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# CHARACTERISTICS OF THE CAUSE, EPISOTOTOLOGICAL FEATURES, CLINICAL SIGNS, DIAGNOSIS AND THERAPY OF MALASSESIOSIS IN DOGS (REVIEW ARTICLE)

**Chupryna M.I.*** (0000-0001-8805-3737), **Ivanchenko I.M**. (OCRID ID 0000-0001-7465-4822), **Severyn R.V.** (OCRID ID 0000-0003-4990-2489), **Basko S.O.** (OCRID ID 0000-0001-8314-2490), **Dadyshko A.O.** (OCRID ID 0009-0003-7727-216X).
State Biotechnological University, Kharkiv (Ukraine);
e-mail: nickchuprina@gmail.com*

***Abstract.*** *The article describes the results of the analysis of scientific literary sources regarding the epizootic situation of Malassezia in dogs. Actual information about the characteristics, ways of spreading and pathogenic agents are given. Special attention is devoted to clinical signs and courses. Taking into account the world experience, the main therapy methods of this disease and its prevention are substantiated and the necessity of conducting regular dispensation of dogs in order to detect the disease in its initial stages is argued.*

**Key words: malassezia dermatitis, animals, *Malassezia pachydermatis*, *Malassezia* spp., cats, dogs, malassezia otitis.**

**Introduction.** *Malasesiosis* is a fungal disease that runs subacute or chronic, characterized by itching, hyperpigmentation and disruption in the structure of the affected skin areas.

Diseases are caused by lipophilic yeast of the genus *Malassezia*, which belong to conditionally pathogenic microorganisms of the skin and are its commensals. The transition from commensalism to a pathogenic lifestyle is a fairly typical phenomenon for the microflora of outer skins of dogs and to a lesser extent – cats, so cases of *Malassezia otitis* and *Malassezia dermatitis* are not uncommon in the pet veterinary clinic (Moraru et al., 2019). *Malassezia dermatitis* (Malesesiosis) is a common secondary pathology accompanying the underlying disease (Schlemmer et al., 2018). If the development of the disease is not stopped at the initial stages, Malasesiosis will take a generalized form and will lead to destructive changes in the skin, which in turn will lead to a decrease in the body's resistance and can cause serious concomitant diseases (Guillot et al., 2020).

**The purpose of the work** was to analyze and systematize the data of the scientific literature on the course and therapy of Malasesiosis in dogs. Based on the data obtained, the main characteristics and etiological aspects of the disease were provided, epizootological features, course and clinical signs of the dog disease were determined and modern methods of diagnosis and therapy were described.

**Materials and Methods.** Studies were carried out by thorough consideration and analysis of modern scientific literary sources on dog malasesiosis.

**Results.** Malassezia yeast, which at one time was considered one species, but according to modern nomenclature, is attributed to a unique cluster consisting of 18 species, living mainly on the skin and mucous membranes of warm-blooded vertebrates (Lorch et al., 2018; Theelen et al., 2018). Over the last decade, based on analysis of the yeast genome of Malassezia, it has been assumed that their ancestors were symbionts of plants or soil, which

gradually adapted to development in the skin ecosystem (Xu et al., 2007). The available quantity of *Malassezia* species (n = 18) is probably limited by the predominance of interest in parasitocenoses in humans and domestic animals. Probably, the number of autonomous *Malassezia* species will increase significantly when researching the skin microbiota of a wider range of wildlife species. So, recently a new independent *Malassezia* species was described from the skin of nine species of bats from the subfamily Myotinae in the eastern and western regions of the United States (Lorch et al., 2018).

**Characteristics of the cause/pathogen.** *Malassezia* yeasts have the appearance of small ovoid, ellipsoidal or cylindrical cells (1.5-6.0 μm x 3.5-8.0 μm). Reproduction is by budding and monopolar blastic development. They have a thick cell wall (0.12μm), whose innermost layer shows a characteristic serrated structure.

The first remarkable feature of *Malassezia* genomes is their small size (~10 Mb), which is probably due to the limited ecological niche of the yeasts. Gene family analyses point to the uniqueness of some characteristics of Malassezia yeasts, in particular their low carbohydrate-degrading capacity due to the reduction of genes, that encode glycosyl hydrolase enzyme; a lipid dependence for growth due to the lack of a fatty acid synthase gene and a contaminant expansion in the number of lipid hydrolysing enzymes (such as secreted lipases, phospholipases and acid sphingomyelinases). They allow Malassezia yeasts to collect and use fatty acids from the skin or mucosal surfaces of their hosts. Structural analysis of the genome also revealed the presence of unique genes with unknown functions, which were probably acquired through horizontal transfer.

The sexual form of *Malassezia* yeasts is still unknown.

The species identification of the *Malassezia* species depends on the lipid composition of the cell and is associated with the inability to synthesize long-chained (C14 or C16) fatty acids de novo. There are some interspecies differences in the lipid metabolism of yeasts that are used to identify them in specific tests. Historically, *M. pachydermatis* was regarded as "lipophilic, but not lipid dependent," because it was the only representative of the genus to grow on Sabouraud`s dextrose agar. Currently, genome sequencing has confirmed that *M. pachydermatis* does not have a fatty acid synthase gene like other members of the genus, but is uniquely able to utilise lipid fractions within the peptone component of Sabouraud`s dextrose agar for growth. Its inability to grow in lipid-free environments is now becoming clear, but *M. pachydermatis* should also be regarded as "lipid-dependent."

Therefore, specific identification of *Malassezia* yeasts should be confirmed by DNA sequencing analysis. The most frequently used loci are the D1/D2 domain of the 26S rRNA gene and the internal transcribed spacer (ITS).

**Epizootological information.** *Malassezia* yeasts belong to the commensal flora of the skin. The skin of puppies is infected from birth, probably due to close contact with their mothers. The primary infection was studied on dogs of the Rottweiler breed: whereas yeast was not detected in the mammary gland or mother`s labia, instead they were found in almost 40% of puppies on the third day of life from areas around the lips. There is a possibility that the infection of the offspring could occur during childbirth when passing through the female's birth canal or from the skin around the anus. In healthy adult dogs, *Malassezia* yeast is usually localized in the interdigital intervals on the skin around the oral cavity or in the armpits. They mainly accumulate in the ear canal, around the anus and in the rectum, as well as in the mouth, sometimes in the vagina and nasal cavity (Bond, 2009).

Early research (Chang et al., 1998) established the zooanthroponotic potential of *Malassezia* yeast in the case of infection of low-weight newborn babies in the intensive care unit. The author made an assumption about the possible infection of the *M. pachydermatis* with lipid emulsions through the hands of medical personnel who had domestic dogs. After primary contamination, *Malassezia* yeast can be stored on incubator surfaces for preterm infants for an extended period of time (Van Belkum et al., 1994).

Also, the case of granuloma on the face caused by *M. pachydermatis* in a dog owner (Fan et al., 2006). In newer research, we find data on the association with *M. pachydermatis* fungemia in a small number of adults with different predisposition potentials (Choudhury and Marte, 2014; Roman et al., 2016; Lee et al., 2019). Because hand infection *M. pachydermatis* is commonplace among dog owners, especially animals prone to allergic conditions and, as a result, with excessive accumulation of *Malassezia* on the body integuments (Morris, 2005), there is a clear need for strict hand hygiene for persons, which contact with domestic dogs and cats, and especially if such animal owners are also prone to allergic conditions or have signs of weakened immunity (Bond et al., 2020).

**Pathogenesis**. Recently, significant advances have been made in understanding the mechanisms of interaction between *Malassezia* yeast and the dog organism (Bond et al., 2020). The growth rate of *Malassezia* colonies on the skin (whether commensal existence or inflammation and disease) depends on both the metabolic activity of the yeast and the innate and adaptive immune defense responses of the host. The yeast synergism with other skin commensals (especially *Staphylococcus*) may also play a role in the effective colonization of animal body integuments, although this fact has not been conclusively proven (Ianiri et al., 2018). In a healthy, with normal resistance of the body, these processes should lead to a balanced homeostatic relationship. The presence of *Malassezia* yeast in the stratum corneum of the skin may increase the negative effects of certain chemicals, immunogens and allergens (carbohydrates, proteins and lipids associated with the cell wall of the fungus); enzymes secreted by yeast itself and used by them in the process of nutrition, on the other hand, act as irritating metabolic by-products of metabolism (Ashbee and Bond, 2010; Sparber and Leibundgut-Landmann, 2017).

It is known, that the presence of *Malassezia* yeasts on the skin, both in normal physiological and excessive numbers, activates the skin immune system in dogs. Malassezia antigens can stimulate innate immune responses, mediated by antibodies and cells, as well as trigger hypersensitivity reactions (Bond et al., 2010). In animals in which an overgrowth of microorganisms has occurred, or in individuals that are predisposed to allergic sensitization, the ensuing inflammatory response can lead to clinical signs such as dermatitis and pruritus. Elevated IgE levels to *Malassezia* yeasts or *Staphylococcus* bacteria in human atopic dermatitis are related to «the skin severity index». To assess whether a similar association occurs in dogs, it was comparable levels of allergen-specific IgE, $IgG_1$, and $IgG_2$ directed against *M. pachydermatis* and *S. pseudintermedius*, with total IgG levels, and correlated them with lesion severity in dogs with atopic dermatitis (Khantavee et al., 2019). It was reported that specific IgE and total IgG against yeasts and bacteria were significantly increased in atopic dogs of all ages. However, no significant relationships were found between the clinical score and any specific immunoglobulin levels for both microbe types.

**Clinical signs**. The skin affected by yeast flora is usually erythematous, often with greasy brown-black allocations covering the lower portion of hairs; intertriginous zones are frequently involved (Bond et al., 2010). Pruritus signs, whilst ranging from minimal to severe, are normally a diagnostic feature. At the same time, hyperpigmentation, lichenification, malodour, traumatic alopecia, and otitis externa is common. In otitis externa, the discharge from the ear canal is ceruminous and rarely purulent, and inflammation commonly extends onto the pinnae. Cases caused by *Malassezia paronychia* are manifested by claw fold erythema and swelling, waxy or crusty brown exudate and red-brown claw staining, and may co-exist with a wider pododermatitis of haired skin. An occasional presentation of frenzied pruritus in dog`s faces with varying, sometimes subtle, erythema of chin / perioral skin, may be misdiagnosed as a neurological disease (Mason, 1992, 1993).

The signs of dermatitis, caused by *Malassezia*, may imitate, or complicate, signs of atopic dermatitis in dogs. Features of concurrent diseases may be evident initially, although they are commonly best to evaluate them after secondary *Malassezia* infection is overcome.

**Diagnosis.** Adhesive tape method has gained wide acceptance in veterinary clinical practice as a rapid and versatile method for discharging stratum corneum cells and their attendant adherent microbes (Maynard et al., 2011). Light microscopical research of tape-strips with biomaterial or dry scrapings stained with modified Wright-Giemsa stain (Diff-Quik or equivalents) is rapid and convenient for assessment the presence and numbers of *Malassezia* yeasts (Moraru et al., 2019). A recently published clinical consensus document presents a detailed diagnostic algorithm for use in the veterinary clinic (Bond et al., 2020). It is impossible to overestimate the importance of such research in the presence of concomitant skin diseases or other negative factors to prevent chronic or recurrent courses.

Cytological examination using swabs from the lesion areas and their reflection on the slide will be advisable mainly when the ear canal is infected. As for the examination of biomaterials from lesion skin areas, in this case, the advantages have the method with the use of tape strips, as the yield of squames and yeast when taking swabs from the skin is inferior to that obtained by tape strips or dry scraps (Bond and Sant, 1993; White et al., 1998; Bensignor and Carlotti, 1999). During the investigation of 30 dogs with otitis externa, cytological specimens obtained using a conventional cotton-tipped swab contained certain numbers of yeasts and bacteria, but fewer inflammatory cells, when compared with samples received by aspiration of material from the horizontal area (Choi et al., 2018). In an effort to increase the sensitivity of taking a cytological sample for the presence of *M. pachydermatis* in the canine ear, a quantitative PCR method was developed, that based on the amplification of the single copy ß-tubulin gene (Puig et al., 2019). The authors consider that the results were accurate and showed the best sensitivity in comparison to cytology. Thus, this method may be useful for diagnosis and therapeutic monitoring, and in studies of pathogenesis and therapeutic product development.

**Therapy.** Most wild-type *Malassezia* yeasts remain susceptible to the commonly-used azole drugs such as itraconazole, ketoconazole and miconazole, although the efficacy of fluconazole may be variable (Velegraki et al., 2004; Cafarchia et al., 2012a,b; Weiler et al., 2013). In *M. pachydermatis* isolates from canine otitis externa, synergistic interactions have been reported between caspofungin and itraconazole or fluconazole (Schlemmer et al., 2019a), whereas amphoterecin B antagonized the activity of itraconazole, but not fluconazole or posaconazole (Alvarez-Perez et al., 2019).

Uncertainty about the susceptibility or resistance of flora yeast to azoles prompted the search for alternative antifungal agents. Authors report of in vitro efficacy against M. pachydermatis of a honey-based gel (Oliveira et al., 2018), monensin and, to a lesser extent, narasin (Chan et al., 2018, 2019). Also, they have explored the potential antifungal activity of essential oils, complex mixtures of highly concentrated aromatic oils (primarily terpenes and/or phenylpropanoids) extracted from plants by steam distillation, hydrodiffusion or high pressure (Manion and Widder, 2017; Bismark et al., 2019). A previous randomized clinical trial reported persistent efficacy of a commercial product from essential oil (Malacalm, Flora Slr Oli essenziali, Lorenzana, Italy), the authors developed and worked out regimens for the drugs usage (twice a day for 1 month) in dogs with *Malassezia* dermatitis.

Using a laboratory method – diffusion reactions in the agar disc, the authors have observed in vitro using winter savory oils, lemon grass, oregano, palmarosa and cinnamon leaf (Bismark et al., 2019). Oregano oil and thyme oil due to their major phenolic components (carvacrol, thymol) had fungicidal effect against *M. pachydermatis* when tested using agar dilution (Sim et al., 2019). There are reports of synergistic interactions between essential oil components and azoles or nystatin against *M. pachydermatis*; carvacrol and miconazole or nystatin, thymol and nystatin (Schlemmer et al., 2019b), and also clotrimazole and essential oils of *Melaleuca alternifolia*, *Mentha piperita*, and *Origanum vulgare* (Bohmova et al., 2019).

Most of the recent studies have been conducted in vitro and the results of clinical trials of drugs and their combinations remain unverified. Determining the comparative effectiveness of drugs is complicated by the lack of agreed standards and testing methods, arbitrary

interpretation criteria and the variability of methods for making essential oils (Bismark et al., 2019).

In our opinion, quite effective was the method of treatment of canine *Malasesia* dermatitis using shampoo containing 2% miconazole and 2% chlorhexidine twice a week (Bond et al., 1995, 2020; Maynard et al., 2011). Also, 3% chlorhexidine shampoo was effective (Maynard et al., 2011; Bond et al., 2020). For dogs, when local therapy is ineffective or too time-consuming, there was appropriate to use ketoconazole in a dose of 5–10 mg/kg orally once or twice daily; and itraconazole in a dose of 5 mg/kg orally once daily or two consecutive days per week (reviewed by Bond et al., 2020).

The presence in clinical practice in dogs of chronic/persistent or recurrent *Malassezia* dermatitis is usually associated with incomplete or uncertain diagnosis, with the inability to eliminate all factors that cause or contribute to the disease. Moreover, the reduced susceptibility of *M. pachydermatis* to commonly used antifungal drugs may develop under both in the «field» and laboratory conditions.

**Prevention**. The basis for the prevention of malasesiosis in dogs is proper care for animals: a full and balanced diet, motion, regular hygiene procedures (grooming and care of the pinnae) – factors that contribute to the overall resistance of the body as a whole, and those that increase skin resistance, prevent chronic contamination of the skin and hair, including increased microbial contamination.

**Conclusions.** Based on the analysis of modern scientific sources on the spread of conditionally pathogenic microflora of the skin of domestic animals, in particular *Malassezia* fungi yeast, and their specific gravity among other pathogens, it was concluded that their significant importance in dermal pathology, which is now growing against the background of deterioration of conditions of keeping dogs, reduction of their resistance, etc. Often the situation is complicated by the presence of the underlying disease of any genesis because yeast fungi are commensal and belong to the resident microflora, so the occurrence of a pathological condition due to them can be randomized and unpredictable. Also, yeast flora has significant zooanthroponotic importance, especially for dog owners who have a predisposition to immune disorders and do not comply with proper veterinary and sanitary standards for keeping companions. Therapy of yeast skin diseases requires perseverance, multi-vector, the usage of complex schemes and new, often alternative, non-traditional, non-widespread drugs and remedies. Methods of diagnosis and detection of *Malassezia* pachydermatis in inflammatory exudates or other biomaterials currently do not have clearly defined criteria and standards, as well as grounds for a final diagnosis. All this prompts us to conduct scientifically substantiated clinical studies both on methods of diagnosis and therapy of various forms of dermal pathologies in domestic animals caused by yeast resident microflora.

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## ХАРАКТЕРИСТИКА ЗБУДНИКА, ЕПІЗООТОЛОГІЧНІ ОСОБЛИВОСТІ, КЛІНІЧНІ ОЗНАКИ, ДІАГНОСТИКА ТА ТЕРАПІЯ МАЛАСЕЗІОЗУ У СОБАК (ОГЛЯДОВА СТАТТЯ)

**Чуприна М.І.** * (0000-0001-8805-3737), **Іванченко І.М.** (OCRID ID 0000-0001-7465-4822), **Северин Р.В.** ( OCRID ID 0000-0003-4990-2489), **Баско С.О.** (OCRID ID 0000-0001-8314-2490), **Додушко А.О.** (OCRID ID 0009-0003-7727-216X),

Державний біотехнологічний університет, м Харків, Україна;

e-mail: nickchuprina@gmail.com*

***Резюме.*** *У статті описано результати аналізу наукових літературних джерел щодо епізоотичної ситуації з маласезіозу у собак. Наведені актуальні відомості про характеристику, шляхи розповсюдження і патогенез збудника. Особливу увагу присвячено клінічним ознакам і перебігу. Із урахуванням світового досвіду обґрунтовано основні методи терапії даного захворюванням і його профілактика, аргументовано необхідність проведення регулярної диспансеризації собак з метою виявлення захворювання на початкових його стадіях.*

**Ключові слова: маласезійний дерматит, тварини, *Malassezia pachydermatis*, *Malassezia* spp., коти, собаки, маласезійний отит.**

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