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PCR amplification and DNA sequence identification of an unusual morphological form of *Demodex cati* in a cat

Joseph A. Bernstein*, Linda A. Frank† and Stephen A. Kania‡

*Long Green Animal Dermatology Center, 13515 Long Green Pike, Baldwin, MD 21013, USA
Departments of †Small Animal Clinical Sciences and ‡Biomedical and Diagnostic Sciences, University of Tennessee, Knoxville, TN 37996, USA
Correspondence: Linda A. Frank, Department of Small Animal Clinical Sciences, University of Tennessee, Knoxville, TN 37996, USA.
E-mail: lfrank@utk.edu

Background – Molecular characterization of *Demodex* mites is being used to identify mite species in dogs. This technique is now being applied to cat *Demodex* species, allowing for better characterization of the mites.

Hypothesis/Objectives – Molecular diagnostics will clarify the existence of diverse *Demodex* mites identified morphologically.

Animals – A cat with generalized demodicosis secondary to chronic steroid treatment for erythroid dysplasia.

Methods – Skin scrapings demonstrated large numbers of follicular mites consistent with *Demodex cati* as well as a morphologically different *Demodex* mite with a blunted abdomen. The 16S rRNA DNA was amplified by PCR, sequenced and compared with available *Demodex* sequences, including *Demodex cati*, *Demodex gatoi* and an unnamed *Demodex* sp.

Results – A single PCR product was obtained, the DNA sequence of which was an exact match with *D. cati*.

Conclusions and clinical importance – The shorter unnamed mite was not a different species in this case, but a different morphological form of *D. cati*. This report demonstrates the utility of molecular diagnostics to clarify the identity of mites that differ morphologically.

Introduction

Feline demodicosis is a parasitic condition affecting the skin of cats. Three mites, *Demodex cati*, *Demodex gatoi* and an unnamed mite, have been described morphologically and, in one case, characterized by molecular diagnostics.[1–5] Recently, molecular studies of canine *Demodex* mites revealed *Demodex cornei*, a short-bodied mite morphologically distinct from *Demodex canis*, to be genetically indistinct from *D. canis*,[6,7] emphasizing the importance of molecular characterization. The purpose of this case report is to describe molecular characterization of two morphologically distinct feline *Demodex* mites.

Case report

An 8-year-old male castrated domestic short hair cat was presented with the complaint of progressive alopecia of 3 months duration. The cat had a history 3 years prior of progressive anaemia and moderate pancytopenia that had responded poorly to prednisone and ciclosporin. PCR testing was negative for *Mycoplasma* spp.; tests for tick-borne diseases, feline leukaemia virus and feline immunodeficiency virus were negative. Bone marrow core biopsies from the ileum demonstrated erythroid hyperplasia, with dysplasia and incomplete maturation as well as myeloid and megakaryocytic hypoplasia. There was no evidence of lymphoid neoplasia. A diagnosis was made of erythroid dysplasia with myelodysplastic syndrome. The disease was treated with oral dexamethasone and epoetin alpha (Epogen®; Amgen, Thousand Oaks, CA, USA). At the time of presentation, the cat was receiving 0.25 mg (0.04 mg/kg) dexamethasone daily.

Dermatological examination revealed marked alopecia over the entire body, with easy epilation of hairs (Figure 1). The cat was not observed to be pruritic. A trichogram and deep skin scrapings both revealed large numbers of *Demodex* mites, and two morphological forms were noted. Long, slender mites consistent with

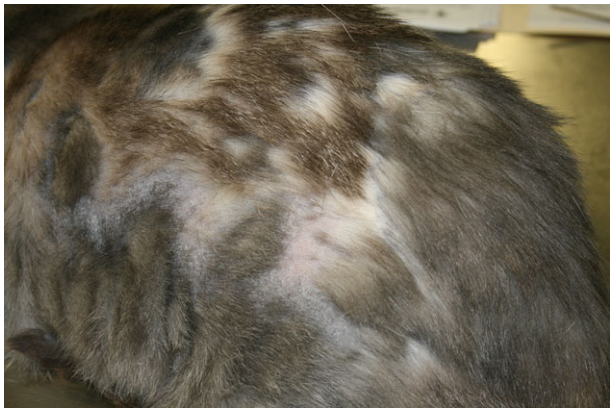

Figure 1. Lateral trunk of an 8-year-old male castrated domestic short hair cat diagnosed with two morphological forms of feline demodicosis.

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D. cati were identified along with mites with blunted abdomens, longer than *D. gatoi* but shorter than *D. cati* (Figure 2). These mites were consistent with the previously reported morphologically distinct *Demodex*.[2,4,5] Scraped samples placed on slides in mineral oil contained both morphological forms. Material from scraped samples in mineral oil and scraped samples placed into a sterile tube underwent molecular analysis to determine whether the unidentified mite population represented a new unnamed species or a morphological variant of *D. cati*.

The cat was treated with weekly topical application of 80 mg moxidectin/imidacloprid (Advantage multi for cats®; Bayer HealthCare LLC, Shawnee Mission, KS, USA) between the scapulae. Survey skin scrapings were performed monthly, and negative skin scrapings were achieved after 4 months. Dexamethasone oral therapy was decreased to every other day. Due to the ongoing steroid therapy, treatment for demodicosis was continued even after two negative consecutive monthly skin scrapings. The frequency of application of moxidectin/imidacloprid was decreased to every 3–4 weeks for maintenance. No recurrence of demodicosis was reported with follow-up of 1 year.

PCR analysis

DNA was extracted using a commercial kit according to the manufacturer's protocol for tissue (DNeasy blood and tissue kit; Qiagen, Valencia, CA, USA). DNA was amplified by PCR as previously described.[8] Briefly, amplification was performed using forward primer ACTGTGCTAA GGTAGCGAAGTCA and reverse primer TCAAAAGCCAACATCGAG to amplify 16S rRNA DNA. Reaction mixtures for PCR consisted of 2 μL of DNA template, 25 pmol of each primer and 25 μL of premix 2× Taq DNA polymerase mastermix (rTaq; Takara Bio, Otsu, Shiga, Japan). PCR amplification parameters were 95°C for 90 s, followed by 35 cycles of 55°C for 30 s, 68°C for 120 s and 94°C for 30 s, with a final cycle consisting of 55°C for 30 s and 68°C for 5 min. The PCR product was visualized on an agarose gel and treated to remove primers. It was sequenced using the forward and reverse PCR primers at the Molecular Biology Resource Facility (University of Tennessee, Knoxville, TN, USA). The sequences were analysed using commercial software (Lasergene 10, SeqMan Pro; DNAStar Inc., Madison, WI, USA). BLAST was used to compare the sequence with all entries available in GenBank.[9]

PCR amplification of DNA extracted from putative *Demodex* mites produced a single band of ~330 bp. A DNA sequence without any background was produced, suggesting the presence of a single amplified product. The product matched 100% with *D. cati* deposited in GenBank (JX193759) over the entire region that was compared. This corresponded to bases 1–329 relative to JX193759.

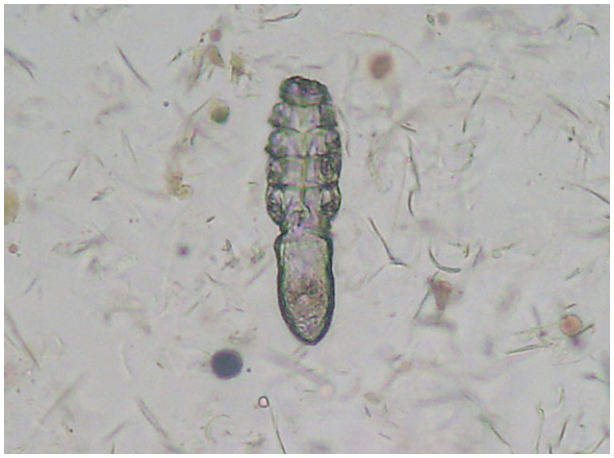

Figure 2. Mite with blunted abdomen detected on deep skin scrapings, morphologically longer than *Demodex gatoi* but shorter than *Demodex cati*.

Discussion

Feline demodicosis is an uncommon dermatological disease of cats. Generalized demodicosis due to *D. cati* is usually associated with underlying immunosuppression, either endogenous or iatrogenic.[2,3,10] In this case, the condition was presumed to be caused by chronic exogenous steroid administration.

To date, three distinct *Demodex* mites in cats have been described based on morphological criteria.[2–5,11] Molecular techniques have been employed to identify canine *Demodex* mite species precisely.[6–8,12] In two studies, morphologically distinct mites, the long-bodied mite (*D. canis*) and one with a short, blunted abdomen (*D. cornei*), were determined both to be *D. canis* based on molecular characterization.[6,7] Recently, molecular techniques have been used for detection and differentiation of feline *Demodex* mites.[1,13]

In the present case, the usefulness of molecular characterization of feline *Demodex* mites was demonstrated. Morphological evaluation suggested that there were two different species of mites, a long-bodied mite and one with a blunted abdomen that was intermediate in length between *D. gatoi* and *D. cati*. The authors suspected this to be the third unnamed species.[4,5] It was not possible to separate the mites physically, so they were processed together, from both the dried scrapings of material and the mites in mineral oil. PCR yielded a single sequence of *D. cati*. It is possible that the primer set used in this study that amplified *D. cati* 16S rRNA DNA may fail to amplify DNA from other species of *Demodex* infecting the cat. However, the primers used are highly conserved among *Demodex* species, including *D. gatoi*, *D. caprae*, *D. brevis*, *D. folliculorum*, *D. canis*, *D. injai* and an unnamed species affecting cats. These include all *Demodex* species for which 16S rRNA gene sequences are currently available in GenBank. Thus, in this case, the authors showed that the mite with the blunted abdomen was likely not to be a distinct species but rather a morphologically different form of *D. cati*, similar to what has been described in the dog, with *D. cornei* shown to be a variant of *D. canis* rather than a distinct species.[6,7] It is likely that as this technique is applied further, more mite species will be identified. This molecular characterization of the mites will aid in determining ancillary diagnostics and treatment approaches for each case, because underlying causes may differ.

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Résumé

Contexte – La caractérisation moléculaire des acariens *Demodex* est utilisée pour identifier les espèces d'acariens chez le chien. Cette technique est à présent utilisée pour les espèces de *Demodex* du chat afin de mieux caractériser les acariens.

Hypothèses/Objectifs – Les diagnostics moléculaires vont clarifier l'existence de plusieurs *Demodex* identifiés morphologiquement.

Sujets – Un chat atteint de démodécie généralisée secondaire à un traitement corticoïde chronique pour dysplasie érythroïde.

Méthodes – Des raclages cutanés not montré un grand nombre d'acariens folliculaires compatibles avec *Demodex cati* ainsi que des *Demodex* morphologiquement différents, présentant un abdomen arrondi. L'ADN 16SrARN a été amplifié par PCR, séquencé et comparé avec les séquences disponibles de *Demodex*, y compris *Demodex cati*, *Demodex gatoi* et *Demodex* sp sans nom.

Résultats – Un seul produit de PCR a été obtenu, dont la séquence d'ADN correspondait exactement à *D. cati.*

Conclusions et importance clinique – Le court acarien sans nom n'est pas une autre espèce dans ce cas mais une forme différente de *D. cati.* Cet article montre l'utilité des diagnostics moléculaires pour clarifier l'identité des acariens qui présentent plusieurs morphologies.

Resumen

Introducción – la caracterización molecular de los ácaros *Demodex* está siendo utilizada para identificar especies de ácaros en perros. Esta técnica está siendo ahora aplicada a las especies de gatos, permitiendo una mejor caracterización de los ácaros.

Hipótesis/Objetivos – el diagnóstico molecular clarificará la posible existencia de diversas especies de ácaros identificados con diferente morfología.

Animales – un gato con de demodicosis generalizada secundaria al tratamiento crónico con esteroides frente a displasia eritroide.

Métodos – los raspados de piel demostraron gran número de ácaros foliculares consistentes con *Demodex cati*, así como un ácaro morfológicamente diferente de tipo *Demodex* con un abdomen redondeado. Se amplificó el DNA del gen 16S rRNA mediante PCR, se secuenció y se comparó con las secuencias existentes de *Demodex*, incluidos *D. cati*, *D. gatoi*, y una especie de Demodex aún sin nombre.

Resultados – se obtuvo un solo producto de PCR, cuya secuencia de DNA fue exacta a la de *Demodex cati.*

Conclusiones e importancia clínica – el ácaro corto sin nombre no es una especie diferente en este caso, sino una variación morfológica de *Demodex cati.* Este artículo demuestra la utilidad del diagnóstico molecular para clarificar la identidad de los ácaros con diferente morfología.

Zusammenfassung

Hintergrund – Die molekulare Charakterisierung von *Demodex* Milben wird verwendet, um Milbenspezies bei Hunden zu identifizieren. Diese Technik wird nun auch bei *Demodex* Spezies der Katzen angewandt, was eine bessere Charakterisierung der Milben erlaubt.

Hypothese/Ziele – Die molekulare Diagnostik wird das Vorkommen verschiedener *Demodex* Milben, die morphologisch identifiziert werden können, verdeutlichen.
Tiere – Eine Katze mit generalisierter Demodikose, die sekundär nach chronischer Steroidbehandlung wegen erythroider Dysplasie auftrat.
Methoden – Hautgeschabsel zeigten eine große Anzahl an Follikelmilben, die wie *Demodex* cati aussahen, sowie eine morphologisch unterschiedliche *Demodex* Milbe mit einem stumpfen Bauch. Die 16S rRNA DNA wurde mittels PCR amplifiziert, sequenziert und mit vorhandenen *Demodex* Sequenzen, die *Demodex cati*, *Demodex gatoi* und eine unbenannte *Demodex* sp beinhalteten, verglichen.
Ergebnisse – Es wurde ein einziges PCR Produkt gewonnen, deren DNA Sequenz mit der von *D. cati* exakt übereinstimmte.
Schlussfolgerungen und klinische Bedeutung – In diesem Fall war die kürzere unbenannte Milbe nicht eine unterschiedliche Spezies, sondern eine unterschiedliche morphologische Form von *D. cati*. Dieser Bericht zeigt die Verwendung von Molekulardiagnostik, um die Identität von Milben abzuklären, die sich morphologisch unterscheiden.

要約

背景 – ニキビダニの分子生物学的な特徴はイヌにおいてダニの種を特定するために利用されている。この方法は現在では、ネコのニキビダニの種に対しても適応されていることでダニの特徴をより知ることができる。

仮説/目的 – 分子学的な診断法により形態学的に識別された様々なニキビダニの存在が明らかになる。

供与動物 – 赤血球異形成のために長期的なステロイド治療で二次的な全身性毛包虫症を生じた1頭のネコ

方法 – 皮膚掻爬検査により*Demodex cati*と一致する多数の毛包内ダニ、形態学的に異なる丸い腹部を持ったニキビダニが検出された。16SrRNA DNAをPCRで増幅し、配列を解析して、*Demodex cati*、*Demodex gatoi*ならびに名前のつけられていない*Demodex sp.*を含む利用可能な毛包虫の配列と比較を行った。

結論 – 1種類のPCR産物が得られ、そのDNA配列は*D. cati*と完全に一致していた。

結論および臨床的な重要性 – この症例において、名前のつけられていない短いダニは異なる種ではなかったが、*D. cati*とは形態学的に異なっていた。この報告は形態学的に異なるダニの正体を明らかにするための分子生物学的な診断法の有用性を立証した。

摘要

背景 – 犬蠕形螨的分子特性被用于辨识虫体种类。为了更好地确定虫体表征，现在这项技术也被用于猫蠕形螨。

假设/目的 – 分子鉴定可以分辨蠕形螨形态学种类。

动物 – 只猫由于红细胞发育不良而长期使用类固醇治疗，导致全身性蠕形螨。

方法 – 刮片检出大量蠕形螨，形态上符合*Demodex cati*但腹部较短。用PCR扩增 16S rRNA DNA，排序并与现有的蠕形螨DNA序列比较，包括*Demodex cati*, *Demodex gatoi* 和 一种未命名的*Demodex sp*。

结果 – 制得的PCR样本DNA序列和*D. cati.*高度吻合。

总结与临床意义 – 该病例中，短的未命名的虫体不是一个新品种， 但是形态和 *D. cati* 有些不同。 本篇报告证明了分子鉴定可用于判断形态学上有差异的虫体。