Biological Models Research and Technology
ISSN 9675-9225X (Online)

BMRT

CASE REPORT

Scopulariopsis brevicaulis fungal outbreak in laboratory rats

Giovanny Augusto Camacho Antevere Mazzarotto[1]*, Rosana Cé Bella Cruz[2], Daniele Masseli Rodrigues Demolin[3], Rovilson Gilioli[3], Marcel Frajblat[4]

[1]*Fundação Instituto Oswaldo Cruz – Fiocruz CE, Eusébio, CE, Brasil.*
[2]*Laboratório de Parasitologia e Micologia, Universidade do Vale do Itajaí – Univali, Itajaí, SC, Brasil.*
[3]*Universidade de Campinas – UNICAMP, Campinas, SP, Brasil.*
[4]*Coordenação de Atividades com Modelos Biológicos Experimentais – CAMBE, Decania do Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro – UFRJ, Rio de Janeiro, RJ, Brasil.*

How to cite: Mazzarotto GACA, Cruz RCB, Demolin DMR, Gilioli R, Frajblat M. *Scopulariopsis brevicaulis* fungal outbreak in laboratory rats. Bio M Res Tech. 2022;2:e00042022. https://doi.org/10.4322/2675-9225.00042022

Abstract

This study describes an outbreak of alopecia in laboratory rats caused by the saprophytic fungi *Scopulariopsis brevicaulis* in a Brazilian lab animal facility. Initially, lesions were identified only in females during the lactation period, which showed circumscribed areas with alopecia without pruritus extensively at the abdomen and the medial side of the thighs. Later during the course of the infection, skin lesions affected both genders reaching about 10% of the colony. Skin scrapings, hair, and environmental samples from the facility were sent for analysis. The cultures were carried out in Sabouraud agar and over seven days fast-growing colonies varying in color from white to brown were observed. Micromorphology showed mycelium and characteristic ringworms of the fungus *S. brevicaulis*. There is a similar report of an outbreak of this fungus in Turkey. To date, this is the first report in the literature of the fungus *S. brevicaulis* causing disease in laboratory rats in Brazil.

Keywords: laboratory animals, mycological examination, rats, *Scopulariopsis brevicaulis*.

INTRODUCTION

Rats (*Rattus norvergicus*) are commonly used as animal models in biomedical research due to their reduced body size, high fertility, and short reproductive cycles[1]. Experimental research has accumulated continuous refinements, and it has motivated the demand for laboratory animals with better health conditions. In an extensive review, Baker[2] described how the presence of undesirable pathogens in animal models would interfere with results in the different fields of research such as pharmacology, toxicology, and virology.

Opportunistic infections are one of the main problems observed in laboratory animal models such as rats and can be caused by a variety of fungi[3]. *Scopulariopsis spp*. is a ubiquitous, saprophytic fungus found worldwide in soil, plant debris, wood, paper, animal matter, air and humid indoor environments[4]. The genus *Scopulariopsis* and predominantly *Scopulariopsis brevicaulis* is known to be an opportunistic pathogen that is thought to cause invasive and noninvasive infections such as onychomycosis, conjunctivitis, keratitis, and endocarditis in animals and humans, therefore, can be considered a zoonotic organism[5-9].

Animal models are widely used in teaching and research, so they must be in perfect physical and biological conditions for biomedical studies. Thus, this work aimed to report the outbreak of hyalohyphomycosis in laboratory rats in Brazil caused by the opportunistic fungi *S. brevicaulis*.

***Corresponding author:** giovanny.mazzarotto@fiocruz.br
Competing interests: No conflicts of interest declared concerning the publication of this article.
Received: February 17, 2022. Accepted: April 17, 2022.

MATERIAL AND METHODS

Animal maintenance

Outbred Wistar rats from a breeding animal facility were maintained in controlled conditions with a temperature of 23 ± 2 °C and humidity of 65 ± 10%. Feed, water, bedding and cages provided to animals were submitted to an autoclaving sterilization process. Air in animal rooms was HEPA filtered and animals were housed in open cages in a controlled light/dark cycle of 12h/12h. Food and water were provided ad libitum. Animal ethical approval was not applicable for the routine veterinary practice in this study, according to Brazilian federal Law 11.794/2008.

Microbiological identification

Animals with alopecia lesions had samples of skin and hair obtained by scraping with scalpels and placed in individually sterile plastic plates. Also, healthy rats without alopecia had samples collected with swabs and placed in test tubes with 0.85% physiological saline solution.

Furthermore, Petri dishes containing Sabouraud medium were exposed to sedimentation of suspended ambient air particles in all animal rooms and corridors of the facility as proposed by Pires et al.[10]. Petri dishes were sent to the mycology laboratory of the University of Vale do Itajaí, Santa Catarina, Brazil, and incubated at room temperature for 14 days for the growth of fungal colonies followed by their identification.

For microbiological examination, part of the samples collected was processed in 30% potassium hydroxide solution (KOH) for 30 min and then examined by light microscopy using objective 400x magnification. The remaining samples were seeded on Sabouraud Dextrose agar plates (SDA) supplemented with chloramphenicol (0.05 mg/mL) and separately incubated at 25 °C for 7 days.

Plates were examined daily, and isolated colonies were subsequently stained with lactophenol blue. Direct mycological examination revealed the presence of hyaline and septate hyphae, suggestive of hyalohyphomycetes. After 5 days the macromorphology of the colony was observed. At first, it was white, membranous, becoming velvety or powdery with numerous conidia and aerial hyphae with light brown coloration. The reverse side of the culture was brown with a central brown hue. After the growth of colonies, their identifications were carried out. The isolated fungal colonies were stained with Lactophenol blue and identified as *S. brevicaulis* based on macroscopic and microscopic characteristics.

RESULTS AND DISCUSSION

Breeding colonies of laboratory rats are used largely in biomedical research to understand mechanisms of disease, prevention of disease development or treatment options, and evaluate health risks present in our living environment[11]. Although breeding conditions for laboratory animals continue to improve, various infectious agents have been affecting animal colonies, especially fungal species[12].

Upon physical examination, the only observed sign was alopecia across several areas of the body of males and females without any other signs of illness. The lesions were initially observed in 2004 in female rats during the lactation period presenting circumscribed and non-pruritic areas of alopecia located in the abdomen region or one of the forelimbs (Figure 1 and 2).

Later, lesions were also observed in males in the head and dorsal regions of male rats (Figure 3 and 4). The animals did not show signs of irritability or any other clinical signs related or not to the infection.

The fungus *S. brevicaulis* was identified as the main pathogen. Macromorphology of the Sabouraud medium showed colony white, membranous, becoming velvety or powdery with a brown coloration on SDA agar after incubation for 7 days at 25 °C. Micromorphology with cotton blue showed septate hyaline hyphae as well as annelophores and anelloconids (Figure 5).

Hyalohyphomycosis consists of a group of fungal infections characterized by the presence of septate hyaline mycelium in tissues. Hyaline fungi causing hyalohyphomycosis belong to Hyphomycetes, Coelomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes. These fungi have similar morphology, causing the same type of tissue reactions[13].

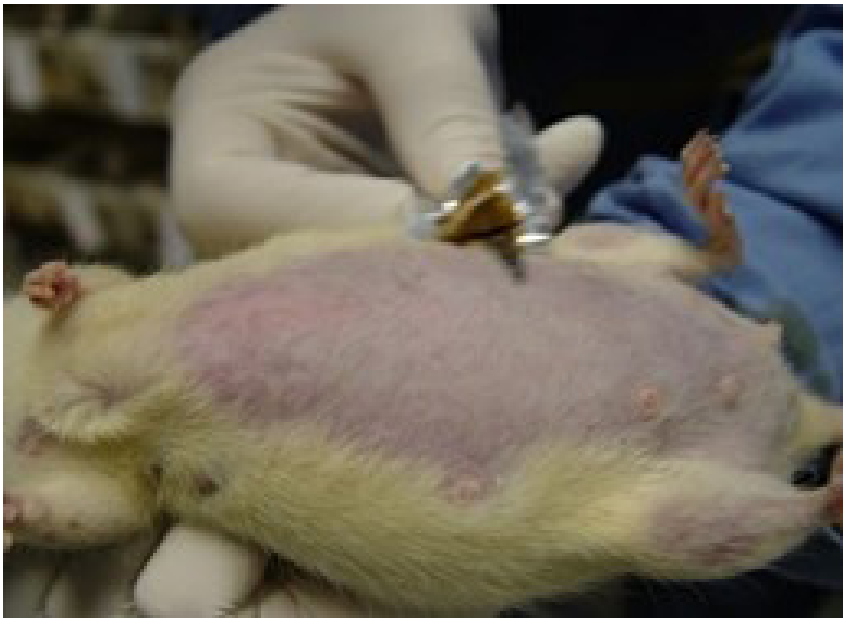

Figure 1. Alopecia observed in the abdominal area and inner legs of the female rat.

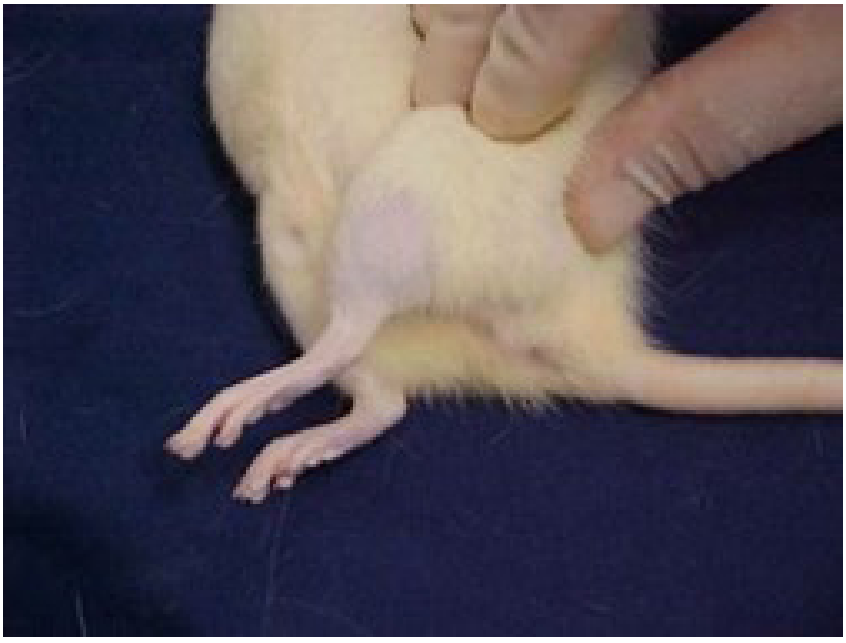

Figure 2. Detail of alopecia in the media leg of female rat.

There are several previous reports of a high incidence of *S. brevicaulis* in humans and other animals such as dogs, cats and large animals[14-16]. Şahan Yapicier et al.[9] reported *S. brevicaulis* causing infection and disease in Wistar rats in Turkey.

Scopulariopsis sp. is a filamentous fungus that inhabits soil, plant material, feathers, and insects and it is distributed worldwide. The most common species is *Scopulariopsis brevicaulis*, which is commonly considered an opportunistic contaminant that can cause infections, including onychomycosis in humans, especially in agricultural workers and immunosuppressed patients[17].

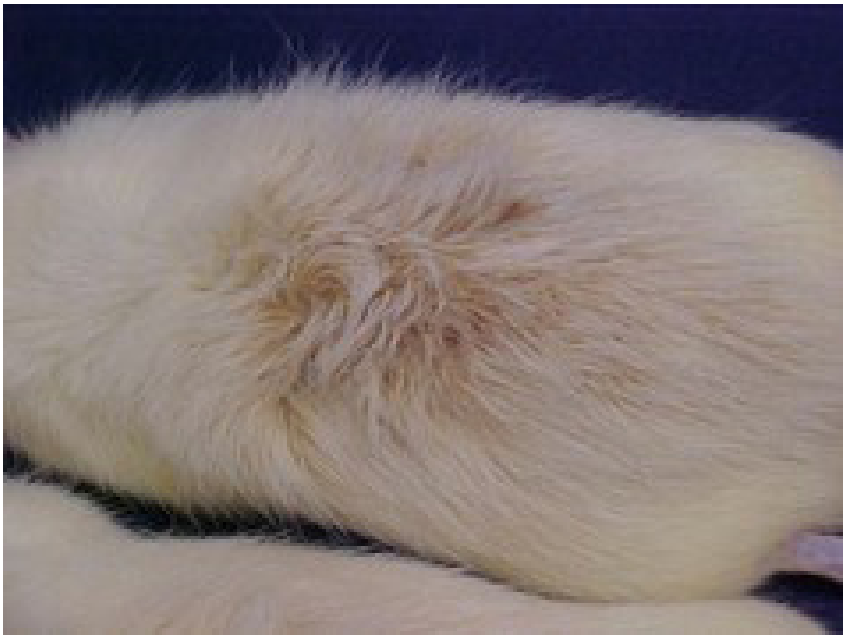

Figure 3. Alopecia in the initial stage observed in the dorsal region in male rat.

Figure 4. Alopecia in advanced stage observed in the dorsal region in older male rat.

There are limited data on clinical and experimental treatment of this fungus for animals and humans[18,19]. In this report, we have treated some animals with topical 10% iodine tincture for 14 days and did not observe any improvement of the lesions (data not shown). According to Şahan Yapicier et al.[9], rats that presented skin lesions by *S. brevicaulis* recovered completely without treatment and had no recurrence of clinical signs at one-month post-sampling. Despite the high antifungal resistance of *S. brevicaulis*, researchers have recommended agents as amphotericin B (AMB)[20] and voriconazole (VRC)[21].

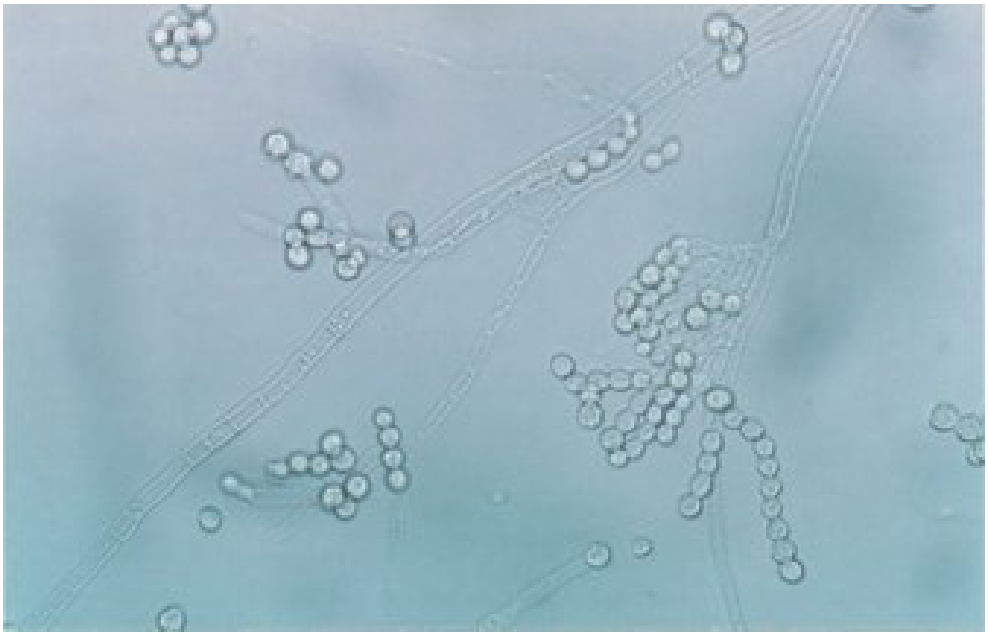

Figure 5. Branching and septated hyphae with conidiophores and conidia in chains seen on lactophenol cotton blue stain.

Environmental contamination with *S. brevicaulis* was observed in the dirty and breeding rooms of the facility. All animals that presented lesions had positive cultures for *S. brevicaulis*. The presence of *S. brevicaulis* was also observed in animals without any clinical sign which indicates the possibility of positive carriers in their coat. Similarly, one study reported that the *S. brevicaulis* was found in 36 animals out of 79 asymptomatic animals analyzed[22].

Infection initially occurred preferentially in pregnant and nursing females, probably due to the lowering of immunity that occurs during the normal course of pregnancy and lactation. The lesions were not observed in young animals. Most of the positive animals were older than 8 weeks.

Gilioli also observed the occurrence of infection by *S. brevicaulis* in Wistar female and male rats maintained under positive pressure Trexler insulator system during an experimental protocol developed in the laboratory of sanitary control at the CEMIB/UNICAMP. The high internal humidity and the accumulation of organic matter, apparently favored the dissemination of the fungus. More cage change frequency and replacement of the insulator air outlet filters corrected excessive internal humidity and the accumulation of organic matter (corn meal, faeces and damp wood shaving) reducing the occurrence of new cases. There was a complete recovery of the animal's fur without the need for treatment with antifungal drugs (data not shown, personal communication).

According to these information, animal age, sex and housing environment conditions were found to be factors in previously described *S. brevicaulis* infections[22]. However, Pires et al.[10] did not observe sex preference in a similar outbreak seen in mice from a breeding facility. Hair loss and skin lesions in both male and female Wistar rats due to *S. brevicaulis* were also observed in Turkey[9].

Since feed, water, bedding, and cages provided to animals were all autoclaved, and air in animal rooms was HEPA filtered, we hypothesized that the appearance of the fungus may have been due to failures in the autoclave equipment, air system failures causing up to 75% humidity, or even carried by the technician into the animal facility.

Recently, on a mailing list of Latin American lab animal facilities, there was a report of similar skin lesions in rats as those related in this work. Interestingly, the foundation animals for this colony came from the animal facility where the *S. brevicaulis* outbreak had occurred. Currently, we are carrying out an investigation aiming to verify if these reported skin lesions are also related to the presence of *S. brevicaulis*.

Despite not being a common skin pathogen in laboratory animals, *S. brevicaulis* should be considered in cases of alopecia without pruritus in older rats or during pregnancy and lactation.

Additional studies should be carried out to understand the range of occurrence of this fungus in animal facilities in Brazil as well as in other lab animal facilities in the world and its impact on research.

Financial support

This work was supported by article 170, an educational grant from the State of Santa Catarina funding agency (FAPESC).

REFERENCES

1. Andrade A, Pinto SC, Oliveira RS, editors. Animais de laboratório: criação e experimentação. Rio de Janeiro: FIOCRUZ; 2002. 388 p.
2. Baker DG. Natural pathogens of laboratory mice, rats and rabbits and their effect on research. Clin Microbiol Rev. 1998;11(2):231-66. http://dx.doi.org/10.1128/CMR.11.2.231. PMid:9564563.
3. Jacoby RO, Lindsey JR. Risks of infection among laboratory rats and mice at major biomedical research institutions. J.ILAR. 1998;39(4):266-71. http://dx.doi.org/10.1093/ilar.39.4.266.
4. Anandan V, Nayak V, Sundaram S, Srikanth P. An association of *Alternaria alternata* and *Scopulariopsis brevicaulis* in cutaneous phaeohyphomycosis. Indian J Dermatol Venereol Leprol. 2008;74(3):244-7. http://dx.doi.org/10.4103/0378-6323.41371. PMid:18583793.
5. Iwen PC, Schutte SD, Florescu DF, Noel-Hurst RK, Sigler L. Invasive *Scopulariopsis brevicaulis* infection in an immunocompromised patient and review of prior cases caused by *Scopulariopsis* and *Microascus* species. J Mycol Med. 2012;50(6):561-9. http://dx.doi.org/10.3109/13693786.2012.675629. PMid:22524638.
6. Cawcutt K, Baddour LM, Burgess M. A case of *Scopulariopsis brevicaulis* endocarditis with a mycotic aneurysm in an immunocompetent host. Case Rep Med. 2015;2015:872871. http://dx.doi.org/10.1155/2015/872871. PMid:25866512.
7. Kordalewska M, Jagielski T, Brillowska-Dąbrowska A. Rapid assays for specific detection of fungi of Scopulariopsis and microascus genera and *Scopulariopsis brevicaulis* species. Mycopathologia. 2016;181(7-8):465-74. http://dx.doi.org/10.1007/s11046-016-0008-5. PMid:27255522.
8. Hampson ECGM, Gibson JS, Barot M, Shapter FM, Greer RM. Identification of bacteria and fungi sampled from the conjunctival surface of normal horses in South-East Queensland, Australia. Vet Ophthalmol. 2019;22(3):265-75. http://dx.doi.org/10.1111/vop.12587. PMid:29963751.
9. Şahan Yapicier Ö, Kaya M, Erol Z, Öztürk D. Wistar Ratlarından Scopulariopsis brevicularis izolasyonu. Etlik Vet Mikrobiyol Derg. 2020;31(2):196-200. http://dx.doi.org/10.35864/evmd.768818.
10. Pires MFC, Coutinho SDA, Purchio A, Kipnis TL, Noronha M. Microbiota fúngica na pelagem de camundongos de linhagens isogências mantidos em um biotério de criação. Rev Fac Med Vet Zootec. 1986;23(2):139-44. http://dx.doi.org/10.11606/issn.2318-3659.v23i2p139-144.
11. Mähler Convenor M, Berard M, Feinstein R, et al. FELASA recommendations for the health monitoring of mouse, rat, hamster, guinea pig, and rabbit colonies in breeding and experimental units. Lab Anim. 2014;48(3):178-92. http://dx.doi.org/10.1177/0023677213516312. PMid:24496575.
12. Park JH, Seok SH, Baek MW, et al. Microbiological monitoring of guinea pigs reared conventionally at two breeding facilities in Korea. Exp Anim. 2006;55(5):427-32. http://dx.doi.org/10.1538/expanim.55.427. PMid:17090958.
13. Neufeld PM. Micologia clinica. Rio de Janeiro: Laboratório de Micologia Clínica, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro; 2003 [citado 2012 jun 23]. http://pt.scribd.com/doc/54693980/12/HIALOHIFOMICOSE
14. Romano C, Gianni C, Difonzo EM. Retrospective study of onychomycosis in Italy: 1985-2000. Mycoses. 2005;48(1):42-4. http://dx.doi.org/10.1111/j.1439-0507.2004.01066.x. PMid:15679665.
15. Costa FVA, Spanamberg A, Araujo R, Werner J, Ferreiro L. Feline sino-orbital fungal infection caused by *Aspergillus* and *Scopulariopsis*. Acta Sci Vet. 2019;47(Suppl. 1):383-341.
16. Sri-Jayantha L, Matthews KG, Scharf V. *Scopulariopsis brevicaulis* rhinosinal infection in a dog. J Am Anim Hosp Assoc. 2019;55(1):e55102. http://dx.doi.org/10.5326/JAAHA-MS-6869. PMid:30427708.
17. Przybysz CH, Scolin E. Avaliação do formoldeído como fungicida no laboratório de anatomia humana. Rev F@pciência. 2009;5(12):121-33.
18. Ozturk D, Adanır R, Turutoglu H. Superficial skin infection with *Scopulariopsis brevicaulis* in two goats. a case re-

port. Bull Vet Inst Pulawy. 2009;53:361-3.

19. Miossec C, Morio F, Lepoivre T, et al. Fatal invasive infection with fungemia due to Microascus cirrosus after heart and lung transplantation in a patient with cystic fibrosis. J Clin Microbiol. 2011;49(7):2743-7. http://dx.doi.org/10.1128/JCM.00127-11. PMid:21543579.

20. Cuenca-Estrella M, Gomez-Lopez A, Buitrago MJ, Mellado E, Garcia-Effron G, Rodriguez-Tudela JL. In vitro activities of 10 combinations of antifungal agents against the multiresistant pathogen *Scopulariopsis brevicaulis.* Antimicrob Agents Chemother. 2006;50(6):2248-50. http://dx.doi.org/10.1128/AAC.00162-06. PMid:16723597.

21. Skóra M, Bulanda M, Jagielski T. In vitro activities of a wide panel of antifungal drugs against various Scopulariopsis and Microascus species. Antimicrob Agents Chemother. 2015;59(9):5827-9. http://dx.doi.org/10.1128/AAC.00978-15. PMid:26100698.

22. Couto MS, Pantoja LDM, Mourão CL, Paixão GC. Fungal microbiota of the hair coat of laboratory animals. Rev Bras Vet. 2010;17(1):52-4. http://dx.doi.org/10.4322/rbcv.2014.143.