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Malassezia dermatitis in dogs and cats

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ABSTRACT

Malassezia are members of the mycobiome of dogs and cats. In the presence of an underlying disease, these yeasts can proliferate, attach to the skin or mucosa to induce a secondary *Malassezia* dermatitis, otitis externa or paronychia. Since allergic dermatitis is one of the most common underlying causes, diagnostic investigation for allergy is often indicated. Cats may suffer from various other underlying problems, especially where *Malassezia* dermatitis is generalised. *Malassezia* dermatitis in dogs and cats is chronic, relapsing and pruritic. Direct cytology from dermatological lesions and the ear canal, showing "peanut-shaped" budding yeasts, facilitates a rapid and reliable diagnosis. Topical treatment includes antiseptic and antifungal azole-based products. Systemic treatment with oral antifungals is indicated only in severe or refractory disease. Identification and treatment of the underlying cause is essential for an optimal response. In this evidence-based narrative review, we discuss the clinical presentation of *Malassezia* dermatitis in dogs and cats, underlying comorbidities, and diagnostic considerations. Treatment is discussed in light of emerging evidence of antifungal resistance and the authors' clinical experience.

Introduction

Malassezia are lipophilic commensal yeasts of the skin and mucosa of cats and dogs (Kennis et al., 1996; Sierra et al., 2000; Bond et al., 2020; Bajwa, 2023). These fungi can be found in many vertebrates and in environmental niches, making them one of the most widespread and extremely adaptable fungal genera known (Breuer-Strosberg et al., 1990; Chermette, 1998; Nakagaki et al., 2000; Crespo et al., 2002b; Arenz et al., 2006; Tondello et al., 2012; Amend, 2014; Theelen et al., 2018; Amend et al., 2019; Pang et al., 2019; Hobi et al., 2022). Of the18 *Malassezia* species currently described, 11 have been detected in cats and nine in dogs by culture or using molecular methods (Hobi et al., 2022).

Malassezia commonly causes dermatitis, otitis externa, and occasionally other clinical presentations such as paronychia and keratitis in dogs and cats (Morris, 1999; Machado et al., 2011; Ledbetter and Starr, 2015; Bajwa, 2017; Guillot and Bond, 2020; Spatz and Richard, 2020; Hobi et al., 2022). Septic fungaemia, an increasing and potentially fatal complication in humans, has yet to be reported in animals (Hobi et al., 2022).

Malassezia can easily be identified on cytological preparations of skin or otic discharge as small "peanuts" or "foot-prints" shaped organisms with a diameter of 3–8 μm (Bond et al., 2020; Bajwa, 2023). Other diagnostic methods such as culture or DNA-based techniques, are typically reserved for research purposes (Bond et al., 2020). Although antifungal susceptibility testing is not widely performed in veterinary practice, antifungal resistance of *Malassezia* isolates has been reported and resistance mechanisms involving efflux pumps, fungal wall sterol metabolism, and biofilm formation are recognized (Uchida et al., 1994; Figueredo et al., 2013; Cafarchia et al., 2015; Bumroongthai et al., 2016; Iatta et al., 2017; Kim et al., 2018; Peano et al., 2020; Hobi et al., 2022).

Treatment for *Malassezia* dermatitis includes application of a topical antiseptic and/or antifungal preparation. Systemic azole antifungal treatment is reserved for severe disease or for cases not responding to topical treatment alone (Bond et al., 2020; Guillot and Bond, 2020; Hobi et al., 2022; Bajwa, 2023). Treatment of any underlying diseases is critical to prevent relapse (Bond et al., 2020; Hobi et al., 2022; Bajwa, 2023).

In this review, we discuss pathophysiological aspects of *Malassezia*-associated disease as well as the clinical presentation of *Malassezia*

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dermatitis in dogs and cats, comorbidities, and diagnostic as well as medical treatment considerations, in light of emerging evidence of resistance to antifungal drugs.

Pathophysiologic aspects of *Malassezia*-associated disease

Malassezia metabolism

Due to the lack of a fatty acid synthetase gene, all *Malassezia* species, including *M. pachydermatis*, are lipophilic and an exogenous lipid source is required for growth (Shifrine and Marr, 1963; Triana et al., 2015). Consequently, *Malassezia* can especially be found in lipid-rich areas of the skin (Park et al., 2021). *M. pachydermatis* is the only species that can grow on Sabouraud's dextrose agar due to its unique ability to use lipid fractions within the peptone (Puig et al., 2017).

The major lipid sources on the skin are triglycerides and free fatty acids produced by the sebaceous glands (Park et al., 2021). Cholesterol and cholesterol esters are produced during the natural cell turnover and degeneration of keratinocytes. These skin lipids positively influence yeast growth (Porro et al., 1977). Overall, lipid synthesis from *Malassezia*-colonized sites, such as skin, is enabled by production of lipase, phospholipase and sphingomyelinase (Triana et al., 2017; Bond et al., 2020).

Malassezia can also assimilate phospholipids and utilise organic and inorganic nitrogen (Riciputo et al., 1996; Boekhout et al., 2010). Nitrogen sources can lead to the formation of indole alkaloids, which serve as ligands for the aryl hydrocarbon receptor (AhR) in humans, influencing the function of various cells expressing this receptor, and thereby modulating different biological responses associated with inflammation, immune homeostasis, skin pathology, skin microflora and carcinogenesis (Magiatis et al., 2013; Mexia et al., 2015; Sparber and LeibundGut-Landmann, 2017). *In vitro* growth of *Malassezia* can be stimulated by addition of asparagine, pyridoxine or thiamine (Boekhout et al., 2010).

Malassezia cannot metabolize sugars but *M. pachydermatis* is able to assimilate certain carbohydrates such as mannitol, glycerol and sorbitol (Boekhout et al., 2010).

The adherence of Malassezia

The interaction of *Malassezia* with keratinocytes is the fundamental starting point for host invasion by the yeast (Boekhout et al., 2010). *In-vitro* adherence of *M. furfur* and *M. pachydermatis* to keratinocytes peaks at approximately 2 hours (Faergemann et al., 1983; Bond and Lloyd, 1996; Boekhout et al., 2010). Trypsin-sensitive cell-surface proteins/glycoproteins also play a role in canine keratinocyte adherence (Boekhout et al., 2010). In addition, an *in-vitro* study showed that *Malassezia* use glycosaminoglycans (GAGs) as surface receptors to bind to keratinocytes, but not to dermal fibroblasts (Ordiales et al., 2021).

How *Malassezia* interact with melanocytes is not completely understood, although their metabolites including azelaic acid, lipoperoxides and malassezin have been shown to play a significant role due to their cytotoxic and apoptotic potential (Boekhout et al., 2010). This could explain the de- to hypopigmented type of human pityriasis versicolor. However, the number of melanocytes does not seem to be affected in diseased skin, rather the melanin amount within the melanosomes and the distribution of melanosomes within the skin differ (Boekhout et al., 2010).

The immune response to Malassezia

Malassezia can proliferate within the stratum corneum, the outermost layer of the epidermis, leading to the accumulation of various metabolites and allergens (Marsella, 2013). Epidermal Langerhans cells and keratinocytes recognize *Malassezia* antigens leading to the formation of antimicrobial peptides and specific cytokines resulting in either a "neutral" commensal state (Il-10, TGF-β) or an inflammatory response (Il-1, Il-6, Il-8, TNFα) (Ruth Ashbee, 2006; Bond et al., 2020). Furthermore, epidermal Langerhans cells (and dermal dendritic cells if antigens penetrate through the epidermis into the dermis) migrate to regional lymph nodes, stimulating naïve T-helper cells to proliferate and differentiate into Th1 cells and/or Th2 cells depending on the cytokine profile (Il-12, Il-4, Il-13). Activated B-lymphocytes differentiate into plasma cells and produce different antibodies (IgG or IgE) depending on their co-stimulatory factors. Il-2 and IFNγ induce IgG, while Il-4 and Il-13 lead to an IgE antibody response (Bond et al., 2020). IgG may be protective, while IgE may lead to sensitization and mast cell activation (Ashbee and Evans, 2002; Boekhout et al., 2010; Bond et al., 2020).

Malassezia species

M. pachydermatis is the predominant species in the healthy skin of dogs and cats, although it is comparatively less frequently isolated from cats (Hajsig et al., 1990; Guillot et al., 1994; Bond et al., 2020). Other species reported in dogs include *M. sympodialis*, *M. furfur*, *M. obtusa*, *M. restricta*, *M. arunalokei*, *M. nana*, *M. slooffiae* and *M. globosa* (Crespo et al., 2002a; Nardoni et al., 2004; Cafarchia et al., 2005a; Cafarchia et al., 2005b; Eidi et al., 2011; Meason-Smith et al., 2020; Bajwa, 2023). In cats, *M. furfur*, *M. yamatoensis*, *M. japonica, M. dermatis*, *M. nana*, *M. obtusa*, *M. slooffiae*, *M. sympodialis* and *M. restricta* (ears) are reported (Nardoni et al., 2005; Åhman et al., 2007; Ahman and Bergstrom, 2009; Bellis et al., 2010; Shokri et al., 2010; Castellá et al., 2011). Overall, these other species tend to occur more often in cats than in dogs (Bond et al., 2020). The *Malassezia* species detected in healthy and diseased animals can be found in Table 1.

Clinical presentation of *Malassezia* dermatitis

Breed predisposition

Malassezia dermatitis is commonly seen in dogs, but is rare in cats (Morris, 1999; Ordeix et al., 2007; Negre et al., 2009; Machado et al., 2011; Bajwa, 2017). There is no clear gender or age predisposition (Bond et al., 2020; Hobi et al., 2022; Bajwa, 2023). In dogs, West Highland White Terriers, American Cocker Spaniels, Dachshunds, Boxer, Poodles, English Setters, Australian Silky Terriers, Shih Tzus and Basset Hounds are all breeds with an increased risk of *Malassezia* dermatitis (Bond et al., 2020; Guillot and Bond, 2020; Hobi et al., 2022; Bajwa, 2023). In cats, the Devon Rex and Sphynx have high burdens of colonizing *Malassezia*, predisposing them to oily seborrhoea and *Malassezia*-related skin diseases (Åhman et al., 2007; Ahman and Bergstrom, 2009).

Underlying diseases

Malassezia typically cause superficial dermatitis, otitis externa and/or paronychia (Bond et al., 2020; Guillot and Bond, 2020; Hobi et al., 2022; Bajwa, 2023). In dogs, this occurs secondary to ectoparasites, allergies (flea bite hypersensitivity, food-induced and environmentally induced atopic dermatitis), endocrinopathies (hyperadrenocorticism, hypothyroidism, diabetes mellitus), superficial pyoderma, keratinization disorders or rarely autoimmune diseases. All these diseases alter the environment of the yeasts in a favourable way (Bond et al., 2020; Guillot and Bond, 2020; Hobi et al., 2022; Bajwa, 2023).

In cats, allergic dermatitis, feline leukaemia virus (FeLV) infection, feline immunodeficiency virus (FIV) infection, thymoma-associated exfoliative dermatitis, superficial necrolytic dermatitis and paraneoplastic alopecia are reported triggers for generalised *Malassezia* dermatitis (Sierra et al., 2000; Crosaz et al., 2013; Guillot and Bond, 2020).

Other predisposing factors such as genetics, environmental humidity, anatomic confirmation (skin fold formation) and drug administration (e.g. immunomodulatory drugs) also contribute to the development of

Table 1
Malassezia species and their distribution pattern in dogs and cats.

Species	Host	Healthy Skin	Diseased Skin	Detected by
M. pachydermatis	Dog	Ears[a], skin, conjunctiva, anus, nose, oral cavity, vulva	MD, OE	C, P, NGS
	Cat	Ears[a], skin, mucosa, anus, claws	MD, OE	C, P, NGS
M. furfur	Dog	Skin	MD, OE	C, P, NGS
	Cat	Ears[a], skin	OE	C, P, NGS
M. sympodialis	Dog	Ears[a], skin, claws, faeces	MD	C, P, NGS
	Cat	Skin, anus	OE	C, P, NGS
M. globosa	Dog	Skin	MD, OE	C, P, NGS
	Cat	Skin	OE	C, P, NGS
M. nana	Dog	NR	MD, OE	P
	Cat	Ears[a], skin, claws, anus	OE	C, P, NGS
M. slooffiae	Dog	NR	MD	P, NGS
	Cat	Skin, claws, anus	OE	C, P, NGS
M. yamatoensis	Dog	NR	NR	NR
	Cat	Skin	NR	C, P, NGS
M. obtusa	Dog	Skin	OE	C, P, NGS
	Cat	Skin	OE	C, P, NGS
M. restricta	Dog	Skin, oral cavity	MD	C, P NGS
	Cat	Skin	OE	C, P, NGS
M. japonica	Dog	NR	NR	NR
	Cat	Skin	NR	C, P, NGS
M. dermatis	Dog	NR	NR	NR
	Cat	Skin	NR	C, P, NGS
M. arunalokei	Dog	Oral	NR	NGS
	Cat	NR	NR	NR

MD, Malassezia dermatitis; OE, Otitis externa; NR, not reported; C, culture-based; P, PCR (typically ITS, D1/D2); NGS, Next generation sequencing
[a] External ear canal and/or medial pinnae

Malassezia dermatitis in cats and dogs (Bond et al., 2020; Guillot and Bond, 2020; Hobi et al., 2022; Bajwa, 2023).

Affected body areas and clinical presentation

Malassezia dermatitis can be localized or generalized (Bond et al., 2020; Bajwa, 2023). Areas commonly involved in dogs include the pinnae, external ear canals, muzzle, ventral neck, ventral body sites, medial thighs and paws (Hobi et al., 2022; Bajwa, 2023). In most cats, the pinnae, face, chin, neck, limbs and ventral abdomen are most frequently involved, while in Sphynx and Devon Rex breeds, the ventral neck, axillae, inguinal areas and paws are predominantly affected (Hobi et al., 2022). In addition, claw folds (in cats) and skin folds (for example in brachycephalic breeds) may be affected (Hobi et al., 2022; O'Neill et al., 2022).

Pruritus is variable in both dogs and cats (Hobi et al., 2022; Bajwa, 2023). Dermatological signs include alopecia, erythema, scales, crusts, greasiness and in more chronic cases, hyperpigmentation and lichenification (Bond et al., 2020; Guillot and Bond, 2020; Hobi et al., 2022; Bajwa, 2023). Otitis externa and paronychia are often associated with a brown to black dry to oily rancid material (Bond et al., 2020; Guillot and Bond, 2020; Hobi et al., 2022; Bajwa, 2023) (Figs. 1, 2 and 3).

Interestingly, in dogs with pyoderma *Malassezia pachydermatis* and *Staphylococcus pseudintermedius* are often found together. This is thought to occur due to a symbiotic relationship between these organisms. *Staphylococci* produce beneficial growth factors and change the environment to favour yeast growth (Mauldin et al., 1997; Miller et al., 2012; Bajwa, 2017). In dogs with atopic dermatitis, *Malassezia* exacerbate clinical signs as an important flare factor of the disease (Bond et al., 2020; Hobi et al., 2022).

Diagnosis and *Malassezia* identification

Malassezia can be identified by several methods, including direct cytologic evaluation, culture and molecular-based methods (Miller et al., 2012; Bond et al., 2020; Bajwa, 2023).

Direct evaluation and interpretation

A useful, practical and rapid method to diagnose *Malassezia* dermatitis is direct microscopy of tape strip, impression smear, cotton swab or skin scrape samples (Miller et al., 2012; Bond et al., 2020; Bajwa, 2023). For better visualization of the fungal elements, potassium hydroxide or special dyes such as lactophenol blue, methylene blue, Gram stain or fluorescence dyes can be added to the clinical sample (Miller et al., 2012; Bond et al., 2020; Bajwa, 2023). *Malassezia* are recognized by their typical uni-polar and often broad-based budding referred to as "foot print", "babushka" or "peanut" shapes (Miller et al., 2012; Bond et al., 2020; Bajwa, 2023) (Fig. 4.).

Due to many influencing factors, there is no clear threshold for the number of *Malassezia* organisms deemed to be pathological on cytology. Some investigators suggest that greater than two organisms per higher power field (hpf) may be considered abnormal (Plant et al., 1992; Mauldin et al., 1997). An increased number of *Malassezia* in the absence of inflammatory cells is defined as "*Malassezia* overgrowth", whilst the presence of inflammatory cells indicates "*Malassezia* dermatitis". The authors suggest not to interpret the number of organisms detected by cytology alone, but to consider alongside with history and clinical presentation. For example, two *Malassezia* organisms per hpf found in a patient with chronic, dry dermatitis with abundant keratosebaceous debris and a rancid odour might be more relevant than a higher number of *Malassezia* organisms in a relatively normal looking area of skin. Any presence of clinical lesions or pruritus in association with even low number of *Malassezia* on cytology should be an indication for topical antiseptic therapy to evaluate, how much the yeast organisms contribute to the clinical signs present.

Isolation in culture

The contact plate technique, where an agar surface is pressed against the test surface, has been used in human and veterinary medicine; it is convenient, easy to perform, rapid and inexpensive (Åhman et al., 2007; Boekhout et al., 2010; Bond et al., 2020). *Malassezia* colonies can be cultured on different growth media. The commonly used modified Dixon's or Sabouraud's dextrose agar (SDA) can be analysed after 3–7 days of incubation at 32–34°C (Boekhout et al., 2010; Bond et al., 2020). The percentage of carbon dioxide can affect the isolation frequency and colony count when SDA is used. Modified Dixon's agar is the preferred medium for *Malassezia*, since lipid supplementation renders it suitable for lipophilic yeasts where species other than *M. pachydermatis* are involved (Bond et al., 2020). Other culture media such as Leeming-Notman, Ushijima or chromogenic *Candida* agar, all supplemented with lipid components, are also suitable (Ushijima et al., 1981; Leeming and Notman, 1987; Kaneko et al., 2007).

Other techniques for culture of *Malassezia*, include collecting skin scrapings, dry or moist swabs, and tape strips (Boekhout et al., 2010; Miller et al., 2012; Bond et al., 2020; Hobi et al., 2022). In one canine study comparing adhesive tape to dry swab sampling in dogs with chronic dermatitis, adhesive tape was the superior method (Omodo-Eluk

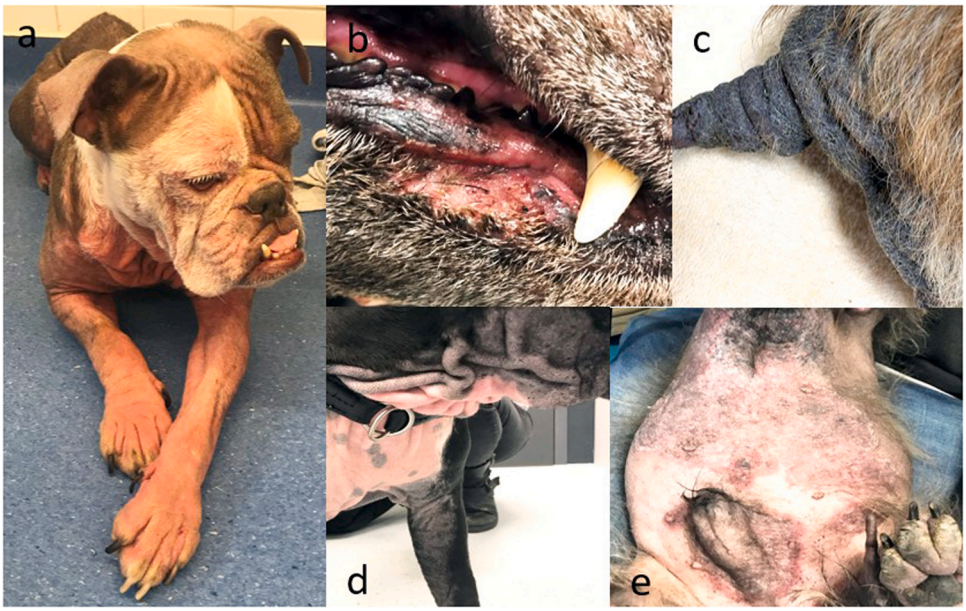

Fig. 1. Chronic *Malassezia* dermatitis in allergic dogs with severe pruritus showing different degrees of alopecia, erythema, lichenification, hyperpigmentation, crusting and accumulation of keratosebaceous debris on the convex pinnae, facial folds, muzzle, ventral chest, distal limbs of a Bulldog (a); the lip fold (b), the tail (c), ventral neck, chest and axilla (d) as well as the paw, ventral chest and abdomen of a Dachshund with Cushing's disease (e).

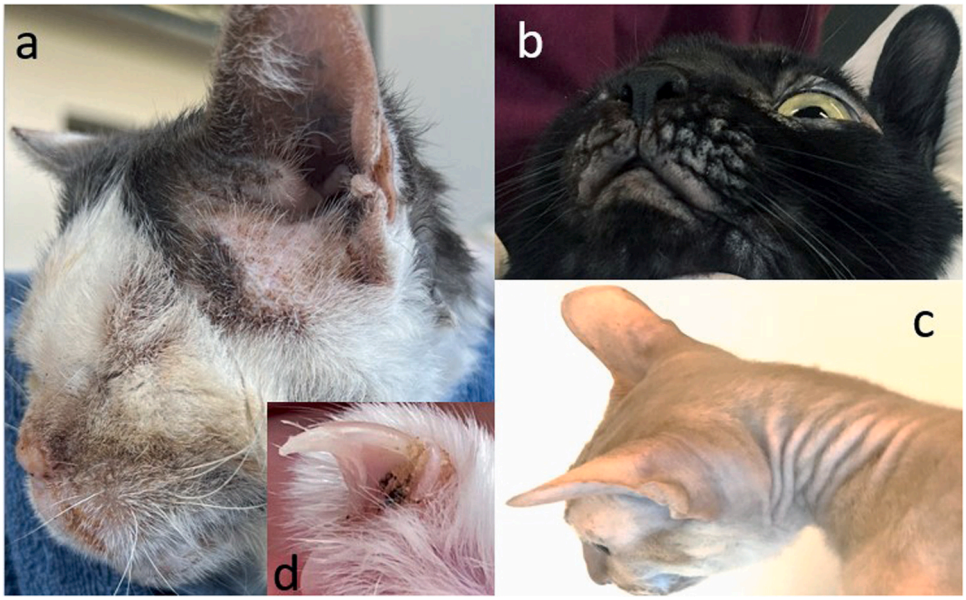

Fig. 2. Allergic cats with *Malassezia* dermatitis indicated by alopecia, mild erythema, crusting and accumulation of black to brown debris on the preorbital area and muzzle (a); alopecia, mild lichenification on the muzzle (b); alopecia, mild erythema and minimal debris on the convex pinnae, face and dorso-lateral neck (c); accumulation of black to brown debris within the nail fold (paronychia, d).

et al., 2003).

Skin biopsies and histopathology

Since *Malassezia* can be easily detected in cytological samples, skin biopsies are typically not performed to confirm a diagnosis of *Malassezia* dermatitis. In histological preparations of skin biopsies from affected patients, *Malassezia* may be absent due to skin preparation and/or sample processing or changes may be non-specific (Bond et al., 2020). Histological changes associated with *Malassezia* dermatitis include pronounced irregular epidermal and infundibular hyperplasia, prominent parakeratotic hyperkeratosis, diffuse intercellular oedema and lymphocytic exocytosis, eosinophilic pustulosis and superficial perivascular to interstitial lymphocyte-dominated dermatitis. If present, unipolar budding yeasts may be found in the surface or infundibular keratin (Mauldin et al., 1997; Gross et al., 2008; Bizikova et al., 2015) (Fig. 5).

Molecular-based identification methods

For the rapid detection of *Malassezia* and species determination, DNA sequence analysis of the ribosomal rRNA genes by PCR-based methods is typically performed (Boekhout et al., 2010; Cafarchia et al., 2011; Bond et al., 2020). These genes are highly conserved among different

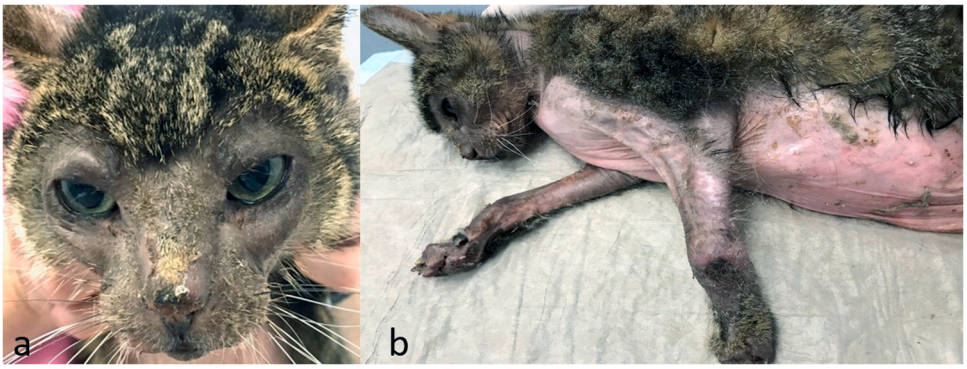

Fig. 3. Paraneoplastic alopecia in a cat with secondary, widespread *Malassezia* dermatitis leading to alopecia, mild crusting and hyperpigmentation on the face (a) as well as prominent alopecia, mild erythema, crusting and accumulation of brown debris on the ventral body and distal limbs; note the shiny/glancy skin characteristic for feline paraneoplastic alopecia (b).

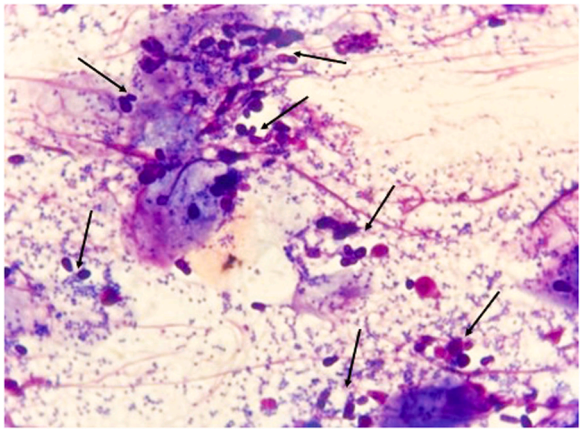

Fig. 4. Cytological impression showing nuclear streaming (thin, purple lines), multiple *Malassezia* yeasts (arrows) and a high number of coccoid bacteria (small, round, purple dots).

Malassezia species (Boekhout et al., 2010). Commonly analysed genes within the rRNA gene cluster include the domain 1 (D1) and 2 (D2) of the 26 S subunit, small subunit 18 S and the internal transcribed spacer (ITS1, ITS2) and 5.8 S regions. Other useful secondary target genes include chitin synthetase 2 (CHS2), RNA polymerase 1 (RPB1), RNA polymerase 2 (RPB2), β-tubulin, translation elongation factor 1 alpha (EF1-alpha), mitochondrial cytochrome B (CYTB) and minichromosome maintenance complex component 7 (MCM7) (Pryce et al., 2003; Boekhout et al., 2010; Cafarchia et al., 2011; Lorch et al., 2018; Theelen et al., 2018).

Treatment

General considerations for Malassezia dermatitis

The underlying primary cause should be identified and corrected, if possible. Depending on the severity and distribution of the skin lesions, topical treatment alone may be adequate, or a combination of topical and systemic treatment may be required (Bajwa, 2017; Bond et al., 2020; Guillot and Bond, 2020; Hobi et al., 2022; Bajwa, 2023).

Topical treatments

There is strong evidence from randomized control trials in dogs to suggest that topical application of a shampoo containing 2% chlorhexidine and 2% miconazole twice weekly is efficacious for treatment of *Malassezia* dermatitis (Negre et al., 2009; Bond et al., 2020). Since this

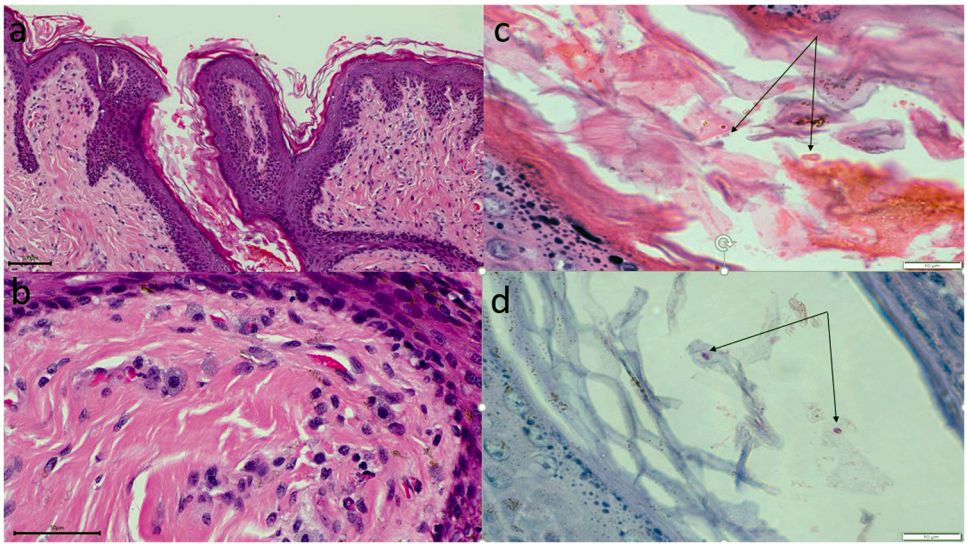

Fig. 5. *Malassezia* dermatitis in a dog demonstrating prominent scalloping of epidermis and follicles, and prominent spongiosis. H&E 100x (a); mild superficial dermal inflammation composed of mast cells, fewer lymphocytes and a few eosinophils. HE 400x (b); occasional yeast organism admixed with the keratin in the follicular lumen. HE 1000x (c), PAS 1000x (d).

shampoo is degreasing and can be irritating, rare side effects including erythema and pruritus may occur (Bond et al., 2020). Consequently, the authors suggest to use a commercially available small-animal hair-coat conditioner following the use of this shampoo treatment.

In a European randomized control study including 67 dogs with pedal or generalized *Malassezia* overgrowth, more than 50% efficacy was observed with a shampoo containing 3% chlorhexidine applied three times to once weekly and evaluated for up to 6 weeks, but adverse effects including erythema and pruritus were common (Maynard et al., 2011). This shampoo could be recommended as second choice for shampoo therapy. In a recent pilot study, the use of a 0.003% colloidal silver nanoparticle-based shampoo showed beneficial effects in dogs with *Malassezia* dermatitis, but prospective, double-blinded studies are needed to confirm this finding (Jones and Bloom, 2022).

Other topical formulations including a shampoo containing 3% chlorhexidine and 0.5% climbazole, 1% and 2% miconazole conditioners, 2% climbazole shampoo, 0.2% enilconazole lotion, essential oil mixtures, various products containing selenium sulphide, piroctone olamine, benzalkonium chloride and triclosan could be used when the recommended topical shampoos do not work, however there are no published prospective randomised clinical trials to demonstrate evidence of efficacy (Bond et al., 2020).

In cats, there are only anecdotal reports regarding the topical treatment of *Malassezia* dermatitis. Based on a consensus panel, the World Association of Veterinary Dermatologist's WAVD guidelines state that products containing chlorhexidine, miconazole or climbazole should be beneficial (Bond et al., 2020).

Alternative topical products

Plant-based products or peptides may be additional or prophylactic options. Antifungal peptides are positively charged protein fragments such as defensins, cathelicidins, lactoferrin or lysozyme, interfering with the permeability of the fungal cell membrane. Various plant components including flavonoids, phenolic compounds, terpenes, sulfur-bases and saponin show *in vitro* activity against different *Malassezia* species (Rhimi et al., 2021). Two *in-vivo* studies performed in Italy showed good improvement of canine *Malassezia* dermatitis and otitis externa using different topical plant/herbal formulations (Nardoni et al., 2014; Nardoni et al., 2017). This was reflected by the significant reduction of clinical signs, reduction of frequency of yeasts occurrence and/or reduction of *Malassezia* numbers. Some dogs with otitis externa developed local adverse effects (erythema, swelling) after contact to one of the mixtures.

Systemic antifungal therapy

Antifungal azoles are characterized by an azole ring with two (imidazoles) or three (triazoles) integrated nitrogen molecules. Azoles are fungistatic and inhibit the enzyme 14-alpha demethylase thus preventing ergosterol synthesis of the fungal wall. This leads to the disruption of the cell membrane, making the fungal cell more vulnerable. It also impairs many membrane-associated enzymes. These agents have an effect on the mammalian cytochrome P450 enzyme system thus interfering with the steroid biosynthesis of the patient (Adams, 2001; Foy and Trepanier, 2010; Miller et al., 2012). Overall, azoles are well tolerated, but interactions with other medications are common hepatotoxicity can be severe (Adams, 2001; Wiebe and Karriker, 2005).

Allylamines are a group of antifungals with a high selectivity for squalene epoxidase, an enzyme being responsible for the synthesis of lanosterol. This specific, reversible, and noncovalent inhibition affects both the fungal membrane structure and function. Allylamines show broad-spectrum activity and can be fungicidal as well as fungistatic. Terbinafine, an oral allylamine, is well tolerated overall (Adams, 2001; Foy and Trepanier, 2010; Miller et al., 2012). Its use for treatment of *Malassezia* dermatitis has been described in single reports and randomized, single blinded clinical trials but further studies are needed to inform treatment recommendations (Guillot et al., 2003; Bond et al., 2020).

Griseofulvin, an oral antifungal drug historically used for the treatment of dermatophytosis is not effective against *Malassezia* yeasts and should be avoided (Miller et al., 2012; Bajwa, 2017; Bond et al., 2020).

Allergen-specific immunotherapy

In a retrospective study from Sweden, the treatment success of 16 atopic dogs with *Malassezia* hypersensitivity, using at least 10 months of subcutaneous immunotherapy with *Malassezia* extract only, independent of the application frequency, was evaluated. A good response, leading to a reduction of pruritus and medication use, was seen in nine (56%) cases. No adverse effects were observed (Åberg et al., 2017). No similar studies have been performed in cats. Thus, there is insufficient evidence to recommend *Malassezia*-specific immunotherapy as routine treatment modality (Nuttall, 2012; Bond et al., 2020).

Treatment protocols

Topical antiseptic and/or antifungal mousses, wipes or sprays, containing 2–3% chlorhexidine and/or azoles, can be applied once to twice daily, while 2–3% chlorhexidine shampoos, with or without 2% miconazole, can be used twice weekly (Miller et al., 2012; Bajwa, 2017; Bond et al., 2020; Bajwa, 2023). Shampoo formulations containing > 2% chlorhexidine, with our without miconazole, should be followed by a conditioner, as they can be drying and irritating (Maynard et al., 2011; Kloos et al., 2013). Topical shampoos and conditioners after a contact time of at least five to ten minutes should be thoroughly rinsed off after use to prevent ingestion. This is particularly important in cats. In addition, body orifices should be avoided (Miller et al., 2012).

In selected cases, where topical treatment is ineffective, or where disease is severe (generalised, chronic or deep lesions), a systemic oral antifungal drug is added to the topical treatment regime (Miller et al., 2012; Bond et al., 2020; Hobi et al., 2022; Bajwa, 2023). Itraconazole is usually used for first line therapy in cats and dogs. Ketoconazole can be used as an alternative in dogs, but is not recommended for the use in cats due to toxicity. In many countries, ketoconazole has been withdrawn from the market due to its high adverse effect profile in humans (Bond et al., 2020). In a clinical trial including 25 dogs with *Malassezia* dermatitis, fluconazole was as effective as ketoconazole, but as cefalexin was part of the treatment regime, interpretation is difficult (Sickafoose et al., 2010). Since itraconazole achieves high, prolonged concentrations in the stratum corneum, oral pulse therapy with a dose of 5 mg/kg q24h in an alternate week protocol, is recommended for first line treatment (Miller et al., 2012; Bond et al., 2020). Ideally, serum liver biomarkers (alanine aminotransferase, aspartate aminotransferase, bilirubin) should be measured before, and intermittently during the treatment, as hepatotoxicity can occur (Wiebe and Karriker, 2005; Miller et al., 2012). Compounded formulations of itraconazole should be avoided, since their bioavailability is low resulting in poor absorption from the gastrointestinal tract and treatment failure (Bond et al., 2020; Bajwa, 2023).

At the same time, to correct any underlying cause of *Malassezia* dermatitis, further diagnostic investigations should be carried out, depending on the clinical presentation (Miller et al., 2012; Bajwa, 2017; Bond et al., 2020; Guillot and Bond, 2020; Hobi et al., 2022; Bajwa, 2023). Since allergy is a common trigger in both dogs and cats, effective flea/parasite prevention, a strict elimination diet over 4–8 weeks, and an allergen-specific immunotherapy in case of atopic disease, are indicated in many patients (Miller et al., 2012; Gedon and Mueller, 2018; Nuttall et al., 2019; Bond et al., 2020; Hobi et al., 2022; Bajwa, 2023).

Recheck examination to evaluate treatment success, is recommended 2–4 weeks after the initial topical and/or systemic treatment (Miller et al., 2012; Bajwa, 2023). A dermatological examination is combined

with a cytological evaluation to determine the remaining *Malassezia* burden (Miller et al., 2012; Bajwa, 2023). It is important to treat patients 7–10 days beyond clinical remission (Miller et al., 2012). Uncomplicated cases easily take 3–4 weeks until clinical remission.

Treatment failure and considerations

It is fundamental to take a detailed history of each patient, since treatment failure is common, and the reasons are multifactorial. The following situations are commonly encountered on recheck examinations after treatment has been initiated:

(1) The *Malassezia* dermatitis has completely resolved, but relapses occur several weeks or months later. In this scenario, it is likely, that the underlying trigger has not yet been identified or corrected and further diagnostic investigations are warranted (e.g. allergy work up, endocrinological testing, imaging, skin biopsies).
(2) The *Malassezia* dermatitis is unchanged compared to pretreatment. In this scenario, treatment might not have been adequate (e.g. only topical therapy alone, incorrect or insufficient treatment), wrong drug formulation (e.g. compound itraconazole), incorrect dosages were used (e.g. itraconazole) or the *Malassezia* isolate may be resistant to the antifungal drug).
(3) The *Malassezia* dermatitis has resolved, but relapses occur some days after clinical remission. In this scenario, most likely microbiological cure was not achieved despite remission of clinical signs and treatment duration was likely not long enough.

Antifungal susceptibility testing

Despite the regular use of oral antifungal medications in general practice, antifungal susceptibility testing of *Malassezia* isolates is rarely performed in veterinary practice and there is no standardization regarding the method, culture medium, inoculum size, incubation time and MIC endpoint criteria, making data interpretation and recommendation difficult (Boekhout et al., 2010; Bond et al., 2020; Hobi et al., 2022).

A research group from Italy assessed the antifungal susceptibility of *M. pachydermatis* in various media and concluded that culture in Sabouraud dextrose broth with 1% tween 80, a stock inoculum suspension of 1–5 $\times 10^6$ CFU/ml, and an incubation time of 48 hours is optimal (Cafarchia et al., 2012a; Cafarchia et al., 2012c). Another method using Christensen's broth with 0.1% Tween 80 and 0.5% Tween 40, an inoculum size of approximately 1–5 $\times 10^5$ yeast cells/ml and an incubation time of 48 hours can be considered as a suitable alternative (Rincón et al., 2006; Peano et al., 2012; Chiavassa et al., 2014).

International standardised guidelines for susceptibility testing of *Malassezia* are urgently needed so that antifungal susceptibility testing can be widely offered to veterinary practitioners. This would facilitate detection of *Malassezia* isolates with increased minimum inhibitory concentrations of antifungals and would inform the selection of appropriate antifungal drugs for therapy.

Antifungal susceptibility

M. pachydermatis, the main species causing *Malassezia* dermatitis and/or otitis externa in small animals, can show azole resistance as demonstrated in two studies using clinical isolates (Angileri et al., 2019; Kano et al., 2019). Reduced azole susceptibility in *Malassezia* may be caused by missense mutations, amino acid alterations, tandem quadruplication, increased expression of *ERG11*, efflux pump alterations caused by overexpression of genes encoding membrane transporters of the ABC transporter family (CDR1/CDR2) or the major facilitator (MDR1) family and also by biofilm formation (Bumroongthai et al., 2016; Kim et al., 2018; Angileri et al., 2019; Kano et al., 2019; Peano et al., 2020; Hobi et al., 2022). A study in atopic dogs from East Asia, using the E-test (Sabouraud dextrose agar with 0.5% Tween 40), found higher MIC values for ketoconazole and itraconazole, while in another study from Italy, implementing the CLSI reference broth microdilution, clinical isolates of diseased dogs showed intermediate to high MIC values of fluconazole, ketoconazole, itraconazole, voriconazole, miconazole and posaconazole, demonstrating that cross-resistance to multiple azole antifungals can occur (Cafarchia et al., 2012b; Watanabe et al., 2014). One report using the M27-A3 protocol indicated less susceptibility (high MICs) towards amphotericin B, clotrimazole, miconazole, fluconazole, and nystatin, associated with *M. pachydermatis* strains of diseased dogs (Weiler et al., 2013).

There are some reports in dogs, where treatment failure due to resistance was suspected clinically and later confirmed *in vitro* via antifungal susceptibility testing. Resistance was encountered to commonly used systemic and topical agents including itraconazole, ketoconazole and clotrimazole (several-fold increase of MIC) (Angileri et al., 2019; Kano et al., 2019; Kano et al., 2020).

Conclusions

Malassezia dermatitis is commonly encountered in dogs, while less frequent in cats. In both species there are breed predispositions. *M. pachydermatis* is most commonly isolated from diseased dogs and cats. Skin lesions include alopecia, erythema, crusts, scales, lichenification and accumulation of keratosebaceous debris, while the ears or nails often show a brown to black, malodorous exudate. Pruritus is variable. *Malassezia* dermatitis is usually triggered by a primary underlying disease. A definitive diagnosis is easily achieved via direct cytology and microscopy. There is a need for a standardized, commercially available culture and susceptibility methodology to inform treatment decisions in case of refractory disease. The focus in patients with *Malassezia* dermatitis should be on topical treatment and identification of the underlying illness. Systemic antifungal treatment should be reserved for severe or refractory disease only, due to the risk of azole resistance.

CRediT authorship contribution statement

Stefan Hobi: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pawel M. Bęczkowski:** Visualization, Validation. **Vanessa R. Barrs:** Validation, Supervision, Formal analysis, Conceptualization. **May Tse:** Visualization, Resources, Investigation. **Ralf Mueller:** Visualization, Validation, Formal analysis.

Declaration of Competing Interest

Ralf Mueller has been a consultant, lectured for or received support for research from the following companies manufacturing or selling antifungal medications: Ceva (topical chlorhexidine), Elanco (Malaseb shampoo, itraconazole), Selectavet (enilconazole) and Virbac (topical chlorhexidine). None of the other authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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