Plant Archives Volume 20 No. 2, 2020 pp. 7657-7662 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DERMATOPHYTE INFECTION PATHOGENESIS ON NEW ZEALAND WHITE RABBIT SKIN, BOGOR, WEST JAVA, INDONESIA

Faisal Jamin[1], Sri Estuningsih[2], Eko Sugeng Pribadi[3] and Ekowati Handharyani[2*]

[1]Faculty of Veterinary Medicine, Universitas Syiah Kuala Jln. Tgk Hasan Krueng Kalee No. 4, Kopelma Darussalam-23111, Banda Aceh, Indonesia.

[2*]Department of Veterinary Clinic Reproduction and Pathology, Faculty of Veterinary Medicine, IPB University, Jalan Agatis Kampus IPB Darmaga, 16680 Bogor, Indonesia.

[3]Department of Animal Disease and Veterinary Public Health, Faculty of Veterinary Medicine, IPB University, Jalan Agatis Kampus IPB Darmaga, 16680 Bogor, Indonesia.

Abstract

Dermatophytes are the most common fungal infection in the world. This research aimed dermatophyte characteristics and to conduct in vivo test on macroconidia's ability in inducing lesions and inflammatory response on rabbit skin. Characterization method includes colony pigmentation, texture, growth and morphological structure. Clinical evaluation was conducted for the presence of erythema, alopecia includes hyperkeratosis, acanthosis and inflammatory cell infiltration. Twenty New Zealand White rabbit were divided into four treatment groups.The result of this research showed that intradermal inoculation of macroconidia from *M. gypseum, Microsporum canis* and *T. mentagrophytes* induced 100% lesions in rabbit model. Lesion severity such as erythema, alopecia, damaged hair, skin scaling and skin itch started to be identified on day 3 to day 6 post inoculation. Thickening epidermis occurred characterized by hyper parakeratosis and acanthosis, while lesions in dermis was characterized by inflammatory cell infiltration. Intradermal inoculation was proven to be very effective to induce infection directly to skin invasion phase.

Key words: dermatophytes, dermatophytosis, pathogenesis, New Zealand White rabbits.

Introduction

Dermatophytosis is a skin disease caused by dermatophyte fungi which can infect human and animal. Dermatophyte is the most common fungal infectious agent in the world (Achterman and White, 2013). Based on its habitat, fungi is classified as anthropophilic, zoophilic and geophilic. Dermatophyte fungi is keratinolytic which can cause damages in skin, hair, feather and nail (Fehr, 2015). Etiologically, *Microsporum gypseum, Microsporum canis* and *Tricophyton mentagrophytes*are the main dermatophytosis agent in dogs and cats (Nardoni *et al.,* 2013). *M. canis*is the main agent of dermatophytosis, followed by *T. mentagrophytes*; which dominates zoophilic dermatophyte in Spain. While in India (Asia); *T. rubrum*is the main dermatophytosis agent followed by *T. mentagrophytes*. *M. gypseum*infection generally occurred through animal or human contact with soil (Hayette and Sachli, 2015). Pathogenesis of dermatophyte infection occurred in three stages: dermatophyte attachment, dermatophyte penetration on keratin layer and immunological reaction. These three stages are influenced by the dermatophyte. Study in virulence and the mechanism of dermatophyte infection that are already performed have not found consistent model for experimental and clinical data on dermatophytosis management. Existing models have shown repeated clinical infection variation in animal and human. The development of dermatophyte infection model in animal is one of the ways to obtain a stable dermatophytosis model as research materials.

****Author for correspondence :*** E-mail: ekowatieko@apps.ipb.ac.id

Materials and Methods

Animal model

The use of animal model for this research is from the Animal Ethical Commitee of IPB University (No 123-2018 IPB, August 2018). Twenty healthy male New Zealand White rabbits with no dermatophytosis weighing

average 1.5 kg was obtained from Animal Husbandry Research Center (Balai Penelitian Ternak-BALITNAK) Bogor, Bogor Regency, Indonesia.

Dermatophyte reculture, characterization, preparation and standarisation

Three dermatophyte isolates: *M. gypseum, M. canis* and *T. Mentagrophytes* were obtained from animal specimen with dermatophytosis. Dermatophyte fungi were reculturized and then identified based on macroscopic and microscopic characteristics. The dermatophytes were grown in Sabouraud Dextrose Agar (SDA) media. The cultures were incubated in ±26ºC and observed for 2 weeks. Identifying characteristics consist of colony pigment, texture and growth and morphological structures of hyphae, microconidia and macroconidia (Adzima *et al.,* 2013). Microscopic examination of hyphae, microconidia and macrocodia were performed on SDA media that were considered positive (with dermatophyte growth) by using Potassium Hydroxide (KOH) 20% and Lacto Phenol Cotton Blue (LPCB) staining. The examination were performed under light microscope (Kalsiet *et al.,* 2019). Macroconidia were obtained from suspension of colony taken from the medium. Colony was ground and washed three times with agitation in fosfat buffered saline (PBS) followed by separation via centrifugation which then resuspended with distilled water to obtain clearer suspension. Macroconidia preparation and standardization were determined with the use of hemocytometer and adjusted to 3×10^6 mL according to method performed by Pihet *et al.,* (2015).

Dermatophyte ultrastructure

Three dermatophyte fungi isolates; *M. gypseum, M. canis,* and *T. Mentagrophytes*, grown in *Sabouraud Dextrose Agar* (SDA) medium were clipped. The clipped dermatophyte specimens were washed 3 times by caccodylate buffer followed by glutaradehydein 4ºC. Samples were then dehydrated with graduated alcohol (50-95%), transffered to be frozen by freezed drier/ vacuum drier to dry and then covered by Au by using ion *coater* device. Interaction between dermatophyte fungi was examined by JSM-5310LV electrone microscope (Jeol, jepang). Scanning electron microscopy (SEM) was used to observe the ultrastructure visualization and to take the image of dermatophyte (Yue *et al.,* 2018).

Skin infection, clinical and histopathological examination

Twenty New Zealand White rabbits were divided into 4 treatment groups, as provided in table 1. Before inoculation, all rabbits were anasthesized through intramuscular route by the combination of ketamine (40 mg/kg BW) and xylazine (10 mg/kg BW). The hair on the dorsal part of rabbit model was cut and trimmed in 5×8 cm^2 size. The remaining hair was gently abraded with sterile scalpel by Jensen and Arendrup, (2012) method modified Karimi and Mikaeili, (2013) method. Data was taken from day 1 up to day 30 post infection (p.i.). Skin reaction was observed on minute 15, 39 and 360 post infection (p.i.). Sampling of skin showing dermatophytosis lesion was conducted in day 10 and day 20 post infection with dermal punch 10 mm biopsy which then processed for histopathological preparates.

Dermatological examination of macroconidia inoculation on model rabbit consist of conventional dermatophyte microscopic examination by potassium hydroxide (KOH) 20% and Lacto Phenol Cotton Blue (LPCB) staining. Clinical evaluation of the lesion involved erythema, edema, alopecia, damaged hari, scaling and itchiness of thee skin. Histopathology image examined consist of hyper keratosis, achantosis and inflammatory cell. Dermatophytosis histopathological examination were performed by hematoxylin eosin (HE) staining, Periodic Acid Schiff (PAS) staining and Grocott's methenamine silver (GMS) staining. Examination was done under olympus optical CO microscope (400x magnification), Ltda BX40 (Tokyo, Japan).

Data analysis

Data analysis of fungi reculture, macroscopic examination, miscroscopic examination, clinical lesion and skin histopathology caused by dermatophyte intradermal inoculation were analyzed descriptively and provided in tables and figures.

Table 1: Intradermal inoculation of dermatophyte macroconidia test on New Zealand White rabbit groups.

No.	Group	No. of rabbit	Dosage	Frequency
1.	Control (D0)	5	0.02 mL	1 time
2.	Dermatophyte *1* (D1)	5	3×10^6/mL macroconidia *M. Gypseum*	1 time
3.	Dermatophyte *2* (D2)	5	3×10^6/mL macroconidia *M. Canis*	1 time
4.	Dermatophyte *3* (D3)	5	3×10^6/mL macroconidia *T. mentagrophytes*	1 time

Results and Discussion

Dermatophyte culture and characterization

Hyphae found in dermatophyte culture on SDA media were unbranched, soft and even. Most of the hyphae found

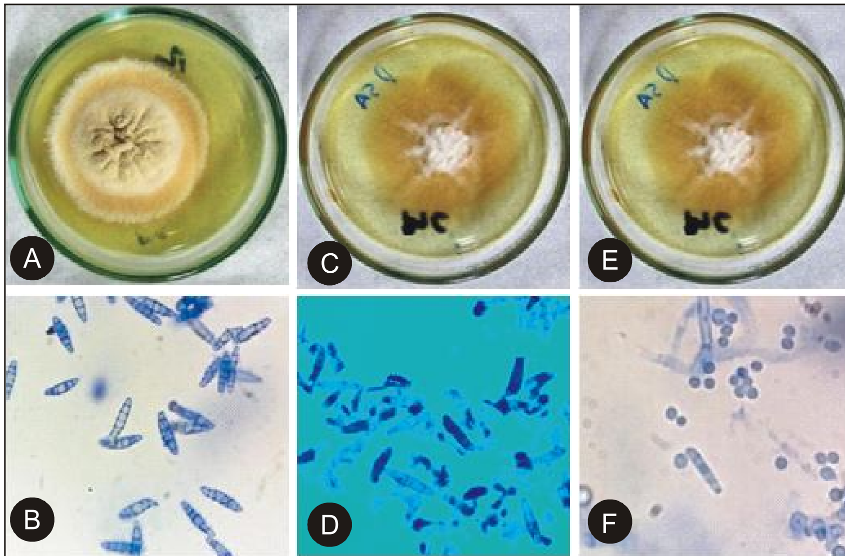

Fig. 1: Dermatophyte fungi macroscopic image on SDA media, (A) *M. gypseum*; (C) *M. canis*, dan; (E) *T. mentagrophytes*. Microscopic image of macroconidia in LPCB staining; (B) *M. gypseum*; (D) *M. canis*, dan; (F) *T. mentagrophytes* (arrow).

were dry such as the ones on fig. 1A, 1C and 1E. The microscopic image of macroconidia and microconidia could be seen on fig. 1B, 1D and 1F) using Potassium Hydroxide (KOH) 20% and Lacto Phenol Cotton Blue (LPCB) staining.

Borman and Summerbell, (2015) stated that *Trichophyton* morphologically has the macroscopic figure on agar media appeared powdery, velvety or waxy. On the other hand, *Microsporum* macroscopic characteristic on agar media appears velvety or powdery with brownish white pigmentation Simpanya, (2000) stated that *Microsporum* genus produced microconidia and macroconidia. The macroconidia appeared to have multiple septate bordered by thin cell wall, echinulate cell wall with spindle form. Microconidia found was pyramid shaped 2-3μm in size. Trichophyton genus produced soft walled macroconidia and microconidia. The macroconidia had thin wall and shaped like cigar Microconidia were pyriform shaped 2-3μm in size with uneven form. Ultrastucture of macroconidia, microconidia and hyphae are profided in fig. 2A and 2B in SEM images (1D, 1E and 1F). Macroconidia of dermatophyte is an important factor as agent of infection infeksi (Shenoy *et al.,* 2008). Microconidia has clavate shape, small, unicellular, with runded thinwall while macroconidia was ellipsoid with 3-6 septa (Mihali *et al.,* 2012).

Clinical lesion and skin histopathology

Biopsy sample examination result and clinical signs for dermatophytosis confirmation showed that all rabbit in the groups showed 100% dermatophytosis. Lesions found were erythema, alopecia, damaged hair, scaling and itchiness which were observed in group D1, D2 and D3. On all group D1, D and D3, erythema was observed on hour 2 p.i. and elevated up to hour 6 p.i. This result showed faster reaction compared to previous researches. Clinical lesion severity of dermatophytosis is determined by width of skin with primary balding. Initial lesion on D1 animals were observed on day 4-5, on D2 animals were observed on day 5-6 and on D3 animals on day 3-5. Intradermal infection of macroconidia confirmed that this method is very effective in elevating direct infection to invasion stage to the skin. Animal model used in this research showed the process of epidermal differentiation and skin permeability which cause primary lesion. Intradermal infection may minimize the probability of similar infection from other microbes around inoculation site. The earliest change on the skin observed were vasodilatation and edema, followed by the spread of erythema and desquamation (Shimamura *et al.,* 2012).

This result agrees with a research by Shimamura *et al*., (2011) which displayed erythema on the skin of animal model. This progress is characterized by inflammatory response which begins to show from day 1 p.i. and will continue until it escalates into alopecia. Clinical lesion can be clearly be observed on fig. 3. Rabbit skin of all test group were covered by yellowish white layer that stick strongly to the epidermis and progressively growing. All rabbits inoculated by *M. gypseum, M. canis* and *T. mentagrophytes* displayed dermatophytosis with regular and circular alopecia (Karimi and Mikaeili, 2013).

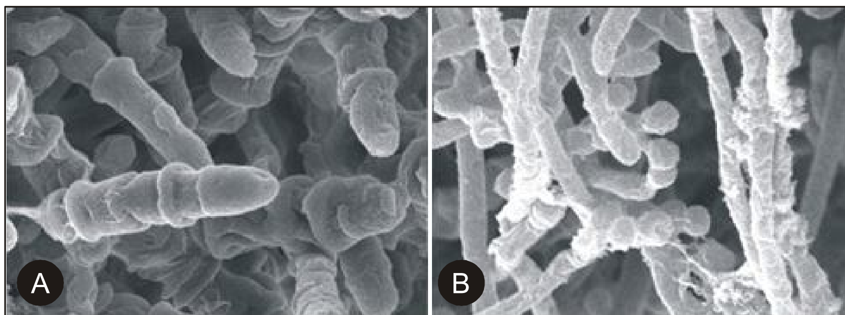

Fig. 2: Two weeks old SDA media culture dermatophyte ultrastucture as picture by SEM; (A) Macroconidia; (B) Microconidia (arrow).

On gross anatomy, the lesion produced by *M. gypseum*, *M. canis* and *T. mentagrophytes* displayed the same characteristics. Aljabre *et al.,* (2015) stated that *in-vitro* attachment of *T. mentagrosphytes* macroconidia has the highest time of 6 hours after contact and germination began in 4 hours after contact. Attachment of *T. mentagrosphytes* macroconidia was observed 6 hours post inoculation and the growing hyphae and could be clearly identified after the next 10 hours. A research by Duek *et al.,* (2004) showed that human skin biopsy of *T. Mentagrophytes* required 12 hours for attachment, 24 hours for germination and 3 days for invasion to stratum corneum. The attachment of *M. Canis* arthroconidia to reconstructed cat interfollicular epidermis could be observed in 2-6 hours post infection (Baldo *et al.,* 2008).

Method in obtaining dermatophytosis model was focused in animal species, dermatophyte selection and infection condition (occlusion or open condition with or without abrasion) of the skin (Shimamura *et al.,* 2012). The right model which simulate dermatophyte infection on host is a requirement to study dermatophytosis infection mechanism. Liang *et al.,* (2016) in an *in-vivo* research stated that dermatophyte infection was only limited to *stratum corneum*. Skin lesion against dermatophyte involved all part of epidermis. Histologically, thickening epidermis with hyperkeratosis and acanthosis could be found. Other than that, inflammatory infiltration of T cell and neutrophils could be observed by the dermis. Histopathological examination of the skin within ringworm zone with Hematoxylin Eosin (HE) staining showed inflammatory reaction characterized by hyperkeratosis, acanthosis and papillary dermis (Fig. 4A and 4B). The hyphae colonies of *M. gypseum, M. canis* and *T. mentagrophytes* were all observed in *stratum corneum* (Fig. 4C, 4D, 4E, 4F, 4G and 4H) using Periodic Acid Schiff (PAS) staining and Grocott methenamine silver (GMS) staining.

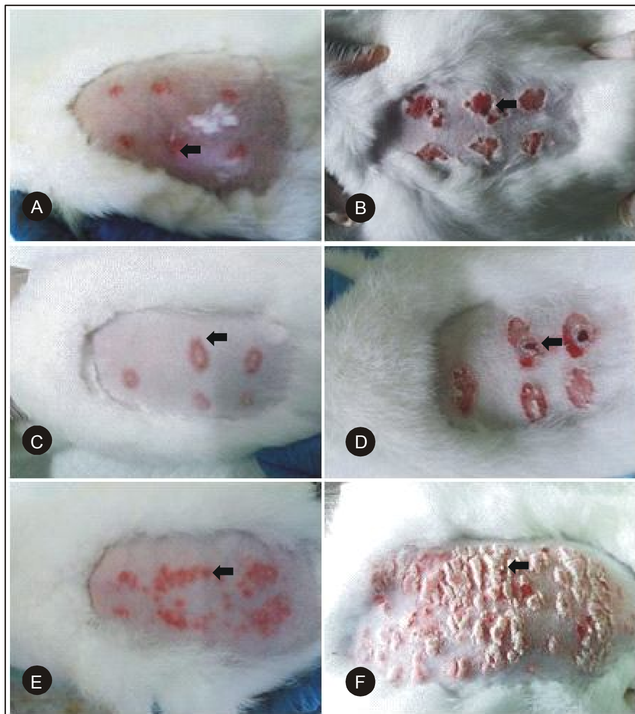

Fig. 3: Primary clinical lesion on day 10 and day 20 in the form of circular and regular alopecia. (A,B) *M. gypseum*; (C, D) *M. canis* and (E,F) *T. mentagrophytes* (arrow).

Infected group displayed invasion on epidermis layer which moved to the surface through keratinocyte and stratum corneum layer according to dermatophytosis pathologic characteristics. HE staining showed hyper keratosis and acanthosis (Fig. 4A and 4B). Fungi presence is hard to observe by HE staining which only shows the damages on skin tissue structure. Periodic Acid Schiff (PAS) staining showed a lot of septate hyphae with in different sized all over the epidermis and Grocott methenamine silver (GMS) staining showed a lot of conidia and septate hyphae stained black with different

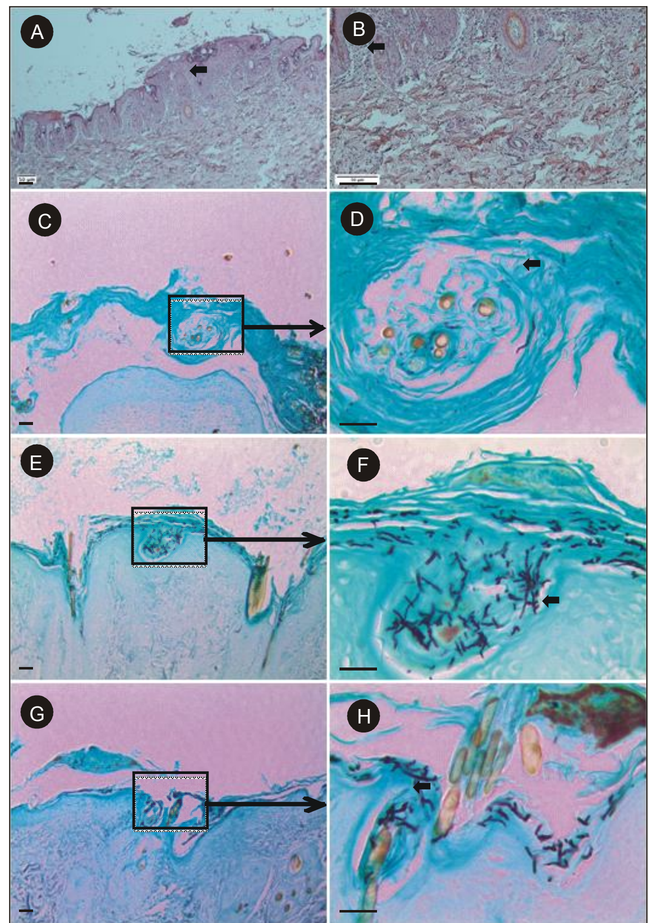

Fig. 4: Rabbit skin histopathology; Hyperkeratosis and acanthosis with HE staining (A,B). Conidia and hyphae of *M. gypseum* (C,D), *M. canis* (E,F), *T. mentagrophytes* (G,H) with GMS staining (arrow); (arrow).

sizes all over epidermis. Microscopically dermatophyte lesion showed varied and noninflammatory pattern on stratum corneum layer or hair follicles (Cavalcanti *et al.,* 2002).

Fungi attachment on host cell is mediated by fungi adhesin and its interaction with host receptor. Adhesin plays an important role for attachment during infection process (Esquenazi *et al.,* 2004). An experiment by Kaufman *et al.,* (2007) utilized *ex-vivo* model which used human skin epidermis explant. In this model maximum attachment ability was reached after 12 hours and germinations after 24 hours, while maximum germination along with penetration to stratum corneum happened after 3 days. Tabart *et al.,* (2007) explained in a different experiment on dermatophyte species infection model on both *in-vitro* and *in-vivo* model that infection severity increases depending on time and the number of attaching spores, where it will be followed by germination and invasion phase to *stratum corneum* by hyphae growth into the skin. Histopathological examination by GMS showed hyphae colony of *M. gypseum*, *M. canis* and *T. mentagrophytes* on skin epidermis. According to Copetti *et al.,* (2006) *M. gypseum, Microsporum cani*s and *T. mentagrophytes* species are responsible of more than 95% of all dermatophytosis cases in pet animals.

Conclusion

Intradermal inoculation of macroconidia induced 100% lesion on rabbit model. Lesions, such as erythema, alopecia, damaged hair, scaling and itchiness on rabbit skin, were began to be identified between day 3 to day 6 post inoculation. Thickening of epidermis was caused by hyperkeratosis, acanthoses and supported by the epidermis and dermis inflammatory cells infiltration.

Acknowledgements

The first author is thankful to Ministry of Research and Technology of Higher Education, Indonesia for providing fellowship for pursuing Ph.D and “Hibah Penelitian Doktoral 2019” (Doctoral Research Grant 2019), Ministry of Research Technology and Higher Education, Republic of Indonesia.

References

Achterman, R.R. and T.C. White (2013). Dermatophytes. *Current Biology,* **23(13):** R551-R552.

Adzima, V., F. Jamin and M. Abrar (2013). Isolation and identification of canine dermatophytosis. Mold in Syiah Kuala Banda Aceh. *Jurnal Medika Veterinaria,***7(1):** 46-48.

Aljabre, S.H., O.M. Alakloby and M.A. Randhawa (2015). Dermatological effects of *Nigella sativa*. *Journal of Dermatology & Dermatologic Surgery,***19(2):** 92-98.

Baldo, A., J. Tabart, S. Vermout, A. Mathy, A. Collard, B. Losson and B. Mignon (2008). Secreted subtilisins of Microsporum canis are involved in adherence of arthroconidia to feline corneocytes. *Journal of medical microbiology,* **57(9):** 1152-1156.

Borman, A.M. and R.C. Summerbell (2015). Trichophyton, Microsporum, Epidermophyton and agents of superficial mycoses. Manual of Clinical Microbiology, Eleventh Edition. *American Society of Microbiology*, 2128-2152.

Cavalcanti, J.N., J.L. Guerra, W. Gambale, B. Corrêa and C.R. Paula (2002). Histopathologic and mycologic aspects of experimental infection of guinea pigs with *Microsporum canis*. *Brazilian Journal of Veterinary Research and Animal Science,* **39(5):** 238-243.

Copetti, M.V., J.M. Santurio, A.S. Cavalheiro, A.A. Boeck, J.S. Argenta, L.C. Aguiar and S.H. Alves (2006). Dermatophytes isolated from dogs and cats suspected of dermatophytosis in Southern Brazil. *Acta Scientiae Veterinariae*, **34(2):** 119-124.

Duek L., G. Kaufman, Y. Ulman and I. Berdicevsky (2004). The pathogenesis of dermatophyte infections in human skin sections. *Journal of Infection,* **48(2):** 175-180.

Esquenazi, D., C.S. Alviano, W. De Souza and S. Rozental (2004). The influence of surface carbohydrates during *in-vitro* infection of mammalian cells by the dermatophyte *Trichophyton rubrum. Research in microbiology,* **155(3):** 144-153.

Fehr, M. (2015). Zoonotic potential of dermatophytosis in small mammals. *Journal of Exotic Pet Medicine*, **24(3):** 308-316.

Hayette, M.P. and R. Sacheli (2015). Dermatophytosis, trends in epidemiology and diagnostic approach. *Current Fungal Infection Reports,* **9(3):** 164-179.

Jensen, R.H. and M.C. Arendrup (2012). Molecular diagnosis of dermatophyte infections. *Current opinion in infectious diseases*, **25(2):** 126-134.

Kalsi, A., R. Thakur and P. Kushwaha (2019). Extensive tinea corporis and tinea cruris et corporis due to Trichophyton interdigitale. *Journal of Dermatolgy and Cosmetology*, **3(1):** 16-20.

Karimi, I. and A. Mikaeili (2013). Animal Models as Tools for Translational Research: Focus on Dermatophytosis. Microbial pathogens and strategies for combating them: science, technology and education 1448-1454.

Kaufman, G., B.A. Horwitz, L. Duek, Y. Ullman and I. Berdicevsky (2007). Infection stages of the dermatophyte pathogen Trichophyton: microscopic characterization and proteolytic enzymes. *Medical Mycology,* **45(2):** 149-155.

Liang, P.P., X.Z. Huang, J.L. Yi, Z.R. Chen, Ma H, C.X. Ye, X. Y. Chen, W. Lai and J. Chen (2016). A *Trichophyton rubrum* infection model based on the reconstructed human epidermis-episkin®. *Chinese Medical Journal,* **129(1):** 54-58.

Mihali, C.V., A. Buruiana, V. Turcus, A. Covaci and A.Ardelean (2012). Comparative studies of morphology and ultra-structure in two common species of dermatophytes: *Microsporum canis and Microsporum gypseum*. *Annals of RSCB,* **17(1):** 85-89.

Nardoni, S., L. Mugnaini, R. Papini, M. Fiaschi and F. Mancianti (2013). Canine and feline dermatophytosis due to *Microsporum gypseum*: a retrospective study of clinical data and therapy outcome with griseofulvin. *Journal de mycologie Medicale,* **23(3):** 164-167.

Pihet, M., N. Clément, C. Kauffmann-lacroix, S. Nail-billaud, A. Marot, F. Pilon and R. Robert (2015). *Diagnosis of dermatophytosis: an evaluation of direct examination using MycetColor® and MycetFluo®. Diagnostic Microbiology and Infectious Disease*, **83(2):** 170-174.

Shenoy, M., S. Teerthanath, V.K. Karnaker, B. Girisha and M.K. Prasad and J. Pinto (2008). Comparison of potassium hydroxide mount and mycological culture with histopathologic examination using periodic acid-Schiff staining of the nail clippings in the diagnosis of onychomycosis. *Indian Journal of Dermatology, Venereology and Leprolog*, **74(3):** 226-229.

Shimamura, T., N. Kubota, S. Nagasaka, T. Suzuki, H. Mukai and K. Shibuya (2011). *Establishment of a novel model of onychomycosis in rabbits for evaluation of antifungal agents*. *Antimicrobial Sgents and Chemotherapy*, **55(7):** 3150-3155.

Shimamura, T., N. Kubota and K. Shibuya (2012). Animal model of dermatophytosis. *Bio. Med. Research International*, 1-11.

Simpanya, M.F. (2000). Dermatophytes: their taxonomy, ecology and pathogenicity. *Rev. Iberoam. Micololgy,* **17:** 1-12.

Tabart, J., A. Baldo, S. Vermout, B. Nusgens, C. Lapiere, B. Losson and B. Mignon (2007). Reconstructed interfollicular feline epidermis as a model for *Microsporum canis* dermatophytosis. *Journal of medical microbiology*, **56(7):** 971-975.

Yue, X., A. Wang and Q. Li (2018). The role of scanning electron microscopy in the direct diagnosis of onychomycosis. *Scanning*, 1-4.