SEVERE ULCERONECROTIC DERMATITIS ASSOCIATED WITH MITE INFESTATION IN THE CRITICALLY ENDANGERED AMARGOSA VOLE (MICROTUS CALIFORNICUS SCRIPSENsis)

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ABSTRACT: The entire range of the critically endangered Amargosa vole (Microtus californicus scirpensis) consists of less than 20 km² of riparian habitat in the Amargosa River drainage of the Mojave Desert in southern California. In September 2010, deformities on ears and chiggers on the ears and genitalia were detected, with some individuals so severely affected that they were missing ear pinnae altogether. Follow-up trapping was performed to determine the presence of mites and mite-associated disease, and molecular characterization was performed on the mites. Of 151 Amargosa voles sampled from February to April of 2011, 60 (39.7%) voles had hard orange mites adhered to some part of their bodies, on ears of 46 (76.7%), on genitalia of 11 (18.3%), and near mammary tissue of 13 (21.7%) voles. Gross lesions were not detected on genitalia, but nearly all individuals examined showed pinnal lesions and deformities, among which included alopecia, swelling, marginal necrosis, and ulceration, as well as scarring, scabbing, and loss of pinna mass covering 25–100% of the pinna. Biopsies revealed parakeratotic hyperkeratosis and acanthosis with diffuse neutrophilic exocytosis and dense necrotic granulocytes in the epidermis and superficial dermis associated with focal erosion and ulceration. In the underlying dermis, there were dense pleocellular inflammatory cell infiltrates composed primarily of necrotic granulocytes and multifocal hemorrhage. In some samples, mite mouthparts could be seen penetrating the superficial epidermis associated with necrosis, and mite fragments were found on the surface epidermis and within hair follicles. Microscopic examination of the mites documented that they were a larval trombiculid in the genus Neotrombicula with anatomical features that most closely resemble Neotrombicula micrati, based on scutal shape, setation, and texture. PCR of 2 mite pools (each consisting of 3 mites from an individual animal) amplified 331 bp amplicons, which had 92.97% homology with the 18S rRNA gene of Leptotrombidium deliensc, although coverage of Trombiculidae in GenBank is sparse. The severity and prevalence of lesions due to this chigger were atypical and distinct. Severe clinical trombiculiasis in this endangered species could negatively impact individual health and fitness.

MATERIALS AND METHODS

Voles were trapped in Sherman live traps at 3 semipermanent grid stations chosen from 23 sampling sites along the Amargosa River in the vicinity of Tecopa Hot Springs in southeastern Inyo County, California (35°53′8.4″ N, 116°14′1.2″ W). In these sites, riparian vegetation predominantly consists of bulrush (Scirpus olneyi), interspersed with a mix of cattails (Typha domingensis), desert salt grass (Distichlis spicata), and rushes (Juncus spp.). Appropriate biosecurity was implemented, including sterilization of traps, footwear, and equipment among sites. Captured individuals were identified to species, sex, and age class, and then ear-tagged, weighed, evaluated for reproductive condition, and released. An overall health examination was conducted including assessment for lesions on the ear pinnae.

Mites were collected by gentle scraping of ear tissue and peri-anal and genital regions and placed into 70% ethanol. Mites were examined on Euarapid-mounted slides (BioQuip, Rancho Dominguez, California) under compound light microscopy to determine whether all mites collected were the same species and for species-specific anatomic features. In order to begin to develop a DNA reference for this organism, the 18S rRNA gene was sequenced from a sample of the mite. In order to extract DNA, mites were air-dried to evaporate ethanol, boiled for 15 min at 100°C in 100 μL of 0.7 M NH4OH, quickly cooled for 30 sec on ice, and then boiled again for 15 min to evaporate ammonia. Newly designed PCR primers 31F1 (CGCGAATGGCTCATTTAAATC) and 344R2 (GCCTTCCTTGAGATGTGGTAG) were used in a PCR reaction using GoTaq Green enzyme and mastermix (Promega, Madison, Wisconsin) in a 25-μL volume. Cycling conditions were 94°C for 5 min then 30 cycles of 94°C each for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, followed by a 7 min extension at 72°C. Following PCR, DNA was purified from agarose gels using a kit (QiaQuick, Qiagen, Valencia, California) and submitted for sequencing using an ABI 3730 sequencer (Davis Sequencing, Davis, California) using the forward primer. Sequenced amplicons were evaluated by BLAST search of GenBank (NCBI; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Biopsy samples of the ears were taken using a biopsy punch at the margin of skin lesions. Skin was fixed in 10% buffered formalin, embedded in paraffin, thinly sectioned, and stained with hematoxylin and eosin as well as periodic acid Schiff (PAS) for fungi. These samples were examined for histopathology. In order to visualize the mites, a description of the newly described causative mite, and a summary of the pathologic changes associated with this infestation.

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RESULTS

One-hundred and fifty-one Amargosa voles were sampled in early 2011 at 3 semipermanent grid stations. Most voles were recorded to gender and age class: These included 71 males and 72 females, with 132 adults, 4 juveniles, and 13 subadults. Among the 3 stations, a total of 60 (39.7%) voles had “hard orange material” adhered to some part of their bodies, including 58/110 (52.7%) at site 1, 2/23 (8.7%) at site 2, and 0/18 (0%) at site 3. The prevalence was 26/71 (36.6%) in males, 32/72 (44.4%) in females, 56/132 (42.4%) in adults, 1/4 (25.0%) in juveniles, and 3/13 (23.1%) in subadults. Among infested voles, mites were found in and around the ears of 46 (76.7%), on genitalia of 11 (18.3%), and near mammary tissue of 13 (21.7%) voles. Gross lesions were not detected on genitalia, but 47% of all individuals examined showed pinnal lesions and deformities, which included alopecia, swelling, marginal necrosis, and ulceration, as well as scarring, scabbing, and loss of pinna mass covering 25–100% of the pinna.

Biopsy samples of ear tissue were assessed from 9 voles, and 1 vole that died in a trap was submitted for full necropsy (animal A). In the ear tissue from animal A, there was parakeratotic hyperkeratosis and acanthosis with light diffuse neutrophilic exocytosis and dense necrotic granulocytes in the epidermis and superficial dermis associated with focal erosion and ulceration. In the underlying dermis, there were dense pleocellular inflammatory cell infiltrates composed primarily of necrotic granulocytes and multifocal hemorrhage. A mite mouthpart penetrating the superficial epidermis was associated with focal necrosis, and mite fragments were present on the surface epidermis and within hair follicles (Fig. 1). The deep ear canal was unremarkable. Lungs were diffusely congested, but this was thought to be associated with attempts in the field for resuscitation. In the biopsy samples, inflammation ranged from mild to severe. Notable lesions occurred in most samples. Vole B tissue was characterized by few clusters of lymphocytes, plasma cells, and granulocytes in the periadnexal dermis. Biopsy C had multiple foci of dense neutrophils and lymphocytes (sometimes necrotic) in the superficial dermis and moderate periadnexal pleocellular inflammatory cell infiltrates. In biopsy D, there were moderate, dense accumulations of granulocytes, many necrotic, admixed with light to moderate numbers of mast cells in the periadnexal dermis. Ulcerative inflammatory dermatitis and intraadnexial mites were identified associated with the orange material observed grossly. Biopsies E and F had acanthosis and orthokeratotic hyperkeratosis of the nonhaired skin with mites present. There were dense pleocellular infiltrates deep into the dermis or even into adipose and muscle with hemorrhage; some islands of cartilage had pyknotic debris. None of the samples contained bacteria sufficient to cause the clinical signs.

Microscopic examination of the mites documented that they were a larval trombiculid in the genus Neotrombicula with anatomical features that most closely resemble Neotrombicula microti, based on scutal shape, setation, and texture (Brennan and Wharton, 1950) (Fig. 2). PCR of 2 mite pools (each consisting of 3 mites from an individual animal) amplified 331 bp amplicons, which had 92–97% homology with the 18S rRNA gene of Leptotrombidium deliense, although coverage of Trombiculidae in GenBank is sparse.

DISCUSSION

The Amargosa vole, with its dependence on very limited, patchily distributed, and highly specialized habitat, is at considerable danger of extinction. Probable factors contributing to endangerment include anthropogenic habitat change, small-population-size–induced demographic stochasticity, genetic impoverishment (Neuwald, 2010), and potentially infectious disease. Here we describe a clinically significant trombiculiasis in this endangered subspecies, which was not identified in the Recovery Plan but could negatively impact individual health and fitness. It is not known whether the severity of the disease associated with this mite is exacerbated by additional host or pathogen factors, but multiple infectious challenges to which these voles may be exposed must be identified and managed in order to contribute to a realistic recovery plan for the species.

Across the class Mammalia, mite infestation ranges from commensal (such as hair follicle mites) or moderately irritating and inflammatory with minimal pathological changes (e.g., “chiggers” on human beings), to being associated with severe dermatopathies, such as mange mites (Eldridge and Edman, 2000). Demodex spp. mites infest hair follicles and sebaceous glands, causing no disease or, when very abundant on some hosts, pathologic lesions including alopecia, epidermal scaling and necrosis, dermal inflammation with microabscesses, granulomas...
attachment to host skin, in order to digest host tissue and then imbibe digested, liquefied tissue fluid. A few case reports have documented dermatitis associated with chiggers. Trombiculiasis was reported in one black bear associated with secondary Staphylococcus intermedium dermatitis ventrally and in a second bear suffering from generalized crusting and scaling dermatitis with concurrent infestation with Eutrombicula splendens and Demodex sp. (Cunningham et al., 2001). Histopathology revealed larval mites near follicular ostia in the stratum corneum containing characteristic mite stylostomes or feeding tubes; some stylostomes extended into the superficial dermis. Inflammation near the dermis was ulceronecrotic with neutrophils, eosinophils, and histiocytes. These bears did not show signs of alopecia or edema, in contrast with infested Amargosa voles. In deer, chigger infestation produced hyperkeratotic, acanthotic dermatitis with neutrophilic, plasmacytic infiltration of epidermis and dermis (Little et al., 1997). In dogs, invasion of deeper skin including follicles was reported in Stravelia cynotis infestation (Seixas et al., 2006). Lesions were characterized by dilated follicles and pseudoepithelial hyperplasia with mononuclear and neutrophilic inflammation.

Chiggers are larval trombiculid mites that are free-living in nymphal and adult life stages (Krantz and Walter, 2009). It is not clear why the Amargosa voles suffer such severe disease from this mite, although plausible contributory factors may include genetic impoverishment of the vole and specific increased susceptibility to destructive inflammation following infestation, increased pathogenicity of a mite recently encountering a host with which it is not coevolved, and microhabitat factors such as increased warmth and moisture that promote inflammation. Neotrombicula microti, which has a broad geographic range encompassing the entire extent of North America, is reportedly more frequent on Microtus and Clethrionomys (Myodes) species voles, compared with other small mammals, with an apparent predilection for hosts in wet areas (Brennan and Wharton, 1950; Wrenn, 1974). The impact of such disease on vole population health is not known, but particularly with genital infestation, there could be reduction in fertility, which this species can ill afford.

This emerging disease is only the first such to be documented in Amargosa voles: A host of other parasites as well as viral and bacterial pathogens could spillover from extant and invasive animals in the Amargosa ecosystem and help drive the vole towards extinction. Notably, trombiculid mites are known vectors for rickettsial pathogens in the Old World (Traub and Wisseman, 1974) but have received minimal attention as disease vectors in the Western Hemisphere. Ongoing infectious disease processes may become more severe with stress put upon populations as they are forced into fewer habitat fragments with increasingly poor quality, and genetic variability in the population continues to erode. Monitoring the Amargosa vole for population health and population-level impacts of this mite will be an important target for work to be performed over the next few years.

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LITERATURE CITED


