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Evaluation of healthy farm and companion rabbits as carriers of dermatophytes

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ABSTRACT

The present study describes the occurrence of dermatophytes and their antifungal susceptibility in healthy rabbits kept on a farm or in a household as companion animals. Out of 614 animals examined, 146 samples (23.78%) were positive for dermatophytes by direct microscopic examination, and the dermatophytes were isolated from 112 samples (112/146, 76.71%). The four predominant species of dermatophytes isolated from healthy rabbits were *Trichophyton mentagrophytes* (37.5%), *Microsporum gypseum* (25%), *Microsporum nanum* (21.43%) and *Microsporum canis* (16.07%). The kits younger than 6 months of age showed a relatively higher occurrence rate, and male animals showed significantly higher occurrence than the females ($P<0.05$). Occurrence of dermatophytes in the studied rabbits was higher in summer and autumn than in spring and winter ($P<0.05$). Itraconazole (0.03-0.5 µg/mL), ketoconazole (0.06-0.5 µg/mL), miconazole at 0.03-0.5 µg/mL and 0.03-.25 µg/mL concentrations showed the lowest MIC values against *T. mentagrophytes*, *M. canis*, *M. nanum* and *M. gypseum*, respectively. However, fluconazole and griseofulvin showed the highest MIC value against the isolates, indicating antifungal resistance.

Key words: antifungal, dermatophytes, *Microsporum, Trichophyton*, rabbit, India

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Introduction

Dermatophytosis caused by three major genera, such as *Trichophyton*, *Microsporum* and *Epidermophyton,* is an endemic skin infection in many countries throughout the world. The infection generally involves companion animals (dogs, cats), domestic animals (cattle and buffaloes), but also human (SAMANTA, 2015). Recently an emerging trend of dermatophytosis has been observed among the rabbit population kept on commercial farms, laboratories, or in households as companion animals (CAFARCHIA et al., 2012). Undiagnosed dermatophytosis not only causes major economic losses on a rabbit farm (CAFARCHIA et al., 2010), but the rabbits also act as a reservoir for zoophilic dermatophytes (VAN ROOIJ et al., 2006).

Animal reservoirs play a major role in transmission of dermatophytes to the human population through the production of arthospores in their skin or hair. People in frequent close contact with the reservoir are always at higher risk (WEITZMAN and SUMMERBELL, 1995). Although the possibility of apparently healthy rabbits as a dermatophyte reservoir was ruled out in Germany (KRÄMER et al., 2012), several reports have described how dermatophytes were carried by healthy rabbits in other parts of the world (GALLO et al., 2005; CAFARCHIA et al., 2010; CAFARCHIA et al., 2012). Further, direct transmission of zoophilic dermatophytes to contact persons was also reported from rabbits with or without skin lesions (SIMALJAKOVÁ et al., 1989; NAKAMURA et al., 2002).

Rearing of rabbits is gaining popularity in India, for meat, fur/skin and wool, besides as companion animals (DAS et al., 2014). A very limited number of reports describing the isolation of dermatophytes (*Trichophyton mentagrophytes, T. simii*) from rabbits with skin lesions have been observed in the study area (MOHAPATRA et al., 1964; SINHA et al., 1989), and no systemic study has been performed to detect the carriage of dermatophytes by healthy rabbits. Further, antifungal resistance is a global problem which compromises the treatment regimen. Antifungal susceptibility tests optimize the therapy, by selecting an effective antifungal agent for the mycosis (CLSI, 2002). Thus the present study was aimed at detecting the occurrence of dermatophytes and their antifungal susceptibility in healthy rabbits kept on a farm or in a household as companion animals, which may act as potent carriers.

Materials and methods

A total of 614 rabbits, belonging to small rabbit farmers and individual owners in West Bengal (India), were examined for evidence of dermatophytosis in 2012-2013. The animals were apparently healthy, of both sexes, and all age groups, of the New Zealand White breed. The animals was divided into two groups according to age i.e. group 1 (0-6 months) and group 2 (>6 months). The samples were collected over four seasons, i.e. spring (March-May), summer (June-August), autumn (September-November) and winter (December-February).

Hairs from unused toothbrushes or hair brushes were collected after brushing the animal skin over the back, shoulders, sides, hindquarters and legs. Both the hair and the brushes were wrapped in brown paper (or coloured paper) and kept in an air-tight container, preferably without moisture, for transport to the laboratory.

The collected samples were primarily subjected to direct microscopic examination by fluorescence microscopy with calcofluor white stain (REBELL and TAPLIN, 1979).

The clinical samples were inoculated into both Sabouraud dextrose agar (SDA, HiMedia, Mumbai, India) with 0.05% chloramphenicol and 0.5% cycloheximide and dermatophyte test medium (DTM, HiMedia, Mumbai, India). The SDA plates were incubated at 28 °C for four weeks, and were observed periodically for the appearance of fungal growth. The DTM tubes were incubated at 28 °C for 10 days to detect any change in colour (ROBERT and PIHET, 2008).

Each of the fungal isolates was identified on the basis of its colony characteristics, and the hyphal and conidial cells it produced. The conidia were identified after lactophenol cotton blue staining on the basis of their size, shape, the presence of septa, the thickness of the conidial wall and the arrangement of conidial cells around the hyphae (PANG et al., 2008).

The antifungal susceptibility of the isolates was tested by broth micro dilution assay, using fluconazole, ketoconazole, itraconazole, miconazole, griseofulvin and amphotericin-B antifungals (HiMedia, Mumbai, India), as described earlier (ARAÚJO et al., 2009).

Differences in occurrence rates of dermatophytosis were compared according to age, sex and season, using the SPSS version 21 (SPSS Inc., Chicago, IL).

Results

Out of the 614 animals examined, 146 samples (23.78%) were positive for dermatophytes by direct microscopic examination (Fig. 1, 2). Out of the 146 direct examination positive samples, dermatophytes were isolated from 112 samples (112/146, 76.71%). The four predominant species of dermatophytes isolated from healthy rabbits were: *Trichophyton mentagrophytes* complex (37.5%), *Microsporum gypseum* (25%), *Microsporum nanum* (21.43%) and *Microsporum canis* (16.07%) (Table 1).

The kits younger than 6 months of age (group 1) showed a relatively higher occurrence of infection, which did not differ significantly from group 2 (>6 months) animals (Table 2). Male animals showed a significantly higher occurrence of dermatophytes than the females ($P<0.05$) in the present study (Table 2). The occurrence of dermatophytes in the studied rabbits was higher in the summer and autumn than in the spring and winter ($P<0.05$), when the temperature and humidity was relatively higher (Table 2).

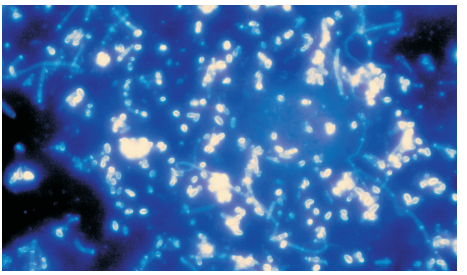

Fig. 1. Fluorescent microscopy (calcofluor white stain) of *Trichophyton mentagrophytes* complex hyphae and conidia isolated from healthy rabbits in India

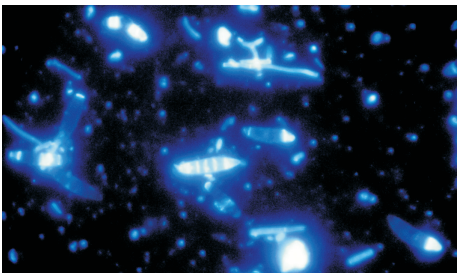

Fig. 2. Fluorescent microscopy (calcofluor white stain) of *Microsporum gypseum* hyphae and macroconidia isolated from healthy rabbits in India

Table 1. Dermatophyte species isolated from rabbits in West Bengal, India

	Rabbit	
Dermatophytes	n	Percentage
T. mentagrophytes	42	37.5
M. gypseum	28	25.0
M. nanum	24	21.4
M. canis	18	16.1
Total	112	18.24

Table 2. Variables of age, sex and season on rabbit dermatophytosis

Variables	No. of positive/ No. of animals examined	Percentage
Age		
0-6	64/343	18.66
>6	48/271	17.71
Sex		
Male	53/270	19.63
Female	48/271	17.15
Season		
Spring	8/154	5.19
Summer	59/156	37.82
Autumn	34/132	25.76
Winter	11/172	6.39

The antifungal susceptibility test of the isolated dermatophytes, by the broth micro dilution method, revealed a minimal inhibitory concentration (MIC) of 6 antifungal agents. Table 3 summarizes the MIC ranges, concentrations inhibiting 50% (MIC50) and 90% (MIC90) of the isolates. Itraconazole (0.03-0.5 µg/mL), ketoconazole (0.06-0.5 µg/mL), miconazole at 0.03-0.5 µg/mL and 0.03-.25 µg/mL concentrations showed the lowest MIC values against *T. mentagrophytes*, *M. canis*, *M. nanum* and *M. gypseum*, respectively. Further, 90% of *T. mentagrophytes*, *M. canis*, *M. nanum* and *M. gypseum* isolates were inhibited by 0.25 µg/mL of itraconazole (MIC_{90}), 0.125 µg/mL of ketoconazole (MIC_{90}), 0.25 µg/mL of miconazole and 0.125 µg/mL of ketoconazole (MIC_{90}), respectively. However, fluconazole and griseofulvin showed the highest MIC value against the isolates, indicating antifungal resistance (Table 3).

Table 3. *In vitro* antifungal susceptibility of dermatophyte isolates from rabbits in West Bengal, India

Species (No. of isolates)	Antifungal agents	MIC (µg/mL)		
		Range	MIC50	MIC90
T. mentagrophytes (42)	Fluconazole	4-64	16	64
	Ketoconazole	0.06-2	0.125	0.25
	Itraconazole	0.03-0.5	0.125	0.25
	Miconazole	0.03-1	0.06	0.125
	Griseofulvin	0.6-1	0.125	0.25
	Amphotericin-B	0.03-1	0.03	0.125
M. canis (18)	Fluconazole	4-64	16	32
	Ketoconazole	0.06-0.5	0.06	0.125
	Itraconazole	0.03-1	0.06	0.125
	Miconazole	0.03-0.5	0.06	0.25
	Griseofulvin	0.06-4	0.125	0.25
	Amphotericin-B	0.03-1	0.06	0.125
M. nanum (24)	Fluconazole	4-64	16	32
	Ketoconazole	0.06-0.5	0.06	0.125
	Itraconazole	0.03-1	0.06	0.125
	Miconazole	0.03-0.5	0.06	0.25
	Griseofulvin	0.06-4	0.125	0.25
	Amphotericin-B	0.03-1	0.06	0.125
M. gypseum (28)	Fluconazole	8-64	16	32
	Ketoconazole	0.03-1	0.06	0.125
	Itraconazole	0.03-2	0.25	0.5
	Miconazole	0.03-0.25	0.06	0.25
	Griseofulvin	0.06-2	0.125	0.5
	Amphotericin-B	0.03-0.5	0.06	0.125

Discussion

The present study was aimed at detecting the carrier status of dematophytes in healthy rabbits kept on a farm or in households. The study detected a moderate occurrence of dermatophytes (23.78%), which is in line with an earlier study of healthy cottontail rabbits (GALLO et al., 2005). However, a higher incidence (33%-61%) of dermatophytosis was observed in rabbits with or without skin lesions in Italy, Jordan and Nigeria (CAFARCHIA et al., 2010; ALI-SHTAYEH et al., 1988; NWEZE, 2011). Probably due to the differences in sample size and geographical location, the occurrence rate of the present study differed from others.

T. mentagrophytes was isolated with maximum frequency from the healthy rabbits in the present study. Similarly, the highest occurrence of *T. mentagrophytes* was detected in healthy and infected rabbits (CAFARCHIA et al., 2010; KRÄMER et al., 2012; CHERMETTE et al., 2008), including the environment of a rabbit farm (MARTINO et al., 2004). Further, *T. mentagrophytes* has also been isolated previously from diseased rabbits in India, where the present study was undertaken (MOHAPATRA et al., 1964). In corroboration with the current work, other dermatophyte species, such as *M. gypseum* and *M. canis,* were also detected earlier in healthy cottontail rabbits[7,] as well as in diseased and healthy rabbits (CAFARCHIA et al., 2010; ALI-SHTAYEH et al., 1988). Isolation of *M. nanum* from rabbits is rare, although some reports were found (REFAI et al., 1970; KRÄMER et al., 2012).

No significant difference was observed in the occurrence rate between young and adult age groups of healthy rabbits. Similarly the occurrence of dermatophytes was detected in kits (>5 months) in a few studies (KRÄMER et al., 2012; KRAEMER et al., 2012) as well as in fattening and finishing stages of growth in another study (CAFARCHIA et al., 2010). So it seems that the age of the animals did not contribute to the carriage of dermatophytes in healthy rabbits. However, male animals showed a significantly higher occurrence of dermatophytes than the females. A similar gender predisposition for the occurrence of dermatophytosis in diseased rabbits was observed earlier in Germany (KRÄMER et al., 2012). However, healthy cottontail rabbits did not show any gender predisposition for the carriage of dermatophytes in Italy (GALLO et al., 2005). Probably due to the differences in breed, the sample size for male and female animals and geographical location, the gender predisposition for occurrence of dermatophytes here differed from other studies.

The present study detected a significantly higher occurrence of dermatophytes in the summer and autumn than in the spring and winter. Similarly, CAFARCHIA et al. (2010) and GALLO et al. (2005) detected a higher dermatophyte prevalence in areas with higher temperatures (>20 °C) and relative humidity (62-65%), or during warmer months (May-September), respectively. It seems that the tropical climate is ideal for the carriage of dermatophytes by healthy rabbits.

The study detected itraconazole, ketoconazole and miconazole as effective, and fluconazole and griseofulvin as resistant antifungals, against the dermatophyte isolates. Similarly, itraconazole, with the lowest MIC activity (0.03-0.5 µg/mL), and fluconazole with the highest MIC activity (0-24 µg/mL), were detected earlier against human dermatophyte isolates (SANTOS et al., 2006; ARAÚJO et al., 2009; AKTAS et al., 2014).

Deramtophytosis is a major public health problem in the world, especially in tropical countries due to the high temperatures and humidity, over-population and poor hygienic conditions. Recently it has been observed as an emerging infection in the study area, and may become an epidemic, especially in people who have direct contact with the animals (GHOSH et al., 2014). Further, as rabbit rearing is gaining popularity in the study area and throughout the world, the present study warns of the need for hygienic maintenance of rabbits and judicial use of antifungals.

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References

AKTAS, A. E., N. YIGIT, A. AKTAS, S. G. GOZUBUYUK (2014): Investigation of *in vitro* activity of five antifungal drugs against Dermatophytes species isolated from clinical samples using the E-test method. Eurasian J. Med. 46, 26-31.

ALI-SHTAYEH, M. S., H. M. ARDA, M. HASSOUNA, S. F. SHAHEEN (1988): Keratinophilic fungi on the hair of cows, donkeys, rabbits, cats, and dogs from the West Bank of Jordan. Mycopathol. 104, 109-121.

ARAÚJO, C. R., K. C. MIRANDA, O. F. L. FERNANDES, A. J. SOARES, M. R. R. SILVA (2009): *In vitro* susceptibility testing of dermatophytes isolated in Goiania, Brazil, against five antifungal agents by broth microdilution method. Rev. Inst. Med. Trop. S. Paulo. 51, 9-12.

CAFARCHIA, C., A. CAMARDA, C. COCCIOLI, L. A. FIGUEREDO, E. CIRCELLA, P. DANESI, G. CAPELLI, D. OTRANTO (2010): Epidemiology and risk factors for dermatophytoses in rabbit farms. Med. Mycol. 48, 975-980.

CAFARCHIA, C., L. A. FIGUEREDO, C. COCCIOLI, A. CAMARDA, D. OTRANTO (2012): Molecular identification and phylogenesis of dermatophytes isolated from rabbit farms and rabbit farm workers. Vet. Microbiol. 154, 395-402.

CHERMETTE, R., L. FERREIRO, J. GUILLOT (2008): Dermatophytoses in animals. Mycopathol. 166, 385-405.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI) (2002): Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Wayne.

DAS, S. K., E. B. CHAKURKAR, N. P. SINGH (2014): Rabbit as an alternative source of meat production. Technical Bulletin No. 45, I.C.A.R. Research Complex for Goa, I.C.A.R.

GALLO, M. G., P. TIZZANI, A. PEANO, L. RAMBOZZI, P. G. MENEGUZ (2005): Eastern cottontail (*Sylvilagus floridanus*) as carrier of dermatophyte fungi. Mycopathol. 160, 163-166.

GHOSH, R. R., R. RAY, T. K. GHOSH, A. P. GHOSH (2014): Clinico-mycological profile of Dermatophytosis in a tertiary care hospital in West Bengal- an Indian scenario. Int. J. Curr. Microbiol. App. Sci. 3, 655-666.

KRAEMER, A., R. S. MUELLER, C. WERCKENTHIN, R. K. STRAUBINGER, J. HEIN (2012): Dermatophytes in pet Guinea pigs and rabbits. Vet. Microbiol. 157, 208-213.

KRÄMER, A., R. S. MÜLLER, J. HEIN (2012): Environmental factors, clinical signs, therapy and zoonotic risk of rabbits with dermatophytosis. Tierärztl. Prax. Ausg K Kleintiere Heimtiere. 40, 425-431.

MARTINO, P. A., F. LUZI, M. VERGA (2004): Microbiological control of the environment in an intensive rabbit rearing; 8th World Rabbit Congress, Puebla, Mexico, 7-10 September, 2004.

MOHAPATRA, L. N., H. C. GUGNANI, K. SHIVRAJAN (1964): Natural infection in laboratory animals due to *Trichophyton mentagrophytes* in India. Mycopathol. Mycol. Appl. 24, 275-280.

NAKAMURA, Y., R. KANO, E. NAKAMURA, K. SAITO, S. WATANABE, A. HASEGAWA (2002): Case report. First report on human ringworm caused by *Arthroderma benhamiae* in Japan transmitted from a rabbit. Mycoses 45, 129-131.

NWEZE, E. I. (2011): Dermatophytoses in domesticated animals. Rev. Inst. Med. Trop. S. Paulo. 53, 95-99.

PANG, S. H., P. REN, X. J. WU (2008): Diagnosis and treatment of rabbit skin mildew. Anim. Sci. Vet. Med. 4, 70-70.

REBELL, G., D. TAPLIN (1979): Dermatophytes: their recognition and identification. Coral Gables, University of Miami Press, Florida.

REFAI, M., A. ALI, I. S. ABDALLAH (1970): Incidence of an experimental infection with *Keratinomyces ajelloi* and *Microsporum nanum* in laboratory animals. Bull. Pharma. Res. Inst. 85/86, 7-8.

ROBERT, R., M. PIHET (2008): Conventional methods for the diagnosis of dermatophytosis. Mycopathol. 166, 295-306.

SAMANTA, I. (2015): Veterinary Mycology. Springer, New Delhi, India.

SANTOS, D. A., M. E. S. BARROS, J. S. HAMDAN (2006): Establishing a method of inoculum preparation for susceptibility testing of *Trichophyton rubrum* and *Trichophyton mentagrophytes*. J. Clin. Microbiol. 44, 98-101.

SIMALJAKOVÁ, M., J. BUCHVALD, B. OLEXOVÁ (1989): *Microsporum canis* infection in rabbits and its transmission to humans. Mycoses 32, 93-96.

SINHA, B. K., B. N. PRASAD, A. K. SINHA (1989): Dermatophytosis due to *Trichophyton simii* in a colony of zoo rabbits. Indian J. Anim. Health 28, 75-76.

VAN ROOIJ, P., M. DETANDT, N. NOLARD (2006): *Trichophyton mentagrophytes* of rabbit origin causing family incidence of kerion: an environmental study. Mycoses 49, 426-430.

WEITZMAN, I., R. C. SUMMERBELL (1995): The dermatophytes. Clin. Microbiol. Rev. 8, 240-259.

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SAŽETAK

U radu je opisan nalaz dermatofita i njihova osjetljivost na protugljivične lijekove u zdravih kunića držanih na farmi ili u domaćinstvu kao kućni ljubimci. Od 614 pretraženih životinja na dermatofite je bilo pozitivno 146 uzoraka (23,78%) izravnom mikroskopskom pretragom, a dermatofiti su bili izdvojeni iz 112 uzoraka (112/146, 76,71%). Dominantne vrste dermatofita izdvojene iz zdravih kunića bile su *Trichophyton mentagrophytes* (37,5%), *Microsporum gypseum* (25%), *Microsporum nanum* (21,43%) i *Microsporum canis* (16,07%). Stopa pojavnosti bila je relativno viša u mladunaca do 6 mjeseci, a u mužjaka je bila značajno viša nego u ženki ($P<0,05$). Dermatofiti su češće bili izdvojeni ljeti i u jesen nego u proljeće i zimi ($P<0,05$). Najmanja minimalna inhibicijska koncentracija (MIK) itrakonazola od 0,03-0,5 µg/mL dokazana je za *T. mentagrophytes*, ketokonazola od 0,06-0,5 µg/mL za *M. canis*, mikonazola od 0,03-0,5 µg/mL za *M. nanum* i od 0,03-0,25 µg/mL za *M. gypseum*. Flukonazol i grizeofulvin pokazivali su najvišu vrijednost MIK što govori o otpornosti izolata na te lijekove.

Ključne riječi: antimikotici, dermatofiti**,** *Microsporum, Trichophyton*, kunić, Indija