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CANINE MALASSEZIA DERMATITIS TREATMENT PLAN IMPROVING

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Abstract. *The paper presents the results of canine Malassezia dermatitis treatment with propolis tincture. To confirm the CMD diagnosis test material was taken with a glue tape from the affected skin of dogs with a generalized form of CMD. The affected ear canals were sampled with sterile cotton swabs. The affected skin was washed off and immediately screened into culture media. In the department's laboratory smears were prepared and stained according to Giemsa, Gram, and alkaline methylene blue techniques. The test material was screened on Sabouraud agar supplemented with polymyxin and lactose, modified Dixon media. Cultures were incubated in a thermostat at 37°C for 24 to 72 hours. The growth pattern of the selected Malassezia cultures was observed. We checked the immunodeficiency state in dogs with a discrete method of immunoglobulins detection in the blood serum using PEG-6000. To determine the level of immunoglobulins and to determine the optimal treatment plan, 4 groups of dogs were formed, 5 dogs in each, which were selected on the analogue principle. The first and the second groups included dogs with signs of external otitis. The third and the fourth had dogs with a generalized form of CMD.*

Microscopy of the smears demonstrated Malassezia yeasts, in the form of small nesting doll-like or ellipsoidal cells with gram-positive staining. Loose colonies of gray-white color with a velvety border were formed in Sabouraud agar medium with glucose. Smooth colonies with wavy margins were formed in Dixon agar, the colonies were gray and white in color with a slightly yellowish tint. The isolated strain had catalase activity.

Dogs in the second and third groups were treated with an immunostimulant. The results of the studies showed that the best treatment plan for dogs with ear canal inflammation was for the second group, where 3 drops of Surolan ear drops in each ear canal twice a day, 2% chlorhexidine solution, and propolis tincture once a day were used. This plan proved to be more effective, as the time of treatment was reduced. For efficacy control apart from the absence of external otitis clinical signs, mycological studies were also considered.

Dogs in the third group had generalized skin lesions with lichenification and hyperpigmentation of the ventral parts of the body. To treat this form of Malassezia dermatitis, we used a complex of drugs: Skingard spray, 3 times a day to the affected skin for 14 days; Apocvel 0.4 - 0.6 mg/kg twice a day to relieve itching and allergic reactions; and propolis tincture for better skin regeneration. Apocvel was used to relieve itching and allergic reactions.

Keywords: canine *Malassezia* dermatitis, treatment plan, *Malassezia pachydermatis*, *Malassezia* spp.

Introduction. Canine *Malassezia* Dermatitis (CMD) is a common infectious disease with normal skin microflora causative agents. In most species, the yeasts live on the skin and do not cause any harm, but under certain conditions, they may acquire pathogenic properties (Moraru, 2019).

The CMD causative agents belong to the genus *Malassezia*, which currently includes more than 18 species (Lorch, 2018; Theelen, 2018). The genus *Malassezia* in dogs most often causes CMD with signs of external otitis, erythema and skin flaking, perianal dermatitis, and pododermatitis (Chupryna, 2024).

The number of dogs infected with CMD is growing steadily every year in Ukraine (Iovenko, 2023). The yeast's growth on the skin increases due to favorable conditions (high humidity, impaired protective function of the corneum stratum, atypical immune response development, etc.) and weakened immune response of dogs (Cafarchia, 2012b).

The common *Malassezia* dermatitis treatment plan includes the use of antifungal shampoos containing 2-4% Chlorhexidine, 2% Miconazole, 2% Ketoconazole, or the use of lotions containing 2% Enilconazole (Bond, 2010; Bond, 2020; Cafarchia, 2012a; Cafarchia, 2012b; Manion, 2017). To relieve itching and allergic reactions, 0.4 - 0.6 mg/kg twice a day of Apoquel or 0.5 - 1 mg/kg of prednisolone are used. To inhibit concomitant microflora 12.5-25 mg/kg twice a day of Sinulox is used. To treat otitis Isotik or Surolan ear drops and 2 - 4% of Chlorhexidine solution externally or Skinguard spray are used (Iovenko, 2020). In cases where topical therapy does not have a significant effect, it is advisable to use Ketoconazole 5-10 mg/kg orally once or twice a day (Velegriaki, 2004).

However, the susceptibility or resistance of yeast flora to azoles has not been proven. This encourages to search for alternative antifungal agents and to development of new treatment plans. The authors informed on the effectiveness of the honey-based gel in vitro against *M. pachydermatis* (Oliveira, 2018). With agar plate diffusion reaction, the effectiveness of essential oils of winter savory, lemongrass, cinnamon leaves, and palmarosa was observed (Manion, 2017). Oregano and thyme oils due to their main phenolic components (carvacrol, thymol) showed fungicidal activity. There is information on the synergistic interaction of essential oil components and azoles or nystatin against *M. pachydermatis*; carvacrol and miconazole or nystatin; thymol and nystatin, and clotrimazole and of *Melaleuca alternifolia*, *Mentha piperita* and *Origanum vulgare* essential oils (Schlemmer, 2018; Schlemmer, 2019). Unfortunately, it is difficult to verify the results because the vast majority of studies of clinical trials were conducted in vitro.

To improve the treatment plan, it was resolved to focus on the increase of innate immune response using a propolis-based immunostimulant. The therapy effectiveness evaluation was based on the results of clinical trials.

The purpose of the study was to improve the *Malassezia dermatitis* infection treatment plan for dogs using an immunostimulant – propolis tincture.

Methods and materials. The study was conducted at the veterinary ambulatory in Ternopil city, the veterinary clinic "Vetexpert" in Kharkiv, and the Department of Epizootiology and Microbiology of the State Biotechnology University.

To confirm the CMD diagnosis test material was taken with a glue tape from the affected skin of dogs with a generalized form of CMD. The affected ear canals were sampled with sterile cotton swabs. The affected skin was washed off and immediately screened into culture media. Purulent exudate washed out of the ear canals was transported in sterile tubes sealed with sterile rubber stoppers to the Department of Epizootiology and Microbiology for mycological studies.

In the department's laboratory smears were prepared and stained according to Giemsa, Gram, and alkaline methylene blue techniques. The test material was screened on Sabouraud agar supplemented with polymyxin and lactose, modified Dixon media. Cultures were incubated in a thermostat at 37°C for 24 to 72 hours. The growth pattern of the selected *Malassezia* cultures was observed.

We checked the immunodeficiency in dogs with a discrete method of immunoglobulins detection in the blood serum using PEG-6000. To determine the level of immunoglobulins and to determine the optimal treatment plan, 4 groups of dogs were

formed, 5 dogs in each, which were selected on the analogue principle. The first and the second groups included dogs with signs of external otitis. The third and fourth groups had dogs with a generalized form of CMD.

2 ml of blood was taken from the ulnar vein of dogs. The blood samples were kept at ambient temperature for 6 hours to obtain blood serum.

Results. Microscopy of the smears showed *Malassezia* yeasts, in the form of small nesting doll-like or ellipsoidal cells, 1.5 - 6.0 μm in width and 3.5 - 8.0 μm in length with gram-positive staining.

Loose colonies of gray and white color with a velvety border were formed in Sabouraud agar medium with glucose. Smooth colonies with wavy margins were formed in Dixon agar, the colonies were gray and white in color with a slightly yellowish tint. The isolated strain had catalase activity.

The results of the study to determine the level of immunoglobulins in the blood serum are shown in Table 1.

Table 1

The immunoglobulin level was determined in the blood serum of dogs with CMD using PEG-6000

Number of dogs in the group	1st group of dogs	2nd group of dogs	3rd group of dogs	4th group of dogs
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	±	+	+	+

Where «+» – immunodeficiency;

«±» – low level of immunoglobulins in the blood serum, which indicates the beginning of immunodeficiency

The results of the study showed that all groups of dogs had immunodeficiency. Thus, in the second, third, and fourth groups, all dogs had immunodeficiency. In the first group, only one dog had a low level of immunoglobulins, and in the other four dogs the immunodeficiency was observed.

The study allows us to assume that dogs with CMD are affected by the development of immunodeficiency. Thus, dogs with immunodeficiency have reduced nonspecific response, as a result, the pathogen agents producing enzymes and toxins can cause inflammatory processes.

The first group was a control group, with dogs with *Malassezia* dermatitis signs in inflammation of the external ear canal. First, the ear canal was carefully cleaned of crusts, sulfur, and purulent matter and washed with a 2% Chlorhexidine solution, which was at ambient temperature. After washing, the ears were thoroughly wiped to keep them dry and clean. Then, Isotik ear drops were used, which contain hydrocortisone, miconazole nitrate, and gentamicin sulfate. The duration of the treatment was 5 days.

Dogs of the second group were treated according to the second plan, with other drugs. Thus, after the external ear canal washing with chlorhexidine and thorough drying, Surolan ear drops containing miconazole nitrate, polymyxin B sulfate, and prednisolone acetate were used. The 3 drops in each ear canal twice a day of Surolan were used 7 days in a row. In addition, dogs of the second group had 1 drop of propolis tincture in the ear canal once a day. Propolis is known for its good immunostimulating properties, anti-inflammatory and antimicrobial effects, and boosting regeneration processes.

The dogs in the third group had generalized skin lesions with lichenification and hyperpigmentation of the ventral parts of the body. Some dogs injured skin due to itching.

The claw beds were brown in color, which is a sign of CMD. To treat this form of *Malassezia* dermatitis, we used a complex of drugs: Skingard spray, 3 times a day to the affected skin for 14 days; Apocvel 0.4 - 0.6 mg/kg twice a day to relieve itching and allergic reactions; and propolis tincture for better skin regeneration.

Propolis tincture also has anti-inflammatory and immunostimulating properties. It was applied with a sponge to the affected skin once a day for 7 days. Apocvel was used to relieve itching and allergic reactions.

The dogs of the fourth group had almost the same clinical signs of *Malassezia* dermatitis as those of the third group. There was a generalized form of CMD, with discoloration of the skin of the ventral parts of the skin. This group was treated with Skingard spray and prednisolone 0.5-1mg/kg to remove allergic reactions.

The results of the studies showed that the best treatment plan for dogs with ear canal inflammation was in the second group, where 3 drops in each ear canal twice a day of Surotan ear drops, 2% chlorhexidine solution, and propolis tincture once a day were used. This plan proved to be more effective, as the time of treatment was reduced. For efficacy control apart from the absence of external otitis clinical signs, mycological studies were also considered. The ear drops with miconazole nitrate, polymyxin B sulfate and prednisone acetate in combination with chlorhexidine and propolis tincture showed good antifungal, anti-inflammatory, and anti-itching effects. This treatment plan reduced the treatment time and was cost-effective, as the cost of treating dogs infected with *Malassezia* dermatitis with signs of external otitis was significantly less, compared to other treatment plans.

As for the first group of dogs, where Isotik ear drops 1 milliliter per day and 2% Chlorhexidine solution were used, also showed a therapeutic effect, but the time of treatment was 7 days on average. This treatment plan was more expensive than the plan proposed for the second group.

In the generalized form of Canine *Malassezia* Dermatitis the most effective was the treatment plan used for the third group of dogs. The therapeutic effect in this group was observed on day 11. Clinical examination showed no signs of skin inflammation, lichenification, or hyperpigmentation in the ventral areas of the body. There were also no signs of itching or allergic reactions. So, chlorhexidine digluconate and ketoconazole in Skingard spray in combination with the antihistamine Apocvel and the propolis tincture showed a good therapeutic effect. The use of propolis tincture as an immunostimulant was justified, as the treatment process was 3 days less. With this respect, due to the reduced time of treatment, the cost-effectiveness was much higher, and this made the course of treatment cheaper.

The use of Skingard spray and prednisolone in group 4 had a lesser therapeutic effect compared to dogs in group 3, the course of treatment was 14 days, and, respectively, the cost-effectiveness was much lower, and the cost of the course of treatment was much higher.

The study showed that the combination of antifungal, antimicrobial, antipruritic drugs and immunostimulants was appropriate in the treatment of *Malassezia* dermatitis in dogs, as the therapeutic effect was significantly better compared to the groups without treatment with propolis-based immunostimulants.

Conclusions:

1. In recent years, Canine *Malassezia* Dermatitis has spread and has been clinically manifested by external otitis, erythema and skin flaking, perianal dermatitis, and pododermatitis.

2. As the results of microscopy and studies of culture, we isolated *Malassezia* yeasts from dogs, which were identified as *M. pachydermatis*.

3. The results of the immunology study showed that all groups of dogs with CMD had immunodeficiency.

4. According to the results of a study to determine the optimal treatment plan for dogs infected with CMD, the group that used Surolan ear drops, chlorhexidine solution, and propolis tincture treatment had better results in the treatment of inflammation of the ear canal.

5. The treatment plan with Skingard spray, Apocvel, and propolis tincture proved to be the most effective in the generalized form of CMD. With this respect, due to the reduced treatment time, the cost-effectiveness was much higher, and this made the course of treatment cheaper.

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УДОСКОНАЛЕННЯ СХЕМИ ЛІКУВАННЯ МАЛАСЕЗІОЗНОГО ДЕРМАТИТУ СОБАК

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Резюме. У статті наведено результати лікування маласезіозного дерматиту собак з використанням настоянки прополісу. Для підтвердження діагнозу МДС, від хворих собак при генералізованій формі перебігу МДС, з уражених поверхонь шкіри, матеріал відбирали клішкою стрічкою. Стерильними ватними тампонами здійснювали відбір вмісту уражених слухових проходів. Робили змиви із уражених поверхонь шкіри і відразу висівали на транспортні поживні середовища. В лабораторії кафедри готували мазки, фарбували за Романовським-Гімзе, Грамом та лужним метиленовим синім. Досліджуваний матеріал висівали на модифікований агар Сабуро з поліміксином та лактозою, середовище Діксона. Посіви інкубували в термостаті за температури 37°C протягом 24 – 72 години. Враховували характер зростання виділених культур маласезій. Наявність у собак імунодефіцитних станів ми перевірили з використанням дискретного методу виявлення імуноглобулінів в сироватці крові за допомогою ПЕГ – 6000. Для визначення рівня імуноглобулінів та для визначення оптимальної схеми лікування було сформовано 4 групи собак, по 5 тварин у групі, які відбирались за принципом аналогів. До собак першої та другої групи були віднесені тварини з ознаками зовнішнього отиту. Собаки третьої та четвертої групи мали генералізовану форму МДС.

При мікроскопії мазків, виявили гриби роду *Malassezia*, у вигляді дрібних матрьошкоподібних, чи еліпсоїдних клітин, які забарвлювалися грампозитивно. На середовище Сабуро з глюкозою, формувалися пухкі колонії сіро-білого кольору, з бархатистою каймою. На агарі Діксона утворювались гладкі колонії з нерівними краями, колонії при цьому були сіро-білого кольору з дещо жовтуватим відтінком. Виділений штам володів каталазною активністю.

Собаки другої та третьої групи, лікували з використанням імуностимулятора. За результатами проведених досліджень, кращою схемою лікування собак, при запаленні слухового проходу були результати другої групи,

де були застосовані вушні краплі Суrolан в дозуванні 3 краплі в кожен слуховий прохід 2 рази на добу, використання 2% розчину хлоргексидину та використання настоянки прополісу 1 раз в день. Така схема виявилася більш ефективною, оскільки курс лікування було скорочено. Для контролю ефективності крім відсутності клінічних проявів зовнішнього отиту, враховували мікологічні дослідження.

Собаки третьої групи мали генералізовані ураження шкіри з явищами ліхеніфікації, гіперпігментації вентральних ділянок тіла. При лікуванні цієї форми маласезіозного дерматиту ми використовували комплекс препаратів, а саме - спрей Скінгард, яким зрошувати уражені ділянки шкіри 3 рази на добу в продовж 14 днів; для усунення свербіжу та для усунення алергічних реакцій використовували препарат апоквел 0,4 - 0,6 мг/кг 2 рази на добу; та для кращої регенерації шкіри використовували настоянку прополісу. Для усунення алергічних реакцій та свербіжу використовували препарат апоквел.

Ключові слова: маласезіозний дерматит собак, схема лікування, *Malassezia pachydermatis*, *Malassezia* spp.

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