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Review Article

Canine malasseziosis: An overview

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Abstract

The present review describes the prevalence, etiology, pathogenesis, clinical manifestations, clinical pathology, diagnosis, treatment and zoonotic importance of Malassezia infections in dogs. *Malassezia* spp. are non-mycelial saprophytic lipophilic yeasts that belong to normal cutaneous or mucosal microbiota of many warm-blooded vertebrates. They may act as an opportunistic agent and plays a role in the development of Malassezia dermatitis and otitis externa in animals. Pathogenicity of Malassezia yeasts depends on the production of various virulence factors like proteases, lipases, phospholipases, lipoxygenases and other enzymes. Treatment of malasseziosis in dogs includes the use of topical therapy and systemic therapy. If predisposing factors cannot be controlled, recurrence is common, administration of routine topical therapy or pulse oral medications can be done. Polymerase Chain Reaction (PCR) is used as an effective diagnostic tool for the identification of *Malassezia* species. Sequencing is the most widely used method to understand the genetic diversity among *Malassezia* spp., including sequencing of the large subunit, internal transcribed spacer 1 region, the intergenic spacer 1 of ribosomal RNA and chitin synthase 2 gene.

Key words: Malasseziosis, Pathogenicity, Polymerase Chain Reaction, Sequencing

Highlights:

- Description of prevalence, etiology, pathogenesis, clinical manifestations, clinical pathology, diagnosis, treatment and zoonotic importance of Malassezia infections in dogs are described.
- Molecular techniques are considered an effective diagnostic tool for identifying many of the currently recognized *Malassezia* species.
- Identification and correction of predisposing factors are necessary to reduce the chance of recurrence of infection.

INTRODUCTION

Skin is the largest organ of the body, and it serves as a protective barrier between the animal's body and its environment. The skin of a dog represents 12% to 24% of its body weight (Moriello, 2016). *Malassezia* spp. are commensals of the normal cutaneous microflora of humans and animals. These yeasts may become opportunistic pathogens causing dermatitis (Figueredo *et al*., 2012). *Malassezia* spp. was commonly isolated from the face, neck, legs, ears and digits of the affected animals (Kumar *et al*., 2011). Dermatitis caused by *Malassezia pachydermatis* is one of the common skin mycosis in dogs and cats (Kano *et al*., 2020). Bardshiri *et al*. (2014) opined that the occurrence of *Malassezia pachydermatis* was more prevalent in adult dogs between one to eight years. The highest percentage of dogs with otitis associated with *Malassezia pachydermatis* was seen in the age group of one to three years (Girao *et al*., 2006). Dog breeds like West Highland White Terriers, English Setters, Shih Tzus, Basset Hounds, American Cocker Spaniels, Boxers, Dachshunds, Poodles and Australian Silky Terriers were at increased risk of Malassezia dermatitis (Guillot and Bond, 2020). Kashif *et al*. (2016) stated that Saint Bernard and Cocker Spaniel having pendulous ears were more prone to Malassezia otitis than breeds having an erect ear, such as German Shepherd

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and Russian breeds. Khosravi *et al*. (2016) reported a higher prevalence of *Malassezia* species in male dogs (57.6%) than in females (42.4%) with dermatitis. Nardoni *et al*. (2004) studied the occurrence of Malassezia yeasts in dogs of both sexes and observed a prevalence rate of 65.3% in males and 61% in females. Nichita and Marcu (2010) were of the view that malasseziosis occur most commonly as a secondary infection and tends to complicate other conditions like allergies, keratinization disorders, skin fold dermatitis, immuno-deficiency and previous antibiotic administration. Morris (1999) reported that allergies like atopy, food allergy, flea allergy and contact allergy, keratinization disorders like seborrhea, bacterial skin diseases, endocrinopathies like hyperadrenocorticism, hypothyroidism and diabetes mellitus, metabolic diseases (zinc-responsive dermatosis and superficial necrolytic dermatitis) and cutaneous or internal neoplasia could predispose dogs to Malassezia dermatitis and otitis externa. Sihelska *et al*. (2017) isolated Malassezia yeasts from 76 dogs out of 300 dogs presented with skin lesions, otitis externa or inflammation of the anal sacs. Among them, 79 isolates were identified as *Malassezia pachydermatis*, four isolates were identified as *Malassezia furfur*, and only one isolate was identified as *Malassezia nana*. The pathogenesis of the genus *Malassezia* depends on the production of enzymes (including lipases, phospholipases and hydrolases) that could contribute to cutaneous inflammation (Lautert *et al*., 2011). Petrokilidou *et al*. (2019) were of the view that the production of lipases by Malassezia yeasts plays a role in the invasion, colonization, persistence and proliferation within host tissues. Velegraki *et al*. (2015) reported that the zymogen proenzyme of the yeast cell wall might initiate damage to keratinocyte integrity through the activation of the complement system and thus lead to epidermal spongiosis, inflammation and pruritus. Cafarchia *et al.* (2007) suggested that the interactions between beta-endorphin and phospholipase activity in *Malassezia pachydermatis* isolates induced pathogenicity and produced skin lesions in dogs.

Clinically, Malassezia dermatitis in canines was characterized by erythematous lesions and greasy exudation in skin folds and toe webs. In chronic cases, the major clinical signs included traumatic alopecia, hyperpigmentation, lichenification and prominent malodour (Bond, 2010). Bond *et al*. (2020) stated that the clinical presentations in dogs with Malassezia dermatitis were pruritus, erythema, hyperpigmentation, lichenification, malodour, traumatic alopecia, and paronychia and otitis externa. Santoro *et al*. (2015) were of the view that the correlation of opportunistic bacteria such as *Staphylococcus pseudintermedius* and fungi such as *Malassezia* spp. in dogs may induce clinical signs of atopic dermatitis. Nardoni *et al*. (2008) opined that the major clinical features of canine Malassezia dermatitis include diffuse, regional or localized alopecia, hyperpigmen-tation, lichenification, erythema, erythematous macules, oily skin and hair, and excessive scaling. Mircean *et al*. (2010) reported skin lesions such as alopecia, hyperpigmentation, scales, follicular pustules, erosions, papules, vesicles, epidermal collarette, nodules, etc. in dogs with Malassezia dermatitis. Maynard *et al*. (2011) indicated that the frequent clinical signs in Malassezia dermatitis were pruritus, erythema, secondary hair loss, excoriations, greasy seborrhoea, scaling, rancid fat odour and hyperpigmentation through a randomized controlled trial on sixty seven dogs. Nayak (2018) reported that the average values of haemoglobin (Hb), packed cell volume (PCV), and total erythrocyte count (TEC) were significantly lower in fungal dermatological conditions in dogs. Dhillon *et al*. (2020) reported normocytic, normochromic anaemia in a hypothyroid dog with recurrent dermatological affections. Ramesh *et al*. (2018) found the presence of moderate leukocytosis, neutrophilia and eosinophilia in dogs with malasseziosis associated with hypothyroidism. Hoskins (2005) remarked that dogs with

superficial necrolytic dermatitis were prone to secondary Malassezia and staphylococcal infections. He also reported increase in serum liver enzymes like ALP and ALT. An increase in the levels of Alkaline Phosphatase (ALP) and Alanine Aminotransferase (ALT) was noticed in a dog diagnosed with recurrent pyoderma associated with Malassezia and hyperadrenocorticism (Reddy and Kumari, 2011). Brar *et al*. (2017) noticed a significant decrease in values of total serum protein in atopic dogs (5.8±0.14 g/dL) as compared to healthy dogs (6.7±0.11 g/dL). A mild decrease in values of albumin and globulin was also observed in these atopic dogs. A significant increase in total protein and globulin values and a reduction of serum albumin values and A:G ratio were observed in dogs affected with mycotic dermatitis (Joshi, 2019).

Impression smears from the wet lesions (interdigital spaces, skin folds) and tape strip impression smears from the dry lesions (alopecia associated with scaling, crusting or lichenification) suggestive of Malassezia dermatitis were used for cytological examination of diagnosis (Mircean *et al*., 2010). Reddy and Sivajyothi (2016) reported that methylene blue-stained tape impression smears from the dry lesions and glass slide impression smear from the wet lesions were used to detect the Malassezia organisms cytologically under the oil immersion objective. Omodo-Eluk *et al.* (2003) concluded that adhesive tape and dry swab sampling techniques in combination with the culture method could be used to detect *Malassezia* spp. from the skin of dogs. Bensignor *et al*. (2002) concluded that Malassezia yeasts were determined by cytology using the tape-strip technique more effectively. He also stated that on cytology, higher yeast counts were obtained on the pinna, followed by the umbilical area, axilla and perianal area. Scott *et al.* (1995) reported that a case could be considered positive for malasseziosis only if more than five organisms were detected microscopically in case of samples from skin and more than ten organisms in case of samples from the ear canal at 40X magnification. Pinchbeck *et al*. (2002) stated that cytologically, at least one body site having a mean of ≥1 yeast organism per oil immersion field (OIF) during microscopic examination of 10 consecutive OIF with corneocytes was considered positive for malasseziosis. Nascente *et al*. (2015) opined that a clinical case of canine malasseziosis could be considered positive by giving scores like '+' for up to five cells per oil immersion field, '++' for six to ten cells per oil immersion field and '+++' for more than ten cells per oil immersion field. Satishkumar *et al.* (2008) reported that *Malassezia pachydermatis* was isolated from clinically affected dogs with seborrheic dermatitis and/or otitis externa on Sabouraud dextrose agar (SDA) with chloramphenicol at 37°C within 3-5 days. He also reported that colonies of *Malassezia pachydermatis* in Sabouraud dextrose agar were raised, doomed or high, convex and smooth with cream colour initially that later became dry, wrinkled and buff to orange beige. Saranya (2011) concluded that Sabouraud's dextrose agar with olive oil added as lipid supplement could be used as a standard culture medium for the identification of Malassezia yeasts. Karlapudi (2017) isolated *Malassezia pachydermatis* from lesions suggestive of otitis in dogs on Sabouraud dextrose agar with 0.05 per cent of chloramphenicol at 37°C for 48 hours and observed that colonies were first white or creamy, turning to dark tan to brown colour within five to seven days. Rathnapriya *et al*. (2016) suggested using Modified Dixon's agar as an ideal culture medium for the isolation of Malassezia yeasts. She also stated a higher isolation rate could be achieved on Modified Dixon's agar compared to Sabouraud dextrose agar with olive oil overlay. Marin *et al.* (2018) also isolated *Malassezia pachydermatis* on Modified Dixon's agar (MDA) at 37°C for five days. Polymerase chain reaction (PCR) is an effective diagnostic tool for the identification of *Malassezia* species. Genetic typing of *Malassezia* species isolated from a dog with or without otitis and seborrhoeic dermatitis was

done by applying the Random Amplified Polymorphic DNA (RAPD) PCR technique (Eidi *et al*., 2011). Harju *et al*. (2004) suggested an inexpensive method for isolating yeast genomic DNA from lipid cultures or directly from colonies. In this, cell lysis was followed by chloroform extraction and precipitation of DNA with ethanol. Machado *et al*. (2011) suggested a method for yeast cell wall lysis with lyticase (200 IU) at 30°C for 30 min in dogs with Malassezia dermatitis. Rodriguez and Vaneechoutte (2019) also reported the most efficient yeast cell lysis with the use of lyticase lysis buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA, 1.2 M sorbitol and 10 mM β-mercaptoethanol) containing 2 U of lyticase/ µL. According to Zia *et al*. (2015), a PCR-RFLP (Restriction Fragment Length Polymorphism) method targeted toward 26S rDNA and with a restriction enzyme, *Hin*6I (*Cfo1*) enzyme was used to identify various *Malassezia* species. Ilahi *et al*. (2018) opined that matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry could be used as a new laboratory diagnostic tool for the rapid and accurate identification of *Malassezia* species. Few phenotypic tests are available to differentiate *Malassezia* species, and some of them may overlap. So, DNA sequencing or other techniques like mass spectrometry may be used for specific identification. Molecular techniques are significant in the accurate identification of many of the currently recognized *Malassezia* species. Particularly, sequencing of D1/D2 domain of the large subunit of the rRNA gene, ITS, IGS, CHS2 and ß-tubulin genes or regions aids in the accurate identification of species and recognition of genotypes that may have relevance for host-adaptation and virulence. Multiplex PCR can be used for rapid and specific identification of *Malassezia* spp. from skin specimens (Bond *et al.,* 2020).

Treatment of malasseziosis in dogs includes the use of topical therapy and systemic therapy. For localized lesions, topical antifungal agents are usually used and are more effective in short-coated animals. Topical prevention of Malassezia dermatitis in dogs can be achieved effectively using 2% chlorhexidine/ 2% miconazole or 3% chlorhexidine shampoo twice weekly, which may be considered the topical treatment of the first choice. In chronic or generalized cases, systemic therapy with azole antifungals can be done. Commonly used azole derivatives in the treatment of malasseziosis include triazoles (like itraconazole, fluconazole, voriconazole, posaconazole and ravuconazole) and imidazoles (miconazole and ketoconazole). Azoles impair ergosterol synthesis in the fungal cell membrane by inhibiting cytochrome P450 sterol C14 alpha-demethylation (Koch *et al*., 2012). Cafarchia *et al.* (2012) documented the highest *in vitro* antifungal susceptibility of *Malassezia pachydermatis* to itraconazole, followed by ketoconazole and posaconazole. Pinchbeck *et al*. (2002) suggested that both once-daily administration and pulse therapy of itraconazole were efficient in the treatment of *Malassezia pachydermatis* dermatitis and otitis in dogs at 5 mg/kg body weight. Identification and correction of underlying predisposing factors are necessary to reduce the chance of recurrence of infection. If predisposing factors cannot be controlled, administration of routine topical therapy or pulse oral medications can be done (Scott *et al*., 2001). Traditional ayurvedic medicine uses various plants to cure skin diseases and infections. Neem compounds appear to have antibacterial, antifungal, anti-inflammatory and immunomodulatory effects. Bioactive compounds from neem like nimbidin, gedunin, and sulphur-containing compounds such as cyclic trisulphide and tetrasulphide have antifungal activity. Mainly, the oil of seed kernels and neem leaf extracts possess antifungal action (Brahmachari, 2004). Salazar *et al.* (2015) reported that terpenoids in neem (*Azadirachta indica)* extracts (both the neem leaves and seed oil extracts) had either fungistatic or fungicidal power on several pathogenic fungi.

From a zoonotic perspective, Morris *et al*. (2005) suggested that *Malassezia*

pachydermatis could be classified as an emerging zoonotic pathogen, especially in immune-compromised individuals. Fan *et al.* (2006) reported a clinical case of *Malassezia pachydermatis*-induced skin infection in an immunocompetent dog owner. Marin *et al.* (2018) reported the ability of *Malassezia pachydermatis* to adapt to new hosts and its potential for zoonotic transmission. Chang *et al.* (1998) remarked that *Malassezia pachydermatis*is a zoonotic pathogen in low birth weight ill infants.

Conclusion

Malassezia yeasts are unique with their strict dependence on lipids, cellular ultrastructure and dominance as eukaryotic inhabitant on the skin of warm-blooded vertebrates. Malassezia populations impart a reservoir of yeasts that might proliferate and induce an inflammatory response under the influence of various host predisposing factors. Molecular techniques are considered as the accurate identification for many of the currently recognized *Malassezia* species. Regular treatment is often required to minimize relapses if predisposing factors are not controlled. Topical treatments are preferred over systemic treatments for long-term therapy because of a lower risk of toxicity. To reduce the risk of zoonosis, good hand hygiene by individuals in contact with pet dogs and cats should be highlighted.

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Author's contribution: AKD: Acquisition of data, analysis and designing of the manuscript.

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