Morphological variability of *Demodex cati* in a feline immunodeficiency virus–positive cat

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CASE DESCRIPTION  
A 17-year-old FIV-positive cat was evaluated because of weight loss during the preceding few months. The cat had a weight loss of 0.5 kg (1.1 lb) during the last month. Because of its FIV-positive status, the cat was confined indoors.

CLINICAL FINDINGS  
A large nonpruritic area of alopecia with hyperpigmentation and comedones was present on the right lateral aspect of the neck. The chin had diffuse alopecia and comedones. Mild alopecia was present on the dorsal aspect of the muzzle. Trichography and microscopic examination of acetate tape imprint preparations and skin scrapings revealed a very morphologically heterogeneous population of *Demodex* mites. Micrometry of adult mites revealed a broad range of body lengths (92.68 to 245.94 µm), which suggested that as many as 3 *Demodex* spp might be present in the skin lesions of this cat.

TREATMENT AND OUTCOME  
Owing to its concurrent disease, no treatment was initiated for the demodicosis, and the cat died spontaneously 14 days after the evaluation. Sequence analysis of the 16S rRNA gene of collected mites was performed. Analysis revealed that the 16S rRNA gene sequence of collected mites appeared 100% identical to the *Demodex cati* 16S rRNA gene sequence deposited in GenBank (JX193759). A similarity of 79.2% and 74.4% was found when the 16S rRNA gene sequence of collected mites was compared with that of *Demodex gatoi* (JX981921) and *Demodex felis* (KF052995), respectively.

CLINICAL RELEVANCE  
Demodicosis in cats is often associated with underlying disease. In cats, FIV infection may lead to an altered immune response and induce species polymorphism of *Demodex* mites. (J Am Vet Med Assoc 2016;249:1308–1312)

A 17-year-old neutered male domestic shorthair cat was evaluated because of weight loss during the preceding few months. The cat had a weight loss of 0.5 kg (1.1 lb) during the last month. Because of its FIV-positive status, the cat was confined indoors. A diagnosis of hypertension had been made 1 year earlier, and the cat had since been treated with a calcium channel blocker (amlodipine [0.5 mg/kg [0.23 mg/lb], PO, q 24 h]).

Results of a general physical examination were considered normal, except the cat had a body condition score of 1/9. A CBC revealed mild nonregenerative anemia (Hct, 21.4% [reference interval, 24% to 45%]). Assessment of a serum sample revealed that the cat was positive for anti-*Toxoplasma gondii* antibody (IgM). Although no signs of a urinary tract infection were evident, urinalysis revealed *Escherichia coli* cystitis. Further investigations were advised, and in the interim, the cat was treated with clindamycin (12.5 mg/kg [5.7 mg/lb], PO, q 12 h) and amoxicillin–clavulanic acid (25 mg/kg [11.4 mg/lb], PO, q 12 h).

On dermatologic examination, a large, irregular area of alopecia with hyperpigmentation and comedones was visible on the right lateral aspect of the neck (Figure 1). Diffuse alopecia and comedones were present on the chin. The dorsal aspect of the muzzle had mild alopecia. No pruritus was evident, and the owner did not recall the cat displaying any pruritus-associated behavior; on the contrary, grooming behavior was diminished.

Trichography and microscopic examination of tape imprints and skin scrapings from the lesions were performed. Large numbers of *Demodex* mites were present in the samples. The morphology and length of the adult mites varied considerably. Some mites had the typical long and slender appearance of *D cati* (Figure 2), whereas other mites appeared remarkably shorter and rounder (Figure 3). Micrometry of all adult mites in the collected samples was performed (Table 1). Given the broad range of body lengths (92.68 to 245.94 µm) among the adult mites, we theorized that multiple *Demodex* spp might be present in this cat. Therefore, material from the skin scraping samples underwent molecular analysis to identify the present *Demodex* spp.

The DNA of adult mites was extracted with a commercial DNA extraction kit, according to a tissue protocol with adjustments. Briefly, mites were scraped into a 1.5-mL vial. The volume of the vial contents was adjusted to 100 µL with cell lysis solution, after
which proteinase K (10 µL) was added, followed by incubation overnight at 56°C. Thereafter, lysis buffer c (100 µL), ethanol (100 µL), and 2 washing buffers d (260 µL each) were added. The DNA was finally eluted with 50 µL of elution buffer e. To amplify the 16S rRNA gene DNA from demodectic mites, PCR amplification was performed with the forward primer 5′CTGTGCTAAGGYAGCGAAGTC-3′ and the reverse primer 5′TCAAAWGCCAACAKCGAGGTAA-3′, according to procedures described by Frank et al. 2 Reaction mixtures for the PCR procedure were composed of 2.5 µL of DNA template, 0.5 µL of the deoxynucleoside triphosphate (10mM each), 0.5 µL of forward primer, 0.5 µL of reverse primer, 2 µL of 25mM MgCl2, 5 µL of 5X PCR buffer, 13.875 µL of nuclease-free water, and 0.125 µL of commercially available polymerase f (5 U/µL). Conditions for PCR amplification were as follows: 95°C for 4 minutes, followed by 35 cycles of 95°C for 30 seconds, 35 cycles of 55°C for 30 seconds, and 35 cycles of 72°C for 45 seconds, followed by a final cycle of 72°C for 10 minutes. The PCR product was visualized on an agarose gel.

After removal of primers, the amplified fragment (350 base pairs) from each slide was cloned in an E coli strain by means of a commercially available vector system g. From each slide, 4 clones were sequenced.

The 16S rRNA gene DNA sequences were analyzed with commercial software h and aligned with sequences available in an online genetic sequence database i for D cati (GenBank No. JX193759), Demodex gatoi (GenBank No. JX981921), and Demodex felis (GenBank No. KF052995) via a multiple-sequence-alignment method h. All extracted 16S rRNA gene DNA sequences matched 100% with that of D cati. The sequences shared 79.2% identity with that of D gatoi and 74.4% with that of D felis.

The owner preferred not to treat the cat for demodicosis. The cat died spontaneously 14 days after the initial evaluation. Necropsy revealed pancreatic amyloidosis, diffuse hepatocytic vacuolar degeneration, glomerulonephritis, interstitial nephritis, and

Figure 1—Photograph of the right lateral aspect of the neck of a 17-year-old FIV-positive cat. Notice the irregular areas of alopecia.

Figure 2—Photomicrograph of a Demodex mite observed in an acetate tape preparation from an affected skin area on the cat in Figure 1. The mite is long (245.97 µm) and slender in appearance. Methylene blue stain; bar = 100 µm.
bacterial pyelonephritis. Skin tissue samples were obtained at necropsy and stored in formaldehyde solution. On histologic examination of the skin tissue samples, lamellar orthokeratotic hyperkeratosis and dyskeratosis of the epidermis were evident. Numerous *Demodex* mites were found within the follicular lumina, and moderate mural lymphocytic folliculitis was observed.

### Discussion

To date, 3 *Demodex* spp have been identified in affected cats. Each species has different morphological, epidemiological, pathogenic, and molecular characteristics.3,4

*Demodex cati* is assumed to be part of the normal skin fauna of cats, and these organisms inhabit hair follicles and sebaceous glands and ducts.3 Follicular inflammation associated with the presence of these mites may lead to alopecia, hyperpigmentation, comedones, papules, pustules, scales, crusts, and erosion or ulceration. Lesions are variably pruritic and located on the head and neck area. *Demodex cati* infestation may also cause ceruminous otitis externa. Generalized demodicosis caused by *D cati*, involving the trunk and distal portions of the limbs, is rare and associated with systemic illnesses or immunosuppression.5-10 *Demodex cati* is a long and slender mite, with a mean ± SD body length of 219 ± 27.4 µm for females and 181.7 ± 17.9 µm for males.11

*Demodex gatoi* is believed to be a contagious parasite that resides in the stratum corneum.5 Infestation is associated with primary pruritic skin disease and self-trauma, but carriers that have no clinical signs have also been described. A few mites can cause severe pruritus, suggesting that hypersensitivity may be the causal factor.2,3,5,12 *Demodex gatoi* mites have a shorter, stubby, and roundish appearance, compared with that of *D cati*; the mean body length is 108.3 ± 4.4 µm for females and 90.6 ± 4.8 µm for males.13

The body length of a third morphotype, referred to as *Demodex* sp or *D felis*, is shorter than that of *D cati*, but longer and more slender than that of *D gatoi*.4,14 It has been suggested that *D felis* is a follicular mite like *D cati*, but additional research is required to clarify its actual location on cats’ skin.3 Case reports of cats with *Demodex* sp infestation describe alopecia with or without mild erythema on the head, ventral aspect of the neck, thorax, and the mediadorsal aspect of the forelimbs.3,14,15 Severity of pruritus associated with *Demodex* sp infestation appears variable.15 Most authors report a body length for *Demodex* sp of approximately 140 µm,14-16 but the length may vary up to approximately 175 µm.9

Concurrent infestation of cats with different *Demodex* spp, classified by morphological characteristics, has been reported.9,10,17 However, several factors can alter mite species morphology; therefore, species designation has to be confirmed by molecular characterization.15,18 Phylogenetic analysis has revealed that all 3 *Demodex* spp affecting cats can be considered distinct species, which makes molecular testing a reliable method for species verification.2,4,14

The present report has described the presence of morphologically different *Demodex* mites in a cat infected with FIV. Molecular characterization was used to determine which different *Demodex* spp were

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**Table 1**—Descriptive statistics of body length measurements (µm) of adult *Demodex* mites observed in various diagnostic samples collected from a 17-year-old FIV-positive cat with irregular areas of alopecia.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate tape imprint</td>
<td>147.31 ± 38.87</td>
<td>136.97</td>
<td>92.68-245.95</td>
</tr>
<tr>
<td>Trichogram</td>
<td>157.12 ± 29.41</td>
<td>157.40</td>
<td>116.70-195.24</td>
</tr>
<tr>
<td>Skin scraping</td>
<td>159.81 ± 23.15</td>
<td>154.59</td>
<td>129.46-192.53</td>
</tr>
</tbody>
</table>

Trichography and microscopic examination of tape imprints and skin scrapings from the lesions were performed. Large numbers of *Demodex* mites were present in the samples, and micrometry was performed to determine the body lengths of all adult mites.

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**Figure 3**—Photomicrograph of another *Demodex* mite observed in an acetate tape preparation from an affected skin area on the cat in Figure 1. This mite is shorter (125.13 µm) and rounder in appearance than the one in Figure 2. Methylene blue stain; bar = 100 µm.
present. Clinical signs in this cat were consistent with lesions described for *D cati* infestation in other cats, namely alopecia, hyperpigmentation, and comedones localized in the head and neck area.\(^5,^5,^10\) *Demodex gatoi* infestation appeared less likely because of the apparent absence of pruritus.\(^5,^5,^12\) However, trichography and microscopic examination of tape imprints and skin scrapings from the lesions revealed a large number of *Demodex* mites with variable phenotypes. Moreover, superficial samples (tape imprints) contained shorter mites than did the deep samples (skin scrapings), which could indicate that different mite species inhabiting different skin regions were present. Histopathologic findings were not straightforward. Epidermal lamellar hyperkeratosis has been described in association with both *D cati* and *D gatoi* infestations,\(^8,^19\) but mural lymphocytic folliculitis and the presence of mites in the follicles are typically found in association with *D cati* demodicosis.\(^20\)

The presence of different *Demodex* spp in cats has been reported previously.\(^9,^15–^17\) Remarkably, in 2 of those cases,\(^9,^17\) infected cats were also FIV positive. Species classification of the mites in all those previous reports was based on morphological characteristics, but no molecular analysis was performed. Analysis of the mitochondrial 16S rDNA partial sequence has proven to be a useful tool with which to differentiate *Demodex* spp.\(^21–^23\) For *Demodex* mites found in cats, all 3 species appear to have distinct genotypes.\(^2,^4\)

Given the morphological diversity of mites in the case described in the present report, we theorized that different species of *Demodex* mites might be present in 1 cat. However, although body lengths of the mites were very variable, all observed mites appeared to be morphological variants of *D cati*.

One explanation for size variation among arthropod parasites is the influence of the host’s immune response.\(^18\) Demodicosis in cats is often associated with underlying disease, and we advise testing of affected cats for immunosuppressive conditions such as FIV infection.\(^10\) Previous studies have described morphologically distinct *Demodex* mites in FIV-positive cats,\(^5,^17\) in cats with concurrent illnesses, and in cats that recently recovered from systemic illnesses.\(^15\) None of those studies involved molecular analysis of mite DNA; hence, the assumption that different *Demodex* spp were present in those cats might be incorrect. Moreover, in 1 case study,\(^21\) 2 morphological forms of *Demodex* mites were detected in a cat that received long-term corticosteroid treatment. However, the product of PCR amplification of 16S rRNA gene DNA from sampled mites matched 100% with the corresponding DNA sequence of *D cati*.\(^24\)

In arthropod parasites, morphological characteristics can be affected by site of infestation.\(^18\) Size of *Demodex canis* mites may vary depending on depth within the skin at which they are located; size increases from that determined for mites collected in superficial tape imprint samples to that determined for mites collected in the deepest skin scrapings.\(^5\) Measurements of body length among mites obtained via different sample collection methods in the present study did not differ remarkably; however, the longest mites were found in the deep skin scraping samples, a finding that appears similar to the body length variation among *D canis*.

Findings in the case described in the present report have highlighted that morphological characteristics are not reliable for differentiation of *Demodex* spp found in cats. Results of molecular testing are necessary to determine whether multiple distinct species are present. Further research is required to assess factors that induce *Demodex* sp polymorphism, but in cats, an altered immune response caused by FIV infection may contribute to this phenomenon.

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The authors thank Bieke Weyn for technical assistance.

**Footnotes**

a. QIAamp DNA mini kit, Qiagen, Hilden, Germany.

b. ATL buffer, Qiagen, Hilden, Germany.

c. Alu buffer, Qiagen, Hilden, Germany.

d. AW1 and AW2 buffer, Qiagen, Hilden, Germany.

e. AE buffer, Qiagen, Hilden, Germany.


g. pGEM-T Easy vector system, Promega, Leiden, The Netherlands.

h. Lasergene, DNASTAR Inc, Madison, Wis.


**References**


2013;24:460–462.

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From this month’s AJVR

Effects of dobutamine hydrochloride on cardiovascular function in horses anesthetized with isoflurane with or without acepromazine maleate premedication

Mara F. Schier et al

OBJECTIVE

To determine the effects of acepromazine maleate premedication on cardiovascular function before and after infusion of dobutamine hydrochloride for 30 minutes in isoflurane-anesthetized horses.

ANIMALS

6 healthy adult horses.

PROCEDURES

Each horse was anesthetized once following premedication with acepromazine (0.02 mg/kg, IV) administered 30 minutes prior to anesthetic induction (ACP+ treatment) and once without premedication (ACP– treatment). Anesthesia was induced with IV administration of xylazine hydrochloride (0.8 mg/kg), ketamine hydrochloride (2.2 mg/kg), and diazepam (0.08 mg/kg). Horses were positioned in right lateral recumbency, and anesthesia was maintained via inhalation of isoflurane delivered in oxygen. End-tidal isoflurane concentration was adjusted to achieve a target mean arterial blood pressure of 60 mm Hg (interquartile range [25th to 75th percentile], 57 to 63 mm Hg) for at least 15 minutes. Cardiac index, oxygen delivery index, and femoral arterial blood flow indices were determined 60 minutes after anesthetic induction (baseline). Dobutamine was then infused to achieve a target mean arterial blood pressure of 80 mm Hg (interquartile range, 76 to 80 mm Hg). Data collection was repeated 30 minutes after the start of dobutamine infusion for comparison with baseline values.

RESULTS

Complete data sets were available from 5 of the 6 horses. Dobutamine administration resulted in significant increases in oxygen delivery and femoral arterial blood flow indices but no significant change in cardiac index for each treatment. However, at baseline or 30 minutes after the start of dobutamine infusion, findings for the ACP+ and ACP– treatments did not differ.

CONCLUSIONS AND CLINICAL RELEVANCE

In isoflurane-anesthetized horses, dobutamine administration increased oxygen delivery and femoral arterial blood flow indices, but these changes were unaffected by premedication with acepromazine. *(Am J Vet Res 2016;77:1318–1324)*