and species of the *Trichophyton mentagrophytes* complex are also pathogenic to humans. *Trichophyton equinum* and *M. canis* frequently cause 'ringworm' in horses, particularly in young animals (Chermette et al., 2008). Other species such as *T. mentagrophytes* or *M. gypseum* have also been isolated from skin lesions, while *T. bullosum* and *M. praecox* from healthy animals and the surrounding environment (De Vroey et al., 1983). The latter two species have also been implicated in human cases (Alanio et al., 2011; Sitterle et al., 2012).

Dermatophyte infection is acquired by direct contact with diseased animals or asymptomatic carriers and/or from the environment. Generally, clinical signs include mild to severe alopecia associated with erythema (Table 1—Chermette et al., 2008). Lesions due to *T. equinum* or *M. canis* are typically dry, with thin powdery scales and hairs broken at their base (Fig. 1—Chermette et al., 2008). Lesions are usually not pruriginous, and kerion and miliary dermatitis may also occur which extends rapidly from the saddle and the girth through the body (Chermette et al., 2008). Infections are clinically indistinguishable from those by *Dermatophilus congolensis* (Chermette et al., 2008).

**Table 1**

Fungal diseases in horses: clinical features, diagnosis and therapy.

Disease	Clinical features	Diagnosis	Therapy	References
<i>Malassezia</i> infections	Alopecia, pruritus and otitis with brown malodour exudates	Skin sample cytology and culture	Miconazole/chlorhexidine	White et al. (2006), Kim et al. (2011)
Chromoblastomycosis	Spherical, sharply circumscribed, firm, brown-black nodules of 2–3 cm in diameter	Histopathology of scrapings or biopsy material	Surgery	Abid et al. (1987), López and Méndez (2007)
Dermatophytosis	Circular alopecia, erythematous and exudative lesions, thin desquamation	Direct microscopy and culture of skin scrap and hair samples	Enilconazole (emulsifiable solution four times at 3–4 days intervals). Griseofulvin (5 mg/kg/day for 2–4 weeks for foal or 10 mg/kg/day for 1–2 weeks for pony). Natamycin (topical 100 p.p.m. suspension), spray, two or three times, with interval of 4 days	Rochette et al. (2003), Chermette et al. (2008)
Geotrichosis	Dry, erythematous, not well-defined circular alopecia, desquamation and pruritus	Culture of skin scrap of lesioned tissue and skin sample for histopathology	Acid peroxygen system based disinfectant BID until the remission of symptoms and negative culture results	Figueredo et al. (2011)
Keratomycosis	Microerosions, corneal ulcers, stromal plaques and abscesses, iris prolapse, blepharospasm and subepithelial infiltrates and opacities	Cytology, culture and histopathology of corneal lesioned tissues	Topically (1.5% amphotericin B, or 1% miconazole, or natamycin, or voriconazole) TID for 2–5 weeks  Topical serum (QID), atropine (BID), and antibiotic (TID). Surgery	Andrew et al. (1998), Brooks et al. (2000), Sansom et al. (2005)
Onychomycosis	Brittle hooves with sand cracks, severe or slight horn fissures around the nail holes, or in the coronary band and in the proximal hoof wall, white line disease	Cytology, culture and histopathology of lesioned tissues	White line disease resection, local cauterization and use of terbinafine hydrochloride as topical antifungal agents	Kuwano et al. (1998), Keller et al. (2000), Faravelli et al. (2004)
<i>Histoplasma capsulatum</i> var. <i>capsulatum</i> infections	Cutaneous form: ulcerated lesions  Respiratory form: anorexia, weight loss, intermittent fever, nasal discharge, rapid breathing and depression. Gastrointestinal form: abdominal discomfort and watery diarrhoea	Histopathology of lesioned tissues or lymph node aspirate. Culture of lesioned tissues, nasal discharge or faeces. Serology and hypersensitivity skin test for histoplasmin	Amphotericin B (0.3, 0.45, and 0.6 mg/kg on days 1, 2, and 3, respectively), followed by 4 days without treatment. Subsequent doses of 0.6 mg/kg administered every other day. The drug was solubilized in 1 l of 5% dextrose in water and administered IV for 1 h	Goetz and Coffman (1984), Cornick (1990), Rezabek et al. (1993), Johnston et al. (1995)
<i>Histoplasma capsulatum</i> var. <i>farciminosum</i> infections	Cutaneous form: granulomatous wound with tendency to ulcerate	Cytology histopathology and culture of exudates. and lesioned tissues	10% Sodium iodide solution (100 ml, IV, repeated weekly for four weeks)	Al-Ani (1999)

Table 1 (Continued)

Disease	Clinical features	Diagnosis	Therapy	References
	Ocular or a naso-lachrymal form: watery discharge from one or both eyes and some swelling of the eyelids, followed by the development of papules and ulcerating button-like growths on the conjunctiva and/or on the nictitating membrane. Respiratory form: yellowish papules or nodules evolving in crater-like granulating ulcers that bleeds easily		Amphotericin B (0.2 mg/kg three times on alternate days)  Griseofulvin in association with local surgical treatment and daily cleaning with 7% iodine solution	
Mycetomas	Subcutaneous mass, sinus and presence of discharge containing black or white grains	Fine needle aspiration cytology of subcutaneous mass. Culture and histopathology of lesioned tissues	2% miconazole cream, and systemic sodium and potassium iodide (SPC infection). Surgical excision and thiabendazole powder topically ( <i>Madurella mycetomatis</i> infection)	Van Amstel et al. (1984), Davis et al. (2000), Ahmed et al. (2004), Elad et al. (2010), Elad (2011)
Phaeohyphomycosis	Black and alopecic skin lesions from 1 to 10 cm in diameter covered or not with small pustules. Nodules that may ulcerate and have draining fistulous tracts	Histopathology, fungal culture and PCR diagnosis of fine needle aspiration or exudates	Surgery and fluconazole, for 10 days (a loading dose of 14 mg/kg given once followed by 5 mg/kg, PO, SID) and potassium iodide (30 mg/kg, PO, SID, for 30 days)	Valentine et al. (2006), Schwarz et al. (2009), Antoniassi et al. (2010), Dicken et al. (2010)
Pythiosis	Cutaneous or subcutaneous form: single or multiple non-healing, rapidly enlarging, tumor-like, nodular masses with multiple draining tracts and serosanguineous discharge. Presence of yellowish gritty coral-like bodies ranging from 0.5 to 1.5 mm in diameter named kunkers Gastrointestinal form: gastrointestinal obstruction, weight loss, anorexia, diarrhea, and colic	Culture, histopathology and PCR diagnosis of the fine needle aspiration or exudates. Serology	Surgery and topical administration of antifungal agents (miconazole, ketoconazole, itraconazole) associated with systemic antimicrobials. Intravenous administration of amphotericin B	Rochette et al. (2003), Bezerra et al. (2010), Gaastra et al. (2010), Doria et al. (2012)
Sporotrichosis	Small, firm reddish non painful, nonpuritic, dermal to subcutaneous nodules 1–5 cm in diameter that may exude a thin seropurulent fluid	Cytology and fungal culture of fine needle aspiration or exudates Histopathology of the nodules	Systemic iodine therapy (i.e., 40 mg/kg, SID, for 3–5 days), followed by 10 g of potassium iodine, PO, until the lesions are solved. Griseofulvin (20–25 mg/kg, PO, for 2 week), and then at 10 mg/kg for 46 days	Rochette et al. (2003), Barros et al. (2011)
Entomophthoramyiasis	Serosanguineous mucopurulent nasal discharge and dyspnea ( <i>Conidiobolus</i> spp)  Progressive ulcerative lesions of the cutaneous or subcutaneous tissues ( <i>Basidiobolus haptosporus</i> ). Exudates contain small, gritty, yellow-white, coral-shaped bodies (0.7–1.7 mm).	Cytology and fungal culture of exudates and/or lesioned tissue. Histopathology of lesioned tissues. Serology	Surgery and potassium iodide (10–20 mg/kg, PO) or organic iodide (1–2 mg/kg). Sodium iodine solution (20% solution, at 20–40 mg/kg). Amphotericin B, IV, SID	Miller and Campbell (1984), Owens et al. (1985), Rochette et al. (2003)

Mucormycosis	Cutaneous form: ulcerated, extensive and pruritic granulomatous lesions. Pulmonary form: apathy, fever lachrymation and dyspnoea. Disseminated form: lethargy, fever neurological signs (i.e., circling and convulsion)	Culture and histopathology of lesioned tissues	Amphotericin B at 40 mg/kg daily for 3 weeks	Guillot et al. (2000), Thirion-Delalande et al. (2005)
Adiaspiromycosis	Chronic weight loss, fever, elevated respiration and abnormal lung sounds	Histopathology of bioptic lung tissue, thoracic radiography	No reported	Pusterla et al. (2002)
Aspergillosis	Sinusitis: nasal discharge, submandibular lymphadenopathy, epiphora and facial swelling and sinonasal polyps  Rhinitis: dyspnea, stertorus breathing, nasal discharge, epistaxis, enlarged submandibular lymph nodes, and head shaking Guttural pouches infections: epistaxis, dysphagia, abnormal head posture, nasal discharge, colic, soft palate paresis, pharyngeal paralysis and laryngeal hemiplegia, depression, and cough Pulmonary form: tachypnea, lung or pleural sounds, weight loss and fever, reduced bronchovesicular sounds bilaterally	Cytology and culture of lesioned tissues or lavage fluid. Histopathology of lesioned tissues. Endoscopy, radiography or ultrasonography findings	Removal of mycotic plaques, followed by lavage with 1% natamycin solution SID for 3–8 days, and then Nystatin powder, insufflated per nares (sinusitis)  Enilconazole solutions (0.2–2%) lavages, BID, for 1.5–3.8 weeks, after mechanical debris of necrotic material, and catheter implantation (Rhinitis). Itraconazole 3 mg/kg PO, BID for 30 days (guttural pouches infections and rhinitis)  Flunixin meglumine (1.1 mg/kg IV, BID for three days), trimethoprim sulfamethoxazole (30 mg/kg PO, BID for 7 days) and voriconazole (10 mg/kg PO, SID for 24 days). Surgery in cases of pneumothorax (pulmonary infections)	Carrasco et al. (1996), Guillot et al. (1997), Tremaine and Dixon (2001a,b), Cafarchia et al. (2012b), Knottenbelt (2002), Hilton et al. (2009)
Blastomycosis	Lethargy, lameness, anorexia, weight loss associated with nasal discharge and/or exudative cutaneous lesions and mammary gland infections	Cytology and histopathology of lesioned tissues or fluids. Fungal culture	Never reported before	Toribio et al. (1999), Dolente et al. (2003), Wilson et al. (2006), Méndez-Angulo et al. (2011)
<i>Candida</i> infections	Oral form: bruxism,ptyalism and white plaque covering the tongue and gingival mucosa  Gastroesophageal and intestinal form: colic, anorexia, depression and watery diarrhea  Genital tract infection: vulval discharge	Cytology, histopathology and fungal culture of lesioned tissues and exudates (e.g., intestine exudates, semen, cervical discharge, gastric, peritoneal and synovial fluid). Endoscopy	Intra-uterine infusion of antimycotic agent (clotrimazole, 500–700 mg, or nystatin, 0.5–2.5 million units, or amphotericin B, 100–200 mg, or fluconazole, 100 mg) during 7–10 days, or even longer on cases of resistant infections (genital tract infection) Perfusion of saline and Ringer's solution. Bicarbonate and potassium supplementation, and plasma transfusion (gastroesophageal and intestinal infection)	Gross and Mayhew (1983), Reilly and Palmer (1994), Sout (2008)
Coccidioidomycosis	Fever, coughing, lameness	Histopathology, microscopic examination and fungal culture of lesioned tissues, lavage fluid or exudates. Serology	Itraconazole, 2.6 mg/kg, PO, BID, for 6 months (osteomyelitis).	Ziemer et al. (1992), Foley and Legendre (1992), Walker (1993), Stoltz et al. (1994), Higgins et al. (2006)

Table 1 (Continued)

Disease	Clinical features	Diagnosis	Therapy	References
	Musculoskeletal pain, abortion, colic with peritoneal effusion or skin lesions		Fluconazole (loading dose of 15 mg/kg followed by 5 mg/kg PO, SID), until remission of symptoms and reduction of Ig-G level (titres <1:4-pulmonary form).	
Cryptococcosis	Respiratory form: cough, abnormal lung sounds, nasal discharge, anorexia, fever, abdominal pain  Meningitis: bilateral blindness, fever, mydriasis, anisocoria  Genital tract infection: placentitis and endometritis	Histopathology, immunohistochemical and fungal culture of lesioned tissues. Latex cryptococcal antigen agglutination test using cerebrospinal fluid (CSF) and serum	Amphotericin B infusions (0.5 mg/kg in 1 l D5W IV over 1 h) pre-treated with flunixin meglumine (50 mg, IV for 1 month, until cumulative dose of 3 g), or flucytosine, or ketoconazole. Fluconazole (5 mg/kg, PO, SID for 4 weeks) and enilconazole solution 10% (50 ml instilled on paranasal sinuses, then 0.5 mg/kg PO SID for 5 days—Respiratory form). Fluconazole (14 mg/kg PO once, followed by 5 mg/kg PO, SID for 6 month) and flunixin meglumine (1.1 mg/kg, IV, bid, for 10 days followed by 0.5 mg/kg, PO, BID for 5 days, and then 0.5 mg/kg, PO, SID for 5 days) (meningitis). Uterine lavage with lactated ringer S and povidone-iodine solutions a week after foaling, and griseofulvin for 14 days (genital tract infection)	Begg et al. (2004), Cruz et al. (2009), McGill et al. (2009)
<i>Pneumocystis</i> spp. infection	Cough, abnormal lung sounds, tachypnea (36–64 breaths/minute), tachycardia (72 beats/minute) and pyrexia (38–41 °C)	Cytology, histopathology broncho-alveolar lavage fluid	Trimethoprim and sulfamethoxazole (30 mg/kg, PO, BID)	Peters et al. (1994), Flaminio et al. (1998), Perron Lepage et al. (1999), Franklin et al. (2002)





Fig. 1. Ringworm due to *Trichophyton equinum* in a horse (Rui Kano, Nihon University College of Bioresource Sciences, Japan).

Laboratory diagnosis consists in the direct microscopic examination of the clinical sample, and in particular of endo and/or ectothrix arthroconidia (Fig. 2, Table 2) followed by *in vitro* culture. Colonies grown onto SAB (supplemented with chloramphenicol 0.05 g/l and cycloheximide 0.5 g/l) are identified to species level based on their macromorphology, as well as of the microscopic characteristics of the hyphae, macroconidia and microconidia. The growth of *T. bulbosum* is very slow and, once isolated, the organism is not clearly distinguishable from *T. verrucosum*; thus, a molecular diagnosis may be required (Sitterle et al., 2012). Horse dermatophytosis usually resolves spontaneously within 1–4 months; however, the treatment is mandatory due to the contagious and the zoonotic nature of this disease. Antifungal therapy includes mostly topical or oral treatments. The treatment of large numbers of horses requires the use of solutions rather than ointments or salves, easily applicable and relatively inexpensive. Natamycin, enilconazole and, in some countries (e.g., Switzerland, USA), griseofulvin (Table 1–Rochette et al., 2003) are the only three registered products for the antifungal therapy of dermatophytoses. The treatment should be continued for 2–4

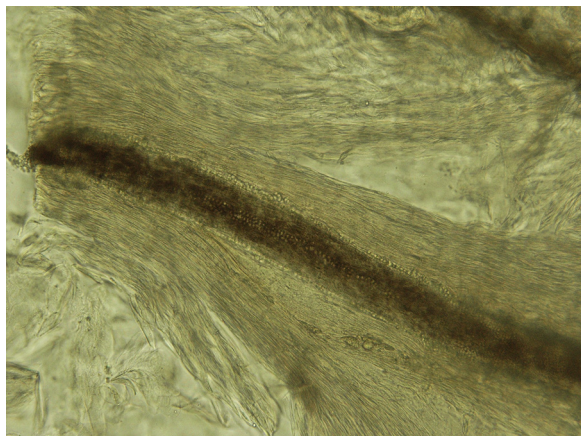


Fig. 2. Infected hair with chains of arthroconidia of a horse with *Trichophyton equinum* infection (Rui Kano, Nihon University College of Bioresource Sciences, Japan).

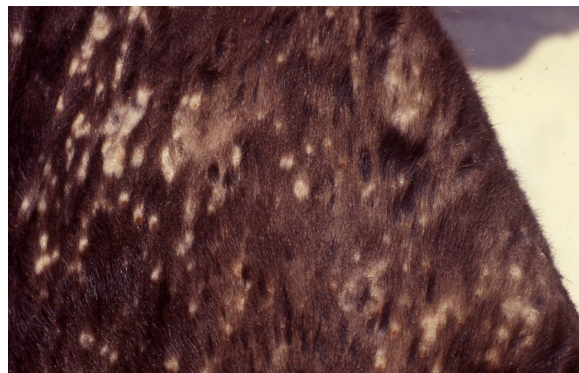


Fig. 3. Alopecia and itchy skin lesion caused by *Geotrichum candidum* in a horse.

weeks after clinical resolution, and until two negative cultures are obtained (Rochette et al., 2003). Hypochlorite bleach and enilconazole environmental sprays may be used for environment decontamination (Rochette et al., 2003).

### 3.3. Geotrichoses

Yeast-like fungi of the genus *Geotrichum* are usually detected in the environment and, in some circumstances (e.g. in immunocompromised hosts), they may cause disseminated or localized skin diseases (Figueredo et al., 2011). *Geotrichum candidum* may cause either dermatomycosis, with lesions mainly localized on the head and neck, or infections of the digestive tract (Mós et al., 1978; Figueredo et al., 2011). However, cutaneous geotrichosis is the most common manifestation of the disease (Table 1; Fig. 3). Diagnosis relies on the microscopic examination of the sample, followed by fungal culture and histological examination (Tables 1 and 2). The latter method allows to visualize fungi in tissues and may overcome the risk of contamination of the culture by the same fungus present in the environment (Figueredo et al., 2011). For recommended treatment strategies, see Table 1.

### 3.4. Keratomycosis

Keratomycosis is a fungal infection of the corneal stroma mainly caused by commensal fungi of the cornea and conjunctiva. *Aspergillus* spp. are the most prevalent aetiological agents, although yeasts of the genus *Candida* have also been found responsible for the infection (Andrew et al., 1998; Brooks et al., 2000, 2012). Tissue invasion usually occurs as a consequence of an injury of the cornea, or following a bacterial infection (Machado et al., 2005).

Horses are prone to develop keratomycosis, due to the innate immunoprotective deficiencies of the tear film and the prominent conformation of the ocular globe, together with the usually high concentration of fungi in stables (Andrew et al., 1998). Treatments with antibiotics and corticosteroids increase the risk of fungal infection, as well as exposure to plant material and dust (Andrew et al.,

**Table 2**  
Fungal diseases in horses: etiological agents and their microscopic features at cytology or histopathology.

Disease	Etiological agent	Cytology and/or histology	Microscopic features of microorganism	References
<i>Malassezia</i> infections	<i>Malassezia furfur</i> , <i>Malassezia slooffiae</i> , <i>Malassezia obtusa</i> , <i>Malassezia globosa</i> , <i>Malassezia restricta</i> , <i>Malassezia pachydermatis</i> .	Moderate hyperplasia of epidermis, mild lymphocytic exocytosis, mild eosinophilic dermatitis, diffuse parakeratosis.	Budding yeasts ranging from 3 to 8 $\mu\text{m}$ in diameter	Nell et al. (2002), Cabañes et al. (2007), Kim et al. (2011)
Chromoblastomycosis	<i>Fonsecaea</i> spp.	Encapsulated granulomas characterized by multinucleated cells, fibrosis, acanthosis, papillomatosis, hyperkeratosis, and pseudo-epitheliomatous hyperplasia	Rounded, thick-walled, brown-coloured muriform or fumagoid cells ranging from 8 to 14 $\mu\text{m}$ in diameter with a single or double septum. Filamentous structures in thick scrapings	Abid et al. (1987)
Dermatophytosis	<i>Microsporum canis</i> , <i>Microsporum gypseum</i> , <i>Trichophyton equinum</i> , <i>Trichophyton mentagrophytes</i>	Infected hairs or hair fragments, present as enlarged structures with a rough and irregular surface. The hair surface demonstrates clusters or chains of fungal arthroconidia.	Chains of spherical, translucent spores (arthroconidia), with a diameter ranging from 2 to 18 $\mu\text{m}$	De Vroey et al. (1983), Chermette et al. (2008)
Geotrichosis	<i>Geotrichum candidum</i>	Never reported before	Septate filamentous fungal hyphae with many branching. Arthroconidia of about 5–17 $\times$ 4–6 $\mu\text{m}$	Figueredo et al. (2011)
Keratomycosis	<i>Aspergillus</i> spp. <i>Candida</i> spp.	Presence of hyphae or yeast cells at cytology	Septated hyphae Budding cells and pseudomycelium.	Andrew et al. (1998), Brooks et al. (2000), Sansom et al. (2005)
Onychomycosis	<i>Scopulariopsis brevicaulis</i>  <i>Scedosporium</i> spp.  <i>Trichophyton</i> spp. <i>Candida</i> spp.	Irregular space from horn wall to white line. Irregular-shaped horn tubules on white line-like tissue in the laminar layers and deterioration of tubular structure	Conidia from 4 to 9 $\mu\text{m}$ and septate hyphae ( <i>Scopularopsis</i> )  Budding unicellular racquet-shaped conidia from the conidiophores ( <i>Scedosporium</i> ). Fungal hyphae ( <i>Trichophyton</i> ). Budding cells and pseudo-mycelium ( <i>Candida</i> )	Kuwano et al. (1998), Keller et al. (2000), Faravelli et al. (2004), Apprigh et al. (2010)
Histoplasmosis	<i>Histoplasma capsulatum</i> var. <i>capsulatum</i>  <i>Histoplasma capsulatum</i> var. <i>farciminosum</i>	Fewer multinucleated giant cells, and low numbers of neutrophils, lymphocytes, and plasma cells  Yeast-like, double-contoured cells at cytology  Polymorphous inflammatory cells consisting of an admixture of neutrophils, lymphocytes, plasma cells, histiocytes, macrophages giant cells and grains	Oval-shaped cells 5 $\mu\text{m}$ in diameter with a 2- $\mu\text{m}$ basophilic central body surrounded by a 1–2- $\mu\text{m}$ thick clear cell wall that stained pink with PAS. Gram positive, ovoid to globose cells (2.5–3.5 $\mu\text{m}$ by 3–4 $\mu\text{m}$ ) within macrophages or extracellularly  Black grains	Goetz and Coffman (1984), Cornick (1990), Rezabek et al. (1993), Johnston et al. (1995)  Al-Ani (1999), Ameni (2006)  Ahmed et al. (2004), Elad et al. (2010), Elad (2011)



Micetoma	<i>Scedosporium/Pseudallescheria</i> complex <i>Madurella mycetomatis</i> , <i>Curvularia verruculosa</i> , <i>Phialophora oxyspora</i> <i>Scedosporium/Pseudallescheria</i> complex		White grains	
Phaeohyphomycosis	<i>Alternaria</i> spp., <i>Drechslera spicifera</i> and <i>Curvularia</i> spp.	Granuloma consisting of a central core of cellular debris and neutrophils surrounded by lymphocytes, epithelioid macrophages, and small numbers of multinucleate giant cells	Irregularly, brown pigmented, septate and branched hyphae ( <i>Drechslera spicifera</i> and <i>Curvularia</i> ) of 10–20 µm, with non-parallel walls ( <i>Alternaria</i> spp.)	Valentine et al. (2006), Antoniassi et al. (2010), Dicken et al. (2010)
Pythiosis	<i>Pythium isidiosum</i>	Pyrogranulomatous inflammatory infiltration with large quantities of eosinophilic granulocytes	Irregular sparsely 90° bracked septate hyaline hyphae 2.6–6.4 mm wide and with a thick cell wall.	Mendoza and Newton (2005), Gaastra et al. (2010)
Sporotrichosis	<i>Sporothrix schenckii</i> complex	Granuloma associated with epithelial hyperplasia, histiocytic plasma cell infiltrate and Splendore-Hoeppli bodies	Few cigar-shaped organisms (1–3 µm) within macrophages	Cafarchia et al. (2007), Barros et al. (2011)
Entomophthoromycosis	<i>Conidiobolus coronatus</i> (i.e., <i>Entomophthora coronata</i> ), <i>Conidiobolus lamprauges</i> , <i>Basidiobolus haptosporus</i>	Eosinophilic granulomas with multinucleated giant cells, fibrosis, and Splendore-Hoeppli bodies	Hyaline, broad (5–12.8 µm in width), thin walled occasionally septate and irregularly branched hyphae.	Owens et al. (1985), Humber et al. (1989), Tan et al. (2010)
Mucormycosis	<i>Rhizopus stolonifer</i> , <i>Absidia corimbifera</i> and <i>Mucor</i> spp.	Large multifocal necrotic eosinophilic areas with macrophages and multinucleated giant cells. Neutrophils and eosinophils in the external zone	Numerous wide (10–20 µm) and thin-walled hyphae without regular septation or branching	Carrasco et al. (1997), Guillot et al. (2000)
Adiaspiromycosis	<i>Emmonsia</i>	Granulomas composed of an outer layer of epithelioid macrophages and occasional giant cells, and an inner neutrophilic focus that surrounded a central spherical fungus	Fungal spherules of 30–80 µm in diameter, composed of fine granular eosinophilic material and surrounded by a thick trilamellar wall capsule (5–20 µm), PAS and GMS positive but mucicarmine negative	Pusterla et al. (2002)
Aspergillosis	<i>Aspergillus</i> spp.	Mucosal hyperaemia, pvascular infiltration, spongiosis, and epithelial infiltration of neutrophils. Presence of fungal structures on exudates and necrotic tissue	Acutely branching fungal hyphae, occasionally conidia and sporulating bodies	Guillot et al. (1997), Ludwig et al. (2005), Cafarchia et al. (2012b)
Blastomycosis	<i>Blastomyces dermatitidis</i>	Chronic inflammation, with abundant fibrous connective tissue with granulomatous and pyogranulomatous foci characterized by lymphoplasmacytic infiltrates and numerous multinucleated giant cells	Spherical yeast cells of 15–17 µm in diameter with thick double-contoured walls	Toribio et al. (1999), Dolente et al. (2003), Wilson et al. (2006), Méndez-Angulo et al. (2011)
<i>Candida</i> infections	<i>Candida</i> spp.	Inflammatory reaction with neutrophils, lymphocytes and plasma cells, yeasts or elongated fungal hyphae	Budding cells and pseudohyphae	Gross and Mayhew (1983), Reilly and Palmer (1994), Sout (2008)
Coccidioidomycosis	<i>Coccidioides immitis</i>	Granulomas or pyogranulomas	Spherules of 20–80 µm containing endospores of 2–5 µm in diameter	Ziemer et al. (1992), Foley and Legendre (1992), Walker (1993), Stoltz et al. (1994), Higgins et al. (2006)
Cryptococcosis	<i>Cryptococcus gattii</i> <i>Cryptococcus neoformans</i>	Pyogranulomatous inflammation and yeast cells	Round, budding yeast cells of 2–20 µm, surrounded by capsule	Begg et al. (2004), Cruz et al. (2009), McGill et al. (2009)
<i>Pneumocystis</i> spp. infection	<i>Pneumocystis</i> sp.	Neutrophils and reactive macrophages containing fungal organisms	Many cluster of round microorganisms of 6 µm in diameter with thin clear capsule and small internal basophilic bodies arranged in ring	Peters et al. (1994), Flaminio et al. (1998), Perron Lepage et al. (1999), Franklin et al. (2002)

1998). Male horses and thoroughbred are more predisposed to the infection (Andrew et al., 1998; Sansom et al., 2005), and summer and autumn are the seasons with the highest prevalence (Andrew et al., 1998; Sansom et al., 2005). Keratomycoses are characterized by different clinical expressions (Table 1—Sansom et al., 2005). The lesions may lead to symptoms such as blepharospasm, pain, chemosis, lacrimation, moderate to severe purulent discharge, hypopyon; infections may also lead to secondary uveitis, iris prolapse and corneal edema (Sansom et al., 2005). The diagnosis of keratomycosis is based on clinical examination (including staining of the corneal tissue with both fluorescein and rose Bengal), cytological examination of corneal scraping, culture of tissue isolated from the cornea, and histopathological observations following keratectomy (Andrew et al., 1998; Brooks et al., 2000).

The cytological examination of the corneal tissue can be performed following Romanowsky staining (Wright and Diff-Quik stains); in most cases, the presence of fungal hyphae can be observed (Andrew et al., 1998; Brooks et al., 2000). However, a negative result is not conclusive and isolation of the etiological agent is required. Histopathological examination should be performed using GMS or PAS staining, which allow the visualization of the hyphae or yeast cells (Tables 1 and 2—Sansom et al., 2005).

The treatment of keratomycosis depends upon the severity of the lesions. Topical and/or surgical intervention, including keratectomy can be recommended in cases of deep stromal involvement. The topical treatment is usually effective in mild cases (Andrew et al., 1998). When unresponsive to the medical therapy, horses may be subjected to surgical removal of the lesions, combined with topical and systemic administration of antifungal agents (Table 1—Brooks et al., 2012). Usually, treatment protocols may take up to 8 weeks, with favourable outcomes achieved in >90% of cases (Andrew et al., 1998; Brooks et al., 2000).

### 3.5. Onychomycosis

Onychomycosis is a fungal infection of the hoof horn, commonly secondary to deterioration and disruption of the horn wall integrity (Faravelli et al., 2004). The disease is caused by keratinopathogenic fungi such as *Scedosporium* spp., *Trichophyton* spp. and *Scopulariopsis brevicaulis*, which invade the horn structures and cause damage (Table 2—Kuwano et al., 1999; Keller et al., 2000). Lesions of the horn may lead to severe consequences such as lameness, poor performances and white line diseases (Faravelli et al., 2004). Species identification requires fungal culture, although frequent contamination of the horns by non-keratinolytic fungi can lead to misdiagnosis; therefore, histological examination of the sample is recommended in order to confirm culture identification. Lesions are characterized by severe sand cracks, separation of the hoof wall, and white line disease (Kuwano et al., 1999). Fungal structures can be observed on damaged tissues (Table 2); deterioration of the tubular structure of the horn wall, disruption of the horny layers, superficial lysis of cornified cells. Presence of fungal elements can also be observed using scanning electron microscopy (SEM)

(Aprich et al., 2010). The treatment of onychomycosis consists in local cauterization and topical application of antifungal agents (Table 1—Kuwano et al., 1998; Keller et al., 2000).

## 4. Subcutaneous mycoses

Subcutaneous mycoses are a heterogeneous group of fungal diseases of the subcutaneous tissues with involvement of the dermis and/or epidermis. Most of these mycoses are localized (e.g., *Alternaria alternata* infections), however some of them can spread slowly to contiguous tissues (i.e., *Basidiobolus haptosporus* and *Conidiobolus coronatus* infections), or through the lymphatic system (i.e., *Histoplasma capsulatum* var. *farciminosum* and *Sporothrix schenckii* infections). In all cases, subcutaneous mycoses are generally chronic and progressive diseases, and their diagnosis and treatment may prove challenging.

### 4.1. Histoplasmosis

Histoplasmosis in horses may be due to infections by two distinct varieties of *Histoplasma capsulatum*, namely *H. capsulatum* var. *capsulatum* (HCC), and *H. capsulatum* var. *farciminosum* (HCF); each of these is characterized by distinct clinical manifestations and geographical distributions (Kasuga et al., 2003).

#### 4.1.1. *Histoplasma capsulatum* var. *capsulatum* infections

HCC is a dimorphic fungus with a worldwide distribution, although it is highly prevalent along the Mississippi and Ohio River valleys of North America (Cano and Hajjeh, 2001). It usually occurs in soil, especially if contaminated with bird and bat droppings (Cano and Hajjeh, 2001). HCC can infect a broad range of hosts, such as humans, dogs, cats, cattle, and many other animal species, including horses. Although skin-test surveys (i.e., delayed hypersensitivity skin test for histoplasmin) have shown that 50–73% of the equine population in endemic areas harbours the infection, only a few case reports are available (Hall, 1979; Goetz and Coffman, 1984; Rezabek et al., 1993; Johnston et al., 1995; Richter et al., 2003; Nunes et al., 2006). Equine histoplasmosis may cause localized infections of lungs, bone marrow, placenta, eyes, colon, cecum, and mesenteric lymph nodes (Hall, 1979; Goetz and Coffman, 1984; Rezabek et al., 1993; Richter et al., 2003; Nunes et al., 2006) and, rarely, disseminated infections of lungs, pleura, spleen, kidney, liver, small intestine, and colon, depending upon the immunological status of the host (Rezabek et al., 1993; Johnston et al., 1995).

The commonest route of infection is via inhalation of microconidia (mould phase), which can easily reach the lower respiratory tract (Cano and Hajjeh, 2001). Nonetheless, the occurrence of gastrointestinal signs without concurrent respiratory involvement may suggest an oral route of infection (Goetz and Coffman, 1984). Analogously, the absence of generalized clinical signs in mares, albeit in presence of infected foals or aborted fetuses, indicates that ascendant infections of the reproductive tract may occur (Rezabek et al., 1993). Once inside the body of the host, the organism transforms into the yeast phase and is

phagocytized by alveolar macrophages, which then disseminate the infection to other organs (Cano and Hajjeh, 2001). Clinical signs of both disseminated and localized histoplasmosis include anorexia and weight loss and emaciation (Table 1). Generalized lymphadenopathy and pleural and peritoneal effusions have also been documented in disseminated forms (Johnston et al., 1995). A mild increase in haematocrit (PCV, 0.53 L/L), neutrophils ( $0.14 \times 10^9/L$ ) and a marked lymphopenia ( $0.14 \times 10^9/L$ ) are the most common hematological abnormalities in disseminated infections (Rezabek et al., 1993; Johnston et al., 1995). A chronic granulomatous inflammatory process can be observed at the histopathological examination of infected tissues stained with both H&E and PAS, while multiple yellow, friable caseous foci surrounded by a thick fibrous capsule are usually observed at necroscopy (Table 2—Goetz and Coffman, 1984; Cornick, 1990; Rezabek et al., 1993; Johnston et al., 1995). HCC are usually detected in multinucleated cells, single free alveolar macrophages, and in endothelial cells (Table 2); however, for identification, culture is necessary. Suitable culture media include SAB, brain heart infusion agar (BHI) or BHI supplemented with blood, penicillin (20 units/ml) and streptomycin (40 units/ml) (Cafarchia et al., 2012a). Serological tests (e.g., agar immunodiffusion) have also been employed for the diagnosis of HCC infection (Rezabek et al., 1993; Johnston et al., 1995). Amphotericin B has been reported successful in the treatment of a 2-year-old Trekehner filly with pulmonary histoplasmosis (Table 1—Cornick, 1990).

#### 4.1.2. *Histoplasma capsulatum* var. *farcinosum* infections

(Syn: *Pseudoglanders*, *Pseudofarcy*, *Equine Histoplasmosis*, *Histoplasmosis farciminosi*, *African Farcy*, *Equine Blastomycosis*, *Equine Cryptococcosis*). HCF is a dimorphic saprobic fungus causing epizootic lymphangitis (EL) in horses, donkeys and mules. This fungus is endemic in some countries of west, north, and northeast Africa, and Asia including India, Pakistan and Japan, where it is mostly diffused in areas characterized by humid and hot climates (Ameni, 2006). Horses under six years of age are more susceptible (Al-Ani, 1999). Fungal spores can be transmitted to healthy animals by direct contact with infected animals or with inanimate objects or fomites, such as grooming equipment, bedding, saddler, etc., and enter the skin through cutaneous abrasions. Biting flies of the genera *Musca* and *Stomoxys* may contribute to spreading the infections, while tick bites most likely represent a predisposing factor for EL in mules (Al-Ani, 1999; Ameni, 2006). Four different forms of HCF infections are described: asymptomatic, cutaneous, conjunctival/ocular and respiratory. The first one, asymptomatic, occurs in patients which present fibrocalcific skin lesions at previous sites of infection and which are positive to intradermal sensitivity or other serological tests (Al-Ani, 1999).

The cutaneous form is characterized by the presence of a granulomatous wound along a lymphatic vessel (mostly along the forelimbs, the chest wall, and the neck), which tends to ulcerate or undergo discharge alternated with closure, prior to healing with the formation of a residual scar (Table 1). Extensive lesions may lead to death;

however, mortality does not usually exceed 15% (Al-Ani, 1999).

The conjunctival/ocular form, most likely spread by biting flies, may occur as conjunctivitis or a nasolacrimal infection (Table 1).

The respiratory form of the disease occurs after inhalation of the pathogen and is characterized by lesions mostly confined to the upper respiratory tract (i.e., nasal mucosa, Table 1). Clinical lesions consist in pyogranulomas, purulent discharge of thickened superficial lymphatic vessels and enlargement of regional lymph nodes. At the hematological findings, leucocytosis, neutrophilia and an increase in the erythrocyte sedimentation rates can be observed (Al-Ani, 1999; Ameni, 2006).

Laboratory tests used in the diagnosis of epizootic lymphangitis include: identification of the yeast form of HCF in smears of exudates or in histological sections of material from lesions (Tables 1 and 2), serological tests (i.e., fluorescent antibody test, enzyme-linked immunosorbent assay and passive hemagglutination tests), skin hypersensitivity test and isolation of the causative agent by culture (Al-Ani, 1999; Ameni, 2006). However, growth in media culture is slow (i.e., up to 8 weeks) and available serological tests are characterized by low sensitivity and/or specificity (Al-Ani, 1999). Treatment of EL is mandatory to prevent spreading of the infection (Table 1), whereas for disease control, culling infected horses and adoption of hygiene measures (e.g., cleaning and disinfection) and insects control are required. In recent years, the use of vaccines (i.e., a killed formalized vaccine, attenuated vaccine, and live vaccine) have been proposed as a strategy to eradicate the infection in endemic areas; the administration of the attenuate vaccine (i.e., vaccine developed by exposure of the causative agent to high temperatures) has resulted in a protection rate of 75.5% over >31 months (Al-Ani, 1999).

#### 4.2. *Mycetoma*

Mycetomas (Syn: *Maduromycosis*) are chronic pyogranulomatous infections of the skin and subcutaneous tissues caused by actinomycetes (actinomycetoma) or fungi (eumycotic mycetoma) (Ahmed et al., 2004; Elad, 2011). These are characterized by tumefaction of the sinus tracts, and drainage with macroscopically visible “grains” of bacterial (actinomycetoma) or fungal colonies (eumycotic mycetoma) (Ahmed et al., 2004; Elad, 2011). The lesions may vary in shape, size, texture, and colour, depending upon the etiological agent; fungal grains are rather firm and composed of microscopically recognizable hyphae, which are either black or whitish in colour (Table 2—Ahmed et al., 2004; Elad, 2011). The most common etiological agents of black-grain mycetoma belong to the genus *Madurella* and are characterized by the presence of non-conidiating hyphae, whereas those responsible for the formation of white grains are ranked within the *Scedosporium/Pseudallescheria* complex (SPC) (Ahmed et al., 2004; Elad, 2011). Mycetomas in horses have been reported mostly in North America, South Africa, and Australia, and in only once in Europe (reviewed in Elad et al., 2010). The most common agents of eumycotic mycetoma in horses belong to SPC and *Madurella* genera





Fig. 4. Eumycotic mycetoma caused by *Madurella mycetomatis* in a horse (Daniel Elad, Kimron Veterinary Institute, Israel).

(i.e., *Madurella mycetomatis*), however *Curvularia verruculosa*, *Phialophora oxyspora* and *Aspergillus* spp. have also been occasionally detected (Elad et al., 2010; Elad, 2011). These organisms can usually be isolated from plant material and soil; in particular, SPC can also be isolated from polluted aquatic environments, salt water and air (Ahmed et al., 2004; Elad, 2011). Infections usually begin from a lesion of the skin caused by sharp objects, such as a thorn (Ahmed et al., 2004; Elad et al., 2010; Elad, 2011). Although the disease incubation time remains unclear, symptoms may appear several months to years post-infection (Ahmed et al., 2004). The duration of the clinical disease, the type of causative agent, the site of infection and, most likely, the immunological status of the host, can affect the presentation of mycetomas (Table 1). The infection is classically characterized by the presence of subcutaneous nodules, increasing in size, which may suppurate and drain a serous, serosanguineous or purulent discharge through multiple sinus tracts (Fig. 4—Ahmed et al., 2004; Elad et al., 2010; Elad, 2011).

Mycetomas can be accurately diagnosed by Fine Needle Aspiration (FNA) cytology (Ahmed et al., 2004). Positive samples are characterized by the presence of polymorphous inflammatory cells (Tables 1 and 2—Ahmed et al., 2004), and grains closely surrounded by neutrophils in cases of *M. mycetomatis* infections (Ahmed et al., 2004), and by eosinophils in SPC infections (Elad, 2011). Since multiple

agents can cause mycetoma, the identification of the specific causative agent is important in order to set out effective treatment strategies. The microorganisms can be identified by fungal culture using media such as SAB, BHI and Malt extract agar (Ahmed et al., 2004). Recently, the use of trypticase soy agar with 5% (vol/vol) horse serum has been proposed to significantly boost the growth of *M. mycetomatis* (Elad et al., 2010). In addition, selective media obtained adding Benomyl or Amphotericin B or dichloran have been proven suitable for the isolation of SPC (Elad, 2011). The accurate identification of the species responsible for the infection relies on the observation of morphological features of the isolated fungi (de Hoog et al., 2000). Until recently, the surgical excision of the affected areas, associated with medical therapy in localized forms, has been the only treatment available for mycetomas (Table 1—Van Amstel et al., 1984; Davis et al., 2000).

#### 4.3. *Phaeohiphomycosis*

*Phaeohiphomycosis* (Syn: *Cerebral chromomycosis*, *chromoblastomycosis*, *cladosporiosis*, *phaeomycotic cyst*, *phaeosporotrichosis*, *subcutaneous mycotic cyst*) is a chronic cutaneous, subcutaneous, and mucosal or systemic infection caused by species of pigmented, saprophytic fungi, which are frequently found in soil, water, and decaying vegetable matter (Revankar, 2006). Infections usually follow contamination of injured tissue by the fungi (Revankar, 2006), and usually involve immunocompromised individuals and animals, including horses (Revankar, 2006). Sporadic cases of equine infections have been reported in young horses in North and South America, Australia, New Zealand and Europe (Valentine et al., 2006; Antoniassi et al., 2010; Dicken et al., 2010). *Alternaria* spp., *Drechslera spicifera* and *Curvularia* spp. are most frequently isolated from equine *phaeohiphomycosis* infections (Table 2); they cause the formation of single or multiple ulcerated cutaneous or subcutaneous nodules in the regions of the head and neck and, occasionally, lymphangitis and regional lymphadenopathy (Table 1—Valentine et al., 2006; Antoniassi et al., 2010; Dicken et al., 2010). The diagnosis usually relies on histopathological examinations followed by fungal culture. Histological staining with H&E is indicative of a fungal granuloma, and the fungi appear as dematiaceous elements (Table 2). Cultures of the excised tissue usually yield a rapidly growing colony whose surface is flat, velvety and blackish. Molecular diagnosis by PCR can prove useful when fungal cultures are unsuccessful (Schwarz et al., 2009).

Treatment is challenging and usually depends upon the individual cases; however, it usually consists in a combination of both surgical (i.e., excision of the lesion; Valentine et al., 2006; Dicken et al., 2010) and long-term medical treatment. Medical therapy may be considered in cases where surgery is not recommended (Table 1—Schwarz et al., 2009).

#### 4.4. *Pythiosis*

*Pythiosis* (Syn: *bursattee* or *bursatte esputia*, *equine phycomycosis*, *granular dermatitidis*, *hyphomycosis destruens*

*equi*, leeches, swamp cancer and summer sores, phycomycosis) is an uncommon cutaneous/subcutaneous disease caused by a fungus-like organism known as *Pythium insidiosum*, which is phylogenetically related to diatomeae and algae (Gaastra et al., 2010). *Pythium* infections especially occur in tropical and subtropical regions characterized by wet, warm climates such as Southeast Asia, Australia, New Zealand, South America, Costa Rica and Guatemala. No predisposing factors (i.e., breed, age or sex) have been described, however immunocompetent horses exposed to warming and fresh water in swampy areas may be at risk of contracting the disease (Mendoza and Newton, 2005; Gaastra et al., 2010).

The infection can be acquired via colonization of traumatic lesions by zoospore and/or hyphae of *P. insidiosum* (Gaastra et al., 2010), or by ingestion of *P. insidiosum*-contaminated water (Bezerra et al., 2010). Following contact with mammalian tissues, the zoospores encyst in the surface of the injured tissue(s) and produce hyphae, which mechanically penetrate into the host tissues (through the activity of secreted proteases) and cause disease (Gaastra et al., 2010). Horse infections usually involve the cutaneous and subcutaneous tissues; however, intestinal forms of the disease have been described (Table 1—Bezerra et al., 2010; Gaastra et al., 2010). The lesions, commonly localized to the limbs and ventral abdomen (Fig. 4), usually contain yellowish gritty coral-like bodies known as “kunkers” and are intensely pruritic with mild to marked lymphadenopathy. In chronic infections (>4 weeks duration), *Pythium* sp. may spread to the underlying bone and cause lameness (Gaastra et al., 2010). The clinical signs of horse pythiosis resemble those of other diseases such as habronemiasis, skin fungal infections caused by *Conidiobolus* and *Basidiobolus* spp., and invasive squamous cell carcinoma (Gaastra et al., 2010). Following sampling, kunkers or bioptic material should be preserved in water or saline solution supplemented with antibiotics (i.e., chloramphenicol, tetracycline, streptomycin and ampicillin) and refrigerated until processing; however, it has been reported that storage at 4–8 °C may inhibit the growth of *P. insidiosum* (Gaastra et al., 2010).

Histological tests may assist the diagnosis of pythiosis, albeit they do not allow the differentiation between pythiosis and infections caused by *Conidiobolus* and *Basidiobolus* (Tables 1 and 2—Mendoza and Newton, 2005; Gaastra et al., 2010). Currently, the gold standard for diagnosis is culture of the infected tissue, followed by morphologic and molecular identification of the etiological agent and detection of anti-*P. insidiosum* antibodies using serological assays (reviewed in Gaastra et al., 2010). Surgical removal of the lesions is considered the treatment of choice for equine pythiosis, especially in cases characterized by small and shallow lesions (Table 1—Rochette et al., 2003; Gaastra et al., 2010). Antifungal therapy is ineffective due to the lack of ergosterol (targeted by antifungal drugs) in the cell membranes of *P. insidiosum*. Recently, an association of ergosterol biosynthesis inhibitors and caspofungin (which inhibits the synthesis of  $\beta$ -glucan, a component of *Pythium* spp. cell walls), as well as the intravenous administration of amphotericin B (in

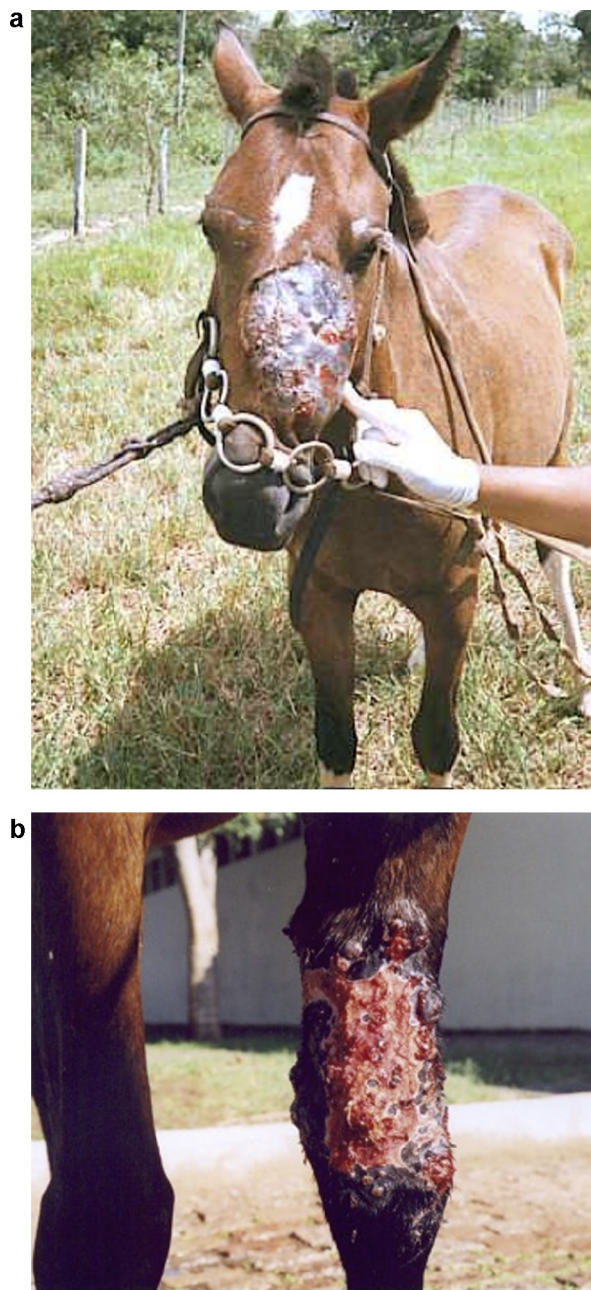


Fig. 5. *Pythium insidiosum* infections of about 3 months localized on face (a) and on legs (b) (Prof. Janio Morais Santurio, LAPEMI, UFSM, RS, Brazil).

correspondence of the lesions) have proven useful in the treatment of equine pythiosis (Gaastra et al., 2010; Doria et al., 2012). To date, two vaccines have been evaluated for the treatment of cutaneous pythiosis in horses (Santos et al., 2011). A cell-mass (CMV) and a soluble concentrated antigen (SCAV) are key components of the first and second vaccine, respectively. While both vaccines were able to cure of *P. insidiosum* infections, CMV lost its effectiveness within two or three weeks after its preparation. In addition, the latter vaccine was effective in horses with recent



lesions (<0.5 months), whereas horses with older lesions (>2 months) did not respond to the vaccine. Thus, in spite of the short-lasting activity (i.e., 1 year) of SCAV, the latter has been proposed as a possible treatment choice in new cases of equine cutaneous pythiosis (Santos et al., 2011).

#### 4.5. Sporotrichosis

Sporotrichosis is an acute or chronic, granulomatous and usually lymphocutaneous infection which affects both humans and animals (Barros et al., 2011). The disease, potentially zoonotic, is characterized by the development of nodular lesions in subcutaneous tissues, skin, and lymph nodes, which later soften and break down forming indolent ulcers. The etiological agents belong to the *Sporothrix schenckii* complex, which are dimorphic fungi found as mould in the environment, especially in plant debris, soil and water, and as yeast in infected tissues (Barros et al., 2011). The disease has been described in horses, dogs, cats, cattle, camels, fowls, swine, rats, mice, hamsters, chimpanzees, and humans (Cafarchia et al., 2007; Barros et al., 2011). Sporotrichosis has been reported worldwide, mainly in areas characterized by high humidity and mild temperatures; in Europe, it is considered endemic in Spain and Italy (Cafarchia et al., 2007; Barros et al., 2011). The main route of infection is through wounded skin (Barros et al., 2011). The organism enters the tissues as spore, and readily transforms into the yeast phase (Barros et al., 2011). In horses, lesions appear approximately in 1–3 months after infection; primary lesions consist in subcutaneous nodules, which ulcerate and drain purulent discharges. Subsequently, fungi spread via the lymphatic vessels and secondary lesions appear along the regional lymphatic tracts (White, 2005). The lesions are usually more prominent along the medial surface of the forelimb or thigh, but can be occasionally observed along the jugular furrow of the neck. Differential diagnoses include ulcerative lymphagitis (i.e., *Corynebacterium pseudotuberculosis*) and EL; the definitive diagnosis requires the cytological examination of exudates stained using Giemsa (Tables 1 and 2) or isolation of *S. schenckii* by fungal culture (Cafarchia et al., 2007; Barros et al., 2011). The histological examination of a biopsy may lead to false negatives due to the small number of yeasts in lesions and it requires PAS-staining (White, 2005; Barros et al., 2011). If sporotrichosis is clinically suspected, fungal cultures are recommended to achieve a definitive diagnosis (Cafarchia et al., 2007). Systemic therapy with iodine or griseofulvin has been successful for the treatment of this affection in horses (Table 1—Rochette et al., 2003).

#### 4.6. Zygomycoses

Zygomycoses (Syn: *Mycosis mucorina*, *Mucormycosis*, *Phycomycoses*, *Mucormycosis*, *Entomophthoramyces*) are caused by fungi of the Zygomycetes class, which includes the orders Mucorales and Entomophthorales. Members of these two orders are characterized by extremely distinct ecological, epidemiological and morphological features, as well as the infections that they cause (i.e., entomophthoramyces and mucormycosis) (Kwon-Chung, 2012).

Entomophthoramyces are subcutaneous zygomycoses caused by *Conidiobolus coronatus* (i.e., *Entomophthora coronata*), *Conidiobolus lamprauges* and *Basidiobolus haptosporus*; these occur in immunocompetent horses and are characterized by localized subcutaneous granulomas (Owens et al., 1985; Humber et al., 1989; Tan et al., 2010). Infective organisms reside in decaying plant material, soil, leaves from deciduous trees, while some (e.g., *Basidiobolus* spp.) are frequently isolated from the intestines of fish, frogs, toads, insects, reptiles and insectivorous bats. These fungi occur in tropical and subtropical areas; infections have been reported in the United States, Costa Rica, Colombia, Brazil, Australia and India (in Humber et al., 1989). The routes of infections by *Conidiobolus* and *Basidiobolus* species remain unclear, although direct contact with spores in the ground, or mechanical infections via insects depositing spores directly on the nostrils are the most plausible (Humber et al., 1989; Tan et al., 2010). In horses, entomophthoramyces are reported mainly as infections of the rhinofacial, nasopharyngeal, oral, head neck, chest or trunk regions (Humber et al., 1989; Tan et al., 2010). In particular, infections by *Basidiobolus* spp. cause cutaneous or subcutaneous lesions localized to the head neck, chest or trunk characterized by a granulomatous mass with an erythematous and hemorrhagic surface (Table 1—Miller and Campbell, 1984; Owens et al., 1985); infections by *Conidiobolus* spp. are usually localized to the nasopharyngeal tract, with or without local dissemination to the tissues of the face, retropharyngeal region, maxillary sinus, guttural pouches, trachea, retro bulbar spaces or cerebrum (Table 1—Miller and Campbell, 1984; Tan et al., 2010).

Diagnosis relies on the microscopic examination and fungal culture of material collected from the lesions (Tables 1 and 2—Miller and Campbell, 1984). Culture media routinely used for fungal isolation are SAB or Potato Dextrose Agar (PDA) and Cornmeal agar. Recently, an immunodiffusion (ID) test has been used for the identification of the etiological agent of entomophthoramyces in horses and humans (Kaufman et al., 1990). In animals, systemic iodine therapy or topical administration of amphotericin B, in association with surgical excision have been successfully employed for the treatment of the infection (Table 1—Owens et al., 1985; Rochette et al., 2003).

Mucormycoses are caused by fungi of the genera *Mucor*, *Rhizopus*, *Rhizomucor*, *Absidia*, *Apophysomyces*, *Cunninghamella*, *Saksenaia*, *Mortierella* and *Syncephalastrum*. Infections by Mucorales are usually angioinvasive diseases of immunocompromised humans and animals, characterized by an acute onset, rapid progression and often fatal outcomes. Mucorales infections have been reported in a broad range of animal species. Reports of the disease in horses are rare and frequently described as mixed infections and/or diagnosed *post mortem* (Carrasco et al., 1997; Astorga et al., 2004). The etiological agents are *Rhizopus stolonifer*, *Absidia corimbifera* and *Mucor* spp. Mucormycoses are typically opportunistic infections; in horses, treatments with corticosteroids and antibiotics might represent a predisposition factor to disseminated



mucormycosis (Carrasco et al., 1997; Guillot et al., 2000). In horses, four clinical manifestations of mucormycoses have been described: cutaneous/subcutaneous (López-Sanromán et al., 2000; Guillot et al., 2000), gastrointestinal (Astorga et al., 2004), pulmonary (Guillot et al., 2000) and disseminated (Table 1—Guillot et al., 2000; Thirion-Delalande et al., 2005). Gastrointestinal mucormycosis had been suspected in a clinical case associated with *Salmonella enteritidis* septicemia (Astorga et al., 2004). Mucorales infections are usually diagnosed by microscopic examination (e.g., following treatment of the sample with 20% KOH or, H&E and PAS staining) (Tables 1 and 2—Guillot et al., 2000; Thirion-Delalande et al., 2005) or by fungal culture of material collected from the lesions (Guillot et al., 2000). However, negative cultures should be subjected to further testing by indirect enzyme immunohistochemical assays (Guillot et al., 2000). Since the most cases of mucormycoses are diagnosed *post mortem*, horses are not subjected to any antifungal treatment. Mucorales are known to be resistant to a range of antifungal drugs; however, they are characterized by variable susceptibility to itraconazole, terbinafine and amphotericin B. The latter agent has proven useful in the treatment of cutaneous mucormycosis in a horse with concomitant *Aspergillus* intestinal infections (Table 1—Guillot et al., 2000).

## 5. Deep mycoses

Deep mycoses may represent a serious threat to horses. These usually affect either the upper or lower respiratory tract and, in most cases, they disseminate via the bloodstream and lymphatic system to visceral organs. Although some forms of the disease are sporadic, others occur in epidemics or are enzootic. In many cases, a diagnosis based on clinical features, endoscopy, radiography or ultrasonography is not specific; the definitive diagnosis relies on the unequivocal detection of the pathogen in the infected tissues. Achieving an early diagnosis is crucial to a positive prognosis.

### 5.1. *Adiaspiromycosis*

Adiaspiromycosis is a pulmonary disease of small mammals and occasionally humans, caused by dimorphic fungi of the genus *Emmonsia*. *Emmonsia crescens* (syn. *Chrysosporium parvum* var. *crescens*) is widespread in Europe, while *Emmonsia parva* (syn. *Chrysosporium parvum* var. *parva*) occurs in the Americas, Central Asia, and Africa. These fungi are ubiquitous in the environment, and common in forest-steppe areas, where they grow on soil and produce 2–4 µm conidia in the saprophytic form; thick-walled spherules are usually described, in their infective stage, as adiaconidia (Cafarchia et al., 2012a).

One disseminate pulmonary infection in a horse has once been reported; infections occur when dust-borne adiaconidia are accidentally inhaled, subsequently reaching the alveoli and eliciting extensive granulomatous reactions resembling mycobacterial granulomas (Pusterla et al., 2002).

Clinical signs include chronic weight loss, tachypnea and abnormal lung sounds. Hematological findings are

indicative of an existing inflammatory infectious process (i.e., leucocytosis, neutrophilia, hyperfibrinogenemia, hyperglobulinemia). The diagnosis usually relies on thoracic radiographs and observation of adiaconidia in bioptic samples via histopathological examination.

Thoracic radiographs are characterized by the presence of diffuse small nodular soft tissue densities with a marked alveolar and interstitial affinity, distributed diffusely throughout the lungs and resembling infections by other fungal organisms (i.e., *Coccidioides immitis*), disseminated neoplasiae (i.e., lymphoma), and/or generalized or localized idiopathic granulomatous diseases. Bronchoalveolar and trans-tracheal wash samples do not aid the diagnosis; samples should be collected by percutaneous lung biopsy (Pusterla et al., 2002).

The bioptic material should be stained with PAS, GMS, and mucicarmin stains (Tables 1 and 2). A PCR assay based on the amplification and analysis of conserved regions of the nuclear 28S rRNA gene from the affected lung tissue has been proposed as a specific diagnostic method (Pusterla et al., 2002). Corticosteroids, antifungal or antimycobacterial agents have proven effective in the treatment of human adiaspiromycosis (Turner et al., 1999), whereas suitable treatment strategies in horses are yet to be defined.

### 5.2. *Aspergilloses*

Equine aspergilloses are opportunistic infections caused by species of the genus *Aspergillus* (*Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus nidulans* and *Aspergillus niger*) (Guillot et al., 1997; Ludwig et al., 2005; Cafarchia et al., 2012b). These fungi inhabit and multiply in the environment (e.g., dead leaves, stored grain, compost piles, hay, and decaying vegetation) and, when inhaled, their spores may cause severe infections, mainly in immunocompromised individuals (e.g., suffering for endocrinopathies and/or neoplasiae, or with a history of prolonged administration of antibiotics and corticosteroids) (Carrasco et al., 1996; Guillot et al., 1997; Cafarchia et al., 2012b). Clinical signs vary according to the localization of the infection, but they usually include pulmonary, bronchopulmonary, guttural pouches signs, sinusitis, and/or rhinitis (Table 1—Carrasco et al., 1996; Sweeney and Habecker, 1999; Tremaine and Dixon, 2001a; Cafarchia et al., 2012b).

The diagnosis heavily relies on anamnesis, clinical examination and the results of accessory tests (e.g., endoscopy, radiography or ultrasonography), fungal culture, as well as histological findings (Tables 1 and 2—Tremaine and Dixon, 2001a; Cafarchia et al., 2012b). The diagnosis of guttural pouches or sinonasal infections relies on the observation, via endoscopy, of intranasal or intrasinus mycotic plaques, as well as a series of positive fungi cultures (Tremaine and Dixon, 2001a; Cafarchia et al., 2012b).

The treatment of sinonasal infections and guttural pouch mycosis usually results in positive outcomes when the diagnosis is precocious (see Table 1—Carrasco et al., 1996; Guillot et al., 1997; Cafarchia et al., 2012b). Aspergillosis of the low-airways is usually characterized

by poor prognoses (Tremaine and Dixon, 2001b; Knottenbelt, 2002).

### 5.3. Blastomycosis

Blastomycosis (Syn: *North American blastomycosis*, *Blastomycetic dermatitis* and *Gilchrist's disease*) is a systemic mycosis, caused by a dimorphic fungus, *Blastomyces dermatitidis*, which grows as a 'mycelial' form at room temperature and as a 'yeast' at 37 °C. The mycelial form grows in sandy, acid soils and along waterways. The disease has been described mainly in dogs and humans and only occasionally in horses (Toribio et al., 1999; Dolente et al., 2003; Wilson et al., 2006; Méndez-Angulo et al., 2011). Blastomycosis is endemic in North America; however, it has been described in some African regions and, only sporadically, in Europe. Inhalation is considered the primary route of infection in horses; however, percutaneous infections cannot be ruled out (Wilson et al., 2006; Méndez-Angulo et al., 2011). Following infection, the conidial spores transform at 37 °C into yeast-phase cells, which multiply within the tissue (i.e., lung) and may disseminate via the bloodstream and lymphatic system to the internal organs. Blastomycosis in horses may be either cutaneous or systemic (Table 1—Toribio et al., 1999; Dolente et al., 2003; Wilson et al., 2006); lungs are primarily affected and the radiography of the thorax may reveal pleural effusion and alveolar disease (Toribio et al., 1999; Dolente et al., 2003; Wilson et al., 2006). Blastomycotic osteomyelitis without involvement of the lungs has been also described (Méndez-Angulo et al., 2011). Hematological abnormalities associated with the infection include anaemia, hyperproteinemia, hyperglobulinemia, hyperfibrinogenemia, and hypoalbuminemia (Toribio et al., 1999; Dolente et al., 2003; Wilson et al., 2006).

Diagnosis of blastomycosis can be achieved by several methods. Histological and/or cytological examination of the affected tissues (i.e., skin, subcutaneous tissues, pericardium, lung, tracheal fluid and pleural tissue) have proven useful (Tables 1 and 2). Histopathological findings include chronic inflammation in all grossly affected sites; yeasts may be clearly visualized with PAS and GMS staining (Tables 1 and 2—Toribio et al., 1999; Dolente et al., 2003; Wilson et al., 2006; Méndez-Angulo et al., 2011). At necropsy, firm nodules (up to 2.0 cm in diameter) may be observed in the lungs and in other affected tissues (Wilson et al., 2006). At the histology, nodules appear as focal concentrations of macrophages and yeast-like round cells with a thin wall (Dolente et al., 2003; Wilson et al., 2006). A positive culture (restricted to biosafety level 3 laboratories), confirms the diagnosis of blastomycosis. Treatment strategies yet to be defined since all clinical cases available in the literature were described *a posteriori* in euthanized horses. However, the administration of amphotericin B and ketoconazole has proven efficacious in humans and dogs (Dolente et al., 2003).

### 5.4. Candida infections

*Candida* is a wide ascomycetous genus comprising hundreds of species; however, only few of them act as

opportunistic pathogens. *Candida albicans* is a major pathogen within the genus, and along with *Candida krusei*, *Candida famata*, and *Candida parapsilosis*, has been described as causative agent of disease in horses. These yeasts are part of the microflora of skin, and upper respiratory, alimentary and genital tracts of humans and warm-blooded animals, and occur in the environment (e.g., soil, water, or on plants and fruits). Risk factors for horse infections include an underlying status of immunodeficiency, prolonged administration of antibiotics and/or corticosteroids, use of intravenous and urinary catheters, or endotracheal tubes and alterations of the normal microbial flora of the skin or of the urogenital and gastrointestinal tracts (Reilly and Palmer, 1994). Candidiasis of horses are characterized by different clinical expressions (Table 1), which can be classified according to the location of the lesions (i.e., lesions on gastrointestinal tract, reproductive tract, and systemic infections) (Gross and Mayhew, 1983; Reilly and Palmer, 1994; Stout, 2008) may also occur. Diagnosis of candidiasis relies on fungal culture from material isolated from lesions and exudates (e.g., intestine exudates, semen, cervical discharge, gastric, peritoneal and synovial fluid—Gross and Mayhew, 1983; Reilly and Palmer, 1994; Stout, 2008), and on its histological examination using the PAS stain (Tables 1 and 2). Cytological examination of the endometrial and gastric fluids may also prove useful (Gross and Mayhew, 1983). Endoscopy can also assist in cases of gastroesophageal candidiasis. Administration of amphotericin B is recommended in animals with disseminated candidiasis; however, fluconazole is also effective, safe, relatively inexpensive, and easier to administer (Reilly and Palmer, 1994). Intra-uterine infusion of antimycotic agents (Table 1) is recommended in cases of infection of the reproductive tract; however, no treatment strategy offers a high likelihood of resolution (Stout, 2008).

### 5.5. Coccidioidomycosis

Coccidioidomycosis (Syn: *Valley fever*, *California fever*, *Desert rheumatism*, *San Joaquin Valley fever*) is a fungal disease caused by *Coccidioides* spp., a dimorphic pathogenic fungus that causes infections characterized by respiratory, dermatological, musculoskeletal, neurological, and ophthalmological clinical signs. Two genetically distinct species have been recently identified, namely *Coccidioides immitis* and *Coccidioides posadasii*; the first species has been identified in California, while the second in Central and South America. A few cases of coccidioidomycosis in horses have been reported, and most of them represent disseminated forms of *C. immitis* infection.

The endemicity of the disease, the horse breed (i.e., Arabians are reportedly more susceptible than Quarter Horses and Thoroughbreds) and, most likely, the pregnancy status in mares (Ziemer et al., 1992) represent risk factors. The infection occurs when dust-borne arthroconidia are inhaled. Upon inhalation, the fungi convert to spherules, which contain endospores that are released by rupture and lead to the formation of new endospore-producing spherules. Approximately 4.06% of horses residing in endemic areas (i.e., Arizona) show sub-clinical

infections (Higgins et al., 2005). Coccidioidomycosis evolves from a primary pulmonary infection to an extrapulmonary disease (Table 1—Ziemer et al., 1992; Higgins et al., 2006). Abortions accompanied by placentitis, fetal infection and osteomyelitis with or without respiratory signs have also been reported (Foley and Legendre, 1992; Stoltz et al., 1994). Mastitis can also occur as a result of disseminated infections (Walker, 1993).

Fungal cultures or biopsies which are positive for *C. immitis* (Tables 1 and 2), together with serological evidence, are useful to diagnose the infection. Microscopic examination of tissues or of transtracheal or bronchoalveolar lavage fluids, lymph nodes and pleural fluid exudates, can be performed using KOH (or KOH-ink), lactophenol cotton blue, H&E, Papanicolaou, PAS, methenamine silver stains (Tables 1 and 2). *Coccidioides* spp. grows within 2–5 days on several media. Fungal culture should be restricted to biosafety level 3 laboratories. Serological tests (i.e., Agar Gel Immunodiffusion (AGID) assays and ELISA for the detection of IgM and IgG antibodies) may assist the diagnosis of coccidioidomycosis. An IgG titre lower than 1:8 may be indicative of an exposure to the organism, albeit without the development of disease (Higgins et al., 2006). Treatments in horses (Table 1) might be required in cases of >10% weight loss, presence of lung infiltrates and IgG titres >1:16 (Foley and Legendre, 1992; Higgins et al., 2006). Treatment is successful when the plasma level of antifungal agents is at least equal to the minimum inhibitory concentration values for *C. immitis* (Higgins et al., 2006).

### 5.6. Cryptococcosis

*Cryptococcus* spp. are encapsulated basidiomycetous yeasts which cause an infection known as *European blastomycosis* or *Torulosis*. Within this genus, *Cryptococcus gattii*, *Cryptococcus neoformans* var. *grubii* and *Cryptococcus neoformans* var. *neoformans* are the main agents of infections in mammals. *Cryptococcus neoformans* inhabits a number of substrates (e.g., avian guano, rotting vegetables, and soil), whereas *C. gattii* can be detected in more than 50 tree species, including *Eucalyptus* spp. The infection occurs following the accident inhalation of basidiospores from the environment, ingestion of desiccated yeast cells or, rarely, via cutaneous inoculation (McGill et al., 2009). Young horses and/or immunocompromised individuals are more predisposed towards developing the disease (McGill et al., 2009). Many clinical forms of cryptococcosis are known, which localize to the higher (i.e., causing rhinitis, sinusitis) and lower respiratory tracts (i.e., pneumonia), as well as reproductive (i.e., endometritis, placentitis, abortion), and digestive tissues (i.e., intestinal cryptococcosis) (Begg et al., 2004; Cruz et al., 2009; McGill et al., 2009). Disseminated infections leading to meningitis have been also reported (McGill et al., 2009). In all cases clinical signs are not pathognomonic, and the diagnosis should rely on the detection and identification of the pathogen in the infected tissues (Table 1). Histologically, blastospores can be stained using PAS and GMS, whereas India ink preparation allows to visualize their gelatinous capsules (Table 2). Fungal

cultures, as well as detection of *Cryptococcus* antigens in biopsy samples (i.e., immunohistochemical identification of cryptococcal epitopes) or in serum and or cerebrospinal fluid (i.e., latex cryptococcal antigen agglutination test—LCAT) may assist the diagnosis of the infection (McGill et al., 2009). Successful treatment strategies have been described in the literature, although prognosis is usually poor (Table 1—Begg et al., 2004; Cruz et al., 2009).

### 5.7. *Pneumocystis* infections

The genus *Pneumocystis* includes non-viable, highly diversified fungal pathogens dwelling in the lungs of several host species in which they induce severe pneumonitis, especially in immunocompromised individuals. *Pneumocystis* species are highly similar in morphology, however genetic characterization of *Pneumocystis* may assist species identification according to the host species (i.e., humans, other primates, rodents, rabbits, insectivores and other mammals) (Dei-Cas et al., 2006). *Pneumocystis carinii* has been reported as the commonest species infecting horses (mainly foals), although preliminary genetic analyses (Peters et al., 1994), suggest that additional studies may be necessary for definitive species identification. Therefore, the etiological agent of horse infections is currently referred to as *Pneumocystis* spp. Although three stages of the lifecycle have been described (i.e., trophic forms, sporocytes and mature cysts), the trophic forms are the most abundant, representing 90–95% of the total population in the host lungs (Dei-Cas et al., 2006). Horse infections by *Pneumocystis* spp. are diffused worldwide, but prevalent in Arabian foals up to three months of age with concurrent immune deficiencies, pulmonary infections (i.e., *Rhodococcus equi*) and/or malnourishment; however, the disease may occur also in immunocompetent animals (Perron Lepage et al., 1999; Franklin et al., 2002). Recently, a decreased proportion of CD4+ and CD8+ T lymphocytes in peripheral blood has been documented as a contributing factor to the development of pneumocystis pneumonia in foals (Flaminio et al., 1998). The inhalation of the pathogen represents the main transmission route. Following inhalation, *Pneumocystis* spp. does not colonise the upper respiratory tract, instead it adheres to alveolar type I cells, therefore impairing the pulmonary function by blocking the alveoli, altering the alveolar microenvironment and consequently triggering the inflammatory response of the hosts, which result into interstitial fibrosis (see Table 1—Peters et al., 1994; Flaminio et al., 1998; Perron Lepage et al., 1999; Franklin et al., 2002). Physical examination may not reveal abnormalities, whereas radiographs may display increased opacities of the interstitial–alveolar pattern with alveolar–interstitial pneumonia.

The diagnosis of the infection relies on the cytological and/or histological examination of pulmonary material/tissues, sampled by broncho-alveolar lavage (BAL) or by post-mortem homogenization of lungs (Table 1). *Pneumocystis* organisms are detected using toluidine blue O (TBO), GMS and methanol–Giemsa or Giemsa like, which stain the cell wall of cystic forms in reddish violet or dark brown. The trophic forms can only be stained with Giemsa, or

Giemsa-like stains (i.e., the nucleus is stained in pinkish-purple while the cytoplasm in blue, Table 2). At the *post mortem* examination of lungs, a proliferative interstitial pneumonia characterized by a large number of macrophages in the alveolar lumen that induces type II pneumocystis change may be observed (Peters et al., 1994; Flaminio et al., 1998; Perron Lepage et al., 1999; Franklin et al., 2002). *Pneumocystis*-specific fluorescein-labelled antibodies have proven useful for the identification of *Pneumocystis* organisms in impression smears or lung-homogenate air-dried smears (Perron Lepage et al., 1999).

## 6. Conclusion

Due to their extreme zoonotic potential and challenging diagnosis, fungal diseases represent a serious threat for horses. Indeed, besides superficial dermatophytosis (ringworm), for which culture and direct microscopic examination of hair shafts and skin scrapings may lead to a definitive diagnosis, that of other fungal infections relies on biopsies and detection of microorganisms at the histological examination. Fungal affections are usually neglected by public health authorities and often by the scientific community; this severely impacts on the availability of epidemiological data and on knowledge of their clinical features.

Similarly, little information is available of the morphological, physiological and molecular features of the etiological agents, as well as of their susceptibility to antifungal compounds both *in vitro* and *in vivo*. Based on these observations, further studies aimed at determining the geographical occurrence of many fungal species and at evaluating novel rapid and specific diagnosis tests are mandatory in order to develop adequate strategies of control and prevention of the diseases caused by these organisms.

In addition, the unequivocal detection of the pathogen in the diseased tissues should be followed by its culture and, possibly, by the molecular characterization of the isolates; this is pivotal in order to expand current knowledge of the etiological agents. Further investigations of the virulence of these pathogens should also be considered, in order to improve current therapeutic and control strategies.

## Conflict of interest

All authors declare to have no conflict of interest.

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