Carriage of Malassezia spp. yeasts in healthy and seborrhoeic Devon Rex cats

S. ÅHMAN*, N. PERRINS†,# & R. BOND†

*Barton Veterinary Hospital, Canterbury, Kent, and †Department of Veterinary Clinical Sciences, Royal Veterinary College, Hatfield, Herts, UK

Skin and anal mucosal carriage of Malassezia spp. yeasts was investigated in 21 healthy Devon Rex cats (DRC) and in 9 seborrhoeic DRC using swabs and contact plates. M. pachydermatis was isolated from 26 cats and lipid-dependent Malassezia spp. isolates were recovered from the claw fold of 5 healthy and 3 seborrhoeic DRC. The frequencies of isolation and population sizes of M. pachydermatis in the axillae, left groin and claw fold in seborrhoeic DRC significantly exceeded (P < 0.05) those of healthy animals. The frequencies of isolation and population sizes of M. pachydermatis in the axillae and groin in both groups of DRC, and the frequencies of isolation and population sizes of M. pachydermatis in the claw fold of the seborrhoeic DRC, exceeded those of healthy Domestic short-haired cats. Using polymerase chain reaction – restriction enzyme analyses (PCR-REA) based on amplification of the large subunit rRNA gene, all eight lipid-dependent isolates had profiles that were indistinguishable from that of M. slooffiae CBS 7956. These data indicate that DRC are frequently colonized by M. pachydermatis and that the claw folds may also be colonized by M. slooffiae. The pathogenic significance of the high Malassezia spp. counts in the seborrhoeic DRC should now be determined.

Keywords Malassezia pachydermatis, Malassezia slooffiae, Devon Rex, cat, skin

Introduction

In common with many mammalian species, the skin of healthy cats is often colonized by members of the genus Malassezia [1–3], including the non-lipid dependent species, M. pachydermatis [1], as well as the lipid-dependent M. sympodialis [1,2], M. globosa [3], M. furfur [4], M. nana [5,6], and more recently, M. slooffiae [7]. Dermatitis associated with Malassezia spp. in cats is frequently reported in those with endocrine and metabolic diseases, neoplasia, and infection with feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) [8–11]. A recent report also suggested that Malassezia spp. overgrowth may be found with feline allergic skin diseases [12], in a manner analogous to that commonly reported in dogs. Although it is not mentioned in standard textbooks on veterinary dermatology, it is the authors’ clinical impression that Devon Rex cats (DRC) are predisposed to a seborrhoeic dermatitis associated with Malassezia spp. [13,14]. In a study of Malassezia carriage in the claw folds of 46 cats of various breeds, the yeast was identified in 18 out of 31 Domestic short-haired (DSH) and Persian cats, but notably in all 15 DRC sampled [15].

We hypothesized that the carriage rates of Malassezia spp. in healthy DRC exceed those of healthy DSH, and that population sizes of the yeast are further
increased in DRC with seborrhoeic dermatitis. The purposes of this study were to compare the frequencies of isolation and population sizes of Malassezia spp. in healthy DRC and seborrhoeic DRC with those of healthy DSH cats in order to determine whether there was an association between yeast carriage and the FIV/FeLV status in DRC.

**Material and methods**

**Devon Rex and Domestic Short-haired cats**

The use of animals was approved by the Royal Veterinary College's Ethics and Welfare Committee, and written informed consent from the owner was obtained before sampling each cat. Twenty-one healthy DRC (aged 1–13 years, 15 female, 6 male) and 9 DRC with clinical signs of localized or generalized seborrhoeic skin disease (aged 1–13 years, 5 female, 4 male) were examined and samples obtained for culture. Cats that had received antifungal or topical therapy in the previous 6 weeks were excluded. Samples from ten healthy DSH cats, comprising one neutered male and 9 neutered females, aged 4–12 years, were collected in the same manner to provide control data.

**Skin sample collection**

The skin from the left and right axilla and groin was collected using contact plates comprising bijou bottle lids filled to the meniscus with modified Dixon’s agar [2]. The plates were applied to the skin for 10 sec, removed and incubated at 32°C for 3–7 days were suspended in 600 ul of sorbitol buffer (1.2 M sorbitol, 0.1 M potassium phosphate). The yeast cell wall was disrupted by incubation with 200 U of lyticase (L2524, Sigma-Aldrich Co. Ltd., Poole, UK) for 30–60 min at 30°C. DNA was extracted using the DNeasy Tissue Kit for Animal Tissues (Qiagen Ltd., Crawley, UK) according to the manufacturer’s instructions. Conventional PCR was performed in a 50 ul reaction volume containing 2.5 units of HotStarTaq DNA polymerase (Qiagen Ltd., Crawley, UK), 10 ul of each primer (0.2 μM each), 1 ul of DNA and 0.4 μl of each primer (0.4 μl of each deoxynucleoside triphosphate, 5 ul of template DNA and 0.4 μM of each primer (Malup 5’-AGCGGAGGAGAAACT-3’ and Maldown 5’-GGCGCGAGTTCTCGAG-3’). The amplification programme comprised a 15 min initial DNA polymerase activation period, 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final extension phase at 72°C for 10 min.

The products of the PCR reaction were subjected to REA using BanI, HaeII and MspI according to the manufacturer’s instructions (Promega, Southampton, UK). Products of PCR and REA fragments were visualized by agarose gel electrophoresis (2% w/v agarose for PCR; 4% w/v for REA) in Tris-borate-ethylene diaminetetraacetic acid (TBE) buffer incorporating ethidium bromide (0.4 μg/ml) at 100V for 30 min and photographed under ultraviolet transillumination. This method was originally reported to differentiate M. pachydermatitis, M. furfur, M. globosa, M. obtusa, M. restricta, M. slooffiae and M. sympodialis [20] but we have recently shown that it also differentiates M. yamatoensis, M. nana, M. dermatis and M. japonica from each other and from the Malassezia spp. evaluated previously [7].

The following cultures obtained from the Central Bureau Schimmelcultures (PO Box 85167, Utrecht, The Netherlands) were used as control for identification to use Cremophor EL or Tween 20, 40, 60 or 80 as lipid sources, as well as their catalase activity were evaluated using the methods described by Guillot et al. [16]. Growth on modified Dixon’s agar at 37°C [17] was also assessed. Since aesculin hydrolysis may assist in the differentiation of M. sympodialis from M. slooffiae and M. furfur [18], lipid-dependent isolates were stab-inoculated into a medium composed of Sabouraud’s dextrose agar supplemented with 0.1% Tween 80 [19], aesculin (1 g/l) and ferric citrate (0.5 g/l) and incubated for 4 d at 32°C.

Lipid-dependent isolates were further evaluated using the PCR-REA method described by Guillot et al. [20]. Briefly, Malassezia spp. colonies that had been grown on modified Dixon’s agar plates at 32°C for 3–7 days were suspended in 600 μl of sorbitol buffer (1.2 M sorbitol, 0.1 M potassium phosphate). The yeast cell wall was disrupted by incubation with 200 U of lyticase (L2524, Sigma-Aldrich Co. Ltd., Poole, UK) for 30–60 min at 30°C. DNA was extracted using the DNeasy Tissue Kit for Animal Tissues (Qiagen Ltd., Crawley, UK) according to the manufacturer’s instructions. Conventional PCR was performed in a 50 ul reaction volume containing 2.5 units of HotStarTaq DNA polymerase (Qiagen Ltd., Crawley, UK), 0.2 mM of each deoxynucleoside triphosphate, 5 ul of template DNA and 0.4 μM of each primer (Malup 5’-AGCGGAGGAGAAACT-3’ and Maldown 5’-GGCGCGAGTTCTCGAG-3’). The amplification programme comprised a 15 min initial DNA polymerase activation period, 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final extension phase at 72°C for 10 min.

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**Blood sample collection and FeLV/FIV analyses**

Whole blood samples were collected via jugular or peripheral venipuncture and transferred into a plain serum tube to allow them to clot for a minimum of 3 h before centrifugation and separation of serum. The FIV and FeLV status of the cats were evaluated using a commercially available in-house immuno-chromatographic test designed to detect the p27 viral antigen of FeLV, and antibodies directed against the gp40 trans-membrane glycoprotein of FIV (SpeedDUO FeLV/FIV, Vetlab Supplies Ltd., Pulborough, UK), according to the manufacturer’s instructions.

**Statistical analyses**

Population sizes of the yeasts were compared between healthy DRC, seborrhoeic DRC and healthy DSH cats using the Mann-Whitney U-tests. Frequency data were compared between groups using Fisher’s exact tests. Analyses were performed using the Unistat v3.0 statistical software package (Unistat Ltd., London, UK) with $P < 0.05$ for significance.

**Results**

All nine seborrhoeic DRC had greasy seborrhoea (seborrhoea oleosa) at multiple sites. In eight of these cases, it involved the axilla, groin, ventral neck and feet. In addition, one had severe, generalized seborrhoea sicca. The ninth cat had greasy seborrhoea affecting the ventral neck and head. All eight cats with axilla and groin involvement, had alopecia and either hyperpigmentation or a reddish-brown surface discoloration of the skin at these sites. Three of these eight cats were also erythematous in the axilla and groin. Furthermore, in all eight cats, there was a greasy, tightly adherent brown exudate on the claws and/or in the claw folds of multiple digits (Fig. 1). This was accompanied by a dark, greasy exudate that matted the hairs on the palmar and plantar aspects of the interdigital skin (Fig. 2).

One of the nine seborrhoeic DRC had non-seasonal, generalized pruritus and had been previously reported to show a partial (approximately 50%) reduction in pruritus following fortnightly treatments with a miconazole and chlorhexidine shampoo (Malaseb, VetXX, Thame, UK). The remaining eight cats reportedly did not display pruritic behaviour and none had received previous treatment for skin disease; indeed, all the owners of non-pruritic cats considered the presenting signs to be normal or near-normal for the breed.

The 21 healthy DRC were unaffected by significant skin disease and the healthy and seborrhoeic DRC showed no signs of otitis externa and none had historical or clinical features suggestive of current systemic disease.

*Malassezia* spp. were isolated from 18 out of 21 healthy DRC, and from all 9 seborrhoeic DRC. *M. pachydermatis* was most frequently recovered and accounted for all *Malassezia* spp. isolates collected from the axilla, groin, ear and anus. Lipid-dependent *Malassezia* yeasts were isolated from the claw fold of five healthy and three seborrhoeic DRC. In four healthy DRC, only lipid-dependent *Malassezia* spp.
were recovered from the claw fold, whereas they were recovered in combination with *M. pachydermatis* in one healthy and three seborrhoeic DRC.

*M. pachydermatis* was isolated at a significantly higher frequency from the left axilla (*P* = 0.02), right axilla (*P* = 0.01), left groin (*P* = 0.01) and claw fold (*P* = 0.0001) of seborrhoeic DRC when compared with healthy DRC, whereas no significant difference was found between these two groups relative to the isolation of *M. pachydermatis* from the left ear, right groin and anus (Table 1). The frequency of isolation of lipid-dependent *Malassezia* spp. from the claw fold did not vary significantly between healthy and seborrhoeic DRC. The population sizes of *M. pachydermatis* in the seborrhoeic DRC were significantly greater (*P* = or <0.001) in the axillae, groin and claw fold when compared with the healthy DRC, whereas populations in the ear and anus were comparable between the two groups (Table 1). While large numbers of *Malassezia* spp. were isolated from the eight DRC with dry scaling without greasy exudation, the single seborrhoeic DRC with dry scaling yielded only one colony of *M. pachydermatis* from the right groin.

When compared with healthy DSH cats, the frequencies of isolation of *M. pachydermatis* from DRC were significantly greater from the left axilla (healthy DRC, *P* = 0.015; seborrhoeic DRC, *P* = 0.0001), right axilla (healthy DRC, *P* = 0.026; seborrhoeic DRC, *P* = 0.0001), left groin (healthy DRC, *P* = 0.046; seborrhoeic DRC, *P* = 0.0001) and right groin (healthy DRC, *P* = 0.0022, seborrhoeic DRC, *P* = 0.0001) (Table 1). However, the rate of recovery of *M. pachydermatis* from the ears and anus did not vary significantly between DSH cats and either group of DRC. However, the frequency of isolation of *M. pachydermatis* from the claw fold was greater in only the seborrhoeic DRC (*P* = 0.001, Table 1). In both groups of DRC, the population sizes of *M. pachydermatis* in the axilla and groin were significantly (*P* < 0.05) greater than those of healthy DSH cats, but populations in the ear and anus were comparable between the three groups of cats (Table 1). The size of *M. pachydermatis* population in the left claw fold was significantly greater (*P* = 0.0005) in the seborrhoeic DRC, but not in healthy DRC when compared with healthy DSH cats. Both the frequencies of isolation and population sizes of lipid-dependent *Malassezia* (*M. slooffiae*) in the claw fold were comparable between the three groups of cats (Table 1).

Two distinct *M. pachydermatis* colony morphologies were noted, i.e., ‘type A’ colonies were large, domed, entire yellow colonies, often surrounded by precipitates in the medium, that grew well when sub-cultured on Sabouraud’s dextrose agar as opposed to ‘type B’ colonies which were smaller, buff-coloured, domed or umbo-nate with precipitates, which grew quite poorly but could be maintained on Sabouraud’s dextrose agar (Fig. 3).

The eight lipid-dependent isolates formed small, yellow, domed colonies (Fig. 3), without precipitates, and were composed of ellipsoidal cells. These isolates failed to grow on Sabouraud’s dextrose agar at 32°C but showed profuse growth on modified Dixon’s agar at 37°C. They were catalase-positive, failed to hydrolyse aesculin, did not assimilate Cremophor EL but grew well in the presence of Tweens 40, 60 and 80. Only one isolate (obtained from cat no. 2) assimilated Tween 20.

### Table 1 Frequency of isolation and population sizes of *Malassezia* spp. from healthy Devon Rex cats, Devon Rex cats with seborrhoeic dermatitis, and healthy Domestic short-haired cats

<table>
<thead>
<tr>
<th>Site</th>
<th>Healthy DRC (<em>n</em> = 21)</th>
<th>Affected DRC (<em>n</em> = 9)</th>
<th>Healthy DSH (<em>n</em> = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freq</td>
<td>Popn size</td>
<td>Freq</td>
</tr>
<tr>
<td>Ear canal</td>
<td>9.5%</td>
<td>0 (0–0)</td>
<td>11%</td>
</tr>
<tr>
<td>Anus</td>
<td>9.5%</td>
<td>0 (0–0)</td>
<td>22%</td>
</tr>
<tr>
<td>Claw MP</td>
<td>9.5%</td>
<td>0 (0–0)</td>
<td>89%bc</td>
</tr>
<tr>
<td>Claw MS</td>
<td>24%</td>
<td>0 (0–0)</td>
<td>33%</td>
</tr>
<tr>
<td>L axilla</td>
<td>43%ce</td>
<td>0 (0–0.3)ec</td>
<td>89%ae</td>
</tr>
<tr>
<td>R axilla</td>
<td>38%ce</td>
<td>0 (0–0.3)ec</td>
<td>89%ae</td>
</tr>
<tr>
<td>L groin</td>
<td>48%ce</td>
<td>0 (0–0.6)ec</td>
<td>100%ae</td>
</tr>
<tr>
<td>R groin</td>
<td>57%ad</td>
<td>0.3 (0.0–0.6)d</td>
<td>89%c</td>
</tr>
</tbody>
</table>

Data refers to *M. pachydermatis* unless indicated.

Population sizes are expressed as median log10 [colony-forming units per swab +1], (lower, upper quartile) for the ear canal, claw and anus, and as median colony-forming units/cm² (lower, upper quartile) for axilla and groin. A value of >80 indicates confluent growth on the contact plate.

DRC, Devon Rex cat; DSH, Domestic short-haired cat; Freq, frequency; Popn, population; L, left; R, right, MP, *M. pachydermatis*; MS, *M. slooffiae*.

Comparison with healthy DRC, *P* < 0.05, b *P* < 0.001

Comparison with healthy DSH, *P* < 0.05, d *P* < 0.01, c *P* < 0.001.

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The type culture of *M. slooffiae* CBS 7956 showed the same phenotype, except that it assimilated, as previously reported, all four Tweens tested [20]. Each of the eight lipid-dependent isolates had PCR-REA patterns that could not be distinguished from that of the *M. slooffiae* CBS 7956 type culture (Fig. 4a–c).

**Blood sample collection and FeLV/FIV status**

Blood was obtained from eight of the nine seborrhoeic DRC and 18 of the 21 healthy DRC were all negative for FeLV and FIV. Samples could not be obtained from the remaining four cats but of these, the three healthy DRC had tested negative for FeLV/FIV within three months of the present study, and one seborrhoeic cat had tested negative 5 years prior to examination.

**Discussion**

These data indicate that both healthy and seborrhoeic DRC are frequently colonized by *M. pachydermatis* at the axilla and groin, whereas *M. pachydermatis* and *M. slooffiae* may be recovered from the claw folds. The Devon Rex breed has a small genetic base having been founded on a single male, ‘Kirlee’, who was mated with his own daughters to produce offspring with loosely curled, velvet-like fur, typical of today’s DRC [21]. In-breeding has continued over generations and it seems likely that this has promoted a genetic profile that in some way favours skin colonization by *Malassezia* spp. Further studies are required to elucidate the biochemical or immunological basis of this susceptibility.

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Although retroviral infection was associated with more frequent isolation of *Malassezia* spp. from cats in a previous study [10], the failure to detect either FeLV antigen or antibodies to FIV in blood samples in our investigation suggests that other factors are responsible for the high colonization rates in these cats. Furthermore, the good general health of the cats in the present study contrasts with previous reports of the association between cutaneous *Malassezia* spp. proliferation in cats and visceral neoplasia and life-threatening internal diseases [8,9,11,14].

There are a number of reports of the isolation of *Malassezia* spp. from the feline ear canal [4,22,23], and a correlation between cytological and cultural assessments of population size and clinical symptoms of otitis externa has been reported [24,25]. In our investigation, the external ear canal was one of the sites, along with the anus, from which *Malassezia* spp. were not commonly recovered in the DRC. The seborrhoeic disorder observed in the affected DRC does not appear to be associated with high *Malassezia* spp. counts in the ear canal, or concurrent otitis externa, unlike the situation in seborrhoeic basset hounds [26,27]. However, the distribution of lesions involving the axilla, groin, ventral neck and paws, and the associated high frequency of yeast isolation from these regions in the affected DRC with greasy exudation more closely parallels the presentation seen in seborrhoeic basset hounds. These dogs are normally pruritic whereas DRC owners did not report pruritus in eight of the nine seborrhoeic cats [26,27]. In addition, the higher populations recovered from the axilla and groin in healthy DRC when compared with healthy DSH cats indicate that the skin microenvironment at these sites in DRC is conducive to yeast colonization. The higher yeast counts in the affected DRC at the axilla and groin suggest that either the greasy seborrhoeic skin disorder is caused by and/or favours further proliferation of *M. pachydermatis*. Therapeutic trials are now indicated to determine whether antifungal therapy can ameliorate or resolve the skin lesions in seborrhoeic DRC. By contrast, the infrequent isolation of *Malassezia* spp. from the one seborrhoeic DRC with only ventral neck and head involvement suggests either a failure to recover yeast from that individual, possibly because sampling sites and areas of clinical involvement in this cat did not match, or that factors other than yeast infection were involved in the pathogenesis of the skin lesions.

The frequent recovery of *Malassezia* spp. from the claw folds in the present study is in accordance with the results of a previous cytological study of DRC [15]. Our data indicate that more than one species of *Malassezia* spp. might be represented at this site.

Phenotypic variation amongst *M. pachydermatis* isolates from dogs has been reported previously [28,29]. Huang *et al.* described a small colony phenotype of *M. pachydermatis* that was difficult to maintain on Sabouraud’s dextrose agar [28]. Bond and Anthony reported *M. pachydermatis* isolates that initially showed poor growth on Sabouraud’s dextrose agar, although many grew better after repeated passage on this medium [29]. These isolates belonged to group Id, one of the seven sequence types (Ia–Ig) identified by partial sequencing of the large subunit ribosomal RNA gene [30]. The present study indicates that phenotypic variability is not uncommon amongst *M. pachydermatis* isolates that colonize the skin and mucosae of Devon Rex cats.

*M. slooffiae* has been isolated previously from the skin of an array of mammalian hosts, including humans [31–33], horses [34,35], cattle [36,37] and pigs [38]. In association with a recent report of the isolation of this species from cats with diabetes mellitus, hyperthyroidism and multicentric lymphoma [7], it would appear that the cat is also a host for this species of lipophilic yeast. The failure to isolate *M. slooffiae* from the axilla and groin of the subject cats might reflect either its absence from this anatomical location and/or the difficulty in detecting varied colony morphology when confluent growth of *Malassezia* spp. occurs on small contact plates. By contrast, the examination of plates inoculated with serial dilutions of the swab-wash samples ensures that isolated colonies are readily identified on the culture medium, improving the opportunity to detect mixed cultures, particularly when smaller, slower-growing lipophilic *Malassezia* spp. might be present in small numbers amongst the larger *M. pachydermatis* colonies. While the type culture *M. slooffiae* CBS 7956 grew well in the presence of Tween 20 in our laboratory, as previously reported by Guillot *et al.* [16], Batra *et al.* noted that some *M. slooffiae* isolates may show weak or no growth in the presence of this substance as a rare deviation from the main pattern [39]. The variable growth of the *M. slooffiae* isolates obtained from the DRC in the presence of Tween 20 is in accordance with recent observations of isolates obtained from cats with diabetes mellitus, hyperthyroidism and lymphoma [7], and indicates that this phenotype is not infrequent amongst strains of *M. slooffiae* that colonize cats.

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