Dermatomicoses in the Western Romania – epidemiological, diagnostic and therapeutic research

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ABSTRACT. In an epidemiological inquiry on 126 dogs and 24 cats, the most frequent dermatomycosis was microsporia. The following fungi species were identified: Microsporum canis, M. gypseum, Trichophyton mentagrophytes, T. concentricum, Malassezia pachidermatis, Candida albicans, Alternaria sp., Aspergillus sp., Penicilium sp. The dermatophytes were identified not only on the animal body, but also on the unsterilized equipment, whereas Malassezia pachidermatis was isolated only on animals. Microsporum had a higher prevalence in Pitbull, Teckel, Rottweiler and German Brac races, and Trichophyton in Boxer and Chow-Chow races, especially in the 1 – 3 years of age category. In cats, microsporia prevalence was higher in the European race, with a growing trend until 3 years of age, and then it reduces.

In cats, the preponderant localization was in descending order: head, neck, ears, and posterior limbs. The symptoms and lesions were alopecia, squamas, crusts, lichenifying, erythema, hyper pigmentation and, less, papule, seborrhea and prurit. In dogs, the lesions were identified especially on anterior limbs, head, thorax and abdomen and rarely on ears, neck, posterior limbs, limbs extremities, dorsal lumbar region and tail. The most frequent lesions were alopecia, squamas and crusts.

The solid Sabouraud medium with lacmoid (original), yields comparable results cu those of classic solid Sabouraud medium with phenol red in the identification of the dermatophytes. Allium sativum extract (especially unguent) has antimycotic effects both in natural and in experimental microsporia.

The Biocan M vaccine, although does not prevent experimental infection, accelerates the healing and negativation of M. canis in cats.

Introduction

Microorganisms that belong to the regnum Fungi produce mycotic diseases. They are in high number in the environment. Most of them have adopted the saprophytic way of life, but some of them are parasitic. As much as 300 species are cited as pathogenic for animals. The mandatory parasitic fungi belong, in most part, to the dermatomycetes category. They do not develop outside their host, but they can survive in the environment and transmit to living organisms by direct or indirect contagious process.

In dogs and cats, superficial mycosis that attack skin, hair and the nails are produced by fungi that belong to the Microsporum and Trichophyton genres, taking into account the fact that they can utilize and metabolize keratin. Other fungi, though, as those from Candida și Malassezia genres, may produce superficial mycosis.

As for the Trichophyton genre, both in dogs and cats, the most frequent is T. Mentagrophytes, with an incidence of 23% and 2% from the total of the dermatomycoses, respectively. Other species cited were T. erinacei, T. verrucosum, T. terrestre, T. rubrum și, mai rar, T. equinum, T. ajelloi, T. tonsurans and T. violaceum. Although trycophytia seems to be more frequent in dogs and rarer in cats, there are data that pose some contradictions.

From the Microsporum genre, the most frequent species, in dogs and cats, are: M. canis, M. gypseum, și M. persicolor, the first species being found in over 80% of the cases. Moreover, for M. canis, the cat is considered the natural host.
The parasites sources are various, depending on the real epidemiological context; they may be auto – or allogenec.

Generally, the biotic potential of fungi, and especially dermatomycetes, is high. Their resistance in the environment and to different chemicals is quite high. Moreover, their external localization facilitates the contamination of the environment and their spreading. The difficulties of precise diagnosis in Romania make the infected animals with sub clinic and oligosymptomatic diseases potential sources of contamination for a long time. The receptivity to mycotic infections is conditioned by intrinsic and extrinsic factors, such as species, race, age, sex, individual factors such as physiologic state, life conditions, medication, has represented the subject to many studies, but not in Romania. That is why research of these factors and of other not presented here is necessary. The establishment of their role and the control of mycotic infection are necessary. The precise diagnosis is hard to get, especially in Romania, where the number of the specialists in the field is very low. That is why the practitioners use therapeutic diagnosis, spending a long time until the right therapy is under way. The same is true for dermatological diseases, thus the necessity of a diagnostic protocol in dermatological diseases.

In order to get a correct diagnosis, several methods should be used: clinical, microscopic, histological, cell culture and experimental exams. We have proposed to assess their value in order to elaborate a diagnostic protocol for specialists.

Efficient methods for parasitologic control of dermatomycosis in carnivores represent a way of preventing their spreading in men and animals. Prophylaxis and therapeutic failures in dermatophyties are caused by uncontrolled or short therapy, long-term use of the same antimycotic agent, therapy without any mycotic sensibility test, empirical treatment, use of immunosuppressors, non-use of adjuvant therapy.

The common antimycotic agents used for therapy of dermatophyties are synthesis or biosynthesis antimycotics, both topical and systemic. The best results are achieved by combined therapy, with the exception of pregnant cats, in which systemic antimycotics are teratogenic, fact that makes the topical therapy the treatment of choice until the offspring and their mother can be treated by combined therapy.

Due to the molecular complexity of fungi, the results obtained by vaccination are not satisfactory, unlike the case of bacteria.

On the basis of the knowledge accumulated regarding the control of the dermatophyties, we have testing of some natural extracts in dogs and cats’ microsporia and some vaccines in cats’ experimental microsporia as a goal.

**Results, discussions and conclusions**

**Dermatomycosis incidence in carnivores**

For the determination of dermatomycosis incidence in carnivores in 2001 – 2002 period, the inquiry analyzed 126 dogs and 24 cats with dermatological diseases. Each animal was performed a clinical exam, a skin smear and a culture in Sabouraud medium.

Dermatological diseases were categorized in mycotic, parasitic and others. In dogs, from the total of 126 skin diseases, the most frequent (55) cases were parasitic, followed by mycotic (36), and other skin diseases (35). The most frequent mycotic disease was microsporia. From the parasitic diseases, the most frequent are: demodicosis, allergic dermatitis from fleas, scabies, cheiletielosis, and ancylostomosis.

In cats, from the total of the 24 cases, 9 were dermatomycosis, 10 parasitic, and 5 were other skin diseases. From those, the most frequent were microsporia and scabies. Flea parasitism and other dermatomycosis were present.

**Sources and transmission pathways**

In order to assess the sources of infection in skin diseases in dogs and cats mycotic and parasitic species were taken into consideration as allogene or autogene sources.

The pathologic samples were cultured on Sabouraud medium to identify the fungi, and examined macroscopically and microscopically for the parasites. In this case, dogs and cats were considered as autogene sources.
In order to identify the allogene sources, samples were taken from the environment of the known infected dogs and from the brushes and seeded in Sabouraud medium.

For the assessment of the transmission pathways, cultures of *Microsporum canis* and *Malassezia pachidermatis* were taken into consideration. The pathologic material of *Microsporum canis* prepared for inoculation was applied on haircut skin or on haircut and scratched skin in dogs and rats.

*M. pachidermatis* pathological material was administered in dogs on the skin after hair cut and skin scratching, intraauricular and subcutaneous. In rats, the material was applied on haircut and scratched skin and intraauricular.

The culture in Sabouraud medium revealed the following fungi: *Microsporum canis, M. gypseum, Trichophyton mentagrophytes, T. concentricum, Malassezia pachidermatis, Candida albicans, Alternaria sp., Aspergillus sp., Penicilium sp.* From the skin parasites, the following were identified: *Demodex canis, Ctenocephalides canis şi Ctenocephalides felis, Cheillettia yasguri, Otodectes cynotis, Sarcoptes canis and Anclylostoma caninum.* Polyparasitism with association between species of mycetes or mycetes and parasites (*Demodex canis, Ctenocephalides canis*) was recorded in 9 cases. This amplifies and diversifies the sources of infection.

In cats, *M. canis* and *T. Mentagrophytes* species of fungi and *Notoedres cati, Otodectes cynotis, Ctenocephalides canis* and *Ctenocephalides felis* species of external parasites were found.

*M. canis* was identified especially in instruments, and less in cages or beds. Although identified in cages, beds and instruments, *T. Mentagrophytes* was not identified in the environment. *M. Pachidermatis* could not be put in evidence in cultures, no matter the source examined. *Candida albicans* was identified in a single case, in the brush and in the cage.

The results obtained reveal the determinant role of autogene fungi sources, and of allogene sources in the case of *Microsporum* and *Trichophyton* genres.

*M. canis* infection in dogs was observed by both transmission pathways, whereas in the rats, the skin scratching was necessary. This proves the high infection potential of *M. canis* for dogs.

*M. pachidermatis* infection could be achieved only in dogs but not in rats. In dogs, cutaneous infection, with or without scratching, and subcutaneous, could not be achieved. Only the intraauricular pathway proved efficient, thus suggesting the insignificant role of allogene sources in the case of *M. pachidermatis* infection.

**Receptivity factors**

In order to assess the way some receptivity factors influence the evolution of skin diseases, race, age and sex were taken into consideration in dogs and cats. 138 samples taken from dogs and 33 from cats were analyzed and the results were correlated with the race, age and sex of the animal.

Mycotic skin diseases were identified in Pitbull, German shepherd, Teckel, Boxer, Doberman, Pinscher, Rottweiler, Chow-Chow, American Staffordshire Terrier, Husky, German Brack, Pekinez, Fox Terrier, Labrador, Sharpei, Caniche, Collie races, in crossbreds and in common race. The incidence of skin infection was higher in short hair races, due to the easier access of fungal spores to the skin.

In dogs, the maximum incidence of dermatomycosis was reached in 1 – 3 years of age, although the infection was identified in all groups of age. No significant differences of the sex influence on the incidence of infection, although the males had slightly higher incidence.

In cats, the mycotic diseases identified were microsporia and trycophytia in the following breeds: Siamese, Birmanese, European and in crossbred case. Although the incidence of microsporia was much higher in the European race, we cannot assert that the race has higher predisposition for the infection, because of the small number of cases.

In cats, the incidence of dermatomycosis rises until the age of 3, and then it reduces. It is possible that an age – dependent resistance to install. The sex does not influence the incidence,
although, as in the dogs’ case, the males had a higher incidence.

Clinical diagnosis

In order to assess the symptoms and the lesions in microsporia, 14 cats and 28 dogs naturally infected with *Microsporum canis* were examined. Clinical diagnosis was set after performing a careful examination of head, ears, anterior and posterior limbs, neck, thorax, abdomen, dorsal lumbar area, and tail.

In cats, the preponderant localization of the lesions was in descending order: head, neck, ears, and posterior limbs. Symptoms and macroscopic lesions were alopecia, squamae, crusts, lichenifying, erythema, hyper pigmentation and, less, papule, seborrhea and prurit.

In dogs, the lesions were identified mostly on anterior limbs, head, thorax, abdomen, and rarely on ears, neck, posterior limbs, limbs extremities, dorsal lumbar region, and tail. The most frequent lesions were alopecia, squamas, and crusts. Other symptoms and lesions had a lower incidence and were represented by erythema, hyper pigmentation, seborrhea, papule, pustules, prurit and sclerosis.

In natural infection with *M. Canis*, the clinical forms with typical, regulated alopecia were in 83.33% of the cases of the dogs and in 61.54% in cats, thus proving that typical lesions are more frequent in dogs than in cats.

Since the next in the incidence order was infection with *Malassezia pachidermatis*, clinical assessment of this skin disease was performed. 23 dogs identified as infected by culture analysis were examined. The preponderant localizations of the lesions were outer ear conduct (95.6%), areas with skin folds and face. Less affected were the thorax, the abdomen, the anterior and posterior limbs, the tail and the dorsal lumbar area. The prurit was present in all the dogs examined. The skin lesions, in descendent order of the incidence, were: erythema, seborrhea, alopecia, hyper pigmentation, crusts, lichenifying, and papule. The lesions were fetid in 34.8% of the cases.

Microscopic and culture diagnosis

189 dogs and 40 cats were considered for direct microscopic and culture examinations. Crusts, squamas and hair mixed with lacto phenol were used to diagnose microsporia, whereas malasseiosis was diagnosed in crusts, serosities, and skin or cerumen secretions from the ear conduct. All smears were stained with methylene blue.

18 dogs (10.53%) from the 171 with lesions and 1 dog (5.55%) from 18 without lesions were diagnosed with *M. canis* infection by direct microscopic exam. 7 cats (20.58%) from the 34 with lesions were diagnosed with *M. canis*, whereas in the 6 cats without lesions the diagnosis was negative.

For the culture, two mediums were used:

a. Solid Sabouraud medium with phenol red as an indicator

b. Solid Sabouraud medium with lacmoid, as an original indicator

The pathologic materials used for microscopic examination were used also for seeding in the two culture mediums.

From the 171 dogs with lesions, 24 (14.03%) were found positive for *M. canis*, and also 4 (22.22%) from the 18 dogs without lesions were detected as carriers.

In cats, 13 (38.23%) from the 34 with lesions have been found positive, and also one (16.66%) from the 6 cats without lesions.

As a conclusion, the culture exam is superior to the direct microscopic exam.

Both culture mediums were optimal, the development of the fungi being detected after 4 – 5 days, when the color of the medium changed to red (in the case of the medium with phenol red) or blue (in the case of the medium with lacmoid). Both mediums were useful for the culture of *Malasesia pachidermatis*, the only modification is that the color has changed later, after 8 – 12 days.
Histological diagnosis

Samples from 12 cats experimentally infected with *M. canis* were fixed in formaldehyde, then embedded in paraffin, sectioned and stained with the PAS reagent. All the slides examined presented spores and fungi. The following lesions were diagnosed: epidermal and follicular hyperkeratosis, edema, absence of *stratum corneum*, hyperplasic and spongious vasculitis, and inflammatory infiltrates, mainly with plasma cells. The most frequent were the epidermal hyperkeratosis, hyperplasic vasculitis and inflammatory infiltrate.

Experimental diagnosis

For the experimental diagnosis, 12 cats, 6 dogs and 4 rats were infected with *M. canis*. For each animal species, the infection was realized after haircut, with or without scratching. Moreover, a group of cats was immunosuppressed with Dexamethasone administered daily in a dose of 0,5 mg/kg intramuscularly. The infection was verified by microscopic and culture examinations.

The cats were the most receptive to the infection with *M. canis*, all 12 being symptomatic. In the immunosuppressed group, the lesions appeared earlier and the evolution was severe.

In the scratched dogs, the infection with *M. canis* has been realized in 100% of the cases, whereas in dogs with no scratching performed, the infection has arisen in 66% of the cases.

Only one of the rats with experimental infection has been identified with positive cultures for *M. canis*.

These results prove that the cat is the most receptive to infection and may be used for experimental diagnosis or in case of doubts.

Generally, from the 189 examined dogs, microsporia was identified in 28 (14,8%) and other dermatological fungal infections in 30 (15,9%). From the total of the 40 cats examined, 14 were infected (35%) with *M. canis*, with infection detected by collating the results of both methods, and 6 (15%) with other fungi.

Testing of therapeutic efficiency of some natural plants extracts in experimental microsporia in cats

To test the antimycotic action of some natural extracts, 9 cats, in 3 groups of three cats each, were experimentally infected in day 0 with *M. canis* after scratching the skin of the dorsal area of the head. The material for inoculation was from a culture in Sabouraud medium originating in a cat with known microsporia. The solution was placed on bald and scratched skin, and the infection site was protected with a bandage and a collar.

*Allium sativum* extract was prepared in the Biochemistry Department of the Veterinary Medicine Faculty Timisoara, whereas the rough antimycotic extract was prepared at the Western University of Timisoara.

Beginning with the 30th day after the infection, the solutions mentioned above were locally applied daily. Clinical exams were performed in days 30, 36, 43, 52 and 60 in order to assess the therapeutic efficiency. In days 30 and 60, both microscopic and culture exams were performed.

At the beginning of the experiment, all 9 infected cats had crusts, white squamas, erythema and scratches on the head.

During the therapy with *Allium sativum*, the crusts began to fall and the hair to regenerate. After 30 days, clinical healing, confirmed by culture, was found in 100% of the cases. In two of the 3 cats, secondary lesions were noticed.

In the group treated with rough antimycotic, the crusts began to fall and the hair to regenerate, but at a slower rate than the first group, thus in the 30th day from the beginning of the therapy, one cat was still presenting small crusts. The healing produced in 66% of the cases, also confirmed by culture.

The untreated group presented some small crusts in day 30, but the culture exam revealed the presence of the fungus in all the cats.

The results obtained with these natural extracts were comparable with those obtained with synthesis antimycotic agents, but the first have the advantage of being ecological. *Allium sativum* extract was superior to the rough
antimycotic extract. Both acted as topical medications, allowing the apparition of secondary lesions. Taking these into consideration, natural extracts may be used in combination with systemic antimycotic agents, and could provide an alternative therapy for pregnant females and newborns.

**Testing of the therapeutic efficacy of a natural extract in natural microsporia in dogs and cats**

In order to assess the antimycotic activity of *Allium sativum* extract, 5 dogs and 11 cats with *M. canis* infection were identified from the Parasitology Clinic of the Veterinary Medicine Faculty and from some medical practice in Timisoara. Watery and cream variants of *Allium sativum* extract were used. After the initial clinical, microscopic and culture assessment, daily topical applications of the extract were performed, with a clinical evaluation at 15 days, and a final clinical, microscopic and culture examination after 30 days of therapy.

The clinical exam performed at 15 days revealed a significant decrease in symptoms and lesions, with some dogs and cats that presented squamas, not crusts, and hair regeneration. After 30 days of therapy, the microscopic and culture exams were negative in all the 3 dogs treated with the watery extract of *Allium sativum*, but one of the dogs presented squamas and microscopic lesions of microsporia.

In the group treated with *Allium sativum* cream extract, the clinical healing, confirmed by microscopic and culture exams, was detected in 100% of the cases. Moreover, no secondary lesions were found. It is possible that the cream form of the extract to prevent the spreading of the fungus.

In cats, the group treated with the watery extract of *Allium sativum* healed clinically in a proportion of 80%. The microscopic and culture exam was negative in all 5 treated cats at 30 days from the beginning of the therapy. Three were secondary lesions in 3 of the cats treated.

The group of cats treated with cream of *Allium sativum* has clinically evolved to healing. One cat presented squamas at the place of the former lesions, and one had secondary lesions. The microscopic and culture exams were negative in all the cases.

Comparing the results with *Allium sativum* extracts with those with synthesis antimycotic agents cited in the literature, we may assert that the topical results are the similar. The cream has proven to be more efficient in the prevention of secondary lesions.

**Efficacy testing of some antimycotic vaccines in cats**

In order to test the efficacy of an antimycotic vaccine in cats’ microsporia, 9 cats, divided in three groups of 3 cats each were used as experimental material. One group was vaccinated with Biocan M (Bioveta), other was immunosuppressed with Dexomethasone, and the third was the control group. Five days after the beginning of the experiment, all the cats were experimentally infected with *M. canis* on scratched skin. Clinical examinations were performed in days 7, 12, 15, 18, 30 and 64, and culture exams in days 18 and 64. At day 7, all the immunosuppressed cats and from the control group had macroscopic skin lesions, whereas from the vaccinated group, only 2 had lesions. In day 12, the immunosuppressed cats had severe lesions. In day 18, all 9 cats had positive cultures for *M. canis*. So the infection with *M. canis* was realized in 100% of the cases no matter the experimental conditions.

In day 64, all the 3 vaccinated cats did not presented any crusts, and the hair began to grow. The culture exam was negative.

In the immunosuppressed group, in day 12, one cat died. In day 64, the remaining 2 cats presented small lesions and positive cultures. Additionally, one of them presented secondary lesions.

In the control group, even lacking the vaccination and therapy, in day 64 no macroscopic lesions could be spotted and the hair was regenerating. One cat had secondary lesions and one had positive cultures.

On the basis of the results obtained, we may assert that Biocan M vaccine accelerates the healing and the negativation processes, although it does not prevent the experimental infection. In the conditions of the experimental infection with
M. canis, the immunosuppression aggravates the symptoms, does not allow spontaneous healing, and favors the apparition of secondary lesions. Experimental infection with M. canis has proven that spontaneous healing can produce in some cases.

The results obtained with Biocan M vaccine are similar to those obtained by other authors and with those obtained with other vaccines. Generally speaking, it is admitted that vaccines either do not protect from infection at all or allow a faster healing of the infection with M. canis.

REZUMAT

Dermatomicoze în zona de vest a României – cercetări epidemiologice, diagnostice și terapeutice

Într-o anchetă epidemiologică pe 126 de câini și 24 de pisici, dermatomicoza cea mai frecventă a fost microsporia. Au fost identificate următoarele specii de micete: Microsporum canis, M. gypseum, Trichophyton mentagrophytes, T. concentricum, Malassezia pachidermatis, Candida albicans, Alternaria sp., Aspergillus sp., Penicillium sp. Dermatofizi au fost identificați nu numai pe corpul carnivorelor ci și pe ustensilele de pansaj, în timp ce Malassezia pachidermatis a fost izolată doar pe animale. Microsporum a avut o prevalență mai mare la rasele de câini Pitbull, Teckel, Rottweiler și Brack German iar Trichophyton la rasele Boxer și Chow-Chow, mai ales la categoria de vârstă 1-3 ani. La pisici, prevalența microsporiei a fost mai mare la rasa Europeană, cu tendință creștere până la vârsta de 3 ani, apoi se reduce.

La pisici, localizarea preponderentă a leziunilor a fost în ordine descrescândă: pe cap, gât, urechi, membre posterioare. Simptomele și leziunile au fost reprezentate de alopecie, scuame, cruste, lichenificare, eritem, hiperpigmentare și, mai puțin, de papule, seboree și prurit. La câini, leziunile au fost identificate mai ales pe membrele anterioare, cap, torace și abdomen și mai rar pe urechi, gât, membre posterioare, extremități membre, regiune dorsal-lombară și coadă. Cele mai frecvente leziuni au fost reprezentate de alopecie, scuame și cruste.

Mediul Sabouraud solid cu lacmoid (original), în identificarea dermatofiziilor, dă rezultate comparabile cu cele ale mediului clasic Sabouraud solid cu roșu fenol.

Extractul de Allium sativum (mai ales forma de ungvent) are efecte antimicotice atât în microsporia naturală cât și în cea experimentală.

Vaccinul Biocan M, deși nu previne infecția experimentală, grăbește procesul de vindecare și negativare a infecției cu M. canis la pisici.

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