Carriage of *Malassezia* spp. yeasts in cats with diabetes mellitus, hyperthyroidism and neoplasia

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The frequencies of isolation and population sizes of *Malassezia* spp. on skin and at mucosal sites in 16 cats with diabetes mellitus, 20 cats with hyperthyroidism and 8 cats with neoplasia did not vary significantly from those of healthy cats when measured with the use of contact plates and a swab technique. *M. pachydermatis* was isolated from nine sites in one cat with feline paraneoplastic alopecia and pancreatic adenocarcinoma, two cats with diabetes mellitus and five cats with hyperthyroidism. A polymerase chain reaction-restriction enzyme analysis (PCR-REA) method that differentiated the 11 species of *Malassezia* spp was used to identify the lipid-dependent isolates that were obtained from two cats with diabetes mellitus, two cats with hyperthyroidism and one cat with multicentric lymphoma. Six isolates had PCR-REA patterns that were indistinguishable from *M. slooffiae* CBS 7956 and three matched *M. nana* CBS 9557. Our data suggests that skin and mucosal counts of *Malassezia* spp. are not routinely increased in cats with diabetes mellitus or hyperthyroidism but we report a further example of an association between feline paraneoplastic alopecia and *Malassezia* spp. proliferation. To the authors’ knowledge, this is the first report of the isolation of *M. slooffiae* from feline skin.

**Keywords**  cat, skin, *Malassezia pachydermatis*, *M. nana*, *M. slooffiae*

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**Introduction**

*Malassezia* spp. (previously *Pityrosporum*) are lipophilic yeasts that are most often isolated from skin and mucosal sites of mammals and birds [1]. Healthy cats are not infrequently colonized by the non lipid-dependent species, *M. pachydermatis*, as well as occasionally by lipid-dependent species, including *M. sympodialis* [2,3], *M. globosa* [3], *M. furfur* [4], and more recently, *M. nana* [5,6]. Metabolic diseases such as diabetes mellitus, immunosuppressive viruses such as FeLV and FIV, and paraneoplastic disorders (pancreatic paraneoplastic alopecia, exfoliative dermatitis with thymoma) may favour infection in this host species [7–10].

Lipid-dependent *Malassezia* species may be differentiated using morphologic and biochemical methods [11–14]. However, molecular techniques may be preferable to these generally time-consuming phenotypic methods since they may lack the ability to reliably discriminate between the newly-described species and lipid-dependent isolates from animals [6–15]. Polymerase chain reaction (PCR) and restriction endonuclease analyses (REA) which usually involve amplification and subsequent restriction of portions of the highly variable ribosomal RNA gene [15–18] are potentially applicable for routine laboratory use. The method of Guillot et al. [17] was developed before the description of *M. dermatis* [19], *M. nana* [5], *M. japonica* [20], and *M. yamatoensis* [21], although further characterization may be needed for the acceptance of these yeasts as distinct species [22].

To the authors’ knowledge, carriage of *Malassezia* spp. has not been evaluated by quantitative culture of...
the skin of cats with metabolic and neoplastic diseases previously or potentially associated with *Malassezia* dermatis in cats. The purpose of the present study was to quantify yeast carriage at skin and mucosal sites in cats with diabetes mellitus, hyperthyroidism and internal neoplasia using contact plates and a swab-wash method, and to compare the results with those obtained previously from healthy cats [2,3]. We also aimed to determine whether the PCR-REA method reported by Guillot *et al.* [17] could be used to differentiate *M. dermatis*, *M. nana*, *M. japonica*, and *M. yamatoensis* and field isolates of lipid-dependent *Malassezia* spp. obtained from cats.

**Materials and methods**

**Subject cats and recruitment**

The use of animals was approved by the Royal Veterinary College’s Ethics and Welfare Committee and the written informed consent of the owner was obtained before sampling each cat. The majority of cats sampled were presented to the Royal Veterinary College’s Queen Mother Hospital for Animals but some diabetic cats were sampled at a small animal practice in Surrey, UK. Inclusion criteria were presence of diabetes mellitus, hyperthyroidism, or internal neoplasia. Diagnostic criteria for diabetes mellitus were consistent clinical signs (principally polydipsia, polyuria, polyphagia and weight loss) plus presence of fasting hyperglycaemia (exceeding the normal range 3.4–8.1 mmol/l) and elevated blood fructosamine concentrations (exceeding the normal range 205–322 umol/l). Diagnostic criteria for hyperthyroidism were consistent clinical signs (principally weight loss, tachycardia, polyphagia, hyperactivity) and elevated plasma total thyroxine concentration (exceeding the normal range 19–65 nmol/l). The diagnosis of a neoplastic disorder was based on varied clinical, ultrasonographic and/or radiographic signs, supported by cytological and/or histological features as appropriate. The animals that were sampled had received no systemic antifungal drugs in the proceeding three weeks and no topical antifungal therapy in the preceding 10 days.

**Sample collection**

The skin of the left and right axilla and groin were sampled using contact plates comprising bijou bottle lids of 1 cm diameter filled to the meniscus with modified Dixon’s agar, as previously described [2]. Results were expressed as colony-forming units (cfu)/cm². The nose, mouth, anus, left and right external ear canal were swabbed for 5 seconds using mini-tipped swabs, as previously described [2]. In addition, the claw fold on digit IV of the left and right thoracic limb was swabbed for 5 seconds using a mini-tipped swab by extending the claw from its fold by applying gentle digital pressure on the phalynx. Results were expressed as cfu/swab.

*M. pachydermatis* was identified based on the gross colonial and microscopical morphology, and on the ability to grow when sub-cultured on Sabouraud’s dextrose agar (65 g/l, Oxoid CM0041, Basingstoke, UK) at 32°C. Lipid-dependent *Malassezia* spp. were identified based on their gross colonial and microscopical morphology, and failure to grow when sub-cultured on Sabouraud’s dextrose agar at 32°C. Their abilities to use Cremophor EL or Tween 20, 40, 60 or 80 as lipid sources and their catalase activity was evaluated using the methods described by Guillot *et al.* [12]. Growth on modified Dixon’s agar at 37°C [11] was also assessed. Since aesculin hydrolysis may assist in the differentiation of *M. sympodialis* from *M. slooffiae* and *M. furfur* [13], lipid dependent isolates were stab-inoculated into a medium composed of Sabouraud’s dextrose agar supplemented with 0.1% Tween 80 [23], 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 with the PCR-REA method described by Guillot *et al.* [17], using 2.5 units of HotStarTaq DNA polymerase (Qiagen Ltd., Crawley, UK); the amplification programme was 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 andMspI 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 products; 4% w/v for REA fragments) 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. The molecular sizes of the fragments were determined by reference to standard curves constructed from the relative distance travelled by the molecular weight markers plotted against the log₁₀ of their molecular weights.

Cultures obtained from the Centraalbureau voor Schimmelcultures (PO Box 85167, Utrecht, The Netherlands) were used as control specimens for yeast identification and comprised *M. pachydermatis* CBS 1879, *M. furfur* CBS 1878, *M. dermatis* CBS 9169, *M. globosa* CBS 7966, *M. japonica* CBS 9432, *M. obtusa* CBS 7876, *M. restricta* CBS 7877, *M. slooffiae* CBS.
7956, M. sympodialis CBS 7222, M. yamatoensis CBS 9725, and M. nana CBS 9557.

Statistical analysis

Population sizes of the yeasts were compared between groups of cats using the Mann-Whitney U-test, and frequency data were compared using Fisher’s exact tests. Data on yeast isolation from the claw fold were compared with that of a group of 10 healthy Domestic short-haired cats (one neutered male, 9 neutered females, aged 4–12 years [median 10 years]), whereas data from other sites were compared with previously published values obtained using the same sampling methods [3]. Tests were performed using the Unistat v3.0 statistical software package (Unistat Ltd., London, UK) with a p value of <0.05 for significance.

Results

Carriage of Malassezia yeasts

Samples from two of 16 cats with diabetes mellitus, i.e., 14 Domestic short-haired cats and two Birmans, aged 7–15 years (median 12.4 years), yielded Malassezia spp. M. slooffiae (described below) was isolated from the claw fold of the left and right fore of one cat (1,160 and 1,480 cfu per swab, respectively), and from the right fore of the second cat (720 cfu per swab). In the latter case, M. pachydermatis was also isolated from the anus of the second cat (80 cfu per swab).

Six of 20 cats with hyperthyroidism, i.e., 18 Domestic short-haired cats and two Domestic long-haired cats, aged between 6 and 16 years (median 11.5 years), were found to be colonized by Malassezia spp. In four cases, M. pachydermatis was isolated from a single site (right groin, 0.32 cfu/cm²; left ear, 120 cfu/swab; anus, 1,600 cfu/swab; claw fold of left fore, 520 cfu/swab). A fifth cat was colonized by M. pachydermatis at the anus (40 cfu/swab), left ear (40 cfu/swab) and left axilla (0.32 cfu/cm²), and by M. slooffiae in the claw fold of the left fore foot (40 cfu/swab). The final cat was colonized by M. slooffiae in the claw fold of the left fore foot (640 cfu/swab), and by M. nana (described below) at the left ear (280 cfu/swab), right ear (80 cfu/swab) and in the claw fold of the right fore foot (2,280 cfu/swab).

Eight cats with neoplasia were sampled, which included 7 Domestic short-haired cats and one Burmese cat, aged between 13 months and 14 years (median 10 years). Final diagnoses were multicentric lymphoma (4 cases), renal lymphoma (one case), scapular osteochondroma (one case), thymoma without exfoliative dermatitis (one case), and pancreatic adenocarcinoma with paraneoplastic alopecia (one case). Malassezia spp. were isolated from two cats with neoplasia. M. slooffiae was recovered from samples of the anus (40 cfu/swab) of one cat with multicentric lymphoma. M. pachydermatis was isolated from all sites sampled except the nose and anus of a cat with a pancreatic adenocarcinoma and dermatological and histopathological features of feline paraneoplastic alopecia [24]. High counts of the yeast were isolated from the axillae and groin (all sites yielded confluent growth [>80 cfu/cm²]), claw fold of the left fore foot (32,000 cfu/swab) and right fore foot (40,000 cfu/swab), whereas lower counts were obtained from the left ear (160 cfu/swab), right ear (320 cfu/swab), and mouth (40 cfu/swab).

The frequencies of isolation and the population sizes of Malassezia spp. in the cats with diabetes mellitus, hyperthyroidism and neoplasia did not vary significantly from those of healthy cats.

Yeast identification

An amplicon of approximately 600 base pairs (bp) was obtained from each of field isolates of lipid-dependent Malassezia spp. and from each of the 11 CBS-derived cultures (data not shown). Agarose gel electrophoresis of the restriction digests using the three enzyme system produced distinct restriction patterns for each of the 11 CBS cultures examined (Fig. 1 a–c). The patterns obtained from the seven CBS cultures closely resembled those previously described by Guillot et al. [17] (Table 1). However, there were some minor variations in the molecular weights of the restriction fragments between those determined in the present study and those reported by Guillot et al. [17], and some of the smaller fragments of less than 100 bp were not reliably visualized. The four new species (M. dermatis, M. nana, M. japonica, and M. yamatoensis) could be differentiated from each other and from the seven previously reported species using the combination of the three restriction enzymes (Fig. 1a–c, Table 1).

Each of the six field isolates identified as M. slooffiae had PCR-REA patterns that could not be distinguished from that of the M. slooffiae CBS 7956 type culture. On primary isolation they formed small yellow colonies composed of ellipsoidal or cylindrical 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 60 and 80. All but one of the isolates assimilated Tween 40 but only one isolate (cat 46, anus) assimilated Tween
20. The *M. slooffiae* type culture CBS 7956, showed the same phenotype, except that it assimilated all four Tweens tested, as previously reported [17].

The field isolates identified as *M. nana* had PCR-REA patterns that could not be distinguished from those of the *M. nana* CBS 9557 type culture. On primary isolation they formed small yellow colonies composed of small ovoid cells. These isolates failed to grow on Sabouraud’s dextrose agar at 32°C. Two isolates showed profuse growth on modified Dixon’s agar at 37°C whereas the other grew poorly at this temperature. 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. None of the isolates assimilated Tween 20, although each showed a ring of slight growth some distance away from the well (Fig. 2), resembling the inhibitory effects of high Tween 20 concentrations described for *M. sympodialis* [12]. The type culture CBS 9557 showed the same phenotype as the field isolates, as described previously [5], although growth at 37°C was quite poor and a ring of distant growth was noted around the well inoculated with Tween 20.

**Discussion**

The results of the present study confirm the previous report of Guillot *et al.* of the value of their PCR-REA method for the discrimination of the seven previously described *Malassezia* spp. [17]. However, in view of occasional slight differences in the resolved molecular weights, and the occasional failure to detect smaller fragments, the PCR-REA patterns of field isolates should be systematically compared with those of a relevant type culture on the same gel, rather than relying on fragment sizes of a field isolate alone for the identification of the yeasts. Our data suggests that this method can also be used to identify the more recently described lipid-dependent members of this genus. A further examination of a wider collection of field isolates of these newer species should now be conducted to verify the current observations.

*M. slooffiae* has been isolated previously from the skin of an array of mammalian hosts, including humans [11], horses [25], cattle [26] and pigs [27], but we are unaware of previous reports of its recovery from cats. *M. nana* has been recently described from a cat with otitis externa in Japan, and from the ear canals of Brazilian cattle with otitis externa and healthy ear canals [5,28]. Recently, Cabanes *et al.* have reported that a small number of lipid-dependent *Malassezia* isolates obtained from cats’ ears had internal transcribed spacer 1 (ITS1) sequences that were identical to that of the *M. nana* AB075224 sequence [6]. Here we provide a further report of the association of this yeast with the ear canal of a diabetic cat, and also report its

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**Fig. 1** Restriction patterns obtained by (a) Ban1, (b) HaeIII, and (c) MspI digestion of an amplicon of the large subunit rRNA gene of 11 species of *Malassezia*. Tracks 1 and 8, molecular weight marker (base pairs); 2, *M. furfur* CBS 1878; 3, *M. globosa* CBS 7966; 4, *M. obtusa* CBS 7876; 5, *M. restricta* CBS 7877; 6, *M. slooffiae* CBS 7956; 7, *M. sympodialis* CBS 7222; 9, *M. dermatis* CBS 9169; 10, *M. japonica* CBS 9432; 11, *M. nana* CBS 9557; 12, *M. yamatoensis* CBS 9725; 13, *M. pachydermatis* CBS 1879.
isolation from the claw fold of this host species. The present and previous studies indicate that the Malassezia flora of feline skin is diverse and includes both *M. pachydermatis* and at least five lipid-dependent species. Whilst Guillot *et al.* [12] reported that *M. slooffiae* assimilates Tween 20, Batra *et al.* reported that rare isolates show either weak or no growth as a deviation from the main pattern [22]. Whilst the type culture CBS 7956 and one field isolate of *M. slooffiae* assimilated Tween 20 in our laboratory, our data suggest that a failure to assimilate this substance may not be frequent amongst isolates obtained from cats that have PCR-REA patterns consistent with *M. slooffiae*. This observation, along with the variable growth at 37°C noted amongst our examples of *M. nana*, support the observations of Cabanes *et al.* that the identification of Malassezia spp. recovered from animals on the basis of physiological tests may be difficult [6].

Our results suggest that neither diabetes mellitus nor hyperthyroidism are routinely associated with the proliferation of Malassezia spp. on feline skin. However, the observation of widespread colonization of a cat with pancreatic adenocarcinoma and paraneoplastic alopecia by *M. pachydermatis* is in accordance with two previous case reports of an association between this syndrome and cutaneous Malassezia spp. proliferation [9,10]. However, in the latter cases the yeast populations were assessed by histology or cytology and not by quantitative culture. This case highlights the need to consider the potential for generalized feline

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**Table 1** Fragment lengths (base pairs, approximate) of restriction enzyme digests (*Ban*I, *Hae*II, and *Msp*I) of an approximately 600 bp amplicon of the large subunit of the ribosomal RNA gene of 11 Malassezia spp. in a previous [17] and the present study

<table>
<thead>
<tr>
<th>Culture</th>
<th>Previous study</th>
<th>Present study</th>
<th>Previous study</th>
<th>Present study</th>
<th>Previous study</th>
<th>Present study</th>
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<tr>
<td><em>M. furfur</em></td>
<td>58, 77, 410</td>
<td>83, 95, 427</td>
<td>73, 109,113,</td>
<td>91, (115),</td>
<td>545</td>
<td>575</td>
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<td>CBS 1878</td>
<td></td>
<td></td>
<td>250</td>
<td>~120, 250</td>
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<tr>
<td><em>M. globosa</em></td>
<td>58, 489</td>
<td>83, 513</td>
<td>547</td>
<td>589</td>
<td>65, 71, 411</td>
<td>89, 427</td>
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<td></td>
<td>109, 220,</td>
<td>120, 204,</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. obtusa</em></td>
<td>58, 521</td>
<td>83, 513</td>
<td>250</td>
<td>251</td>
<td>65, 514</td>
<td>81, 500</td>
</tr>
<tr>
<td>CBS 7876</td>
<td>544</td>
<td>589</td>
<td>544</td>
<td>589</td>
<td>544</td>
<td>575</td>
</tr>
<tr>
<td>CBS 7877</td>
<td>27, 47, 58,</td>
<td>(57), ~83,</td>
<td>87, 101,</td>
<td>90, ~125,</td>
<td>65, 101,</td>
<td>81, 112,</td>
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<td><em>M. slooffiae</em></td>
<td>337</td>
<td>~95, ~340</td>
<td>109, 250</td>
<td>~260</td>
<td>381</td>
<td>~385</td>
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<td>CBS 7956</td>
<td>58, 75, 408</td>
<td>(76), ~90,</td>
<td>182, 359</td>
<td>209, 380</td>
<td>53, 65, 98,</td>
<td>83, 110,</td>
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<td><em>M. sympodialis</em></td>
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<td>58, 500</td>
<td>109, 184,</td>
<td>120, 209,</td>
<td>325</td>
<td>339</td>
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<tr>
<td><em>M. pachydermatis</em></td>
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<td>83, 500</td>
<td>250</td>
<td>257</td>
<td>543</td>
<td>562</td>
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<tr>
<td><em>M. dermatis</em></td>
<td>CBS 9169</td>
<td>ND</td>
<td>80, 520</td>
<td>204, 389</td>
<td>ND</td>
<td>(80), ~105,</td>
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<tr>
<td>CBS 9432</td>
<td>ND</td>
<td>~83, ~505</td>
<td>ND</td>
<td>120, 214,</td>
<td>ND</td>
<td>~53, ~520</td>
</tr>
<tr>
<td><em>M. japonica</em></td>
<td>CBS 9557</td>
<td>ND ~78, ~525</td>
<td>ND</td>
<td>263</td>
<td>ND</td>
<td>(55), ~105,</td>
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<tr>
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<td>80, 510</td>
<td>93, 123, 269</td>
<td>ND</td>
<td>~417</td>
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<tr>
<td><em>M. yamatoensis</em></td>
<td></td>
<td></td>
<td></td>
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<td>580</td>
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() represents small fragments not routinely visualized; ~ indicates occasional small variation is resolved size noted; ND, not done.

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Fig. 2 Growth around wells inoculated with Tweens (clockwise from top right: Tween 20, 40, 60, 80) and Cremophor EL (centre) of a field isolate of *Malassezia nana* obtained from the left ear of a hyperthyroid cat incorporated into Sabouraud’s dextrose agar.
Malassezia dermatitis to be associated with underlying life-threatening systemic diseases [29].

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References


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