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Cutaneous carriage of *Malassezia* species in healthy and seborrhoeic Sphynx cats and a comparison to carriage in Devon Rex cats

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Cutaneous carriage of *Malassezia* species yeast was investigated in 32 Sphynx cats, and in 10 domestic shorthair (DSH) cats. Samples for mycological culture were taken using contact plates and swabs at seven sites in each cat (left and right axillae and groin, left ear, claw fold on left front paw and the interdigital palmar web of the left front paw). *Malassezia* species were isolated from 26/32 Sphynx cats (81%) and from 0/10 DSH control cats. In five cases *Malassezia* species yeasts were isolated at a single site, in the remaining 21 Sphynx cats at multiple sites. A total of 73 *Malassezia* species isolates were made, of which 68 were *Malassezia pachydermatis* and five were lipid-dependent *Malassezia*. Five out of the 32 Sphynx had greasy seborrhoea, and all seborrhoeic cats had *M pachydermatis* isolated from their skin, at multiple sites. None of the 32 Sphynx had *Malassezia* species isolated from the ears. The difference in population sizes between Sphynx and DSH cats was significant ($P \leq 0.05$) for the axillae, groins and claw fold. The difference in frequency of isolation was significant ($P \leq 0.05$) for the axillae and right groin. The level of cutaneous carriage of *Malassezia* species in Sphynx was similar to that previously reported for Devon Rex cats (DRC) [Åhman S, Perrins N, Bond R. Carriage of *Malassezia* species yeasts in healthy and seborrhoeic Devon Rex cats. *Med Mycol* 2007; **45**: 449–455]. The poor recovery of *Malassezia* species from ears in both Sphynx and DRC, has clinical implications for dermatological sampling in these breeds.

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Malassezia species are part of the commensal skin flora of cats similar to the situation in many other mammals.[2–4] Various *Malassezia* species have been isolated from cats, including *M pachydermatis*[2], *M sympodialis*[3], *M sloffiae*[5], *M furfur*[6], *M nana*[7] and *M globosa*[4]. It has been shown that cutaneous colonisation with large numbers of *Malassezia species* yeasts and/or *Malassezia*-associated dermatitis in cats may be related to endocrine, immunosuppressive, neoplastic and allergic disease.[8–11] In dogs, certain breeds carry high numbers of *Malassezia* species and may be predisposed to *Malassezia* dermatitis.[12,13] Similarly, Devon Rex cats (DRC) were recently shown to carry significantly more *Malassezia* species on their skin, than did healthy DSH cats, and they were also predisposed to *Malassezia*-associated seborrhoeic dermatitis.[1]

The Sphynx is an almost hairless cat breed (Fig 1). The skin is soft with very few, fine down-like hairs.[14–16] Although not mentioned in standard textbooks on veterinary dermatology, it is the authors' clinical impression that Sphynx skin often has a greasy exudate, which to a varying degree accumulates on the surface as a thin sticky, dark brown or reddish-brown layer. Breeders and judges have long been aware of this and mention it as a common and normal feature of the breed.[15–17] Because of the greasy exudate accumulating on the skin, Sphynx owners are often recommended to bath their cat frequently.[15,16] In the Sphynx, accumulation of greasy material may be particularly noticeable around the claws and in the palmar and/or plantar interdigital web (Figs 2 and 3).[15,16] This greasy exudate is macroscopically similar in appearance to that of seborrhoeic DRC.[1] The DRC was used for many years to outcross Sphynx, and it is probably fair to assume that they share some genetic material.[14,15,17]

We hypothesised that the carriage rates of *Malassezia* species yeasts in Sphynx cats would be increased, similarly to those of DRC, and exceed those of DSH cats. The purpose of this study was to compare the

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Fig 1. Typical Sphynx cat.

frequency of isolation and population sizes of *Malassezia* species recovered from Sphynx cats to that of DSH cats. In addition, to facilitate comparison between breeds, the results from the Sphynx cats were also compared to those of DRC sampled previously in an identical way.[1]

Materials and methods

The study was approved by The Swedish Committee for Animal Research Ethics (Djurförsöksetiska nämnden). The cats were recruited on a voluntary basis via a Swedish internet based Sphynx cat network, and via the patient database at Djurakuten small animal hospital, Stockholm, Sweden. A written informed consent was obtained from the owners of 32 Sphynx cats coming from 13 private homes and one breeder. They were aged between 7 months and 5 years, 14 were male and 18 female and all considered by their owners to be in general good health. The owners were asked to fill out a questionnaire regarding the cats current and previous health, diet, housing and any over-the-counter or prescription drugs or treatments given in the previous 6 months.

Fig 2. Greasy exudate around the claws in a seborrhoeic Sphynx cat.

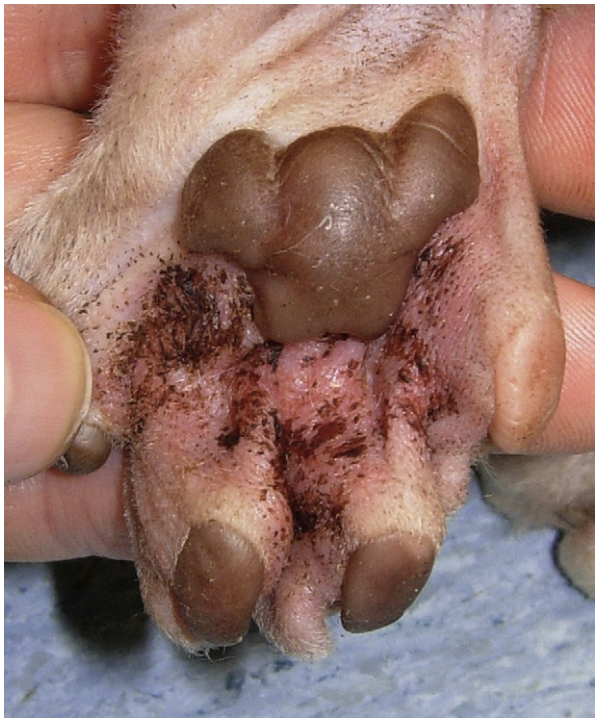

Fig 3. Greasy exudate in palmar interdigital web in a seborrhoeic Sphynx cat.

Inclusion criteria were: purebred Sphynx cat, any sex, over 6 months, feline immunodeficiency virus/feline leukaemia virus-negative, and in good general health. Exclusion criteria were: any topical therapy for the previous 2 weeks, systemic treatment with antibiotic, antifungal, or immunosuppressive drugs, including all glucocorticosteroids for the previous 6 weeks, and obvious signs of ill health. Routine ear cleaning with dry cotton buds or wipes, but without the use of ear cleaning products was permitted. Ten healthy DSH cats (aged 1–5 years, 3 males and 7 females) from one shelter and two private homes were used as control group and sampled in an identical way.

At examination, presence of greasy seborrhoea was assessed by one and the same examiner, using a 4-graded scale developed for evaluating the extent and severity of seborrhoea in DRC in each of the commonly affected areas, namely the left and right axillae, left and right groin, claw folds and interdigital palmar/plantar skin.[1,18] Grade 1 was defined as absence of seborrhoeic exudates and normal skin; grade 2 as mild accumulations of tightly adherent brown greasy

exudate on the interdigital skin or around claws, mild erythema and/or mild exudation in the axilla and groin; grade 3 as obvious, moderate exudate accumulation on the interdigital skin or around claws, and moderate erythema and/or greasy exudation in the axillae and groin. Grade 4, finally, was defined as severe, marked exudate accumulation on the interdigital skin or around the claws and marked erythema and/or greasy exudation in the axilla and groin. Scores from each of the six areas were added to generate an overall extent and severity score ranging from 6 (normal, no seborrhoea) to 24 (severe greasy seborrhoea at all sites). A total score of 6–10 was considered within normal limits for a Sphynx cat, whereas an animal with a total score higher than 10 was considered seborrhoeic.

Skin sample collection

The skin of the left and right groin and axillae were sampled with small contact plates (19 mm ∅) filled to the meniscus with modified Dixon's agar.[19] The plates were pressed on to the skin for 10 s, and then incubated at 32°C (±1°C) for 7 days. Yeast counts from these areas were expressed as colony-forming units (cfu) per cm^2. The left ear, one claw fold on the front left paw and the palmar interdigital web of the front left paw was sampled with a mini-tipped swab that was rubbed over the area for 5 s.[3] Swab tips were cut off into wash fluid (0.075 M phosphate-buffered saline, pH 7.9, with 0.1% Triton X-100). Serial tenfold dilutions were prepared with the same wash fluid and spread on to modified Dixon's agar and incubated at 32°C (±1°C) for 7 days. Yeast counts from these sites were expressed as log10 (cfu $swab^{-1}$ +1).

Yeast identification

Identification of *M pachydermatis* was based on gross colonial and microscopic morphology, and by the ability to grow on Sabouraud's dextrose agar. Identification of lipid-dependent *Malassezia* species was based on gross colonial and microscopic morphology, and by the inability to grow on Sabouraud's dextrose agar. Further identification of lipid-dependent isolates was based on their ability to use Cremophor EL or Tween 20, 40, 60, or 80 as a lipid source.[20,21] Additionally, catalase activity, ability to hydrolyse aesculin, and ability to grow on modified Dixon's agar was evaluated.[20,22,23] Type cultures obtained from the Central Bureau Schimmelcultures were used as control specimens for the yeast identification.

Statistical analyses

Population sizes of *Malassezia* species were compared between groups of cats using the Mann–Whitney *U*-tests. Frequency of isolation was compared between groups using Fisher's exact test. Data from Sphynx cats and the control group of 10 healthy DSH cats were compared. Additionally, in a comparison between Sphynx cats and DRC, all control cat data from both studies were pooled to give a control group of 25 healthy DSH. Statistical calculations were done using SAS statistical software package (SAS version 9.2, SAS, Cary, USA), with $P \leq 0.05$ for significance. We have used $\ll$ to mark when P is considerably smaller, by several orders of magnitude.

Results

Malassezia species were isolated from 26/32 Sphynx cats (81%) and from 0/10 DSH control cats. A total of 73 *Malassezia* species isolates were made from the Sphynx cats. In five cats *Malassezia* species yeasts were isolated at a single site, in the remaining 21 at multiple sites. Five cats had prominent greasy seborrhoea (severity score ranging from 14–22, median 20), and 27 Sphynx cats had skin considered normal for this breed (severity score ranging from 6–10, median 6). Five out of the five seborrhoeic Sphynx cats had *M pachydermatis* isolated from their skin, at multiple sites. In no case was *Malassezia* species isolated from the ears.

The overwhelming majority (68/73) of the isolates were *M pachydermatis.* Only five isolates from five different cats were lipid-dependent *Malassezia* species, namely *M sloffiae* from the axillae of one cat; *M sympodialis* from the axillae of one cat and the claw fold of another. In two cases we were not able to reliably identify the lipid-dependent subspecies.

Table 1. Frequency of isolation and *P*-values from Fischer's exact tests

Site	Sphynx			DSH	*P*-values			
	Total (T)	Normal (N)	Seborrhoeic (S)		T vs DSH	S vs N	N vs DSH	S vs DSH
Left axillae	44%	37%	80%	0	0.02	0.14	0.04	<0.01
Right axillae	56%	48%	100%	0	<0.01	0.05	0.01	≪0.01
Left groin	31%	19%	100%	0	0.08	<0.01	0.30	≪0.01
Right groin	53%	48%	80%	0	<0.01	0.34	0.01	<0.01
Ear	0%	0%	0%	0	–	–	–	–
Claw	31%	33%	20%	0	0.08	1.0	0.08	0.33
Palm	12%	7%	40%	0	0.56	0.11	1.0	0.10

Table 2. Median (lower, upper quartile) population size and *P*-values from Mann–Whitney *U*-tests

Site	Sphynx			DSH	*P*-values			
	Total (T)	Normal (N)	Seborrhoeic (S)		T vs DSH	S vs N	N vs DSH	S vs DSH
Left axillae	0 (0, 1.6)	0 (0, 1.1)	3.5 (3.5, 6.0)	0 (0, 0)	0.01	0.01	0.03	<0.01
Right axillae	0.4 (0, 0.4)	0 (0, 1.1)	7.4 (5.3, 9.9)	0 (0, 0)	<0.01	≪0.01	0.01	≪0.01
Left groin	0 (0, 0.4)	0 (0, 0)	7.4 (7.1, 28.2)	0 (0, 0)	0.05	≪0.01	0.16	≪0.01
Right groin	0.4 (0, 0.5)	0 (0, 0.4)	3.5 (2.5, 5.6)	0 (0, 0)	0.01	0.01	0.01	<0.01
Ear	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)	1.0	1.0	1.0	1.0
Claw	0 (0, 2.7)	0 (0, 2.7)	0 (0, 0)	0 (0, 0)	0.05	0.80	0.04	0.20
Palm	0 (0, 0)	0 (0, 0)	0 (0, 3.0)	0 (0, 0)	0.26	0.04	0.41	0.05

No lipid-dependent isolates were made from the five seborrhoeic Sphynx cats.

The difference in frequency of isolation between Sphynx and DSH cats was significant in several (axillae and right groin, $P \leq 0.02$) but not all of the sampled sites. When comparing the five Sphynx cats with greasy seborrhoea with the remaining Sphynx, the former had a higher frequency of isolation at 5/7 sites (Table 1).

The difference in population sizes between Sphynx and DSH was significant in 5/7 sampled sites (axillae, groins and claw fold, $P \leq 0.05$). The five seborrhoeic Sphynx cats also had significantly higher population sizes in 5/7 sampled sites (axillae, groins and palmar interdigital web, $P \leq 0.04$) than did the remaining Sphynx (Table 2).

When comparing Sphynx cats to DRC and the pooled group of DSH control cats, both Sphynx and DRC had a significantly higher frequency of isolation ($P \leq 0.02$) and higher population sizes ($P < <0.01$) than DSH at all sampled sites except the ear (Tables 3 and 4). In the pooled group of DSH control cats, there were a total of four *Malassezia* isolates from three different DSH cats, originating from the earlier DRC study.[1] There was no statistical difference in yeast carriage between the two DSH control groups pooled for data analysis. Between the Devon Rex and Sphynx groups there was little difference, only at the site of the left groin did DRC have significantly more isolates and higher population sizes than Sphynx cats ($P = 0.02$) (Tables 3 and 4).

Finally, comparing seborrhoeic Sphynx and DRC, and non-seborrhoeic Sphynx and DRC we found the main difference at the claw fold site, where seborrhoeic DRC had significantly more isolates (89% vs 20%, $P = 0.02$), as well as higher population sizes ($P = 0.01$) than did seborrhoeic Sphynx cats (Table 5).

Discussion

The Sphynx cat is a relatively young breed, based on a few hairless kittens found in the late 1960s.[14,15] The Sphynx has histologically small and curved hair follicles, which form small dysplastic hairs most of which do not penetrate the epidermis.[24] In the 60s and 70s, the breed had problems with fatal seizures in kittens and in an attempt to expand the gene pool, DRC was used to outcross the Sphynx.[14,15,17] Within both breeds there are many individuals with a varying degree of dark brown, greasy exudate around their claws, and at other sites, including axillae, groin and sometimes ears.[1,15,16,18] Many Sphynx owners bathe their cats regularly, often every 7–14 days, to remove the excessive greasiness from body, claws and ears.[15,16] For many Sphynx cats, greasiness becomes increasingly obvious the longer it has been since their last bath.[15,16] At examination of the participating Sphynx, the extent of seborrhoeic changes was assessed and a severity score generated. Based on this score, they were categorised into two groups; one seborrhoeic and one within normal limits of the breed. All cats had a minimum of 2 weeks without a bath

Table 3. Frequency of isolation and *P*-values from Fischer's exact tests

Site	Sphynx ($n = 32$)	Devon Rex ($n = 30$)	DSH ($n = 25$)	*P*-values		
				Sphynx vs DRC	Sphynx vs DSH	DRC vs DSH
Left axillae	44%	57%	4%	0.45	≪0.01	≪0.01
Right axillae	56%	53%	0%	1.0	≪0.01	≪0.01
Left groin	31%	63%	1%	0.02	0.02	≪0.01
Right groin	53%	67%	1%	0.31	≪0.01	≪0.01
Ear	0%	3%	1%	0.11	0.44	0.62
Claw	31%	47%	0%	0.30	<0.01	≪0.01

Table 4. Median (lower, upper quartile) population size and *P*-values from Mann–Whitney *U*-tests

Site	Sphynx ($n = 32$)	Devon Rex ($n = 30$)	DSH ($n = 25$)	*P*-values		
				Sphynx vs DRC	Sphynx vs DSH	DRC vs DSH
L axillae	0 (0, 1.6)	0.3 (0, 11.8)	0 (0, 0)	0.26	$\ll 0.01$	$\ll 0.01$
R axillae	0.4 (0, 1.4)	0.3 (0, 11.2)	0 (0, 0)	0.67	$\ll 0.01$	$\ll 0.01$
L groin	0 (0, 0.4)	0.3 (0, 25.5)	0 (0, 0)	0.02	< 0.01	$\ll 0.01$
R groin	0.4 (0, 0.5)	0.6 (0, 25.5)	0 (0, 0)	0.06	$\ll 0.01$	$\ll 0.01$
Ear	0 (0, 0)	0 (0, 0)	0 (0, 0)	0.07	0.27	0.43
Claw	0 (0, 2.7)	0 (0, 3.9)	0 (0, 0)	0.03	< 0.01	$\ll 0.01$

prior to sampling, however, the exact number of days from the last bath (range 2–8 weeks) to the date of examination was not correlated to the degree of greasy seborrhoea.

The results confirm our hypothesis that Sphynx cats carry significantly higher numbers of *Malassezia* species yeasts than DSH cats. The high level of cutaneous carriage of *Malassezia* species in the examined group of Sphynx cats was at most sites similar to that reported in DRC.[1] In Sphynx and DRC cats with greasy seborrhoea the highest frequency of isolation and population sizes was recorded. This provides further support for an association between greasy seborrhoea and cutaneous carriage of high numbers of *Malassezia* species in these breeds. It was recently shown that the greasiness of the skin resolved in seborrhoeic DRC cats treated with itraconazole, and that the yeast count reduction corresponded well with the resolution of greasy exudate, again supporting the association.[18] Furthermore, in DRC a correlation between the presence of dark brown greasy exudate on the claws and in the claw folds and the *Malassezia* species population was recently reported.[25] Sphynx cats likewise often present with a greasy exudate on claws and in the claw fold (Fig 2) and our study showed frequent recovery of *Malassezia* species yeasts from these sites.[15,16] Our study indicates that a relation between the greasy exudate on claws or in the claw fold and the presence of *Malassezia* species might also be true in the Sphynx breed, though recovered yeast populations were not as large as in DRC.

In the comparison between Sphynx cats (living and sampled in Sweden) and DRC (living and sampled in the UK) all DSH control cats, both Swedish and UK, were pooled for data analysis. However, we do not know if potential differences in, for example, diet, housing and genetics vary significantly between UK and Swedish DSH cats, or between the Swedish Sphynx and UK DRC. Nor do we know if such difference might influence the cutaneous yeast colonisation and recovery. A study that evaluated the recovery of yeast from feline ears set in relation to variations in lifestyle, sex and age found no significant differences.[26] Furthermore, statistical analysis showed no significant difference in yeast recovery between the two DSH groups, and although this is not a complete proof that no difference exist, we have assumed that for the purpose of this study, the healthy DSH control cats are similar enough to be pooled for data analysis.

The majority of the 73 isolates were *M pachydermatis*. Five isolates were lipid-dependent *Malassezia* species. With the methods used, we were able to reliably identify 3/5 lipid-dependent *Malassezia* species isolated. Further identification, eg, with polymerase chain reaction technique, was beyond the scope of this study, and not considered necessary for our purposes. In humans most pathogenic *Malassezia* species are lipid-dependent.[27] In an italian study with 151 cats (no Sphynx or DRC) the recovery of lipid-dependent *Malassezia* species was not significantly different from healthy ears or in otitis externa.[27] It is currently not known if the various *Malassezia* species may have different clinical properties in cats.

Table 5. Frequency of isolation and *P*-values from Fischer's exact tests

Site	Seborrhoeic			Normal		
	Sphynx ($n = 5$)	Devon Rex ($n = 9$)	*P*-value	Sphynx ($n = 27$)	Devon Rex ($n = 21$)	*P*-value
Left axillae	80%	89%	1.0	37%	43%	0.77
Right axillae	100%	89%	1.0	48%	38%	0.56
Left groin	100%	100%	–	19%	48%	0.06
Right groin	80%	89%	1.0	48%	57%	0.57
Ear	0%	11%	1.0	0%	10%	0.19
Claw	20%	89%	0.02	33%	29%	0.76

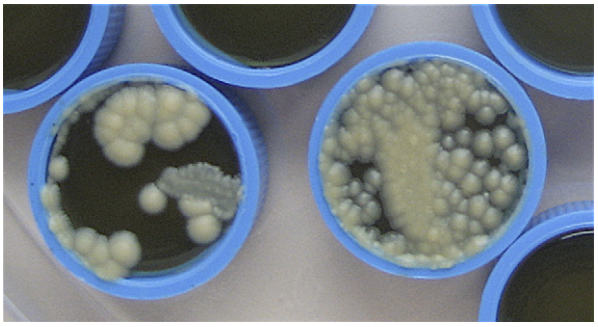

Fig 4. Contact plate with heavy growth of yeast colonies.

Notably, in the seborrhoeic Sphynx cats, no lipid-dependent isolates were made. In previously sampled DRC, lipid-dependent *Malassezia* species were recovered from 3/9 seborrhoeic cats, however, always in combination with *M pachydermatis*, whereas in non-seborrhoeic DRC 4/5 cats with lipid-dependent isolates had those exclusively.[1] A possible interpretation is that lipid-dependent *Malassezia* species are less important than *M pachydermatis* in the pathogenesis of *Malassezia*-associated seborrhoeic dermatitis in these cat breeds. However, it may also reflect the difficulties in reliably evaluating all yeast colonies on a small growth plate when there is heavy, or even confluent growth (Fig 4). Nevertheless, based on these results, we find no support for lipid-dependent *Malassezia* species to be more pathogenic than *M pachydermatis* in cats with greasy seborrhoea.

A number of publications on *Malassezia* species have focused on yeast isolates from the ears, with or without otitis externa, in various animals, including cats, dogs and cattle.[6,12,26,28–31] Interestingly, in our material no Sphynx had *Malassezia* species isolated from the ears. This might reflect that the Sphynx owners were permitted routine ear care and cleaning, thereby removing some greasy debris that would have been easy to collect and culture, or that this population of Sphynx cats did not have a significant aural colonisation of *Malassezia* species. In the compared group of DRC, the situation was similar with only few *Malassezia* species recovered from aural samples, not significantly more than in the DSH control group.[1] Unlike the situation in Basset Hounds and Cocker Spaniels, where the breeds are commonly affected by both *Malassezia* species-associated seborrhoeic dermatitis and otitis, seborrhoeic Sphynx and DRC was neither associated with high counts of *Malassezia* species in the ear, nor with signs of otitis externa.[1,12,13,32,33] This has clinical implications when sampling these feline breeds. The ear is a common site when sampling for yeast involvement, probably as a result of its frequent involvement in predisposed canine breeds, eg, Basset Hounds, Cocker Spaniels and West Highland White Terriers.[12,13,32] However, in Sphynx and DRC, the axillae, groin and claw fold might be more representative sites, and increase the chance of yeast recovery and a correct assessment of the clinical significance of such yeast when investigating overt skin disease.

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