DERMATOPHYTOSIS IN CATS AND DOGS: MOLECULAR IDENTIFICATION AND EPIDEMIOLOGY

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ABSTRACT

Dermatophyte species are strongly related group of filamentous pathogenic fungi. They are keratinophilic and keratinolytic, and responsible for superficial infection designated as dermatophytosis or ringworm. The most commonly used diagnostic methods, even including fungal culture, have some negative properties. This situation encourages the searching and development of new diagnostic and identification methods. Among the most optimal and promising are methods of molecular biology based on the PCR technique. There are studies to develop the molecular methods for identification / detection of dermatophytosis from clinical samples. However, mostly are not adapted to veterinary samples. Those that are specific for samples from animals still have limitations due to the type of specimens or pathogen species of the pathogen, sensitivity and accuracy vary from species to species-carrier. In face of globalization and evolutionary changes, it is also important to pay attention to another aspect - epidemiology. Epidemiological data should be one of the bases in the conduct of therapeutic and diagnostic procedures, especially when it concerns pathogenic fungi, which are common everywhere. Epidemiological data should be periodically updated and reflect a current situation. The main goal of this work was to optimize the selected protocol for identifying dermatophytes from clinical specimens of dogs and cats, as well as updating epidemiological data on dogs and cats dermatophytosis.

Keywords: dermatophytosis, diagnostic, identification, epidemiology

INTRODUCTION

Dermatophytosis

By far, dermatophytosis is the most common superficial cutaneous fungal infection of humans and other animals, including cats and dogs (Frymus *et al*., 2013; Dabrowska *et al*., 2014; Titova, 2017). Disease affects keratinized tissues (hair, skin, nails) of living animals and human. Dermatophyte infections are generally superficial and limited by non-living cornified layers, but immunocompromised patients can experience severe, disseminated disease (Weitzman *et al*., 1995). Dermatophytosis is treatable but has a high rate of recurrence or/and complications in form of secondary bacterial or fungal infections. Host reactions to dermatophyte infection may range from mild to severe and depend on the species or strain virulence, anatomic locations of the lesions, environmental factors (Weitzman *et al*., 1995). The clinical signs of dermatophytosis reflect the pathogenesis of the disease - it invades keratinized structures. There can be any combination of hair loss, papules, scales, crusts, erythema, follicular plugging, hyperpigmentation and changes in nail growth / appearance. Typical lesions are asymmetrical. Pruritus is variable, but in general is minimal to absent (Menelaos, 2006; Mattei *et al*., 2014; Moriello *et al*., 2017). Also, it was confirmed *in vitro* biofilm formation by two of the most prevalent species worldwide: *Trichophyton rubrum* and *T. mentagrophytes* (Costa-Orlandi *et al*., 2014). Dermatophytosis is zoonotic disease and easily transmissible by direct contact with infected animals and humans or by indirect contact with contaminated fomites (Bernardo *et al*., 2005; Aneja *et al*., 2012; Frymus *et al*., 2013).

Dermatophytes

This group of fungi mostly belonging to the genera *Microsporum*, *Trichophyton*, *Epidermophyton* and *Arthroderma* (Varrier *et al*., 2012; Moriello *et al*., 2017). The

causative agents of dermatophytosis in dogs and cats are mainly related to genera *Microsporum* and *Trichophyton* (Frymus *et al.*, 2013; Moriello *et al.*, 2017). Concerning their natural habitat, host preference and transmission route, dermatophytes can be divided into anthropophilic, zoophilic and geophilic species. Anthropophilic dermatophytes are primarily associated with humans and rarely infect other animals. Zoophilic dermatophytes usually infect animals or are associated with animals but occasionally infect humans. Geophilic dermatophytes are primarily associated with keratinous materials such as hair, feathers, hooves, and horns after these materials have been dissociated from living animals and are in the process of decomposition, these species may cause human and animal infection (**Table 1**) (Weitzman *et al.*, 1995; Cafarchia *et al.*, 2013; Zhan *et al.*, 2016;). The most frequently detected dermatophytes in skin lesions of dogs and cats are *Microsporum canis*, *M. gypseum*, *Trichophyton terrestre* and *T.mentagrophytes* complex strains (Bernardo *et al.*, 2005; Cafarchia et al., 2013;).

Table 1 - Zoophilic and Geophilic dermatophytes species in animals. Adapted from Mattei *et al.*, 2014

Dermatophyts species	Main source	Others
Microsporum canis	Cat, dog, horse	All mammals
Microsporum gallinae	poultry	Dog, cat
Microsporum gypseum	Soil	All mammals
Microsporumn nanum	Soil	Pig
Microsporum persicolor	Microtid rodents	Dog, cat
Trichophyton equinum	Horse	Cat, dog (rare)
Trichophyton erinacei	Hedgehog	Dog
Trichophyton mentagrophyts	Rodents	All mammals
Trichophyton simii	Primate	Fowl, dog, cat
Trichophyton verrucosum	Cattle, other ruminants	All mammals

IDENTIFICATION AND DIAGNOSIS

Dermatophytosis diagnosis is based on the history of the patient, physical examination, microscopic examination of scraping and hairs from the lesions in conjunction with fungal cultures, histology of the tissues and other techniques like a Wood´s lamp examination.

Conventional methods of identification and diagnosis of dermatophytes, general review

Among the routine diagnosis techniques, the <u>direct microscopic examination</u> is a simple and rapid method to detect dermatophytes on hairs and scales. Used to confirm the presence of a dermatophyte infection it involves the examination for

hyphae and/or fungal spores, allow the rapid confirmation of infection. Hairs and scales are mounted in solutions (DMSO, potassium hydroxide, mineral oil of varying concentrations) to aid in visualization of fungal elements by microscopic examination (Moriello *et al.*, 2017). This technique originates false-positive result, especially in presence of saprophytic fungi or due to the wrong interpretation of structural elements. Rendering sensitivity relatively poor (Frymus *et al.*, 2013).

Dermoscopy (epiluminescence microscopy**)** is a noninvasive method, performed using an illuminated camera, allowing the *in vivo* evaluation the colors and microstructures of the epidermis, dermo-epidermal junction and papillary dermis not visible to the naked eye. It is based on the identification of specific diagnostic patterns, such as comma-like hairs in infected cats. Is a clinical tool, being a frequently used with or without concurrent use of Wood´s lamp, to identify hairs for culture and/or direct examination (Dong *et al.*, 2016 Moriello *et al.*, 2017).

Wood´s examination is performing by using a Wood´s ultraviolet lamp to detect the presence of dermatophyte fungi by emission of fluorescence. Fluorescence occurs when light of shorter wavelengths initially emitted by the lamp are absorbed, and radiation with of longer wavelengths is emitted (longer than 400 nm in this case). Many microorganisms produce phosphors as result of their growth on skin and/or on hairs, and this characteristic can aid in detection or confirmation of infection. Fluorescence develops as early as day 5 – 7, and usually by day 10 – 14 post-infection. Apart from *Trichophyton schoenleinii*, dermatophytes that produce fluorescence are members of the *Microsporum* genus. The characteristic green fluorescence observed on *M. canis*, infected hair shafts is due to the production of a water-soluble chemical metabolite (pteridine) located within the cortex or medulla of the hair. Nevertheless, there are some strains of *M. canis* that show negative florescence. The sensitivity of the exam depends on the distance between lamp and skin, that must be close, not more than 10-12 cm. Also, some drugs can destroy fluorescence (Moriello *et al.*, 2017).

Fungal cultures are often stated as the gold standard method for dermatophyte diagnosis. This method is very sensitive and can determine the species (Frymus *et al.*, 2013). Some of the culture media that can be used: Sabouraud Dextrose Agar (SDA), Dermatophyte Test Medium (DTM), Mycosel, Cooke Rose Bengal Agar (CRB agar). They are selective media for growth and isolation of fungi, usually incubated for 2 – 3 weeks at +25º to +30ºC, allowing colony development and subsequent identification, based on macro and microscopic examination. However, false-positive and false-negative result can also occur; due to peculiarities of sampling techniques, storage and incubation of cultures and result interpretation (Moriello *et al.*, 2017).

Molecular identification methods based on PCR technique, general review

There are many published reports on the identification of *Microsporum* and *Trichophyton* via PCR, but studies on the use of PCR on clinical specimens from veterinary patients are scarce. Usually these data employs for scientific research and have no validation for regular utilization in clinic.

One-step PCR is conferred to be highly accurate (AUC>90) for the testing of samples from dogs, but only moderately accurate (AUC=78,6) for cats (Cafarchia *et al.*, 2013).

Nested-PCR is described as accurate (AUC=93,6) for dermatophyte identification from samples from cats, and achieved high specificity for dog's sample (AUC=94,1 – 94,4). Sensitivity vary between 94,9 and 100% for detection in samples from cats and dogs respectively. Technique non-already applied to the differentiation of *M*icrosporum *canis* from *Trichophyton interdigitale*, and geophilic dermatophytes (Cafarchia *et al.*, 2013).

Real-time PCR presents sensitivity (100%, with confidence interval 95%) and specificity (88,5%, with confidence interval 95%) for diagnosis of *M. canis* in cats. In a study performed by Jacobson at al. at 2017 no false-negative results were observed, but false-positive results were relatively common (Jacobson *et al.*, 2017).

Identification by Real-time multiplex PCR is commonly applied to clinical specimens from humans for the accurate identification of casual and common agents. In comparison with the conventional 'gold standard' procedures for dermatophyte diagnostics, positive predictive value of the multiplex assay is 95,7%, while the negative predictive value is it 100% (Arabatzis *et al*., 2007).

A Real-time LightCycler PCR protocol followed by **RFLP** is also available for clinical specimens from humans. Using seven primer sets specific for fungal DNA. Two subsequent LightCycler PCR reactions and one RFLP reaction allow the differentiation of dermatophytes and non-dermatophyte molds and the sub classification of yeasts. However, melting point detection is much more sensitive than detection of bands on agarose gels after restriction digestion (Gutzmer *et al*., 2004).

Materials and methods

The PCR-protocol chosen for optimization and validation was developed and described by Dabrowska I. and colleagues in 2014 (Dabrowska *et al*., 2014). They based on the work by Brillowska-Dabrowska A. and colleagues from 2007 (Brillowska-Dabrowska *et al*., 2007).

Conducted research.

Reference dermatophyte strains – T. mentagrophytes and *M. canis*, CECT collection. Cryopreserved, recovered on CECT medium 87 and 72, after that plated on Sabouraud Dextrose Agar. *Fungal DNA isolation* - performed using NZY Plant/Fungi gDNA Isolation kit. *PCR Pan-Dermatophyte protocol parameters* - set of primers detecting a DNA fragment encoding chitin synthase1 of dermatophytes, panDerm_for (5'GAAGAAGATTGTCGTTTGCATCGTCTC3') and panDerm_rev (5'CTCGAGGTCAAAAGCACGCCAGAG3') . *PCR mixture composition*: 13,2 µl of PCR water, 0,3 µl of each primer at 50 µM, 0,5 µl of genomic/analyzed DNA and 10 µl of Master Mix (Supreme NZY Taq II 2x Green Master Mix). *Time-temperature profile*: initial denaturation for 3 min at 95°C followed by 45 s at 94°C, 45 s at 58°C and finally 45 s at 72°C for a total of 35 cycles. Final elongation step for 10 min at 72ºC. *Electrophorese parameters* - 1,5% gel agarose (0,75 g Canvax AgarPure Agarose LE and 50 ml of TBE water). Conditions: 5 µl of the PCR product + 0,5µl of GreenSafe, for 30 minutes, 90 V, 500 A. For procedure was used NZY DNA Ladder VII as molecular-weight size marker.

Research phases:

- Verification of the effectiveness of the protocols and ingredients;
- Examination of the specificity. For this step, beyond the reference strains of species *T. mentagrophytes* and *M. canis* was extracted DNA from the others pathogenic fungi, could be identified frequently in the skin lesions similar to dermatophytosis - *Fusarium spp., Rhodotorula spp., Penicillium spp., Candida spp., Alternaria spp., Chrysosporium spp., Aspergillus niger* and *Aspergillus flavus*. Were analyzed 10 DNA samples, 2 of them from dermatophytes. Were detected 2 of 2, without false-negative and false-positive results;
- Examination of the sensitivity, working directly with clinical samples, was based on counts of CFU on the plates for each suspension. Also, it was added some passes (pre-preparing of the sample – disinfection with Iodine and posterior washing; and incubation prior DNA extraction). Results of this countis was not presented as they are not definitive.

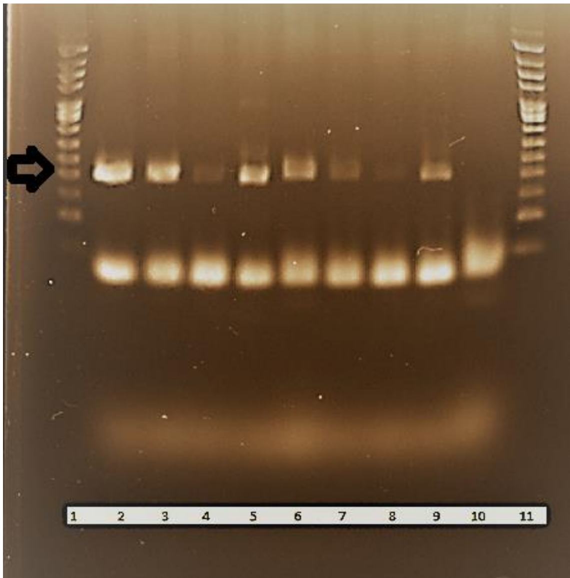

Figure 1 - Testing the protocol and DNA extraction kits (NZYTech and Canvax):

1 and 11 – Ladder; 2 – *Trichophyton mentagrophytes* NZYTech (DNA 1:1); 3 – *T. mentagrophytes* Canvax (DNA 1:1); 4 – *Microsporum canis* NZYTech (DNA 1:1); 5 – *M. canis* Canvax (DNA 1:1); 6 - *T. mentagrophytes* NZYTech (DNA 10^{-1}); 7 – *T. mentagrophytes* Canvax (DNA 10^{-1}); 8 – *M. canis* NZYTech (DNA 10^{-1}); 9 – *M. canis* Canvax (DNA 10^{-1}); 10 – Negative control.

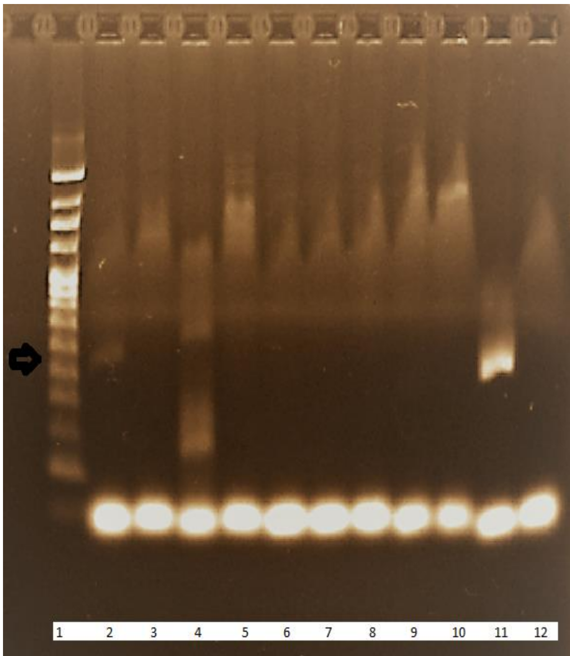

Figure 2 - Testing specificity of the protocol.

1 – Ladder; 2 – *M. canis*; 3 – *Fusarium sp.*; 4 – *Rhodotorula sp.*; 5 – *Penicillium sp.*; 6 – *Candida sp.*; 7 - *Alternaria sp.*; 8 – *Aspergillus niger*; 9 – *Aspergillus flavus*; 10 – *Chrysosporium sp.*; 11 – *T. mentagrophytes*; 12 – Negative control

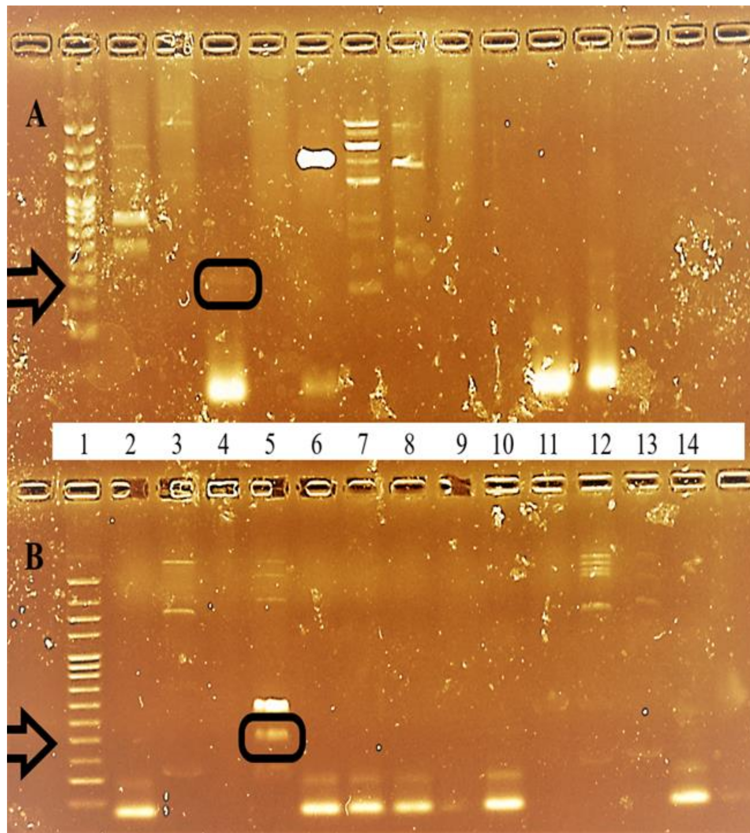

Figure 3 - Testing sensitivity for direct detection in cats´ hair sample, image of agarose gel electrophoresis

A - (*T.mentagrophytes*) 1 – Ladder; 2 - Reference strain; 3 – Original suspension (Cats´ hair incubated with reference strain suspended in normal saline to 0,5 McFarland scale); 4 - 1st suspension (10^{-1}); 5 – 2nd suspension (10^{-2}); 6 – 3rd suspension (10^{-3}); 7 – 4th suspension (10^{-4}); 8 – 5th suspension (10^{-5}); 9 – 6th suspension (10^{-6}); 10 – 7th suspension (10^{-7}); 11 – 8th suspension (10^{-8}); 12 – Negative control.

B - (*M.canis*) 1 – Ladder; 2 - Reference strain; 3 – Original suspension (Cats´ hair incubated with reference strain suspended in normal saline to 0,5 McFarland scale); 4 – unoccupied; 5 - 1st suspension (10^{-1}); 6 – 2nd suspension (10^{-2}); 7 – 3rd suspension (10^{-3}); 8 – 4th suspension (10^{-4}); 9 – unoccupied; 10 - 5th suspension (10^{-5}); 11 – 6th suspension (10^{-6}); 12 – 7th suspension (10^{-7}); 13 – 8th suspension (10^{-8}); 14 – Negative control.

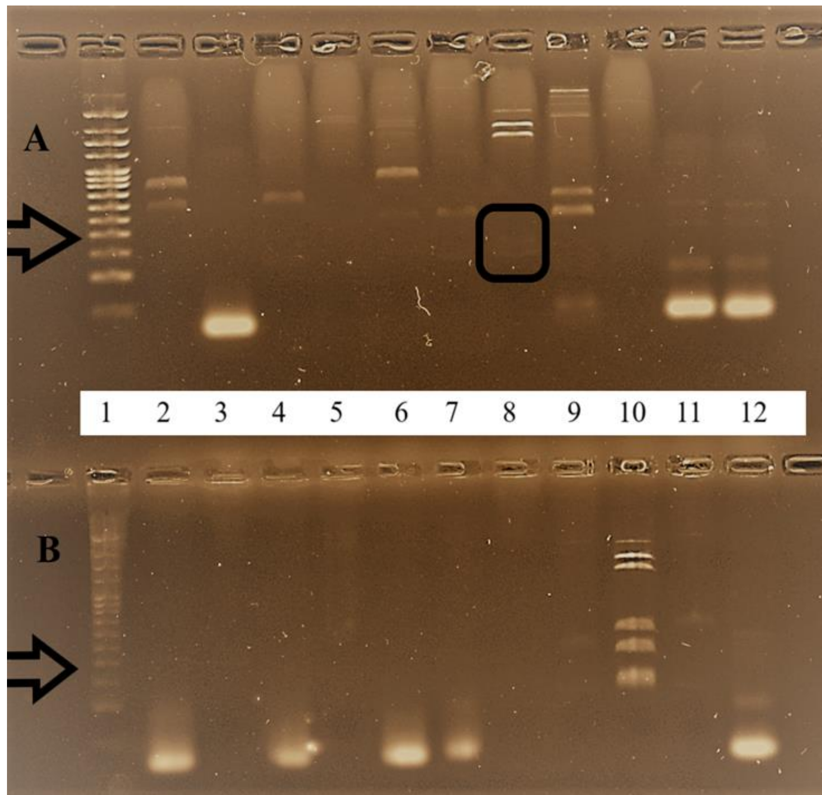

Figure 2.5 - Testing sensitivity for direct detection in dogs´ hair sample, image of agarose gel electrophoresis

A - (*T.mentagrophytes*) 1 – Ladder; 2 - Reference strain; 3 – Original suspension (Dogs´ hair incubated with reference strain suspended in normal saline to 0,5 McFarland scale); 4 – 1st suspension (10^{-1}); 5 – 2nd suspension (10^{-2}); 6 – 3rd suspension (10^{-3}); 7 – 4th suspension (10^{-4}); 8 – 5th suspension (10^{-5}); 9 – 6th suspension (10^{-6}); 10 – 7th suspension (10^{-7}); 11 – 8th suspension (10^{-8}); 12 – Negative control.

B - (*M.canis*) 1 – Ladder; 2 - Reference strain; 3 – Original suspension (Dogs´ hair incubated with reference strain suspended in normal saline to 0,5 McFarland scale); 4 – 1st suspension (10^{-1}); 5 – 2nd suspension (10^{-2}); 6 – 3rd suspension (10^{-3}); 7 – 4th suspension (10^{-4}); 8 – 5th suspension (10^{-5}); 9 – 6th suspension (10^{-6}); 10 – 7th suspension (10^{-7}); 11 – 8th suspension (10^{-8}); 12 – Negative control.

EPIDEMIOLOGY

The challenges that animal and public health have in common that they require identification, quantification and intensive examination of multiple, directly or indirectly causal, and often interacting, disease determinants.

Descriptive epidemiology

Materials and methods.

In this study, 1988 dogs and 805 cats with clinically suggestive ringworm lesions were investigated for the presence of dermatophytes by fungal culture, the gold standard diagnosis technique for the present time. Samples were collected during a 16-year period (2001-2016) and included hairs and scales plucked from the lesion's periphery. All samples were inoculated in Sabouraud dextrose agar supplemented with cycloheximide and chloramphenicol, incubated for 21 days at 28ºC and observed daily for the growth of dermatophytes. Identification of the dermatophyte species was performed by micro and macroscopic examination of colonies and smears.

Results.

Out of the 1988 dogs included in this study, 1795 animals (90,29%) were negative, and only 193 animals (9,71%) were proved to be positive for dermatophytes. As expected, species identified belonged to the genera *Microsporum* (n=139 / 72,02%) and

Trichophyton (n=54 / 27,98%). The most frequently identified dermatophyte species are *Microsporum canis* (n=101 / 52,33%), *Trichophyton mentagrophytes* (n=27 / 13,99%) and *Microsporum gypseum* (n=14 / 7,25%).

Regarding cats, 638 animals (79,25%) are negative, the percentage of positive animals was higher - 167 animals (20,75%) positive for dermatophytes. In resemblance to the dogs, dermatophyte species identified belongs to the genera *Microsporum* (n=142 / 85,03%) and *Trichophyton* (n=25 / 14,97%). In these cases, *M. canis* (n=123 / 73,65%), *T. mentagrophytes* (n=18 / 10,78%) and *Microsporum nanum* (n=7, 4,19%) were the most frequently identified species.

Analytical epidemiology

Materials and methods.

It was used an R-Studio software, R version 3.4.3 (2017-11-30), to estimate a Chi-test values. For calculating the confidence limits (CL) for the proportions was used site EpiTools – epidemiological calculators, by method of Wilson, confidence level 0,95.

It was analyzed the same pool of dates used in Descriptive Epidemiology – samples from dogs and cats with clinically suggested ringworm lesions, collected from 2001 till 2016.

Evaluated factors: age, breed, gender and season of the year.

Results

Dog´s age. The results of calculations (for this population X-squared 16,954 and P-value = 0.004589) suggest that there is an association between the age of the dog and the possibility of having dermatophytosis. Thus, the age could be considered a risk factor that influences susceptibility of the dogs to dermatophytes. Row Percentage of ill animals in this data set is 24,2% less than 2 month of age, 12,8% for 2 to 6 month, and 10,0% for 6 to 12 months of age. Prevalence / Proportion, respectively, is 0,24; 0,13 and 0,1, with Confidence Level=0,95. Corresponding indicators at the age of more than one year are lower.

Cat´s age. The results of calculations (for this population X-squared 111,28 and P-value $<2,2^{-16}$) strongly suggest that there is an association between the age of the cat and the possibility of having dermatophytosis. Thus, the age could be considered a risk factor that influences susceptibility of the cats to dermatophytes. Row Percentage of ill animals in this data set is 78,9% less than 2 month of age, 63,6% for 2 to 6 month, and 23,2% for 6 to 12 months of age. Prevalence / Proportion, respectively, is 0,79; 0,64 and 0,23, with Confidence Level=0,95. Corresponding indicators at the age of more than one year are lower.

Dog breeds. The results of calculations (for this population X-squared 15,004 and P-value = 0,05907) are slightly above the boundary value, that suggest that there is association between the breed of the dog and the possibility of having dermatophytosis. Basing on this information, breed couldn´t be considered a risk factor that influences susceptibility of the dogs to dermatophytes, however this possibility not totally excluded. The Row Percentage of ill animals in this data set is 7,1% for Boxers, 10,0% for French Bulldogs, 16,2% for Poodles, 5,2% for Huskies, 7,8% for Labradors, 7,1% for German Shepherds, 20% for Retrievers, 11,1% for Rottweilers and 28,6% for Yorkshires. Prevalence / Proportion is 0,07; 0,10; 0,16; 0,05; 0,08; 0,07; 0;20; 0,11 and 0,20 respectively, with Confidence Level=0,95.

Cat breeds. The results of calculations (for this population X-squared 19,582 and P-value = 0.001497) strongly suggest that there is an association between the breed of the cat and the possibility of having dermatophytosis. Thus, breed could be considered a risk factor that influences susceptibility of the cats to dermatophytes. Row Percentage of ill animals in this data set is 23,1 % for Norwegian forest cat, 36,2% for Persian, and 12,5% for Siamese. Prevalence / Proportion, respectively, is 0,23; 0,36 and 0,113, with Confidence Level=0,95.

Dog´s gender. In this case, X-squared = 0.0728, P-value = 0.7873, Row percentage for Female Dogs 9,4 and for Male 9,7 and

Prevalence / Proportion for Female 0,09 and for Male 0,1, with Confidence Level 0,95. It suggest that there are no association between dog gender and susceptibility to dermatophytosis. So, gender in dogs could not be considered a risk factor for this disease.

Cat´s gender. In this case, X-squared = 6,2723 and P-value = 0.01226, Row percentage for Female Cat´s 17,2% and for Male 24,5 and Prevalence / Proportion for Female 0,17 and for Male 0,2. It suggest that there is possibility to have an association between cat's gender and susceptibility to dermatophytosis. So, gender could be considered a risk factor for this disease in cats.

Season of the year for dogs. In this case, X-squared = 11,288, P-value = 0.4195, Prevalence / Proportion summarily for Winter 0,09, Spring 0.1, Summer 0,09 and Autumn 0,10, with confidence Level 0,95. It suggest that there are no association between season of the year and dogs´ susceptibility to dermatophytosis. So, it could not be considered a risk factor for this disease.

Season of the year for cats. In this case, X-squared = 39,346, P-value = 0.00004625. Prevalence / Proportion summarily for Winter 0,17, Spring 0,17, Summer 0,14 and Autumn 0,29, with confidence Level 0,95. It strongly suggest that there is an association between season of the year and cats´ susceptibility to dermatophytosis, with special stand out of the Autumn. So, time of the year should be considered a risk factor in cats for this disease

DISCUSSION

Dermatophytes are not life threatening microbial agents, but they are distributed around the world and cause acute or chronic mycotic infections with high morbidity, but not mortality (Behzadi *et al.*, 2014). Epidemiological, etiological and clinical patterns of fungal infections caused by dermatophytes are changed in many aspects. From one point of view, it leads to changes and the emergence of alternatives in the therapeutic and diagnostic protocols. From another, explains and promotes the search for new methods of identification, processing and periodical updating of the epidemiological information (Skerlev *et al.*, 2010; Zhan *et al*, 2016).

Identification and detection tools, based on PCR technique, have a high degree of sensitivity and specificity, and can be performed in a short time (Verrier *et al.*, 2012; Carfarchia *et al.*, 2013; Dabrowska *et al.*, 2014; Jacobson *et al.*, 2017; Moriello *et al.*, 2017). By these indicators they are better than the usual methods of diagnosis and identification.

Method, described by Briilowska-Dabrowska and colleagues in 2007, and later used by Dabrowska and coworkers in 2014, was optimized and applied for dermatophyte detection directly from veterinarian clinical specimens. It shows high degree of specificity and promising sensitivity (**Figure 2 and 3**), clearly above all the conventional diagnostic and identification methods commonly used in practice. However, this relatively simple and rapid method, based on one-step PCR technique still needs some additional work. DNA extraction, Pan Dermatophyte PCR and further electrophoresis realization guaranteed detection of the presence of the dermatophytes in clinical specimens, but do not distinguish between detected dermatophyte species.

Obtained epidemiological data are also very important to provide a complete view on a clinical case. In general terms evaluation of mentioned potential risk factors - age, breed, gender and season of the year, and descriptive part, coincides with already published studies.

But this compatibility is not complete, there are some differences – changing of the frequency of isolation of some species (we can find a similar pattern in studies of human dermatophytosis). It also looks like that disease incidence also increase in some recent years (Skerlev et al., 2010; Zhan et al., 2016).

Those changes and incompatibility to preview studies could be explained considering following factors:

- Growing globalization and consequently reduction of restriction criteria for circulation of

- persons and goods (last two particularly prevailing in actual EU);
- The high-dose immunosuppressive therapies widely used, the application of preventive antibiotic therapy against bacterial infections that can promote fungal infections;
- Ubiquity of the dermatophytes;
- Increasing spread of exotic animals (new companion animals) and strong relations between humans and dogs and cats.

All referred factors lead to growing number of mycosis in general terms, and dermatophytosis particularly. Changing of the natural habitat and emergence of new hosts may cause modifying in pathogenicity and consequently clinical signs. During interpretation of results of epidemiological calculation, it is also important to have in account an influence of the anthropogenic factor – conditions of animal welfare, social and cultural characteristics of the owners, breeders work.

Only after final calculations and recording all factors mentioned above it is possible come to conclusions about epidemiological situations in relations of the dermatophytosis.

CONCLUSIONS

One-step PCR was shown as highly sensitive, specific and rapid method of identification of the dermatophytes in veterinary clinical samples. This makes diagnosis more accurate and rapid, and therefore allows to treat a disease faster and cheaper. It needs some additional work because this method does not distinguish between dermatophyte species, and still has no validation in accordance to ISO/FDIS 16140:2000(E) and its actual versions .

Updated epidemiological data, in parallel with diagnostic techniques, are also important tools for choosing a method of treatment and monitoring the general situation with dermatophytosis.

Dermatophytosis is not a fatal disease, however reminding such characteristic’s as transmissibility between animals and humans and ubiquity of fungi it is very important to have modern and efficient tools for disease control.

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