

# FIRST REPORT OF THE USE OF AFOXOLANER/ MILBEMYCIN OXIME IN AN AFRICAN PYGMY HEDGEHOG (*ATELERIX ALBIVENTRIS*) WITH DEMODICOSIS CAUSED BY *DEMODEX CANIS* IDENTIFIED BY MOLECULAR TECHNIQUES



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## Abstract

An African hedgehog patient with generalised erythema and periauricular scaling was presented for consultation; previously, it had been treated with ivermectin but no improvement was noted. Scrapes were performed from the skin and the mite morphologically corresponded to *Demodex* spp. Demodicosis in African hedgehogs is fairly rare; this is the reason why a molecular diagnosis was added to the microscopic diagnosis for this clinical case. The common treatments for mites in hedgehogs are ivermectin, selamectin, flipronil, and amitraz; the application of a single dose of afoxolaner/milbemycin oxime has not been previously reported. The molecular and phylogenetic analysis showed that the species causing the demodicosis in the patient was *Demodex canis*. The single oral dose of afoxolaner/milbemycin oxime reduced the number of mites to 0 in subsequent samples 7 days after the treatment and there were no lesions after 30 days. Copyright 2018 Elsevier Inc. All rights reserved.

**Key words:** *Demodex*; African hedgehog; PCR; afoxolaner; milbemycin oxime

**E**xotic species such as hedgehogs are becoming popular companion animals.<sup>1</sup> Therefore, they are more commonly seen in veterinary clinics. Acariasis is fairly common in these animals, usually those belonging to the Sarcoptidae (*Sarcoptes* and *Notoedres*) and Psoroptidae (*Caparinia*, *Otodectes*, and *Chorioptes*) families<sup>2</sup> or even infestations of local species such as *Demodex erinacei*, *Trombicula autumnalis*, and *Chorioptes* spp.<sup>3</sup>

In most mammals, *Demodex* behaves as an opportunistic pathogen, and has the potential to change from a feeder to a parasite if the cutaneous environment is beneficial to the parasite's proliferation.<sup>4</sup> The most common species is *Demodex canis*, causing the dermatological disease

known as demodicosis, which generally affects young dogs as well as older animals. The diagnosis can be confirmed by the presence of mites in deep skin samples.<sup>5</sup> Sequencing of the genome has proved to be a very effective taxonomic tool in phylogenetic studies and it has been used to

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Conflicts of Interest: There are no conflicts of interest.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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1557-5063/14/2101-\$30.00

<https://doi.org/10.1053/j.jepm.2018.06.007>

classify *Demodex* mites. The mitochondrial 16S rDNA gene is the main sequence.<sup>6</sup>

Traditionally, the common treatments for mites like *Demodex* in hedgehogs have included: ivermectin injections and amitraz in spray form. Other treatments have included imidacloprid at 10% + moxidectin at 1% in hedgehogs for *Caparinia tripilis* mites<sup>7</sup> and the application of selamectin topically (spot on) for fleas at 30 mg and mites (*Demodex erinacei*) at 45 mg. There was a noticeable improvement, with the absence of mites reported after 7 days; additionally, the animal experienced a complete recovery after 1-month.<sup>8,9</sup>

There have been developments in ectoparasiticides of the isoxazoline class with systemic oral administration.<sup>10</sup> In recent reports, fluralaner has been used in hedgehogs to treat capariniasis with an acaricide effect at 14 days after administration and up to 120 days afterwards.<sup>11</sup> Afoxolaner, an isoxazoline, was administered monthly to protect dogs from ticks and fleas, and has shown excellent results against mites like *Otodectes cynotis*, *Sarcoptes scabiei*, and *D. canis*.<sup>12</sup>

This molecule has a high specificity due to a unique union site in the chlorine channels regulated by the mite's GABA, which has no known relevant connection to the GABA receptor in mammals. In addition, it also has a wide safety margin.<sup>13</sup> Its effectiveness against mites, the efficacy of the combination of afoxolaner with milbemycin oxime in a chewable form against common endoparasites has also been demonstrated in another study.<sup>14</sup> Based on this evidence, the current study reports the case of a hedgehog with *D. canis* treated successfully with afoxolaner/milbemycin oxime.

## CASE REPORT

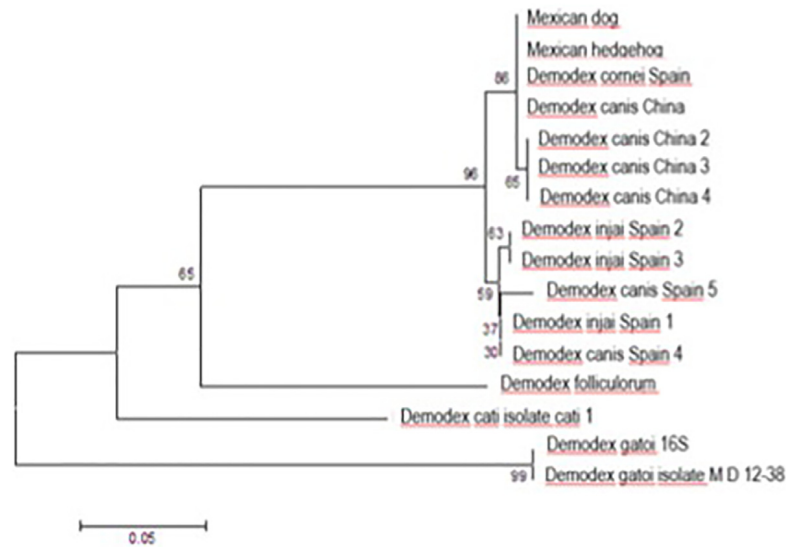
The patient, a 4-year-old male African hedgehog weighing 600 grams, presented with generalised erythema and periauricular scaling. The scaling was also present in the thoracic and pelvic areas as well as the central portion of the abdomen. It was also evident that the quills were easily removed and that papules affected the periauricular, ventral abdomen and feet. The owners have had this hedgehog from the age of 2 months. The hedgehog was in constant contact only with a dog and its environment, as it inhabited the same house as the owner. The presence of mites was identified in the patient, which had been treated 15 days before presentation with a subcutaneous single dose of ivermectin (Iverfull) at 300 µg/kg for skin lesions with no success. Skin scrapings were taken and mites were found which morphologically corresponded to *Demodex* spp. Mites were found in all of the scraping areas. The

samples were taken from 5 distinct areas, between the forelimbs, the hind limbs, the cervical region, ventral region of the abdomen, and the face (subsequent controls were taken from the same areas); samples were processed for the molecular identification of *Demodex* mites to the species level. The DNA was obtained from the skin scraping using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI USA), following the manufacturer's instructions. The absorbance of the DNA samples was measured with a Nano Droop 2000c (Thermo Scientific, WILM USA) to assess the quantity and quality. Molecular identification of the *Demodex* samples was achieved through the identification of a 340 base pair fragment of the 16S gene from the mitochondrial DNA, which was amplified using the following primers: Dem Forward 5'GAGGTATTTTACTGCTAAGG 3' and Dem Reverse 5'TCAAAGCCAACATCGAGG 3'. (Dem = *Demodex*). The conditions applied for the amplification were: 95°C for 10 minutes, 30 cycles of 94°C for 40 seconds, 55°C for 40 seconds, and 72°C for 45 seconds, and then a final extension stage at 72°C for 5 minutes. The products obtained from the amplification were visualised on a 3% agarose gel. Gel-purified PCR fragments were directly sequenced in an ABI Prism 3100 Genetic Analyser (Applied Biosystems, Foster City, CA USA). All of the obtained sequences were compared by multiple alignments to develop molecular and phylogenetic analysis using the MEGA 6 software (PSU, USA).<sup>15</sup>

The obtained sequence was deposited in GenBank under accession number MG703576; it showed a 99% identify with sample HE817764, reported as *Demodex cornei*, and with JF784000, reported as *D. canis*. Molecular and phylogenetic analyses and the morphological characteristics helped to rule out *D. cornei*. The phylogenetic tree shows a close relationship between the sample obtained from the hedgehog in this study and those identified as *D. canis* from China and *D. cornei* from Spain (Fig. 1).

A single dose of 2.5 mg/kg afoxolaner with 40 mg/kg milbemycin oxime, with no other treatment, was administered. The tablet was macerated and mixed with saline solution until a percentage division was made to provide the calculated dose for the hedgehog.

Skin samples were collected on the 7<sup>th</sup>, 14<sup>th</sup>, and 28<sup>th</sup> days after administering the dose of afoxolaner/milbemycin oxime (Nexgard Spectra, Merial). There was no evidence of erythema on day 14, and there were no lesions after 30 days. The study was reviewed and endorsed by the Ethics Committee of the Autonomous University of the State of Mexico.



**FIGURE 1.** Phylogenetic tree constructed by the neighbour joining method, from the partial sequences of mitochondrial 16S rDNA of *Demodex* from hedgehog and a dog from México.

## RESULTS

The molecular and phylogenetic analysis showed that the species causing the demodicosis in this African hedgehog was *D. canis*. The mites probably spread from the subclinically affected carrier dog to the hedgehog. The single oral dose of afoxolaner/milbemycin oxime reduced the number of mites to 0 in subsequent samples, 7 days after the treatment, and there were no lesions after 30 days.

## CONCLUSIONS

*D. canis* can cause demodicosis in African hedgehogs. This case represents the first report of *D. canis* on an African hedgehog to the best of the author's knowledge. The diagnosis was previously confirmed using molecular nucleic acid techniques and morphology characteristics. The infestation responded well to the use of afoxolaner/milbemycin oxime at a dose of 2.5 mg/kg.

## CONTRIBUTORS

All the authors have materially participated in the research and article preparation and have approved the final article.

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