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Antifungal Susceptibility Testing to Different Antifungal Agents to Isolats of *M. canis* from Dogs

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Abstract: In this study, presence of superficial dermatomyces and *Candida* have been analysed on fungus infected suspicious dogs which are fed at various shelters brought to the Istanbul University Department of Microbiology and special clinics. The analyis has been performed with reference to the macrodilution method suggested at the NCCLS M38-A document and effect of fluconazole, ketoconazole, itraconzole, terbinafine, griseofulvin and miconazole have been analysed on the isolated agents. For this purpose, samples of skin scrapings and hairs have been collected from 85 dogs with skin lesion. After the mycologic analysis of the samples, 24 *M. canis* and one *M. nanum* strain have been isolated. It has been determined as a conclusion of this study that the dominant superficial dermatophyte species in dogs is *M. canis* and that terbinafine is the most effective antifungal against *Microsporum species* and hence considered an alternative for the azoles used in treatments.

Key words: Antifungal susceptibility, *M.canis*, superficial dermatomyces, azoles, terbinafine

INTRODUCTION

In the keratin-rich tissues, the species *Microsporum*, *Trichophyton* and *Epidermohyton* which cause superficial fungal infections are significant cutaneous mycosis factors defined as dermaphytosis (Weitzman and Summerbell, 1995). Due to the fact that interest in and contact with pets are growing today, the importance of zoonotical fungal infections has increased (Chinelli *et al*., 2003; Kozak *et al*., 2003; Mancianti *et al*., 2003; Spiewak, 1998).

Researchers have reported that epidemiological data in fungal infections are limited and have associated it with the fact that reporting thereof is not obligatory although they are contagious. Researches have emphasized that such data are very valuable and significant in terms of discovering the resources of infections, active isolation and identification, starting actions for treatment within a very short time and achievement of success (Weitzman and Summerbell, 1995).

Researchers have stated that due the fact that the variety of medications is limited and there is not much resistance, there was no need for antifungal sensitivity tests until the 1980s, when use of azoles was initiated (Hospenthal *et al*., 2004). As a result of increase in the number of antifungal drugs available today, occurrence of resistant strains and increasing incidence of fungal infections, studies on sensitivity and standardization has gained importance (Epsinel-Ingroff, 2002).

Studies aimed for development of a reference method which can be used worldwide have increased recently (Pujol *et al*., 2002). M38-P has been suggested in 1998 and M38-A in 2002 to be used for filamentous fungi (NCCLS, new title Clinical and Laboratory Standards Institute = CLSI). No reference method has been suggested for dermatophytes yet (National Committe for Clinical Laboratory Standard, 1997; 1998; 2002a; 2002b).

In this study, it is aimed to determine the types of superficial dominant dermatophytes on the skin scrap and hair samples collected from the dogs kept in various shelters, suspected to have fungal infections and brought to the University of Istanbul, Faculty of Veterinary Medicine, Laboratory of the Department of Microbiology and to private clinics and to research the effectiveness of flukonazole, ketokonazole, itrakonazole, terbinafine, griseofulvine and mycokonazole using the method of macrodilution to isolated factors.

MATERIALS AND METHODS

Samples: In this study,

- Samples of skin scraps and hairs collected from 85 dogs kept in various shelters in Istanbul, suspected to have fungal infections in the clinic examination and brought to private clinics and to the University of Istanbul, Faculty of Veterinary Medicine, Laboratory of the Department of Microbiology were used for isolation of factor.

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- Factors isolated as a result of culture were used in the antifungal sensitivity tests.

Media: For isolation of superficial dermatophytes, Sabouraud's Dextrose Agar (SDA, Acumedia 7150 A) and Dermatophyte Test Medium (DTM, MERCK 1.10896) was used, for isolation of the *Candida* strains, actidion-free SDA was used and for antifungal sensitivity tests, RPMI-1640 (Sigma, R6504) liquid medium was used.

Antifungals: Sensitivity of the isolated factors towards ketokonazole (Ilsan), mycokonazole (Ilsan), itrakonazole (Ulkar), flukonazole (Eczacibaþi), griseofulvine (Sanofi Synthelabo) and terbinafine (Santafarma) was investigated.

Reference strains: In this study, the strains *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as control strains (Mancianti *et al*., 1997). The strains were obtained from the University of Istanbul, Faculty of Medicine, Research and Implementation Center of Microorganism Culture Collections.

Isolation and identification: The collected samples were sown into the SDA and actidion-free SDA. The SDA was incubated for 4 weeks at 26°C. The cultures were checked every 5 weeks. The actidion-free SDA media were incubated for 7 days at 37°C. The cultures were checked daily for generation. At the end of the incubation period, the suspected colonies isolated in SDA were passed to DTM and to SDA and were incubated in the same medium. The cultures, which were incubated at 26°C and 37°C, were evaluated in terms of reproduction condition, colony form, topography, top side and bottom side appearance of the colony and existence of pleomorphism. Preparations were prepared with the samples taken from various places of the generating colonies between micro-slides and cover glasses painted with lactophenol cotton blue. The preparations were then examined with 400 × enlargement. Hifas, macroconidium and fungi-related structures were seen in the examination.

Antifungal sensitivity test

Preparation of stock solution: In this study, stock solution was prepared from the selected antifungals using Dimethyl Sulfoxide (DMSO) as solvent according to the NCCLS M38-A standards. Antifungal was weighed to be 100 times of the final concentration in an amount that can be dissolved in 1 mL of DMSO according to the below formula.

$$\text{Weight (mg)} = \frac{\text{Volume (mL)} \times \text{Concentration}(\mu g\,mL^{-1})}{\text{Potency}\ (\mu g\,mg^{-1})}$$

One millilitter DMSO was added into the sterile tubes containing antifungal and the tubes were stored at -20°C until they were used. Double dilutions were made from this solution in the predetermined concentration ranges (ketokonazole 4-0.008 μg mL^{-1}, mykonazole 1-0.001 μg mL^{-1}, itrakonazole 1-0.001 μg mL^{-1}, flukonazole 128-0.25 μg mL^{-1}, griseofulvine 4-0.008 μg mL^{-1}, terbinafine 0.06-0.0001 μg mL^{-1}) (National Committee for Clinical Labortary Standard, 2002).

Preparation of inoculums: The seven-day old colonies in the SDA medium were collected into sterile tubes with 1 mL of 85% saline suspension. They were then left for 15 min for precipitation of the heavy particles and the homogenous conidia suspension on top was transferred to another sterile tube and was mixed for 15 min. The suspension concentration was adjusted according to the McFarland 0.5 tube. Finally, the suspension was diluted as RPMI-1640 medium with a percentage of 1:100 (National Committee for Clinical Labortary Standard, 2002).

Liquid macrodilution test: A 10 μL from each antifungal stock solution in the tubes were put in the predetermined concentration ranges. Total 90 μL of RPMI-1640 medium was added into each tube and 1/10 dilution was obtained. Following these steps 900 μL of the conidia suspension was added into the tubes (1/100 dilution). A tube containing medium and conidia suspension was added to the test as the positive control and another tube containing antifungal and medium was added as the negative control. Inoculated macrodilution tubes were then incubated for seven days at 26°C (National Committee for Clinical Labortary Standard, 2002a).

Determination of Minimum Inhibitor Concentration (MIC) final points: Test results were determined through comparison with the positive control tube and based on a 0-4 scale (0: no reproduction [MIC 0], 1: 75-80% reduction in reproduction [MIC 1], 2: 50% reduction in reproduction [MIC 2], 3: 25% reduction reproduction [MIC 3], 4: no reduction in reproduction [MIC 4]).

The test tubes were then compared with the control tube at the end of the incubation time in order to determine the lowest concentration where inhibition of 100%, 75-80% and 50% were seen in terms of reproductions. The dilution, which showed the lowest inhibition, was determined separately as the Minimum Inhibitor Concentration (MIC).

RESULTS

Isolation and identification findings: Tota 24 *M. canis* (28.24%) and one *M. nanum* (1.18%) were isolated from

Table 1: Inhibition final points and arithmetical averages according to MIC-0

	MIC μg mL^{-1} (n = 25)																					MIC range μg mL^{-1}	Arithmetical av.
	0.0001	0.0002	0.0005	0.009	0.002	0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128		
MIC-0																							
F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	7	8	7*	≥16-128≤	67.2
K	-	-	-	-	-	-	-	-	-	-	1	2	3	5*	10	4	-	-	-	-	-	≥0.125-4≤	1.73
G	-	-	-	-	-	-	-	-	-	-	-	-	1	8	13*	3	-	-	-	-	-	≥0.5-4≤	1.86
M	-	-	-	-	-	-	-	-	-	-	-	9*	6	10	-	-	-	-	-	-	-	≥0.25-1≤	0.61
I	-	-	-	-	-	-	-	-	-	3*	2	2	6	12	-	-	-	-	-	-	-	≥0.06-1≤	0.64
T	-	-	-	-	5	8	6	3*	3	-	-	-	-	-	-	-	-	-	-	-	-	≥0.002-0.03≤	0.009

Table 2: Inhibition final points and arithmetical averages according to MIC-1

	MIK μg mL^{-1} (n = 25)																					Mik range μg mL^{-1}	Arithmetical
	0.0001	0.000	0.0005	0.009	0.002	0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128		
MIK-1																							
F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	6	4	4	4	3*	-	≥2-64≤	19.3
K	-	-	-	-	-	-	-	-	-	2	5	5*	4	7	-	-	-	-	-	-	-	≥0.0.6-1≤	0.48
G	-	-	-	-	-	-	-	-	3	2	3	6	8	3*	-	-	-	-	-	-	-	≥0.03-1≤	0.36
M	-	-	-	-	-	-	1	2*	1	3	10	5	2	-	-	-	-	-	-	-	-	≥0.008-0.5≤	0.16
I	-	-	-	-	1	-	-	3*	4	3	7	6	-	-	-	-	-	-	-	-	-	≥0.002-0.25≤	0.11
T	-	2	5	8	5	4*	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	≥0.0002-0.015≤	0.009

Table 3: Inhibition final points and arithmetical averages according to MIC-2

	MIK μg mL^{-1} (n = 25)																					Mik range μg mL^{-1}	Arithmetical ort
	0.0001	0.0002	0.0005	0.009	0.002	0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128		
MIK-2																							
F	-	-	-	-	-	-	-	-	-	-	-	4	1	5	5	3	4*	3	-	-	-	≥0.25-16≤	4.34
K	-	-	-	-	-	-	2	1	5	10*	3	2	2	-	-	-	-	-	-	-	-	≥0.008-0.5≤	0.11
G	-	-	-	-	-	-	-	4	8	7*	5	1	-	-	-	-	-	-	-	-	-	≥0.015-0.25≤	0.06
M	-	-	-	-	-	3	4*	9	6	2	-	-	1	-	-	-	-	-	-	-	-	≥0.004-0.5≤	0.04
I	-	-	-	-	1*	2	9	4	5	3	1	-	-	-	-	-	-	-	-	-	-	≥0.0002-0.125≤	0.02
T	4	8	7	4*	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	≥0.0001-0.002≤	0.0005

*Shows the MIC values for one isolated *M. nanum* strain. F = Flukonazole, K = Ketokonazole, M = Mykonazole, I = Itrakonazole, G = Griseofulvine, T = Terbinafine

the total of 85 collected samples. It has determined that the dominant superficial dermatophyte strain in dogs is *M. canis*.

Antifungal sensitivity findings: Inhibition final points and arithmetical averages according to MIC-0, MIC-1, MIC-2 range of *Microsporum* sp. isolates for flukonazole, ketokonazole, mykonazole, itrakonazole, griseofulvine, terbinafine are available from Table 1-3.

As a result of the MIC-0, MIC-1 and MIC-2 evaluations, respectively (≥0.002-0.03≤ μg mL^{-1}, ≥0.0002-0.015≤ μg mL^{-1}, ≥0.0001-0.002≤ μg mL^{-1}), the most effective antifungal was determined to be terbinafine in terms of its MIC ranges (0.009, 0.002 and 0.0005 μg mL^{-1}) and arithmetical averages.

It was determined that flukonazole was the least effective antifungal in terms of its three MIC ranges (≥16-128≤ μg mL^{-1}, ≥2-64≤ μg mL^{-1}, ≥0.25-16≤ μg mL^{-1}) and arithmetical averages (67.2, 19.3 and 4.34 μg mL^{-1}).

According to the arithmetical averages in terms of MIC-0, it was determined that, respectively mykonazole, itrakonazole, ketokonazole, griseofulvine were placed between terbinafine and flukonazole (Table 1). According to the arithmetical averages in MIC-1 and MIC-2, the above order was changed to itrakonazole, mykonazole, griseofulvine, ketokonazole following terbinafine (Table 2 and 3).

DISCUSSION

It has been reported that the incidence of dermal diseases in humans and animals caused by superficial dermatophytose factors are increasing today (Mancianti *et al*., 1997, 2003) and that the most commonly isolated factor in cats and dogs is *M. canis* (Cabanes *et al*., 1997; Sparkes *et al*., 1993). In this study, 28.24% *M. canis* and 1.18% *M. nanum* has been isolated from samples of skin scraps and hairs collected from 85 dogs with skin lesions. The fact that the *M. canis* isolation rate is in the first place has been found compatible by the researches.

Various researches abaout antifungal susceptibility testing have conducted studies in order to determine such methods and conditions (Epsinel-Ingroff, 2002). It has been reported that different results were attained in the labs in the studies conducted for standardization. It is estimated that such different results are due to host-related factors, drug interactions, medium composition,

pH, preparation of inoculums, incubation temperature, application differences such as reading time and inhibition final points, as well as fungi-specific varieties in colony characteristics and reproduction time (Favre *et al*., 2003; Fernandez-Torres *et al*., 2001; Pujol *et al*., 2002). In this study, *in vitro* sensitivities of 24 *M. canis* and one *M. nanum* isolates towards flukonazole, ketokonazole, mykonazole, itrakonazole, griseofulvine and terbinafine have been evaluated using the NCCLS M38-A reference method. The inhibition final points have been separately determined as 50%, 75-80% and 100% for each antifungal. It has been revealed that the most effective and the least effective antifungals did not change in the 3 final points defined as MIC-0, MIC-1 and MIC-2, but the places of the ones between those two changed. This shows that different inhibition final points may be required in the evaluations conducted according to the antifungals used.

It has been reported in many studies that terbinafine, one of the antifungals used against *Microsporum* sp., forms inhibition of quite low concentrations (Fernandez-Torres *et al*., 2001; Hofbauer *et al*., 2002). Fernandez *et al*. (2001) have stated that terbinafine is the one of the most effective ones among those (vorikonazole, klotrimazole, amfoterisine-B, mykanozole, UR-9825, flukonazole, ketokonazole, itrakonazole, G-1 and terbinafine) they used in their study and that the MIC-2 inhibition final points for 105 *M. canis* strains was in the 0.007-16 μg mL^{-1} range. Hofbauer *et al*. (2002) compared the effectiveness of terbinafine and griseofulvine in 275 *M. canis* strains and revealed that terbinafine was the effective antifungal and was in the 0.002-0.25 μg mL^{-1} value range according to MIC-0. Jessup *et al*. (2000) conducted a study which investigated the effects of flukonazole, itrakonazole, griseofulvine and terbinafine on 217 dermatophytes (*M. canis*, n = 8) and reported that terbinafine was effective with the lowest concentrations among the 4 antifungals according to the MIC-1 final point evaluation system. The said study also revealed that the most effective antifungal among flukonazole, ketokonazole, mykonazole, itrakonazole, griseofulvine and terbinafine tested against the *Microsporum* sp. was terbinafine according to the 3 MIC-0, MIC-1 and MIC-2 final inhibition points. It was revealed that the result obtained was compatible with those of the other studies and that lower concentrations however formed inhibition in this study.

Fernandez-Torres *et al*. (2001) have stated that among the antifungals they used the MIC values of flukonazole for 105 *M. canis* strains was 0.06->64 μg mL^{-1}. Jessup *et al*. (2000) reported that flukonazole had the highest MIC values among those used against 217 dermatophyte strains (*M.canis*, n = 8). In the other studies conducted for dermatophytes, researchers revealed the same result for flukozanole (Pujol *et al*., 2002). In this study, which also revealed that flukozanole was the least effective antifungal in all the 3 inhibition final points defined as MIC-0, MIC-1 and MIC-2, it is seen that the results obtained are compatible with the results of the other researches.

The studies conducted in order to reveal the MIC value ranges of ketokonazole on *M. canis* using various MIC inhibition final points (MIC-0 and MIC-2) provided different results (Favre *et al*., 2003; Fernandez-Torres *et al*., 2001; Pujol *et al*., 2002). Pujol *et al*. (2002) revealed the 0.06-0.25 μg mL^{-1} according to MIC-2, Fernandez-Torres *et al*. (2001) revealed the 0.01-1 μg mL^{-1} value according to MIC-2, Favre *et al*. (2003) revealed the 1 μg mL^{-1} value according to MIC-1. In this study, the value obtained for ketokonazole according to MIC-0 was 0.125-4μg mL^{-1}, 0.06-1 μg mL^{-1} according to MIC-1 and 0.008-0.5 μg mL^{-1} according to MIC-2 and it has been determined that these values are lower than the MIC values determined in the researches. It is estimated that this may be caused by the differences in application conditions in the other studies.

Fernandez-Torres *et al*. (2001) found results in the 0.01-0.5 μg mL^{-1} range for mykonazole according to the 50% inhibition final point (MIC-2). It has been determined that the results they found are higher (MIC-2: 0.004-0.5 μg mL^{-1}) than the ones in this study.

As for the studies conducted for the effectiveness of itrakonazole, another antifungal of the azoles group, on *M. canis*, Favre *et al*. (2003) found the 0.06-2 μg mL^{-1} MIC values according to MIC-1 and Fernandez-Torres *et al*. (2001) found the 0.01-4 μg mL^{-1} MIC values according to MIC-2. In this study, it is revealed that the concentration ranges determined according to MIC-0, MIC-1 and MIC-2 are, respectively 0.06-1 μg mL^{-1}, 0.002-0.25 μg mL^{-1}, 0.002-0.125 μg mL^{-1} and that the results obtained are compatible in MIC-0 and that they have lower values in MIC-1 and MIC-2 (high effectiveness).

As for the evaluation of effectiveness of griseofulvine on *M. canis*, for the 100% inhibition final point (MIC-0), Hofbauer *et al*. (2002) reported that they found the 0.5->16 μg mL^{-1} MIC values. Favre *et al*. (2001) found the effectiveness range according to MIC-1 as 0.025-0.25 μg mL^{-1}. In this study, the effectiveness of griseofulvine according to MIC-0, MIC-1 and MIC-2 are found to be 0.5-4 μg mL^{-1}, 0.03-1 μg mL^{-1}, 0.015-0.25 μg mL^{-1}, respectively and these values are compatible with Favre *et al*. (2003) according to MIC-1.

CONCLUSION

It has been determined as a conclusion of this study that the dominant superficial dermatophyte species in

dogs is *M. canis* and that terbinafine is the most effective antifungal against *Microsporum species* and hence considered an alternative for the azoles usedin treatments.

The correct antifungal should be chosen for the treatment of dermatophyte infections, which are significant in terms of human and animal life in order to achieve success in treatment, to limit pervasion and to prevent such conditions from getting chronic. A standard method is needed to be used in antifungal selections which will provide obtainment of compatible results between and among laboratories. Therefore, parallel *in vivo* and *in vitro* studies should be conducted on this subject and the results obtained should be evaluated.

REFERENCES

Cabanes, F.J., M.L. Abarca and M.R. Bragulat, 1997. Dermatophytes isolated from domestic animals in Barcelona, Spain. Mycopahologia, 137: 107-113.

Chinelli, P.A.V., A.A. Sofiatti, R.S. Nunes and J.E.C. Martins, 2003. Dermatophyte agent in the city of Sao Paulo, from 1992-2002. Rev. Inst. Med. Trop., 45 (5): 259-263.

Epsinel-Ingroff, A., 2000. Clinical utility of *in vitro* antifungal susceptibility testing. Rev. Esp. Quimio, 13: 161-166.

Epsinel-Ingroff, A., V. Chaturvedi, A. Fothergill and M.G. Rinaldi, 2002. Optimal testing conditions for determining MICs and minimum fungicidal concentrations of new and established antifungal agents for uncommon molds: NCCLS collaborative study. J. Clin. Microbiol., 40 (10): 3776-3781.

Favre, B., B. Hofbauer, K. Hildering and N.S. Ryders, 2003. Comparison of *in vitro* activities of 17 antifungal drugs against a panel of 20 dermatophytes by using a microdilution assay. J. Clin. Microbiol., 41 (10): 4817-4819.

Fernandez-Torres, B., A.J. Carrillo, E. Martin, A. Del Palacio, K.M. Moore, A. Valverde, M. Serrano and J. Guarro, 2001. *In vitro* activities of 10 antifungal drugs against 508 dermatophyte strains. Antimicrob. Agent. Chemother., 45 (9): 2524-2528.

Hofbauer, B., I. Leitner and N.S. Ryder, 2002. *In vitro* susceptibility of *Microsporum canis* and other dermatophyte isolates from veterinary infections during therapy with terbinafine or griseofulvin. Med. Mycol., 40: 179-183.

Hospenthal, D.R., C.K. Murray and M.G. Rinaldi, 2004. The role of antifungal susceptibility testing in the therapy of candidiasis. Diagn. Microbiol. Infect. Dis., 48: 153-160.

Jessup, C.J., J. Warner, N. Isham, I. Hasan and M.A. Ghannoum, 2000. Antifungal susceptibility testing of dermatophytes: Establishing a medium for inducing conidial growth and evoluation of susceptibility of clinical isolates. J. Clin. Microbiol., 38 (1): 341-344.

Kozak, M., J. Bilek, V. Beladicova, K. Beladicova, Z. Baranova and A. Bugarsky, 2003. Study of the dermatophytes in dogs and the risk of human infection. Bratisl Lek Listy, 104 (7-8): 211-217.

Mancianti, F., C. Zullino and R. Papini, 1997. Itraconazole susceptibility of feline isolates of Microsporum canis. Mycoses, 40: 313-315.

Mancianti, F., S. Nardoni, M. Corazzo, P. D' Achille and C. Ponticelli, 2003. Environmental detection of *Microsporum canis* arthrospores in the household of infected cats and dogs. J. Feline Med. Surg., 5: 323-328.

National Committee for Clinical Laboratory Standards, 1997. Refence method for broth dilution antifungal susceptibility testing for yeast; approved standard. M27-A. National Committee for Clinical Laboratory Standards. Villanova, Pa.

National Committee for Clinical Laboratory Standards, 1998. Refence method for broth dilution antifungal susceptibility testing of conidium forming filamentious fungi; proposed Standard M38-P. National Committee for Clinical Laboratory Standards. Wayne, Pa.

National Committee for Clinical Laboratory Standards, 2002a. Refence method for broth dilution antifungal susceptibility testing of filamentious fungi; Approved Standard M38-A. National Committee for Clinical Laboratory Standards. Wayne, Pa.

National Committee for Clinical Laboratory Standards, 2002b. Refence method for broth dilution antifungal susceptibility testing for yeast; approved Standard-second edition. M27-A2. National Committee for Clinical Laboratory Standards. Wayne, Pa.

Pujol, I., J. Capilla, B. Fenandez-Torres, M. Ortoneda and J. Guarro, Use of sensititre colorimetric microdilution panel for antifungal susceptibility testing of dermatophytes. J. Clin. Microbiol., 40: 2618-2621.

Sparkes, A.H., T.J. Gruffydd-Jones, S.E. Shaw, A.I. Wright and C.R. Stokes, 1993. Epidemiological and diagnostic features of canine and feline dermatophytosis in The United Kingdom From 1956-1991. Vet. Rec., 133: 57-61.

Spiewak, R., 1998. Zoophilic and geophilic fungi as a cause of skin disease in farmers. Ann. Agric. Environ. Med., 5: 97-102.

Weitzman, I. and R.C. Summerbell, 1995. The dermatophytes. Clin. Microbiol. Rev., 8: 240-259.