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Effectiveness of Farnesol for treatment of dog otitis complicated by *Malassezia pachydermatis*

Ifarajimi R. Olabode[1] ✉, Nadezhda P. Sachivkina[1], Ekaterina V. Kiseleva[2, 3], Aleksey I. Shurov[1]

[1]RUDN University, *Moscow, Russian Federation*
[2]Pskov State University, *Pskov, Russian Federation*
[3]Nikulin Circus on Tsvetnoy Boulevard, *Moscow, Russian Federation*
✉ 1042205126@rudn.ru

Annotation. The study involved 30 dogs with otitis complicated by *Malassezia pachydermatis*. The animals were divided into 2 groups: experimental (n = 15) and control (n = 15). The experimental group was prescribed veterinary ear drops Surolan + a solution of the drug Farnesol; the control group — Surolan. The study showed that when Farnesol is added to the treatment regimen for fungal otitis etiology in dogs, small changes in the clinical composition of the blood are recorded, characterized by an increase in the number of erythrocytes and hemoglobin, a decrease in the number of leukocytes, with a decrease in the indicators of eosinophils and rod-shaped neutrophils in the experiment compared with the control. In addition, the complete absence of *Malassezia pachydermatis* in the smears of the ear contents after two weeks of therapy with a combined drug was proved. That is, in the experimental group, there were no cases of the presence of yeast-like fungi (YLF) in the smear after combined therapy with Surolan + Farnesol. And in the control group of 15 animals, YLF were observed in two with microscopy of ear exudate smears, but not in high concentration. The use of a combination of Surolan and Farnesol in dogs of the experimental group led to a decrease in hyperemia, itching, edema, ear fetid fluid on the 5th…7th days of treatment, and complete clinical recovery of the animals occurred by the 10th…14th day of therapy. In the control group, only Surolan was used, and improvement of clinical condition occurred on days 12…14, and recovery only after completion of the full course. As a result, both treatment regimens were successful, but the scheme with Farnesol gave faster results due to the wide spectrum of action of this drug: both antimicrobial and anti-inflammatory.

Keywords: yeast-like fungi, mycosis, clinical blood test, phytotherapy, Surolan

Conflicts of interest. The authors declare that there is no conflict of interest. The authors have no advertising purposes. Used drugs were purchased: Surolan in a veterinary pharmacy, Farnezol — in the Moscow office of Sigma-Aldrich.

Authors contribution. NP Sachivkina, EV Kiseleva developed and designed the experiments; IR Olabode collected the data; IR Olabode, AI Shurov analyzed the data; NP Sachivkina wrote the paper. All authors read and approved the final manuscript.

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Introduction

Inflammatory processes of the middle ear complicated by fungal infection are a common pathology and, according to O.G. Dutova and co-authors, "otitis media account for 20 % of all diseases encountered in veterinary practice. It has also been found that otitis media of the outer ear in dogs and cats are five times more common than in other animal species" [1]. At the same time, there is a persistent trend worldwide towards an annual increase in the incidence of opportunistic mycoses in animals. According to many scientists, dermatomycoses (microsporia, trichophytia) are replaced by diseases caused by opportunistic fungi, one of these are yeast-like fungi (YLF) of the genera *Candida* and *Malassezia* [1–4]. But, although fungi of the genus *Malassezia* are the most common etiological agents of infectious otitis media in animals, it must be borne in mind that these diseases can also be caused by other types of bacteria and fungi, which indicates the need for qualified species identification of microorganisms isolated from animals suspected of having Malassezia infection.

The pathogenic properties of YLF of *Malassezia* genus and their clinical role in infectious diseases of domestic animals are still the topics of ongoing discussions. According to A. Puigdemont: "The factors due to which the transition of a microorganism from a non-pathogenic to a clearly pathogenic form capable of causing a disease has not been fully clarified" [5]. There is no consensus on whether *Malassezia* infections can be considered as independent diseases, or the development of these fungi becomes widespread as an aggravating factor against the background of other pathologies. In veterinary medicine, the clinical role of fungi of *Malassezia* genus in superficial, and deep mycoses has been established relatively recently — in the last 2–3 decades. However, it was these studies that made a significant contribution to understanding of pathogenesis of *Malassezia* infections. At the moment, most modern researchers share the opinion that pathogenicity of *Malassezia* spp. is "opportunistic" in nature, i.e. DPG is able to exhibit virulent properties only against background of significant predisposing factors. It follows from the work of Jacques Guillot that "Under favorable conditions (increased sebum secretion and humidity, violation of the epidermal barrier), they (*Malassezia* spp.) actively multiply, the yeast form of the fungus turns into mycelial, *Malassezia* is introduced into the epidermis, showing pathogenic properties" [6]. However, according to another theory proposed by R. Bond in 1996 and J. Korbelik in 2018, "the transformation from the yeast phase to the mycelial phase is not due to the special pathogenicity of the latter, but is only a consequence of lipid metabolism disorders in the host body. This is based on the fact that *M. pachydermatis* view is not able to transform into a mycelial form" [7, 8].

According to C. Cafarchia with co-authors, “The development of the pathological process in malassesiosis is associated with a multiple increase in the population of microorganisms in the lesion. The population of YLF in sick animals increases by 100…10,000 times. Moreover, an increase in the number of fungi of *Malassezia* genus is noted not only on the surface of skin, but also on mucous membranes of nasal cavity, vulva and prepuce, i. e. the factors predisposing to this are systemic in nature” [9]. On the one hand, the primary factor for increasing YLF population is a violation of physical, chemical and immunological mechanisms of host defense, which normally limit fungal colonization of skin. On the other hand, as reflected in our work, “in the launch of the pathological process, the key role is played by communication of microorganisms within the population carried out by means of “signaling molecules” (QS). It is assumed that when population reaches a certain number, “sense of quorum” arises in it, which serves as starting signal for activation of pathogenicity factors and, as a consequence, leading to the development of an infectious process” [10].

At the same time, significant virulence factors of *Malassezia* fungi are hydrolytic enzymes described in detail in the work of M. Park. In 2021, “causing the invasion of the fungus into the host tissue. Lipolytic enzymes are able to hydrolyze skin secretion lipids to free fatty acids. In turn, free fatty acids inhibit the growth of other microorganisms, increasing the competitiveness of YLF” [11].

Recently, in the scientific literature, close attention has been paid to the study of herbal medicines, the possibility of their use for the treatment of infectious (fungal and bacterial) diseases [12, 13]. According to the literature and our own research, Farnesol ($C_{15}H_{26}O$) has proven its antimycotic efficacy in in vivo and in vitro models [14–19]. Chemically, Farnesol is an acyclic sesquiterpene alcohol, is a thermally stable molecule that is not exposed to extreme pH values, which is especially important in the development of YLF infection. Farnesol as a QS molecule participates in the regulation of various physiological processes in unicellular fungi, including filamentation, biofilm formation, drug susceptibility and apoptosis. This compound is produced by many microorganisms, and is also contained in various essential oils of plants, for example, in the flowers of Linden, Lat. *Tilia europaea* [20].

Today, in veterinary medicine, only a few doctors recommend phytopreparations for the treatment of otitis and dermatitis, and there is still relatively little data on their effectiveness in the scientific literature, especially in Russian. Therefore, the development of targeted therapy using alternative means can become one of the directions in solving global problems of infectious animal diseases, as well as increased resistance of microorganisms [21], since without proper treatment, serious complications of otitis can occur in animals, namely: extensive hematomas of the auricle, injuries as a result of combing, calcification of the fibrous tissue of the ear, abscess of the parotid space, damage to the eardrum (hyperplasia, thickening, stretching), the transition of external to medium otitis.

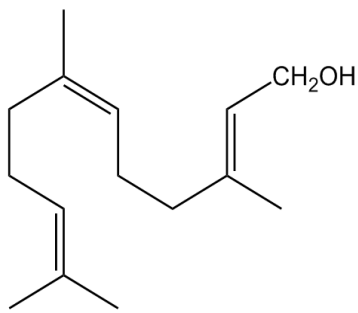
The purpose of the study — to determine the effectiveness of the use of Farnesol for the treatment of dog otitis complicated by *Malassezia pachydermatis*.

Materials and methods

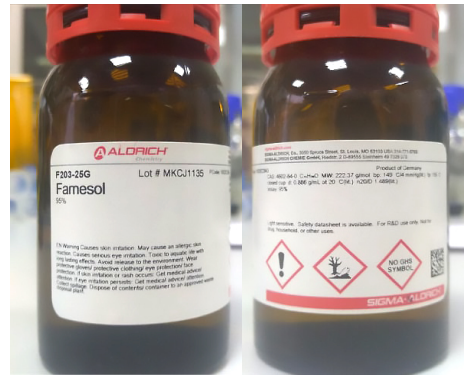
In the center of veterinary Innovative Medicine of RUDN University (Moscow) for the period 2020–2023, 76 cases of dog otitis were investigated, of which 30 cases were caused by *Malassezia pachydermatis*. Preliminary identification of strains to the genus level was carried out by phenotypic signs using microscopy of a smear of ear contents and staining with Gentian violet. Ear exudate was applied to the surface of the nutrient medium "Saburo agar, chloramphenicol 2" and cultured at 37 °C for 48 hours. Species identification was carried out by the MALDI-TOF-MS method on an Autoflex III mass spectrometer (Bruker Daltonics, Germany) using flex Control software. For each isolate, spectra of the expressed proteins were recorded in 4 repetitions. The obtained spectra were compared with MALDI Biotyper 3 mass spectrum profile library [16, 19, 22].

The study involved 30 dogs of different breeds, gender and age from 1 to 10 years. All the animals had apartment maintenance with walking. The diet consisted of dry food. Treatments for ectoparasites (external) and endoparasites (internal) were carried out in all participants of the experiment regularly and on time. From anamnesis: there was itching in the ears, an unpleasant smell for several weeks. At the receptions, it was noted: hyperemia of the auricles, stenosis of the auditory canal, in some cases alopecia, a large amount of yellow-brown discharge with a sharp sour smell.

The animals were divided into 2 groups: experimental (n = 15) and control (n = 15). The experimental group was assigned ear drops veterinary Surolan manufactured by Elanco Animal Health (Indiana, USA). The form is a suspension, the active substance is polymyxin B, prednisone, miconazole, 2 times a day, for 14 days. Plus, a solution of the drug Farnesol (Far) (Sigma-Adrich, USA), which was prepared in advance at a concentration of farnesol 100 microns per ml, dilution was carried out in sterile saline solution (SS) pH 7.0 (Fig. 1). Animal owners were given 10 ml of the drug in a plastic bottle with spray. The instructions for use were explained: after applying Surolan drops, apply Farnesol in the form of a spray twice a day, too. The control was a group of animals that were prescribed only the drug Surolan.



a



b

Fig. 1. Farnesol: ***a*** — the chemical formula; ***b*** — the appearance of the drug (Sigma-Aldrich, USA)
Source: ***a*** — internet, b — own photo, by I.R. Olabode

Venous blood to determine the indicators of general clinical analysis was taken at the initial admission and 2 weeks after the start of treatment from the internal femoral vein or lateral subcutaneous vein of the lower leg.

Blood tests were performed using the Mindray BC-2800Vet hematological analyzer (Mindray, China) [23, 24].

In whole blood, number of erythrocytes and leukocytes, hemoglobin, as well as the content of leukocytes were determined. In the study, a quantitative counter of shaped elements of animal blood was used, the percentage of different types of leukocytes was calculated in stained blood smears by a unified method.

The results obtained were compared in the experimental and control groups with an assessment of the reliability of the differences. The parameters given in the tables had the following designations: *M* is the average, *m* is the error of the average, *n* is the volume of the analyzed subgroup, *p* is the achieved level of significance. In all cases, the critical value of the significance level (p) was assumed to be 0.05.

Research results and discussion

The data available to indicate “the wide prevalence of *Malassezia* infections and the diverse species composition of the etiological agents of these diseases in domestic animals. Fungi of *Malassezia* genus do not have strict specificity with respect to the type of host organism, and both zoophilic and anthropophilic species can act as pathogens in animals. Of great practical importance is the fact that the disease can be caused by the association of several species of *Malassezia* genus” [25, 26]. These data emphasize the importance of a thorough mycological examination in the diagnosis of *Malassezia* infections.

In our work, several clinical forms of external otitis were noted. The first form, erythematous, was characterized, of course, by erythema, edema, the intensity of itching and scratching were different in different dogs and did not fit one general description. The second form, erythematous-ceruminous, manifested itself with erythema, severe itching, abundant discharge of earwax (cerumen) of yellow-brown color, most often with an unpleasant odor. The third form, ceruminous, was characterized, naturally, by abundant secretion of ear secretions, but without signs of inflammation (erythema and edema). The fourth, proliferative, was characterized by hyperplasia of sebaceous glands, formation of papules similar to a calendula flower, which is characteristic of prolonged, chronic cases. The fifth form, purulent, was manifested by abundant purulent discharge from the ear, during palpation the ear was painful, sometimes crepitation was heard. In most cases, we observed Malassezia-otitis media with erythematous-ceruminous form (Fig. 2, 3).

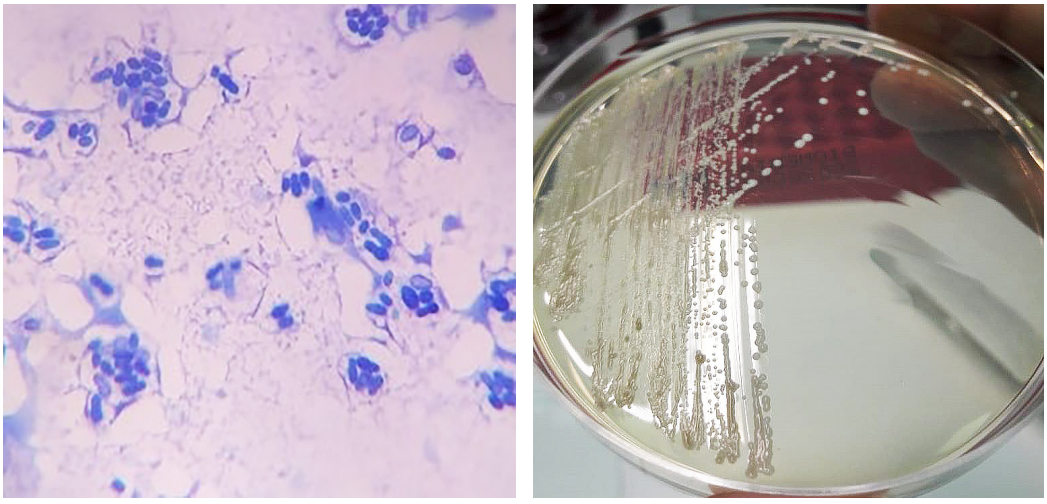
During microbiological examination of the culture of YLF separated from the ears of sick dogs included in the experiment were assigned to *Malassezia* genus by phenotypic characteristics (Fig. 4, a). When cultivating the material on the surface of the nutrient medium “Saburo agar, chloramphenicol 2” after 48 hours of cultivation at 37 °C, a typical growth of S forms of mucous, separately located colonies was observed, d = 3…5 mm; or merged colonies of milky-white color (Fig. 4, b).

Fig. 2. Clinical picture of *Malassezia* otitis characterized by erythema, alopecia and scratching
Source: own photo, by I.R. Olabode

Fig. 3. Clinical picture of *Malassezia* otitis with the release of abundant ear secretions (cerumen) of yellow-brown color with a crumbly or waxy coating
Source: own photo, by I.R. Olabode

Then the strains were identified to the species by the MALDI-TOF/TOF MS method as *Malassezia pachydermatis* — the most frequently isolated causative agent of invasive malasseziosis in dogs. The Score values were at least 2.00 for all studied strains.

If the culture was not identified as *Malassezia pachydermatis*, then the data from these animals were not included in the work. This article presents the results of an experiment with 100 % identification of *Malassezia pachydermatis*. There were precedents for the identification of another type of microorganism and, despite the fact that the animals were prescribed therapy and blood was taken for research, these results were not taken into account.



a b

Fig. 4. *Malassezia pachydermatis*: a — morphology; b — cultural properties

Source: own photo, by I.R. Olabode

Two weeks later, the animals of both groups were repeatedly swabbed with ear contents. It is worth noting that in the experimental group there was not a single case of presence of DPG in the smear after combined therapy with Surolan + Farnesol. And in the control group of 15 animals, YLF was observed in two with microscopy of ear exudate smears, but not in high concentration. The use of drugs Surolan + Farnesol in animals of the experimental group reduced the signs of hyperemia, edema, itching, the amount of exudation on the 5…7 days of treatment, and full clinical recovery of animals occurred on the 10…14 days. When using only Surolan in the control group, the improvement of the clinical condition occurred on days 12…14, and the final recovery followed after a full course of treatment. Analyzing the results obtained, we can say that both treatment regimens were effective, but the scheme used in the experimental group gave faster results due to the wide spectrum of action of the drug Farnesol in relation to microorganisms that are most often the causative agents of otitis media.

The study of this issue by our scientific group showed that "the mechanism of intrapopulation communication of microorganisms is associated with such a phenomenon as the formation of biofilms — supra-organizational structures that provide protective and trophic functions. Biofilms are differentiated communities of microorganisms formed by a single microbial agent or a mixture of fungal and bacterial species. Biofilms attach to biotic or abiotic surfaces, and their structure contributes to the innate physical and chemical resistance of microorganisms" [17–19, 27]. It is known that the ability to form biofilms is one of the pathogenicity factors of *Candida* genus [28]. And in 2007, F.T. Cannizzo found that "fungi of *Malassezia* genus are also capable of forming biofilms on the surface of various substrates" [29]. Also H. Yang with colleagues, he established that "symbiotic relationships of YLF and skin-dwelling bacteria (in particular, staphy-

lococci) play an important role in the pathogenesis of the disease. Staphylococci also produce lipase, which disrupts the secretory function of the skin and creates favorable conditions for the growth of both organisms, while such conditions are unfavorable for other competitive microorganisms" [30]. In addition to staphylococci, other types of bacteria and microscopic fungi can play a role in the pathogenesis of the disease. Thus, "along with *M. pachydermatis*, bacteria — *Staphylococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Streptococcus* spp., as well as fungi — *Candida* spp. and *Aspergillus* spp. were isolated from dogs with otitis" [31]. Treatment includes local and complex therapy with azole-type drugs, most often in the form of shampoos containing miconazole and chlorhexidine, or oral administration of antimycotic agents — itraconazole and / or ketoconazole. Treatment of concomitant animal diseases of infectious and non-infectious origin is very important to minimize relapses of malacesiosis. For many years, it has been believed that *Malassezia* strains from dogs and cats are sensitive to azoles, with the exception of fluconazole. However, mycological studies on the appearance of clinical strains of DPG with resistance to azole-type drugs are increasingly being published. This may be due to mutations or duplication of the ERG11 gene [32]. That is why the resistance of *Malassezia pachydermatis* to azole drugs is constantly monitored by scientists around the world [31–34]. These observations have caused increased interest in alternative antifungal drugs of local action, such as miramistin, chlorhexidine and various essential oils [12, 13, 30–32].

Clinical blood testing is one of the most important diagnostic methods that displays the reaction of hematopoietic organs to the influence of various physiological and pathological factors, it also allows to monitor the effectiveness of therapy. Clinical blood parameters of dogs with *Malassezia* otitis before treatment were characterized by low values of the number of red blood cells — $5.94 \pm 0.72\ 10^{12}$/l, which cannot be called anemia, but is a borderline value. Also, a decrease in hemoglobin to 127.38 ± 9.34 g/l was observed in sick animals (Table). The number of leukocytes in the blood of sick animals is within the normal range of $13.14 \pm 3.85\ 10^{9}$/l, but after therapy, the number of leukocytes became slightly lower. After treatment, the amount of hemoglobin in the blood of dogs increased to values of 168.54 ± 8.34 g/l in the experiment and 137.60 ± 9.34 g/l in the control. There is a significant difference of 1.2 times between these indicators, which indicates a positive effect of Farnesol in the treatment of *Malassezia* otitis.

A small eosinophilia (but within the normal range), which can be observed before the treatment of malacious otitis, is characteristic of many infectious and invasive diseases, as well as intoxication. Also, an increase in rod-shaped neutrophils of 9.63 ± 2.03 was observed in dogs before treatment, which is higher than the physiological norm and also indicates an inflammatory process with small values of segmented 56.28 ± 5.26. After two weeks of treatment, we can observe an improvement in the clinical parameters of the blood of dogs and even see a statistically significant difference between the experience and the control with respect to rod-shaped neutrophils (1.59 ± 0.32 in the experiment and 2.25 ± 0.34 in the control), eosinophils (5.86 ± 1.72 and 8.32 ± 1.62) and hemoglobin levels (168.54 ± 8.14 and 137.60 ± 9.34). We characterize this process as a pronounced inflammatory reaction,

manifested by vivid clinical signs, as well as cellular dynamics, which is an important criterion for the indication of the disease.

Clinical indicators of dog blood in the experiment

Indicators	Reference values	Before treatment (n = 30)	After treatment	
			Experience (n = 15)	Control (n = 15)
Red blood cells, 10^{12}/l	5.5…8.5	5.94 ± 0.72	7.91 ± 0.87	7.12 ± 1.04
Hematocrit,%	37…55	38.12 ± 3.98	51.54 ± 3.86	46.02 ± 4.37
Hemoglobin, g/l	120…180	127.38 ± 9.34	168.54 ± 8.14*	137.60 ± 9.34*
Leukocytes, 10^{9}/l	6…17	13.14 ± 3.85	8.54 ± 1.98	11.14 ± 2.61
Rod-shaped neutrophils,%	0…3	9.63 ± 2.03	1.59 ± 0.32*	2.25 ± 0.34*
Segmented neutrophils,%	60…70	56.28 ± 5.26	61.20 ± 5.45	58.82 ± 5.25
Eosinophils,%	2…12	10.41 ± 3.16	5.86 ± 1.72*	8.32 ± 1.62*
Monocytes,%	3…10	4.27 ± 0.84	2.69 ± 0.35	3.13 ± 0.64
Basophils,%	0…1	0.06 ± 0.02	0	0
Lymphocytes,%	12…30	22.41 ± 3.34	28.66 ± 2.24	27.48 ± 2.57

Note. * — statistically significant difference between experience and control.

Thus, when Farnesol is added to the treatment regimen for fungal otitis etiology in dogs, small changes in the clinical composition of blood are recorded, characterized by an increase in the number of erythrocytes and hemoglobin, a decrease in the number of leukocytes, with a decrease in the indicators of eosinophils and rod-shaped neutrophils in the experiment compared with the control.

Due to the fact that YLF of *Malassezia* genus often enter into symbiotic relationships with pathogenic and opportunistic bacteria, Farnesol therapy is of particular interest, since the antibacterial activity of this drug has long been recognized worldwide [14–20, 33–36]. Thus, the local application of Farnesol in polyinfection may have an effect on several microorganisms at once. In vivo data suggest that in combination with some antifungal drugs, Farnesol may have an adjuvant antimycotic effect [18, 19, 37–43].

Conclusion

Inflammation of the auditory canal develops at any age, it is characterized by a long, protracted course, leading to irreversible changes, in addition, the causative agents of otitis cause great harm to dog breeding. Both one and both ears can be involved in the pathological process. In most cases, both ears are affected in animals (bilateral otitis). The most typical clinical picture of *Malassezia* otitis is characterized by erythema, copious discharge of ear secretions, itching and scratching, and a specific unpleasant odor. Hyperkeratosis, lichenization of the skin of the auricles and the mouth of the auditory canal are also often observed — signs characteristic of chronic

inflammatory processes. As a rule, the disease is persistent, with periodic improvements and exacerbations.

Several degrees of severity of *Malassezia* otitis have been established, depending on the intensity of fungal contamination, the degree of ear inflammation and hyperkeratosis. With a small colonization by yeast-like fungi, there are no systemic manifestations of the disease: the inner surface of the auditory canal is slightly reddened, dry, crumbling or wax-like plaque may be observed during otological examination. With an average colonization of *Malassezia*, inflammation, swelling of the skin of the auricle increases, erythema becomes more pronounced. Pustules and papules appear on the surface of the skin, which after a few days are opened and covered with crusts. With a significant contamination of YLF, the inflammation is of a draining nature, the inner surface of the ear turns into a continuous oozing ulcer. In this case, fungi are always present in the smear, neutrophilic exudation is expressed, a lot of fibrin and tissue detritus. With the chronization of the process, the lichenization of the skin of the external auditory canal occurs, and the skin color acquires a bluish hue. Despite the fact that the process is fading, keratinized cells are detected in large quantities in the cytological smear, as a rule, leukocytes are always present and a large amount of *Malassezia* remains.

In this work, the effective antifungal effect of Farnesol against *Malassezia* infection in the local therapy of otitis media in dogs has been proven. Synergy in a pair of Farnesol/Surolan led to complete eradication of YLF with two-week therapy. Also during the study, it was shown that when Farnesol is added to the treatment regimen of fungal etiology otitis in dogs, small changes in the clinical composition of blood are recorded, characterized by an increase in the number of erythrocytes and hemoglobin, a decrease in the number of leukocytes. At the same time, the indices of eosinophils and rod-shaped neutrophils in the experiment significantly decreased compared with the control, which indicates the positive effect of Farnesol in the treatment of *Malassezia* otitis.

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About authors:

Olabode Ifarajimi Rapheal — postgraduate student, Department of Veterinary Medicine, Agrarian and Technological Institute, RUDN University, 6 Miklukho-Maklaya st., Moscow, 117198, Russian Federation; e-mail: 1042205126@rudn.ru

ORCID: 0000–0003–2395–7350

Sachivkina Nadezhda Pavlovna — Candidate of Biological Sciences, Associate Professor, Department of Microbiology, Medical Institute, RUDN University, 8 Miklukho-Maklaya st., Moscow, 117198, Russian Federation; e-mail: sachivkina@yandex.ru

ORCHID: 0000–0003–1100–929X; SPIN: 1172–3163

Kiseleva Ekaterina Vasilyevna — veterinarian, Nikulin Circus on Tsvetnoy Boulevard, 13 Tsvetnoy Boulevard, Moscow, 127051, Russian Federation; postgraduate student, Department of Zoology and Animal Ecology, Pskov State University, 21 Sovetskaya st., Pskov, 180000, Russian Federation; e-mail: kirsanovakaterina@yandex.ru

ORCID: 0000–0002–4907–9384;

Shurov Alexey Igorevich — postgraduate student, Department of Veterinary Medicine, veterinarian, Center for Veterinary Innovative Medicine, Agrarian and Technological Institute, RUDN University, 8 Miklukho-Maklaya st., Moscow, 117198, Russian Federation; e-mail: shurov_ai@rudn.university

ORCID: 0000–0003–2289–9814; SPIN: 9286–9577

Эффективность применения Фарнезола для лечения отитов собак, осложненных *Malassezia pachydermatis*

И.Р. Олабодэ[1] ✉, Н.П. Сачивкина[1], Е.В. Киселёва[2, 3], А.И. Щуров[1]

[1]Российский университет дружбы народов, *г. Москва, Российская Федерация*

[2]Псковский государственный университет, *г. Псков, Российская Федерация*

[3]Цирк Никулина на Цветном бульваре, *г. Москва, Российская Федерация*

✉ 1042205126@rudn.ru

Аннотация. В исследовании было задействовано 30 собак с отитом, осложненным *Malassezia pachydermatis*. Животных разделили на 2 группы: опытную (n = 15) и контрольную (n = 15). Опытной группе были назначены капли ушные ветеринарные Суролан + раствор препарата Фарнезол; контрольной — Суролан. В ходе исследования показано, что при добавлении Фарнезола в схему лечения отитов грибковой этиологии у собак регистрируются небольшие изменения клинического состава крови, характеризующиеся увеличением количества эритроцитов и гемоглобина, снижением числа лейкоцитов; в то же время снижаются показатели эозинофилов и палочкоядерных нейтрофилов в опыте по сравнению с контролем. Кроме того, доказано полное отсутствие *Malassezia pachydermatis* в мазках ушного содержимого после двухнедельной терапии сочетанным препаратом. Т.е. в опытной группе не было зарегистрировано ни одного случая присутствия дрожжеподобных грибов (ДПГ) в мазке после сочетанной терапии Суролан + Фарнезол, а в контрольной группе из 15 животных у двух при микроскопии мазков ушного экссудата ДПГ наблюдались, но не в большой концентрации. Использование комбинации Суролана и Фарнезола у собак опытной группы привело к уменьшению гиперемии, зуда, отека, ушной зловонной жидкости на 5…7 дни лечения, а полное клиническое выздоровление животных наступало к 10…14 дню терапии. В контрольной группе использовался только Суролан и улучшение клинического состояния наступало на 12…14 дни, а выздоровление — только по завершении полного курса. В итоге обе схемы лечения ока-

зались успешными, однако схема с Фарнезолом дала более быстрые результаты, обусловленные широким спектром действия этого препарата: антимикробным и противовоспалительным.

Ключевые слова: дрожжеподобные грибы, микозы, клинический анализ крови, фитотерапия, Суролан

Заявление о конфликте интересов. Авторы заявляют об отсутствии конфликта интересов. У авторов нет рекламных целей. Используемые препараты приобретались: Суролан — в ветеринарной аптеке, Фарнезол — в московском представительстве фирмы Sigma-Aldrich.

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Об авторах:

Олободэ Ифараджими Рафиль — аспирант департамента ветеринарной медицины аграрно-технологического института, Российский университет дружбы народов, Российская Федерация, 117198, г. Москва, ул. Миклухо-Маклая, д. 6; e-mail: 1042205126@rudn.ru
ORCID: 0000–0003–2395–7350

Сачивкина Надежда Павловна — кандидат биологических наук, доцент, доцент кафедры микробиологии им. В.С. Киктенко медицинского института, Российский университет дружбы народов, Российская Федерация, 117198, г. Москва, ул. Миклухо-Маклая, д. 8; e-mail: sachivkina@yandex.ru
ORCID: 0000–0003–1100–929X; SPIN-код: 1172–3163

Киселева Екатерина Васильевна — ветеринарный врач, Цирк Никулина на Цветном бульваре, Российская Федерация, 127051, г. Москва, Цветной бульвар, д. 13; аспирант кафедры зоологии и экологии животных, Псковский государственный университет, Российская Федерация, 180000, г. Псков, ул. Советская, д. 21; e-mail: kirsanovakaterina@yandex.ru
ORCID: 0000–0002–4907–9384

Щуров Алексей Игоревич — аспирант департамента ветеринарной медицины, врач центра ветеринарной инновационной медицины, аграрно-технологический институт, Российский университет дружбы народов, Российская Федерация, 117198, г. Москва, ул. Миклухо-Маклая, д. 8 корп. 2; e-mail: shurov_ai@rudn.university
ORCID: 0000–0003–2289–9814; SPIN-код: 9286–9577