



## Pet rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*) are vehicles of pathogenic and allergenic fungi

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### Abstract

Nowadays, rabbits and guinea pigs are frequently adopted as companion animals, representing a vehicle for the dissemination of potentially pathogenic and allergenic fungi to their tutors. This study aimed to characterize the cutaneous mycobiota of these species and evaluate the association between mycological cultures results and several variables related to these animals' husbandry. Hair and scales samples (n=102) were collected from 32 rabbits and 19 guinea pigs: 51 by pulling hairs surrounding lesions and collecting scales (if lesions present) or along the body of the animal (if absent); the other samples were collected using Mackenzie's technique. Samples were inoculated in Sabouraud Chloramphenicol Agar and Dermatophyte Test Media and observed daily during the incubation period. Isolated fungal species were identified based on their macro and microscopic morphology. A questionnaire was provided to the animal's tutors to collect information on animal husbandry. The most frequently isolated species corresponded to saprophytic fungi, such as *Aspergillus* spp., *Penicillium* spp., *Scopulariopsis* spp.; yeasts such as *Candida* sp. and *Rhodotorula* sp. were also found. Statistical analysis showed that a positive mycological culture was related with animal's age and the administration of ongoing medication, while the number of isolated fungal species was related with animal's species and outdoor access. These fungi have already been reported as responsible for mycotic infections in humans and animals, including dogs and cats, although they usually affect immunocompromised individuals. Therefore, these animals can represent a zoonotic risk, which may be related with animals age, species, ongoing medication and outdoor access.

### Background

Fungi are ubiquitous microorganisms. The great majority are saprophytic, playing an important role in the environment, contributing to the decaying of organic matter [1]. Similarly, most pathogenic fungi are exogenous, being also present in the environment, water or soil, but can also belong to the animals mycobiota and cause disease (mycosis) when the host presents an organic imbalance [2]. Finally, fungi can also have a potential allergenic effect [3]. Pets can be potential vehicles of pathogenic and allergenic fungi. The most frequent mycosis in pet rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*) is dermatophytosis, with *Trichophyton mentagrophytes* and, less frequently, *Microsporum canis* being the most common associated species in these animals [4]. Typical dermatophytosis lesions are circular, irregular or diffuse alopecia, localized or generalized, presenting scaling, crusting and erythema of the borders. Lesions develop most

frequently on the head and extremities in both species and also in the thorax of guinea pigs. These animals can also present asymptomatic or subclinical disease or develop a carrier state [4-9], representing a potential dissemination vector of zoonotic infections to their tutors, especially to immunocompromised individuals and children [6-8,10]. Pet rabbits and guinea pigs can also present non-dermatophytic mycosis. Despite of being rare, cases of cutaneous cryptococcosis in guinea pig [11], cheilitis by *Candida* sp. in guinea pigs [12-14], pulmonary and cutaneous aspergillosis in rabbits [12] and infections by *Malassezia* sp. in pet rabbits and guinea pigs were already reported [15-19]. Some studies have been carried regarding the characterization of the cutaneous mycobiota of rabbits [6,8,20,21] and guinea pigs [6,8,20-24], but such studies are not available in Portugal, where there is no information on the mycobiota of these pets, its role on these animal health, and on the influence of husbandry on fungal colonization.

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Such data would contribute to evaluate the role of these pets as potential carriers of fungal species with zoonotic potential. Therefore, the present study aimed to characterize the skin and hair mycobiota of pet rabbits and guinea pigs presented for consultation in the University Teaching Hospital of Faculty of Veterinary Medicine, University of Lisbon, Portugal, and to determine the association between animals related factors and their colonization by fungi.

## Materials and methods

### Inclusion criteria

This study included 32 (62.7%) rabbits and 19 (37.3%) guinea pigs. Animals were presented to medical consultation at the University Teaching Hospital of Faculty of Veterinary Medicine, University of Lisbon, Portugal, between February 5, 2018 and July 6, 2018. No selection was performed based on animals age, gender and health status.

### Sample collection

In each animal, samples were collected by two methods. The first method consisted of pulling hairs and collecting scales from the periphery of lesions, if present, with a sterile Halsted Mosquito haemostatic forceps; in animals with no lesions, samples were collected from the area's most frequently associated with dermatophytosis lesions in the rabbit and guinea pig, such as the head and extremities in both species and the thorax in guinea pigs [4,5]. Samples were also collected through the Mackenzie's technique, which consists in passing a sterilized toothbrush through the entire coat of the animal thirty times or until it has hairs and visible skin residues [25]. A total of 102 samples were collected, 51 by the pulling method and 51 by Mackenzie's technique. Samples were collected by trained veterinarians following standard routine procedures with consent of the owner, and no ethics committee approval was needed.

### Sample processing

Samples were inoculated in two different culture media, Sabouraud Chloramphenicol Agar (SCA) and Dermatophyte Test Media (DTM). All samples were incubated at a temperature of 25-27°C, for 21-days (SCA) or 14-days (DTM) [25,26]. During the incubation period, inoculated media were observed daily. Colonies obtained were identified at the genus level based on their macro and microscopic morphology [27].

### Statistical analysis

A questionnaire was provided to the tutors with the goal of collecting detailed information about the history, husbandry and characterization of the animals. The statistical analysis was performed in the R software [28]. Fit models were used to evaluate the relation between dependent variables (positivity for dermatophytes, positivity in mycological culture and number of isolated fungal species) and independent variables (species, gender, age, origin, dental disease, contact with other animals, hygiene, outdoor access, food and ongoing medication), accessed through anamnesis and the questionnaire. Two different regression models were used, since dependent variables with different structures were observed. First, for the variable's positivity for dermatophytes and positivity in the mycological culture, a logistic regression model was used, which considered the value 1 to represent the presence of dermatophytes or fungal species and the value 0 to represent their absence. Second, a

linear regression model was used for the variable number of isolated fungal species.

For each independent variable, the regression coefficient, standard deviation, and p-value were determined, after defining a reference category, used for comparison. The variable species was divided into two categories, rabbit and guinea pig, with the reference category corresponding to guinea pig; the variable outdoor access was divided into five categories, street, backyard, public garden, terrace and none, with the reference category corresponding to none; the variable ongoing medication refers to the medication the animal was taking at the time of sampling and was divided into eight categories, anti-inflammatory, antinematodal drugs, antibiotic, antifungal, analgesic, vitamins, probiotics and none, with the reference category corresponding to none.

## Results

### Population characterization

Of the 51 animals sampled, 23 (45.1%) were males and 27 (54.9%) females. Of the 32 rabbits, 18 (56.25%) were male and 14 (43.75%) were female; in its turn, of the 19 guinea pigs, 6 (31.6%) were male and 13 (68.4%) were female. Animal's age was between one month and a half (0.2 years) and 10 years and 2 months (10.3 years), with an average of 3.70 years and a median of 3.10 years. Animals were presented for consultation due to several reasons. Approximately half of the animals (n=24; 47.1%) were presented for a preventive medicine consultation, with the remaining being presented for consultation due to problems in other organic systems. The most frequent consultations were of dermatology, neurology, and dentistry, with the same number of cases (9.8% each). Despite that, dermatological signs were found in 14 animals presented for non-related consultations. In total, 19 animals (37.3%) showed diverse dermatological lesions at the moment of sampling. The most frequent were scaling (n=7, 28.0%), alopecic lesions (n=6, 24.0%), and pododermatitis (n=5, 20.0%), followed by moist dermatitis (n=2, 8.0%) and mites, pruritus, lipoma, otitis externa and thinned skin (n=1 each, 4.0% each). The majority of the animals (n=36; 70.6%) was not taking any medication at the time of sampling, with the following exceptions: 7 animals (13.7%) were taking anti-inflammatories, 8 animals (15.7%) antibiotics, 5 animals (9.8%) antinematodal drugs, 2 animals (3.9%) vitamins and 1 animal (2.0%) probiotics.

Of the animals included in this study, 43.1% (n=22) had contact with individuals at risk, such as children (n=10, 19.6%), elderly (n=10, 19.6%) and pregnant women (n=2, 3.9%). Most were exclusively indoor (n=40, 78.4%), as only 10 animals (n=11, 21.6%) had occasional access to the street, most of which to a backyard (n=7, 13.7%) and the others to the public garden (n=2, 3.9%), terrace (n=1, 2.0%) and street (n=1, 2.0%).

### Isolated fungal species

Eleven fungal species were isolated from the collected samples. It was not possible to isolate any dermatophytes. Nine genera of saprophytic moulds were identified, representing 93.5% of the total number of fungal isolates obtained, and also 2 species of yeasts, representing 6.5% of the isolates obtained (Table 1). It was possible to obtain 157 saprophytic moulds isolates, belonging to the following genera: *Aspergillus* (n=45, 26.8%), *Scopulariopsis* (n=26, 15.5%), *Penicillium* (n=25, 14.9%), *Mucor* (n=12, 7.1%), *Rhizopus* (n=12, 7.1%), *Cladosporium* (n=8, 4.8%), *Alternaria* (n=7, 4.2%), *Phoma* (n=2, 1.2%) and

**Table 1.** Absolute Frequency (AF) and Relative Frequency (RF) of the fungal species identified in this

	Species	AF	RF (%)
Filamentous fungi	<i>Alternaria</i> sp.	7	4,2
	<i>Aspergillus</i> spp.	45	26.8
	<i>Chaetomium</i> sp.	1	0,6
	<i>Cladosporium</i> sp.	8	4.8
	<i>Mucor</i> spp.	12	7.1
	<i>Penicillium</i> spp.	25	14.9
	<i>Phoma</i> sp.	2	1.2
	<i>Rhizopus</i> sp.	12	7.1
	<i>Scopulariopsis</i> spp.	26	15.5
	Non-identified filamentous fungi	19	11.3
Yeasts	<i>Candida</i> sp.	9	5.4
	<i>Rhodotorula</i> sp	2	1.2
Total		168	100.0

**Table 2.** Results of the analysis between the positivity in mycological culture and the independent variables. The table represents the results obtained after regression analysis between the dependent variable positivity in mycological culture and the independent variables. Symbols presented in front of the p-value indicates that the variable is significant for the most usual levels of statistical significance: “.” – 0.1; “\*” – 0.05; “\*\*” – 0.01; “\*\*\*” – 0.001.

Variable of interest (dependent variable)	Independent variable			Standard deviation	
Positivity in mycological culture	Species	Categories	Coefficient		p-value
	Gender	Male	0.08808	0.12620	0.4942
	Age	–	0.04851	0.02499	0.0647 .
	Origin	Petshop	0.53630	0.29783	0.0854 .
		Breeder	0.46008	0.46299	0.35808
		Particular	0.44375	0.40295	0.2821
	Dental disease	Presence	-0.07853	0.12799	0.5462
	Contact with other animals	Dog	0.72085	0.45056	0.1220
		Cat	-0.43221	0.49383	0.3907
		Rabbit	0.36465	0.25930	0.1728
		Guinea pig	0.33537	0.27617	0.2381
		Chinchilla	0.12792	0.66845	0.8489
	Food	Pellets	0.48166	0.47429	0.3115
		Hay	0.39097	0.45443	0.3910
		Fruit	0.47627	0.45909	0.3012
		Vegetables	0.48032	0.44924	0.2867
		Bread	0.38270	0.55330	0.4904
		Street	0.17066	0.35405	0.6354
	Outdoor access	Backyard	0.34288	0.20971	0.1150
		Public garden	0.79459	0.47662	0.0977 .
		Terrace	-0.20724	0.36657	0.5780
	Hygiene Ongoing medication	Yes	0.05344	0.21650	0.8074
		Anti-inflammatory	0.44386	0.21230	0.0444 *
		Antinematodal drug	0.65259	0.32102	0.0460 *
		Antibiotic	0.50278	0.22179	0.0296 *
		Antifungal	-0.08474	0.53469	0.8744
Analgesic		0.10099	0.31883	0.7524	
Vitamins		-0.23892	0.34387	0.4902	
Probiotics		-0.17014	0.53340	0.7504	

**Table 3.** Results of the analysis between the number of isolated fungal species and the independent variables. The table represents the results of the regression analysis between the dependent variable number of isolated fungal species and the independent variables. Symbols presented in front of the p-value indicates that the variable is significant for the most usual levels of statistical significance: “.” – 0.1; “\*” – 0.05; “\*\*” – 0.01; “\*\*\*” – 0.001.

Variable of interest (dependent variable)	Independent variable	Categories	Coefficient	Standard deviation	p-value
Number of isolated fungal species	Species	Rabbit	-0.944045	0.484200	0.064839 .
	Genre	Male	0.002295	0.255143	0.992933
	Age	–	0.048328	0.049690	0.342146
	Origin	Petshop	0.274939	0.591464	0.646941
	Dental disease	Breeder	1.382482	0.918278	0.146825
		Particular	-0.090275	0.798079	0.910995
		Presence	-0.300154	0.258755	0.260123
	Contact with other animals	Dog	1.815347	0.889183	0.053652 .
		Cat	0.107732	0.981259	0.913665
		Rabbit	0.460553	0.513964	0.380614
		Guinea pig	0.638197	0.553708	0.263399
		Chinchilla	-1.010326	1.252532	0.423662
	Food	Pellets	1.328621	0.825614	0.109752
		Hay	0.916870	0.790083	0.247789
		Fruit	0.385158	0.799038	0.630519
		Vegetables	0.488230	0.782261	0.533530
		Bread	0.542895	0.948621	0.568140
	Outdoor access	Street	0.380810	0.714589	0.601004
		Backyard	1.012497	0.414648	0.023260 *
		Public garden	1.486831	0.847660	0.081777 .
		Terrace	0.201894	0.735818	0.786843
	Hygiene	Yes	-0.378714	0.438532	0.397827
	Ongoing medication	Anti-inflammatory	0.461022	0.411607	0.271631
		Antinematodal drug	0.953670	0.596988	0.114909
		Antibiotic	0.683885	0.427860	0.119800
Antifungal		0.913216	0.970479	0.349053	
Analgesic		0.214098	0.592782	0.719223	
Vitamins		-0.715989	0.646202	0.273205	
Probiotics		-0.049822	0.970504	0.959165	

*Chaetomium* (n=1, 0.6%). It was not possible to identify 11.3% of the colonies obtained (n=19). It was also possible to obtain 11 yeast isolates, identified as *Candida* sp. (n=9, 5.4%) and *Rhodotorula* sp.n=2, 1.2%)

### Statistical analysis

Statistical analysis results are presented on Table 2 and 3. The number of isolated fungal species was significantly related with the animal species; in fact, since the regression coefficient for the rabbit category was found to be negative, rabbit samples are more likely to originate cultures with a lower number of fungal species than guinea pig samples (reference category).

Animal age was significantly related with a positive mycological culture; the coefficient for the age category was found to be positive indicating that older animals are more likely to produce a positive fungal culture. Results from animals'

which origin was a petshop were significantly related with a positive mycological culture. However, all these animals were adults at the time of sample collection, a long time period after their last contact with the petshop, which reduces the relevance of this relation for further discussion.

The number of isolated fungal species is related with the outdoor access. The coefficients of the categories backyard and public garden were found to be positive, suggesting a higher probability that samples obtained from animals with outdoor access, especially those who have access to a backyard, will originate cultures with a higher number of fungal species than those obtained from animals without outdoor access (reference category). A positive mycological culture was also related to outdoor access, specifically the public garden. However, only one observation contributed to this result, and therefore this result was not considered for further discussion. The same was

observed for the number of isolated fungal species and the contact with other animals (dogs).

Finally, a positive mycological culture was found to be related with animals ongoing medication. The coefficients of the categories anti-inflammatory, antinematodal drugs and antibiotic were found to be positive, indicating that animals taking these medications have a higher probability of originating a positive mycological culture than samples obtained from animals that were not taking any medication (reference category).

## Discussion

Pet rabbits and guinea pigs can be potential vehicles for zoonotic or allergenic fungi to their tutors. Results revealed that the cutaneous mycobiota of the rabbits and guinea pigs under study was mainly composed by environmental filamentous saprophytic fungi and yeasts, as described in other studies performed in these animal species [6,7,21,22], as well as in others, such as dogs and cats [21,22,29-36]. Saprophytic fungi were expected to be found in these animals because of their permanent contact with organic matter present in the hay, food and substrate and with the environment. The yeast species identified were also expected, as they are commensals of animals and humans microbiota (*Candida*) [37] or humid environments (*Rhodotorula*) [38]. As far as we know, this study allowed the isolation of three fungal genera in rabbits and guinea pigs for the first time, namely *Chaetomium* sp., *Phoma* sp. and *Rhodotorula* sp., which were already isolated from dogs and cats [29,30,33,34,39]. It was not possible to isolate dermatophytes from the animals under study; however, the other fungi identified were already reported as responsible for cutaneous lesions in humans, dogs and cats. In humans, *Aspergillus* sp., *Alternaria* sp., *Candida* sp., *Chaetomium* sp., *Cladosporium* sp., *Mucor* sp., *Penicillium* sp., *Phoma* sp., *Rhizopus* sp. and *Scopulariopsis* sp. have been reported to be responsible for cutaneous infections [40-46]. In dogs and cats, *Alternaria* sp., *Aspergillus* sp., *Candida* sp., *Cladosporium* sp., *Mucor* sp. and *Penicillium* sp. have also been reported as responsible for cutaneous infections [40,47-50]. Most of them were not previously associated with infections in pet rabbits and guinea pigs, except for *Aspergillus* sp., that was already related with cutaneous infections in rabbits [12]. However, it is important to refer that *Scopulariopsis* sp. was isolated from one animal under study, which had alopecia and crusting lesions on the extremities. Several factors need to be taken into consideration before relating this species to infection, namely the concentration of isolated fungi, the clinical status of the animal and the possibility of contamination of the sample or culture [51]. Although the hypothesis of colonization of a pre-existing wound cannot be ruled out, results seem to indicate that this fungus may have been responsible for this lesion, especially since its association with lesions very similar to dermatophytosis in humans has been described [45]. In fact, identified species may have the ability to cause disease in rabbits and guinea pigs, not only in animals with immunosuppression or deficient nutritional status, but also in healthy individuals after abrasive or perforating injury [40]. Therefore, veterinarians should consider non-dermatophytic fungal infections as a differential diagnosis for cutaneous lesions. Many of the variables under study are frequently referred as predisposing factors for dermatophytosis in guinea pigs and rabbits [4]. Dermatophytes were not isolated, but the association between positive mycological cultures and number of fungal species isolated with several independent variables was evaluated. It was possible to observe that samples collected from guinea pig are likely to originate cultures with a

higher number of fungal species than those from rabbits, which seems to indicate that the self-cleaning habits of guinea pigs are less efficient than the ones from rabbits; also, unlike rabbits, mutual grooming is less frequent between different guinea pigs [52]. Self-cleaning allows the maintenance of animal hygiene by removing dirt and parasites [53]. If not performed, the skin surface presents a higher probability of fungi colonization and dermatological disease development, which may also explain the higher tendency of guinea pigs for dermatophytosis [4,6,8,20,21,54]. In fact, a study in cats with dermatophytosis reported that collared cats show more generalized lesions comparing with animals without Elizabethan collar that could perform their self-cleaning, suggesting that the cleaning behaviour may limit the development of lesions [55].

Age was found to be related with positive mycological cultures. Older animals may suffer from diseases that could impair cleaning behaviours, such as dental disease associated with oral pain, or musculoskeletal disorders that reduce mobility and cause pain [56,57]. Regarding outdoor access, it was already reported that fungal species present in cats' hair may vary depending on the environment [58]. Considering that the organic matter present in gardens and backyards constitutes a relevant substrate for saprophytic fungi, it is expected that samples collected from animals with outdoor access to originate cultures with a higher number of fungal species. Prolonged antimicrobial therapy can influence the composition of the commensal microbiota of mucosa and facilitate yeast proliferation [37], being reported that the skin and hair microbiota may vary according to the immune state [58]. In this study, samples from animals which were under drug therapy (anti-inflammatory, antinematodal drugs and antibiotic) presented a higher probability of originating positive cultures. These animals were mainly diagnosed with advanced dental disease or presented neurological signs compatible with *Encephalitozoon cuniculi*. These two situations generally promote a decrease in body condition, poor nutritional status, and an altered degree of activity [55-57], which may explain this result. Concluding, this is the first study regarding cutaneous mycobiota of pet rabbits and guinea pigs performed in Portugal. Despite of not being possible to isolate dermatophytes, results showed that these animals can carry several filamentous fungi and yeasts in their hair and skin. Three of these genera were isolated for the first time in these animals, namely *Chaetomium*, *Phoma* and *Rhodotorula*. All the isolated fungi are frequently present in the environment and usually do not cause disease. However, infections related with most of the identified fungi were already described in animals and humans, especially in immunocompromised individuals. Therefore, these animals can represent a zoonotic risk, which may be related with animals age, species, ongoing medication and outdoor access.

## Conclusion

Currently, pet rabbits and guinea pigs are frequently adopted as companion animals, being a potential vehicle for the dissemination of pathogenic and allergenic fungi to their owners. A characterization of animal's skin mycobiota and its relationship with their medical records was performed. Results showed that these animals can carry several filamentous fungi and yeasts in their hair and skin. The most frequently isolated species corresponded to saprophytic fungi, such as *Aspergillus* spp., *Penicillium* spp., *Scopulariopsis* spp.; *Candida* sp. and *Rhodotorula* sp. In addition, the three fungal genera *Chaetomium* sp., *Phoma* sp. and *Rhodotorula* sp. were identified for the first time in pet rabbits and guinea pigs. Animal's age or the presence

of ongoing medication were related to a positive mycological culture, while the number of isolated fungal species was related with the animal's species and outdoor access. In conclusion, infections related with most of the identified fungi were already described in animals and humans, reinforcing the importance of the mycological assessment in these animals, as they may represent a potential zoonotic risk.

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