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Prominent animal mycoses from various regions of the world

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The mycoses selected for presentation in this section are relatively common diseases of companion animals or livestock in certain areas of the world. Malasseziosis is arguably the most frequent mycosis of dogs (as otitis externa and dermatitis) throughout the world, although its diagnosis is often overlooked. Protothecosis is also geographically widespread, particularly in cattle where severe mastitis is a result of adventitious infection from the environment. In contrast, coccidioidomycosis and pythiosis are geographically limited in their occurrence (coccidioidomycosis by geographic region and pythiosis by climate), but within regions where they do occur, their presence in animals is not unusual. It was our intention to review recent developments in each of these diseases.

Keywords coccidioidomycosis, malasseziosis, protothecosis, pythiosis

Malassezia infections in animals

Malassezia yeasts have morphological and physiological characteristics which make them easily recognizable from all other fungi. Their bottle-shaped appearance is related to an enteroblastic unipolar budding. *Malassezia* yeasts show a specific requirement for lipids and this may account for their absence in the environment. In fact, the yeasts are universally and exclusively present as commensals among the normal skin flora of warm-blooded vertebrates [1]. The non-lipid dependent species *Malassezia pachydermatis* is frequently recovered from wild and domestic carnivores, including dogs, cats, bears, pinnipeds, ferrets and foxes [2]. It has also been isolated from animal species as diverse as dromedaries, rhinoceros, pigs, primates, horses and birds [2–5]. Other *Malassezia* species are lipid-dependent and appear more anthropophilic. However, *M. furfur* may be isolated from the skin and feathers of birds [6], *M. slooffiae* from pigs and herbivores [6], *M. globosa* from cats [7,8] and cattle [9], and *M. sympodialis* from cats [7]. Moreover, wild mammals such as okapi, rhinoceros and elephants were shown to harbor specific *Malassezia* yeasts that should be described in the near future [6]. Whenever alteration in the skin microclimate or host defense occurs, *Malassezia* yeasts may behave as opportunistic pathogens. Dermatological lesions related to these yeasts were first reported in humans about 150 years ago [1], whereas the first description of an animal case of *Malassezia* dermatitis was made in 1925 [10]. Lesions of exfoliative dermatitis in an Indian rhinoceros were then attributed to the presence of bottle-shaped yeasts.

In 1955, Gustafson [11] first isolated *Malassezia* yeasts from the external ear canal of both healthy dogs and dogs with otitis externa. Subsequent investigations demonstrated that ceruminous otitis externa should be considered as the commonest clinical presentation of *Malassezia*-associated skin diseases in dogs, cats and other carnivores such as ferrets or fennecs [12,13]. *Malassezia*-associated otitis externa have also been described in cattle, okapi, pigs and dromedaries [2,4,9,14].

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Clinical signs include erythema and pruritus with waxy, moist and brown exudate. The presence of ear mites (*Otodectes cynotis* in carnivores and *Raillietia* spp. in cattle) causes inflammation that may lead to a secondary yeast infection [9,15].

In 1983, Dufait [16] first identified *M. pachydermatis* as a cause of more widespread dermatitis in dogs. He described a series of 50 dogs with seborrheic dermatitis from which the yeasts could be readily recovered by cytology or culture and which responded to antifungal therapy. Since then, many articles have been published describing the condition in dogs and cats. Recently, we observed *Malassezia* infections in pinnipeds, horses and birds. Although some animals develop a generalized disease, the condition is usually localized or multifocal. In dogs, the most often affected areas are the ventral abdomen and the ventral cervical region. The muzzle, footpads and perineal regions are also commonly affected. Skin lesions consist of erythema, alopecia, lichenification and hyperpigmentation. Pruritus is a constant manifestation and the disease seems more prevalent during the warmer months. In cats, chin acne localized to generalized alopecia and erythema have been reported [15,17]. In pinnipeds, lesions may consist of wheals on both flanks and chest [5]. In horses, we observed cutaneous lesions on the head, the tail-base or the mane. Lesions were characterized by erythema, scales and crusts with secondary seborrhea. We also diagnosed *Malassezia* dermatitis in two canaries that lived together and simultaneously developed feather loss around the eyes [18].

Whereas pityriasis versicolor is a common dermatomycosis in humans throughout the world, this *Malassezia*-associated dermatitis was hardly ever diagnosed in animals. The first case reported in literature concerned a milking goat, which developed discoloration of the teats and udder [19].

The factors favoring proliferation of *Malassezia* yeasts on animal skin remain poorly understood. They presumably reflect disturbances of the normal physical, chemical or immunological mechanisms, which restrict microbial colonization of the skin. In dogs, canine breed predilections have been identified and the most common underlying causes of secondary yeast dermatitis or otitis seem to be hypersensitivity conditions, such as flea allergy dermatitis, food hypersensitivity or very frequently atopy. Previous antimicrobial or corticosteroid therapy is a frequently associated finding. In cats, cases of generalized *Malassezia* dermatitis are seldom reported but may be associated with concurrent systemic diseases [17]. A recent investigation demonstrated that *Malassezia* yeasts were more frequently recovered from the skin of retrovirus-infected cats, compared with noninfected cats [20].

Diagnostic criteria for *Malassezia* dermatitis and otitis in animals have not yet been firmly established. We suggest that *Malassezia* infections should be suspected whenever an animal with elevated *Malassezia* populations on lesional skin shows a good clinical and mycological response to the appropriate antifungal therapy. Cytology is the most useful method to identify the yeasts on the skin. Different cytological techniques have been described with variable results. Most of these are impression methods using glass slides, cotton swabs, skin scrapings and tape-strip preparations. For culture, the use of lipid-supplemented media, especially modified Dixon's medium [21], was shown to be very advantageous. Incubation temperatures between 27 and 32 °C should be applied. Higher temperatures (up to 37 °C) may enhance the growth of *M. pachydermatis* but prevent that of most lipid-dependent species, especially *M. globosa* and *M. restricta*. When small numbers of yeasts are observed by cytological examination or culture, a firm diagnosis of *Malassezia* infection is not possible. Histological data and skin test reactivity clearly indicate that hypersensitivity to *Malassezia* yeasts may develop in animals, especially dogs. In such cases, observing only occasional yeasts by cytology or culture is significant.

Skin biopsy is useful in the diagnosis of animal *Malassezia* infections. Microscopic lesions typically include parakeratotic hyperkeratosis, irregular epidermal hyperplasia, intercellular edema and lymphocytic superficial perivascular or interstitial dermatitis [22]. However, *Malassezia* yeasts are not always visible in the epidermal stratum corneum, even in cases where large numbers have been seen by cytology or culturing.

Therapeutic options for animal *Malassezia* dermatitis or otitis include systemic therapy and/or topical therapy with a variety of agents, especially azole derivatives (enilconazole and miconazole). In dogs with generalized or multifocal dermatitis, ketoconazole is used orally at 10 mg kg^{-1} every 12 h for several weeks. Itraconazole is also effective orally at 5 mg kg^{-1} every 24 h. Other systemic antifungal drugs are not effective (griseofulvin) or have not been evaluated for the treatment of animal *Malassezia* infections (fluconazole, terbinafine, etc). For topical treatment, a 2% miconazole/2% chlorhexidine shampoo was shown to have a good efficacy in dogs [23]. We recently obtained a favorable response in horses and pinnipeds with topical applications of enilconazole (0·2 or 2% solution) twice a week. The identification and the correction of the predisposing factors may prevent, or reduce, the frequency of relapse. In the two cases of periocular *Malassezia* dermatitis in birds, specific therapy could not be applied but elimination of *Dermanyssus* mites, which were supposed to act as predisposing factors, led to complete recovery.

In animals, *Malassezia* yeasts can be isolated from various mucosal sites (mouth, rectum, anus, anal sacs or vagina) but deep infections are never reported. On the contrary, systemic *Malassezia* diseases are regularly diagnosed in immunocompromised humans. Most cases involve premature infants receiving broad-spectrum antibiotics and parenteral lipid emulsions for several weeks [24]. Some cases are caused by *M. pachydermatis*, the species which belong to the normal cutaneous flora of pet carnivores. In a recent study conducted in an intensive care nursery, *M. pachydermatis* isolates from patients, health care workers and health care workers' pets were shown to be indistinguishable [25]. Consequently, animals could sometimes be suspected as a source of *Malassezia* yeasts for humans.

Protothecosis in animals

Protothecosis is caused by species of algae belonging to the genus *Prototheca* [26]. The genus includes unicellular, non-motile, asexual, achlorophyllous algae with distinctive cellular morphology. The production of asexual endospores in sporangia is a characteristic of the genus [27]. Within the genus, three species, *Prototheca stagnora*, *P. wickerhamii* and *P. zopfii*, are presently recognized [26]. Only the latter two have so far been reported to be pathogenic for animals [26,28] and man [26,29,30]. In humans, protothecosis is usually restricted to the skin and bursae. In animals, single cases have been reported in deer and cats [26,31], a few systemic cases in dogs [28,32,33] and numerous cases in cattle [26,31]. In cows, all infections, with one exception [34], have been restricted to the mammary glands and corresponding lymph nodes [35–43]. The udder is the target for ascending infection, which is sometimes iatrogenic in origin; a long-lasting infection often results [35–37]. In dairy herds, *P. zopfii* may infect one [36] or several cows [40–42]. The consequence of the infection is usually culling of the infected animals.

Prototheca species have been isolated from a wide range of environmental sources and sites characterized by wetness, and the presence of organic matter [43]. Experimental infection has shown *P. zopfii* to be a facultative pathogenic organism that infects bovine mammary glands by an ascending route [37]. From electron microscopic observations it has been found that algal cells in the form of sporangiospores and sporangia are contained by macrophages in the interstitium, sequestered between alveolar epithelial cells, and in the lumen of alveoli [37]. Such observations suggest that algae may proliferate intracellularly and be spread to regional lymph nodes by macrophages [37]. This theory would explain the chronicity of the infection and the negative outcome of therapeutic efforts.

Most infected cows develop clinical mastitis and develop positive California mastitis test scores of 4 to 5 [44]. However, the secretion from affected quarters usually has a normal appearance [44]. Affected glands have a slightly increased density, and apart from enlargement of the involved lymph nodes, no clinical lesions are usually recorded [40]. At post mortem examination, specific macroscopic lesions are not usually recorded [37].

The microscopic morphology of *Prototheca* spp. is the same *in vivo* and *in vitro*, i.e. single cells (endospores) are ovoid to spherical with a diameter 5–15 μm. The mother cells are spherical with a diameter up to 25 μm. Within mother cells the endospores are easily recognized [45]. The organisms are faintly stained by haematoxylin and eosin (H&E), whereas a strong reaction is seen in sections stained by periodic acid-Schiff (PAS) and Grocott's methenamine silver stain [46].

Within affected quarters, a heterogeneous picture of algal proliferation and cellular reaction is present. Within lobules all alveoli are usually not affected. Tissue reaction in infected alveoli and excretory ducts may vary from an acute to a chronic type. In the early stages of infection of alveoli, protothecal cells multiply heavily within the lumen and in the epithelial lining of the alveoli. CD68-positive cells (i.e., activated macrophages [46]), containing algae within the epithelial lining of the alveoli and excretory ducts, are often vacuolated [46]. Within the lumen, multiplication takes place in the cytoplasm of sequestered macrophages in which different stages of development and degeneration of the algae can be seen [46]. In the early stages of infection, algal cells may also be seen within the cytoplasm of neutrophils that have been excreted into the lumen of alveoli [46]. Many chronically infected alveoli are involuted, and epithelial hyperplasia is frequent. The chronic inflammatory reaction is, apart from the proliferation of fibroblastic tissue, dominated by infiltration by macrophages, lymphocytes, plasma cells and occasional eosinophils. However, only rarely epithelioid and giant cells may also be formed [39,40].

Macrophages containing degenerated algae (i.e., empty spherical cell walls without cytoplasmic organelles) are frequently sequestered between epithelial cells and in the lumen of alveoli. In the epithelial lining of the excretory ducts, algal cells are contained by intra-epithelial macrophages [46]. Many CD68-positive macrophages, filled with a homogeneous PAS positive material (which does not react with specific antibodies in a peroxidase anti-peroxidase (PAP) staining technique for *P. zopfii*) are often seen around alveoli [46]. Within the regional lymph nodes, an accumulation of macrophages contain-

ing single and endosporulating forms of algae will be located in the para-cortical and para-trabecular sinuses [46].

In vivo and *in vitro* an organism can be recognized as *Prototheca* species if characteristic endosporulating cells are visible [45]; some may resemble small *Coccidioides immitis*-like spherules. However, if these structures are not visible in tissue sections, the nonsporulating cells may resemble a number of other fungi, e.g. *Blastomyces dermatitis*, *Cryptococcus neoformans*, *Paracoccidioides brasiliensis*, and some stages of *C. immitis* [45]. Several bovine infections due to green algae (i.e., species of *Chlorella*) have been misdiagnosed as protothecosis [47–49]. Therefore, to obtain an accurate diagnosis of protothecosis, it is essential to cultivate the organism and document its nature *in situ*, i.e. demonstrate an absence of chloroplasts [47] and/or specific immunohistochemical reactivity [34,45].

Sudman & Kaplan [45] originally introduced immunohistochemical staining of *Prototheca*. They developed a battery of specific antibodies that was both sensitive and specific after heterologous absorption. Since then we have also developed specific antibodies towards *P. zopfii* which have been applied in a PAP technique. This technique was originally developed for the study of experimental murine protothecosis [50], but has since been used for diagnostic purposes in cases of bovine mammary protothecosis [37].

Cultivation is readily accomplished on Sabouraud agar and most bacteriological isolation mediums. Following isolation, cultures with characteristic cell morphology, as observed in lactophenol cotton blue [36], must be examined for nutrient assimilation, fermentation and sensitivity to clotrimazole (Table 1). Moreover, preparations of cells should be stained by India ink and examined microscopically for capsule formation to exclude *C. neoformans*.

We have examined the ability of 16 strains of *P. zopfii* from bovine secretions to use the carbon sources listed in Table 1 for growth. This was determined using modified prototheca isolation medium (PIM) [51]. The isolates were also characterized using the API 20 C AUX assimilation kit [52]. Fermentation tests, using a 0·5% concentration of the carbohydrates used for assimilation, were

Table 1 Assimilation and fermentation results with 16 *Prototheca zopfii* isolates of bovine mammary origin on PIM medium and in meat extract medium, respectively [19]

	Assimilation						Fermentation	
		Reading on day						
Carbon source	pH of PIM medium	2	3	4	10	21	8	21
Cellobiose	7·0	−	−	−	−	−	−	−
Erythritol	7·0	−	−	−	−	−	−	−
Fructose	7·0	+	+	+	+	+	+	+
Galactose	7·0	−	−	−	−	−	−	−
Glucose	7·0	+	+	+	+	+	+	+
Glycerol	7·0	d	+	+	+	+	+/−	+/−
Inositol	7·0	−	−	−	−	−	−	−
Lactose	7·0	−	−	−	−	−	−	−
Maltose	7·0	−	−	−	−	w	−	−
D(+) mannose	7·0	−	−	−	−	w	−	−
Raffinose	7·0	−	−	−	−	−	−	−
D(−) sorbitol	7·0	−	−	−	−	−	−	−
Sucrose	7·0	−	−	−	−	−	−	−
Trehalose	7·0	−	−	−	−	−	−	−
D(+) xylose	7·0	−	−	−	−	−	−	−
Methanol	7·0	−	−	−	–	−	−	−
Ethanol	7·0	+	+	+	+	+	+	+
n-propanol	7·0	−	w	+	+	+	+	+
iso-propanol	7·0	−	−	−	−	−	−	−
n-butanol	7·0	−	−	−	w	+	+	+
Sodium acetate	7·0	+	+	+	+	+	−	−
Blind test	7·0	−	−	−	−	−	−	−
Sodium acetate	5·1	+	+	+	+	+		

d, different results among isolates; w, weakly positive reaction; +, positive reaction; −, negative reaction; +/−: positive with 11 and 14 isolates, respectively.

performed in meat extract medium [53]. The minimum inhibitory concentration of clotrimazole was determined by agar dilution technique and by agar diffusion using drug impregnated tablets containing 10 μg per tablet. All cultured cells were globose to slightly oval, and ranged in size 4–25 μm. The larger cells were sporangia with endospores. This morphology is highly suggestive of *P. zopfii* [51].

All isolates were able to grow at 25, 30 and 37 °C. Capsule formation was never observed. The results of assimilation on PIM medium and the fermentation tests are given in Table 1. Both tests had a uniform pattern, however, in the fermentation test two isolates remained glycerol negative. These results agree with those of Pore [51] and Pore *et al*. [54] for *P. zopfii*. From Table 1, it is seen that all isolates assimilated acetate on PIM medium adjusted to pH 7·0 as well as at pH 5·1 (*P. wickerhamii* is not able to grow at pH 5·1 [51]). All isolates had identical reactions in the API 20 C Aux kit, i.e. only positive for glucose and glycerol. Administration of clotrimazole did not affect the growth of these algae. A simple test to differentiate *P. zopfii* from *P. wickerhamii* by determining susceptibility to clotrimazole was published by Casal & Gutierrez [55], who found no inhibition with 21 strains studied. However, Pore [51] found 20% of *P. zopfii* isolates ($n = 133$) unable to grow in the presence of 50 μg ml^{-1} of clotrimazole.

Information about the development of immunity against *Prototheca* organisms in animals is sparse. However, a few studies concerning antibody determination in serum of cows with mammary protothecosis have been performed. Using electrophoretic assays with ultrasound-treated *P. zopfii* cells as antigen, it was found that 40–42% of cows with *Prototheca* mastitis had precipitating antibodies [56,57]. A highly sensitive enzyme-linked immunosorbent assay (ELISA) technique for the monitoring of antibodies in the serum of cows was recently developed [37]. This technique detected antibodies in both diseased and non-diseased cows, which parallels the serological response with other opportunistic pathogens of cows such as *Aspergillus fumigatus* [58]. Thus, it is likely that under normal farm conditions cows are regularly exposed to algae through ephemeral contacts. This supposition is supported by the frequent isolation of *P. zopfii* from the environment of dairy herds including bovine feces [43,59]. In conclusion, although a strong serological response may be present in infected cows, the titer in single samples of serum does not appear valid for the diagnosis of bovine mammary protothecosis.

Although isolates of *P. zopfii* are sensitive to a number of therapeutic agents *in vitro*, e.g. gentamicin, polymyxin, nystatin and amphotericin B, many authors have confirmed that treatment of infected animals will not affect the course of the disease [38,42]. Therefore, the disease should be controlled by improving conditions of animal management, by culling infected cows, and possibly by dipping the teats of the cows after each milking [40], and by thorough disinfection of the teat end prior to medication infusion.

Pythiosis

Pythiosis is a granulomatous disease that occurs in tropical, subtropical and temperate areas, affecting horses, cats, dogs, cattle and humans [60,61]. It is caused by the oomycete, *Pythium insidiosum* [62]. The equine species is the most commonly affected; cutaneous, subcutaneous and gastrointestinal lesions form, characterized by the formation of eosinophilic granuloma, with the presence of necrotic masses called 'kunkers' [60,61,63,64].

P. insidiosum belongs to the Kingdom Stramenophila, Phylum Oomycota, Family Pythiaceae, Genera *Pythium* and species *insidiosum* [65]. The organism has several peculiar characteristics: (i) biflagellate zoospores (asexual reproduction); (ii) oogamic sexual reproduction; (iii) cell wall composed of β-glucan, cellulose and hydroxyproline; (iv) diploid thallus; (v) tubular cristate mitochondria; and (vi) molecular and biochemical characteristics such as an alternative route for lysine synthesis [66,67]. This genus contains 120 known species distributed throughout the world, mostly soil inhabitants and plant pathogens. Identification of *Pythium* species is based on the morphological characteristics of the zoosporangia, zoospores, oogonia and antheridium [66,67]. The induction of sporulation of *P. insidiosum* in water was first accomplished by Austwick & Copeland [68]. Attempts to induce zoosporogenesis in a liquid medium using different ions at several concentrations showed that zoospore formation and liberation are influenced by ionic concentration, especially K^+, Ca^{2+} and Mg^{2+} ions [69]. A rapid method to induce *P. insidiosum* zoosporogenesis was described [70] and consisted of sowing the organism in a poor medium with fragments of grass, thus promoting the colonization of the fragments. Afterwards, the colonized grass was transferred to an induction medium composed of sterile distilled water and ions, promoting the formation of the zoosporangia on the borders of the hyphae.

Pythiosis occurs in tropical, subtropical and temperate areas, being reported in several countries including Argentina, Australia, Brazil, Colombia, Costa Rica, Egypt, USA, Greece, Haiti, India, Indonesia, Japan, Papua New Guinea and Thailand [61,71,72]. This disease has many common names, including hyphomycosis, zygomycosis,

granular dermatitis, 'bursattee', Florida 'leeches', phycomycotic granuloma and 'swamp cancer' [71,72]; in Brazil, the most popular name is 'fashion wound'. The term hyphomycosis is not accurate because it includes other diseases caused by fungi, especially *Basidiobolus ranarum* and *B. haptosporus*, in addition to some Mucorales [73].

The environmental conditions are decisive to the development of the organism. According to Miller & Campbell [64], temperatures between 30 and 40 °C and water accumulation in swamps and ponds (rainy season) are necessary for the production of motile zoospores. On the basis of epidemiological data, the authors believe that the incubation period lasts several weeks.

In 1983, Miller [74] proposed an ecological cycle to describe the environmental behavior and the infectious chain of this organism. The common cycle is based on the colonization of aquatic plants that serve as substrata for development and reproduction, forming the zoosporangia. The zoospores in the water move until they find another plant or animal where they can encyst and emit the germinative tube, beginning a new mycelium and completing their cycle. *In vitro* analyses have demonstrated zoospore chemotaxis for hair, animal and plant tissue. The zoospore chemotatic behavior was attributed to some unknown substance present in animal and plant tissues. Another important factor was the liberation of an amorphous substance by the zoospore after its encystment in response to the chemotatic factors of the host. This substance acts as an adhesive, binding the zoospore to the host surface and allowing the formation of germ tubes, which then invade the host tissues [75]. These findings sustained the infection theory, suggesting that horses in contact with polluted waters could attract zoospores, which would germinate on small skin lesions [74,75]. However, other authors suggest the possibility of zoospore penetration through the hair follicle, based on observation of hyphae inside naturally infected bovine follicles and on the fact that chemotaxis is much more active in the region inside the hair follicle [76,77]. The necessity of a skin lesion to zoospore germination can be questioned; differences in individual susceptibility to the infection are suspected. Most reports of human infection are in thalassemic individuals [78]. We suspect there are characteristics that make some animals more susceptible. There is a strong need for more investigation into this aspect. There are no reports of direct contagion among animals or between animals and human beings [61].

The horse is the animal most affected by pythiosis. There is no breed, sex or age predisposition [63,72]. Cutaneous lesions are very frequent and they usually reach the extremities and the ventral portion of the abdominal wall, probably due to the greater amount of time spent in contact with zoospore polluted waters [64,79]. Clinical symptoms include granulomatous ulcerative lesions with large tissue masses (5–500 nm) with irregular borders. These masses contain hyphae covered by necrotic cells producing white-yellowish masses called 'kunkers'. These masses vary 2–10 mm in diameter; they are irregular, ramified and granular, and occupy sinus tracts in the granular tissue. The size of the lesions depends on where they are located and on how long the animal has been carrying the infection. Hemorrhagic suppurating secretions issue from the sinus tract. The animal often exhibits intense itching and often mutilates the lesion attempting to alleviate the discomfort. Lameness is quite frequent in horses with leg lesions [60,61,64,71]. Most reported cases describe only one lesion per animal; however, multiple lesions can occur [63,64,79].

Intestinal pythiosis is the second most common form of *P. insidiosum* infection in horses. All reported cases were presented as a colic episode, in which the cause was the existence of residual masses, narrowing and/or obstructing the intestinal lumen. Necropsy examination revealed intestinal ulceration and nodular masses located on the jejunal wall, some measuring approximately 20 cm in diameter [79].

The diagnosis of pythiosis was formerly based on clinical and histopathological characteristics, on the isolation of the agent, and on identifying the agent in culture by morphological and reproductive characteristics; however, the early diagnosis of this disease is difficult. Techniques including immunohistochemistry and serology (ELISA and immunodiffusion) help support a correct and early diagnosis [61].

The treatment of infections caused by *P. insidiosum* in animals and human beings is complex due to the singular characteristics of the etiological agent. *P. insidiosum* is a pseudofungus, as it differs from the true fungi in the production of biflagellate zoospores and in the composition of its cell wall. The true fungi contain chitin and β-glucan in their wall, while *Pythium* contains cellulose and β-glucan. The plasma membrane is free from sterols (as ergosterol), a pathway where most of the antifungal drugs act [72]. This has an important consequence on the treatment of pythiosis, with no efficient antifungal drug for the disease because of these characteristics [80]. The phytopathogens in the genus *Pythium* are sensitive to common plant fungicides; however, these compounds are toxic to mammals, disabling their use for pythiosis treatment [78]. A commonly used plant fungicide, Metalaxil, was used in the treatment of pythiosis in dogs; the results were inconsistent, partly due to the toxicity of the compound [72].

Successful results using different kinds of treatment vary due to the size of the lesion and how long the animal has been infected. The age and the nutritional condition of the animal also influence the results. The usual treatment for equine pythiosis is surgical removal. Systemic antifungical applications present many side effects and high costs as well as low efficiency [80]. Surgical intervention requires removal of the whole affected area along with a safety margin to prevent recrudescence. This approach is hindered by the involved anatomical structures, especially on the extremities [79]. The surgical treatment is successful only with small and superficial lesions, where it is possible to retreat the affected areas with a margin of safety.

There is little knowledge of the mechanisms involved in infection by *P. insidiosum*, possibly because of the differences between *P. insidiosum* and true pathogenic fungi. According to Miller [80], equine pythiosis is a progressive infection, even in immunocompetent horses, suggesting an inadequate immunological response or some inhibitory factor inducing failure of the immune system. This author believes that although antigenic, the hyphae are not completely recognized by the host because of the dense inflammatory reaction. Mendoza *et al.* [61] proposed a possible immunological mechanism generated by equine pythosis. As soon as the infection starts, *P. insidiosum* releases soluble antigens stimulating the production of immunoglobulin (Ig)E. These immunoglobulins link to the hyphal surface, activating mastocytes, which liberate their chemotaxic factors in order to attract eosinophils to the site of infection. The eosinophils attach to the Fc portion of IgE in the hypha and eventually degranulate, protecting the hypha from the immune system. This mechanism is similar to one related to parasitic infections. According to the authors, there is evidence of the existence of dispersed soluble antigens at the site of the lesion, and on the inside of the 'kunker' bodies.

Regarding the possible mechanisms of immunotherapy action, it is believed that the cytoplasmic antigens, exposed to the immune system of the animal through vaccination, enhance both humoral and cellular responses capable of fighting the natural infection. The existence of the humoral response is proven by immunodiffusion tests and by alteration of the inflammatory infiltrate [61]. Miller [80] proposed that immunotherapy can inhibit natural infection through the inoculation of hyphal fragments and their cytoplasmic factors. He has observed histopathological results that demonstrated hyphal lysis inside the kunkers in immunologically stimulated animals.

Santurio [77] has developed immunotherapy for the treatment of equine pythiosis called Pitium Vac, based on methods proposed by Miller [64]. In recent years, this immunotherapy has been tested in Brazil, mainly in Mato Grosso do Sul and Rio Grande do Sul, with promising results. Monteiro [65] described an effectiveness test for the treatment of equine pythiosis using Pitium Vac.

Present concepts of coccidiodomycosis in animals

The history of coccidioidomycosis, one of the most studied and oldest described major fungal infections, began in the city of Buenos Aires at the Rawson Hospital, where Dr Bengolea asked Alejandro Posadas to perform histopathological studies on a patient named Domingo Ezcurra; the patient had exhibited chronic lesions in the skin and lymph nodes. Although this case had been clinically misdiagnosed as a cutaneous lymphoma, Posadas and his professor Roberto Wernicke reported, after scrupulous study, a new disease that was thought to be caused by a parasite. Experimental infections in several animal species (monkey, rats, rabbits, guinea pigs, dogs and cats) were also carried out by Posadas; the results of these studies were published in Anales del Círculo Médico Argentino (1892), describing the condition as 'psorospermosis' [81–83].

In 1894, just 2 years after Posadas' initial description, Rixford described the first North Americam case of coccidioidomycosis. In 1896, he and Gilchrist, at the Cooper Medical College, created the name *C. immitis*. In 1900, Ophüls and Moffitt reported *C. immitis* to be a fungus, described its life cycle and fulfilled Koch's postulates. The recovery of *C. immitis* three decades later from the soil under a bunkhouse in Delano, California, USA, used by four Filipino workers who had developed severe disease, completed our general understanding of the natural life cycle and source of disseminated coccidioidomycosis [84].

As a result of its geographic distribution this disease is often termed an endemic mycosis. *C. immitis* is found in soil in certain areas of the western hemisphere (from 40° N latitude in northern California to 40° S latitude in Argentina), including southwestern USA, Mexico, some parts of the Central America, Colombia, Venezuela, Paraguay and Argentina [82]. Recently, it was found in a new area located in Brazil [85].

C. immitis exists in the environment as a mould, growing as branching, septate hyphae, bearing specialized conidia called arthroconidia. In immature colonies (less than 5–10 days old) arthroconidia may just begin to form and may readily be confused with conidia of several other saprobic fungi (*Malbranchea*, *Arthroderma*, *Uncinocarpus*, *Geotrichum*, *Oidodendron*, *Auxarthron*) [86]. In mature colonies, the arthroconidia of *C. immitis* ap-

pear alternatively in chains, separated by blank empty cells, and are quiet distinctive. Once mature, arthroconidia are extremely hardy and may remain viable for extend periods of time. In its natural environment the fungus remains in the mould phase; wind-dispersed arthroconidia germinate to form new hyphae and arthroconidia under suitable environmental conditions [87].

Although *C. immitis* is usually found in soil, its hardy arthroconidia are easily airborne and inhaled by animals and humans who become accidental hosts. It is a weak environmental competitor and does not grow as well in soil harboring other fungi or bacteria [84]. However, in its arthroconidial form it is able to withstand extremes of heat, desiccation and soil salinity, and requires little in terms of nutrition. *C. immitis* thrives in the soils of the desert with minimal competition from other microorganisms. Survival of arthroconidia may be enhanced in loose soil at rodent burrows under bushes [88].

In humans, coccidioidomycosis may be overt or occult. It may be without apparent detriment to the host, or it may progress to death in an insidious or fulminant manner [89]. By the late 1930s it was realized that the full spectrum of clinical disease caused by *C. immitis* included a broad range of symptoms. Thus, although patients previously diagnosed with *C. immitis* infection had severe disease, it is now recognized that the vast majority presented milder forms of the disease and many remained asymptomatic [89]. Starting in 1918 with description of coccidioidomycosis lesions in cattle [90] the disease has been found in sheep, swine, horses, burros, domestic cats, dogs, coyotes, desert rodents, a variety of nonhuman primates, many zoo animals, some uncommon pet and companion animals, and a snake [91]. It is diagnosed most frequently in dogs as either uncomplicated primary infections or disseminations [92,93]. Lesions found in slaughtered cattle, sheep and swine reflect self-limiting pulmonary disease but may lead to carcass condemnation if confused with tuberculosis. Unlike most herbivores, llamas and horses sometimes develop progressive coccidioidomycosis [91,94]. Disease signs in animals relate to dissemination and vary with location and duration of infection [94]. Commonly listed are fever, cough, variable appetite, weight loss, pain, muscle atrophy, bone enlargements, draining skin lesions, loss of eyesight and central nervous system signs [91,94]. In humans, the infection also causes a wide variety of lesions [89,95]. Domestic cats are infected, and dissemination, although rare, occurs more frequently than the single published report would indicate [96]. Wild animals are probably vastly under-diagnosed as victims of uncomplicated primary or disseminated coccidioidomycosis [97].

Coccidioidomycosis is reported in wild marine mammals [98,99]. Disseminated coccidioidomycosis has been diagnosed in six southern sea otters (*Enhydra lutris nereis*) found dead or dying along the Pacific coast of California. The pathological findings in disseminated coccidioidomycosis in sea otters were similar to those described in other species, but pleuritis was more striking and consistent. The pronounced and uniform involvement of the respiratory system indicates that the likely route of exposure for sea otters was inhalation, as in other species. The uniformity of disseminated lesions suggests that sea otters have a low resistance to the agent [98]. The definitive mechanism for marine mammal exposure to a soil-associated fungus is unknown but likely reflects soil contamination of the water.

Since the first case of coccidioidomycosis in dogs was described in 1939, the dog has been recognized as one of the most susceptible species [91,93]. Among all animals, dogs show the most recognizable signs of coccidioidal disease, although acute primary infections often go unnoticed by the owners [91,100].

Medical records and laboratory data from 218 cases of canine coccidioidomycosis diagnosed at the University of California, Davis, CA, USA were reviewed. The diagnoses were based on clinical signs in conjunction with positive qualitative and quantitative immunodiffusion serological testing, histopathology, and mycology. Forty-three breeds were represented, as well as numerous mixed-breed dogs. Large and medium sized dogs predominated. Coccidioidomycosis occurred frequently in young adult dogs. Males were affected more commonly than females. Clinical signs noted at presentation included fever, lethargy, partial anorexia with associated weight loss, exercise intolerance, cough and lameness. Primary pulmonary infection occurred most commonly. Evidence of extrapulmonary dissemination occurred most commonly to appendicular bone and overlying skin, but also included visceral (hepatic, splenic, renal), pericardial/myocardial, central nervous system, ocular and prostatic involvement. Extrapulmonary dissemination was associated with a poorer prognosis and increased incidence of euthanasia or death [101].

Laboratory confirmation of infection can be obtained by histopathological or cytological methods, cultural isolation and serological testing. The most direct method of diagnosis is the demonstration of endosporulating spherules in lesion materials [102]. Microscopic examination of exudates, cultures or tissue sections are enhanced by the use of KOH (or KOH-ink), lactophenol cotton blue, H&E, Papanicolau, PAS, methenamine silver or trichrome stains. Culture of specimens is recommended along with histopathological studies in order to verify the

presence of *C. immitis* if microscopic findings are equivocal and if the fungus is viable [103].

Accurate diagnosis of coccidioidomycosis in dogs is essential in veterinary medicine. A positive diagnosis of coccidioidomycosis carries the possibility of serious consequences, from expensive and prolonged treatment to euthanasia. Detection of antibodies to *C. immitis* via complement fixation has been the gold standard assay for serological diagnosis of coccidioidomycosis [103], although precipitins may afford more timely information. Diagnosis of canine coccidioidomycosis is based upon the presence of clinical signs compatible with the infection and positive cultural and serological findings [104]. Early in the course of disease (2–5 weeks), a positive precipitin test for IgM antibody develops and reflects increased IgM levels. Subsequent (8–10 weeks) positive complement fixation (CF) testing marks the presence of IgG antibodies. Canine anti-complement factors can interfere with CF testing. Persistence or reappearance of a positive precipitin test signaling IgM can indicate dissemination. CF titers may persist at low levels (1:4) during recuperation. Negative serological tests may occur in an infected individual with fulminating disease [105].

The high prevalence of *C. immitis* infection in endemic areas and the nonspecificity of clinical signs in canines creates the need for the consideration of coccidioidomycosis as a possible diagnosis in any canine exposed in endemic areas and showing signs of illness. The initial infection is believed to be respiratory, but the disease may disseminate to almost any site in the body, causing signs referable to the organ of involvement [106]. Adaptation of the enzyme immunoassay (EIA) for the detection of antibodies against *C. immitis* in canines has provided a sensitive and specific assay with decreased turnaround time, and a clinically valuable diagnostic aid for the practicing veterinarian. The EIA methodology also provides another tool to study the immunological response to *C. immitis* in the canine [104]. Because of occasional serological 'misses' involving detection of antibody, methods for the detection of antigen have been explored. Utilizing EIA technology, antigen was detected early in the course of coccidioidomycosis. Further refinement may bring this method into general clinical use for the early detection of coccidioidomycosis [107].

Therapy of coccidioidomycosis involves the use of amphotericin B, ketoconazole or itraconazole, either as sole agents, in combination or in succession [101]. Numerous clinical reports exist in the veterinary literature concerning canine coccidioidomycosis, with variable recommendations for length of therapy based on the assessment of a clinical cure [101]. Relapse occurs commonly [91]. Although texts continue to list nephorotoxic amphotericin as the treatment of choice, veterinarians practicing in the Southwest strongly favor ketoconazole. Therapy is often started upon receiving diagnostic evidence of infection, typically positive serology, even though titers may be low. One difficulty relates to drug procurement and cost. Ketoconazole is not approved for animal use and is employed as an 'extra label use' drug [91]. The need for new classes of antifungal drugs with new mechanisms of action is apparent, particularly against the increasing numbers of cases of life-threatening mycoses exemplified by a recent epidemic of coccidioidomycosis. A number of compounds are currently in development [108].

Early on, it was believed that the dust cloud resulting from an earthquake might be responsible for an apparent rise in cases of coccidioidomycosis. This would be consistent with reports of outbreaks described by Smith *et al.* [109] who discussed the co-occurrence of dust and coccidioidomycosis, and the reports by Flynn *et al.* [110] describing a coccidioidomycosis epidemic following a windstorm in 1977 [109–111]. Coccidioidomycosis has proven to be as common in animals as it is in humans. The disease is diagnosed most often in animals that receive man's closest attention as a result of their roles as pets, companions or food sources. While little is known of the vulnerability of free-living wild animals, those confined to zoos or other captive environments and domesticated exotic animals are recognized as having varying degrees of susceptibility once infected [91,112,113]. Recent studies in northeastern Brazil have found *C. immitis* infection in free-living armadillos (*Dasypus novemcinctus*) and infection of dogs and hunters of the armadillos [114,115]. These studies establish the presence of *C. immitis* in Brazil.

Contributors

The contributors to this symposium were: **J. Guillot**, Malassezia *infections in animals*; **J. M. Santurio**, *Pythiosis*; **H. E. Jensen**, *Protothecosis in animals*; **L. Ferreiro**, *Present concepts of coccidiodomycosis in animals*. The co-convenors were **A. C. Pier** and **F. C. Cabañes**.

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