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# DEVELOPMENT AND VALIDATION OF A FECAL PCR ASSAY FOR *NOTOEDRES CATI* AND APPLICATION TO NOTOEDRIC MANGE CASES IN BOBCATS (*LYNX RUFUS*) IN NORTHERN CALIFORNIA, USA

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ABSTRACT: Notoedric mange in felids is a devastating disease caused by a hypersensitivity reaction to the mite *Notoedres cati.* The burrowing of the mite causes intense pruritis resulting in self-mutilation, secondary bacterial infection, and often death of affected felids if left untreated. Our understanding of how notoedric mange is maintained in felid populations, and the true geographic extent of infestations, has been hampered because wild felids are elusive and, thus, traditional diagnostic methods are difficult to implement. To create a noninvasive diagnostic test, we developed and validated a novel PCR assay to detect *N. cati* DNA in fecal samples of bobcats (*Lynx rufus*) and used this assay to investigate a recent outbreak of mange in northern California, United States. Although the fecal PCR assay was 100% specific and could detect as few as 1.9 mites/200 μg of feces, it had a moderate sensitivity of 52.6%, potentially due to intermittent shedding of mites in feces or fecal PCR inhibitors. In a field investigation, 12% (95% confidence interval [CI]: 0.06, 0.23) of fecal samples ($n$=65) collected from Rancho San Antonia County Park and Open Space Preserve in Santa Clara County, California were PCR-positive for *N. cati*. When this estimate was adjusted for test sensitivity, the corrected proportion for fecal samples containing *N. cati* was 23% (95% CI: 0.14, 0.36), suggesting widespread mange in this area. This novel PCR assay will be an important tool to assess the distribution and spread of notoedric mange in bobcats and could be validated to test other wild felids such as mountain lions (*Puma concolor*). The assay could also be used to detect notoedric mange in domestic cats (*Felis catus*), particularly feral cats, which may also suffer from mange and could represent an important contributor to mange in peri-urban bobcat populations.

*Key words:* Felid, ITS-2, mites, PCR, scat, skin disease.

## INTRODUCTION

Notoedric mange is a transmissible skin disease caused by parasitic *Notoedres cati* mites of the family Sarcoptidae (Guaguere et al., 1999; Gross, 2005; Paterson, 2008). Notoedric mange in felids is extremely contagious (Guaguere et al., 1999; Scott et al., 2001), and infestation with this burrowing skin mite induces intense pruritis (Gross, 2005) resulting in self-mutilation, secondary bacterial infection, and often death if untreated (Bowman et al., 2002; Paterson, 2008). *Notoedres cati* has been reported in a range of mammals that includes mostly wild and domestic felids but occasionally other species including domestic dogs (*Canis lupus familiaris*) and humans (Lavoipierre, 1964; Scott and Horn, 1987; Guaguere et al., 1999; Bowman et al., 2002; Verde, 2005; Leone, 2007). Since 2001, notoedric mange has been identified as the primary cause of death in 19 bobcats (*Lynx rufus*) in southern California, United States (Riley et al., 2007), with cases regularly identified by local researchers and wildlife rehabilitators (Serieys, unpubl. data). These bobcats typically present with cachexia, profound hypoglycemia, and purulent exudative dermatitis covering up to 90% of the body. Microscopic assessment of

skin scrapings shows numerous mites, in contrast with sarcoptic mange where mites typically are sparse (Stephenson, Foley, and Serieys, unpubl. data). In 2010, three bobcats with severe skin disease and emaciation were reported dead or moribund in the Midpeninsula Regional Open Space District in Santa Clara County, California. Necropsy of two of the bobcats confirmed severe notoedric mange.

Little is known about the spatial ecology of notoedric mange and why it occurs in certain geographic areas. *Notoedres cati* is an obligate ectoparasite that spends all of its life stages on the host and cannot survive for long off the vertebrate host (Scott et al., 2001; Paterson, 2008) and requires direct host-to-host contact for spread. In North America, cases have been described in domestic cats (*Felis catus*) in Florida (Foley, 1991) and southern California (Thompson, 2012), in ocelots (*Leopardus pardalis*; Pence et al., 1995) and bobcats (Pence et al., 1982) in south Texas, the endangered Florida panther (*Puma concolor coryi*; Maehr et al., 1995) in Florida, and in mountain lions (*Puma concolor*; Riley et al., 2007; Uzal et al., 2007) and bobcats (Riley et al., 2007) in southern California. Most of these publications represent individual animal case reports; population-based assessments have not been published, even from southern California where mange is anecdotally common (Thompson, 2012). Our understanding of how notoedric mange is maintained in felid populations, and the true geographic extent of infestations, has been hampered because wild felids are elusive and, thus, traditional diagnostic methods are difficult to implement. While clinical signs are often suggestive of notoedric mange, skin scraping (abrasion of the top layer of the epidermis for microscopic examination) is the preferred method to confirm a diagnosis (Guaguere et al., 1999; Scott et al., 2001; Bowman et al., 2002; Gross, 2005). However, performing skin scrapings or biopsy to detect infections in free-ranging bobcats requires capture and immobilization of a trap-shy species, which requires considerable time and resources and can potentially cause morbidity to the animals. In contrast, scat transects can be performed relatively easily and feces assayed for mites. Due to mite-induced pruritis, infested felids spend much time grooming, thereby ingesting the mites which are then passed in the feces. Whole mites can be detected on fecal flotation, but the sensitivity of this test is unknown and mites must be passed intact in the feces in order to be identified (Bowman et al., 2002). A fecal PCR assay for detection of mite DNA would be useful because minute quantities of mite DNA might be detectable in a fecal sample so that mites need not be intact to be detected. PCR would also allow for species identification even between morphologically similar species.

Notoedric mange, combined with increasing urbanization, fragmentation of habitat, and increased overlap with humans and domestic cats, could have long-term negative impacts on bobcat populations. In order to understand the ecology of notoedric mange and its impacts on bobcat and other wild felid populations, we sought to develop a noninvasive tool for assessing mange in bobcat populations. Our goals were to develop and validate a fecal PCR assay for detection of notoedric mange in bobcats and use this assay to evaluate a recently detected cluster of notoedric mange cases in a bobcat population in northern California.

## MATERIALS AND METHODS

### PCR development

A PCR test for *N. cati* was designed and validated. Primers were designed to amplify a 241 base-pair fragment of the *ITS-2* of *N. cati* (GenBank accession AF251801, National Center for Biotechnology Information, Bethesda, Maryland, USA) using Primer-BLAST (National Center for Biotechnology Information, 2012). The forward primer, NCITS2F, is 5′-GAACGCACATTGCAGCCATTGGA-3′ and the reverse primer, NCITS2R, is 5′-AGAACCCTCTGAATCGCACCGA-3′. Mites used as a

positive control for primer development were obtained from a heavily infested bobcat from Santa Clara County, California. Mites were identified as *N. cati* based on morphologic criteria including length and shape of stalks on anterior legs, lack of extension of posterior legs beyond the body, presence of body striations, and the dorsal position of the anus (Lavoipierre, 1964; Klompen, 1992; Paterson, 2008). DNA was extracted from several *N. cati* mites using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) following the animal tissues (spin-column) protocol with overnight incubation with proteinase K at 56 C. The PCR reactions were performed using GoTaq® Green Master Mix (Promega, Madison, Wisconsin, USA) per manufacturer instructions. The final 25 µL reaction volume contained 1.0 µM of each primer, 4.5 µL water, and 3 µL of DNA. Thermal cycling conditions were 94 C for 3 min, then 35 cycles at 94 C for 30 sec, 58.3 C for 30 sec, and 72 C for 1 min followed by 5 min at 72 C. Water-containing negative control reactions were included in each run. Results of PCR were assessed by electrophoresis and UV-transillumination of GelStar® (Lonza, Rockland, Maine, USA) stained 1% agarose gels.

#### DNA extraction from feces

DNA extraction from feces was performed using the QIAamp DNA Mini stool kit (Qiagen) following the stool pathogen detection protocol with a few modifications to enhance breakdown of the mite exoskeleton. Briefly, 250–300-mg samples of stool were placed in microcentrifuge tubes and 1.4 mL of Qiagen Buffer ASL were added to each sample and the samples thoroughly homogenized. Next, 20 µL of proteinase K were added and the suspension heated overnight at 70 C. Samples were vortex mixed and centrifuged to pellet stool particles. Supernatants were pipetted into new microcentrifuge tubes and a Qiagen InhibitEX Tablet added to each. Samples were vortex mixed, centrifuged, and the supernatants transferred to new microcentrifuge tubes. Then, 200 µL of Qiagen Buffer AL were added and the samples were vortex mixed and incubated at 70 C for 10 min. Next, 200 µL of 100% ethanol were added to the lysates and vortex mixed, and samples were transferred to a QIAamp spin column and centrifuged. DNA was washed with 500 µL of Qiagen Buffer AW1 and then AW2. DNA was eluted with 100 µL of Qiagen Buffer AE and frozen until PCR was performed.

#### 18S DNA quality assessment

A proprietary eukaryotic 18S rRNA real-time PCR assay (Applied Biosystems, Foster City, California, USA) was performed per manufacturer instructions to ensure suitability of DNA for amplification. PCR reactions of 12 µL total volume consisted of 6 µL Fermentas Maxima® Probe/ROX qPCR Master Mix (ThermoScientific, Glen Burnie, Maryland, USA), 0.2 µL 18S Primer probe mix (Applied Biosystems), 4.8 µL water, and 1 µL DNA. TaqMan® PCR was performed in a thermal cycler (OneStepPlus, Applied Biosystems) using cycling conditions of 50 C for 2 min, 95 C for 10 min, and 40 cycles at 95 C for 15 sec followed by 60 C for 1 min. Results were considered positive when the cycle threshold value was $<$40 with a characteristic amplification plot.

#### Samples for test validation

Samples from bobcats known to be infested with mange or to be uninfested with mange were collected. Where possible, the bobcats were assessed by clinical examination, skin scraping, fecal flotation, and fecal PCR, although all tests were not available for all bobcats. From December 2007 through February 2012, samples were collected throughout California by collaborators including field researchers, wildlife rehabilitators, the California Department of Fish and Wildlife, and the Folsom Zoo (Table 1). Clinical signs of mange were recorded, if present. Skin scrapings were performed at the time of capture for live animals or at the time of necropsy. Skin scrapings were performed on the head, just in front of the ears (sites reported to have the highest density of mites in infested animals [Foley, 1991; Guaguere et al., 1999; Scott et al., 2001; Gross, 2005]) of animals without clinical signs. Skin tissue was scraped with a sterile surgical blade into mineral oil and examined microscopically under 100× magnification for *N. cati*. Mites were identified as *N. cati* based on the morphologic criteria described above. Fecal samples were frozen intact or 1 g of fecal material was placed in 10 mL formalin and thoroughly homogenized prior to storage until fecal examination could be performed. For formalin-stored feces, samples were centrifuged to pellet fecal material, supernatant was discarded, and the remaining fecal sample was examined using a modified McMaster egg-counting technique using Fecasol® solution (Evsco Pharmaceuticals, Buena, New Jersey, USA) and a Paracount-EPG™ Fecal Analysis Kit (Chalex Corporation, Wallowa, Oregon, USA) per

TABLE 1. Source and county (California, USA) of origin for bobcats (*Lynx rufus*) from which samples were collected for the evaluation of a fecal PCR assay to detect notoedric mange, December 2010–July 2011. Bobcats that had clinical signs of mange and a positive skin scraping or fecal floatation were classified as infested, while those with no clinical signs and a negative skin scraping or fecal floatation were classified as uninfested.

| Source | County | Purpose | Infested | Uninfested |
|---|---|---|---|---|
| Santa Monica Mountains | Los Angeles | Field study | 14 | 33 |
| Hoopa Valley | Humboldt | Field study | 0 | 2 |
| Folsom Zoo | Sacramento | Captive | 0 | 3 |
| Department of Fish and Wildlife | Santa Barbara | Necropsy | 2 | 0 |
| Department of Fish and Wildlife | Sacramento | Necropsy | 0 | 1 |
| Department of Fish and Wildlife | Santa Clara | Necropsy | 1 | 0 |
| Lindsay Wildlife Museum | Contra Costa | Rehabilitation | 1 | 0 |
| Wildlife Care of Silicon Valley | Santa Clara | Rehabilitation | 1 | 0 |

manufacture's instructions. For non–formalin-stored feces, a 500 mg sample was added to 2 mL zinc sulfate, homogenized, and examined microscopically at 80× magnification for *N. cati*.

An infested (positive) bobcat was defined as an animal with clinical signs of mange (skin crusting or alopecia on the head) and either a skin scraping or a fecal examination demonstrating *N. cati*. An uninfested (negative) bobcat was defined as an animal having no clinical signs of mange and either a skin scraping or fecal examination with no *N. cati* present. Because the incubation period for mange is unknown, any animal with evidence of mange infestation within 1 yr of sample collection was excluded as a negative case.

#### Assessment of analytic sensitivity and specificity

Analytic sensitivity was determined by PCR of fecal samples containing known numbers of mites. A fecal sample with 300 mites per gram, based on McMaster's quantitative fecal analysis, was serially diluted to 1:64 in distilled water and PCR was performed to calculate the minimum number of mites that could be detected using the PCR assay. A set of astigmatid mite species was used to test the specificity of the PCR assay. Mites used were *Sarcoptes scabiei*, *Psoroptes cuniculi*, and *Notoedres centrifera*. DNA was extracted using the QIAgen DNeasy Blood and Tissue kit and PCR performed as described above. A general PCR protocol for sarcoptid mites was also performed as described (Zahler et al., 1999) to confirm that the samples were positive for mite DNA.

#### Field sample collection

Rancho San Antonio County Park and Open Space Preserve (RSA) is a 165-acre preserve located in the coast range of Santa Clara County, California (centered at 37°20′1.58″N, 122°6′34.57″W). Elevation ranges from 0–600 m and vegetation consists of chaparral, old and disturbed coast live oak (*Quercus agrifolia*) grassland, and mixed conifer with redwood (*Sequoia sempervirens*). This preserve is part of a larger network of connected preserves managed by the Midpeninsula Regional Open Space District, but RSA is the most heavily used preserve for recreation in the district and is open free of charge 365 days per year. Dogs are not allowed on the preserve and neither pet cats nor known feral cats occupy the preserve, although the entire northern boundary of the preserve is adjacent to residences. From the hundreds of hikers and preserve employees who use the site daily, observations of normal bobcats were common until late winter 2010, when three dead or dying bobcats were collected, and staff reported that there were no longer bobcat sightings. Population density and home range sizes of bobcats at this site were not assessed, and bobcats are known to have high variance in home range and density across areas, from a 0.052–330 $km^2$ home range and 1–38 bobcats/65 $km^2$ (Nowell and Jackson, 1996). Bobcat home range and population density estimates have not been performed in RSA; however, based on frequent sightings until late 2010, preserve staff believed that the bobcat population was relatively robust.

Fecal samples were collected throughout RSA from December 2010 through July 2011, both opportunistically when feces were encountered during the course of other fieldwork and systematically in two large-scale transects where all the major accessible trails throughout the preserve were covered in a 2-day period. Major trails in the southwestern area of the preserve could not be accessed

because of rain and mud damage to the trails. All fecal samples that were consistent with bobcat feces in size and composition were collected. DNA extraction was performed as described above. Only fecal samples confirmed to be from a bobcat, using feline PCR (Wengert et al., 2012), were analyzed. The new fecal PCR assay was performed to determine if *N. cati* DNA was present in the samples. Locations where fecal samples were found were plotted on a map of RSA using ArcGIS (Environmental Systems Research Institute, Inc., Redlands, California, USA).

### Evaluation of test performance

Test results were entered into a computer spreadsheet program (Excel 2011, Microsoft Corporation, Redmond, Washington, USA). Test sensitivity (probability that a mange-infested bobcat had a positive PCR test result), specificity (probability that an uninfested bobcat had a negative PCR test result), and positive and negative predictive values (probability that a positive PCR test result represents a truly infested bobcat and probability that a negative PCR test result represents a truly uninfested bobcat, respectively) were calculated (Woodward, 2005). The kappa statistic was calculated to assess the strength of test agreement between fecal flotation and fecal PCR (Gordis, 2009). Mange infestation prevalence (proportion of fecal PCR test positives out of the total) for fecal samples collected in RSA along with the 95% CI for the prevalence estimate were calculated with the statistical package "R" (R-Development Core Team, 2012). To adjust for imperfect PCR test sensitivity, as determined by the validation study, a second corrected prevalence estimate and 95% CI were calculated (Rogan and Gladen, 1978).

## RESULTS

### Test validation

Fifty-eight bobcats met the criteria for inclusion as mange infested or uninfested. Of these, 19 were classified as infested and 39 as uninfested. Some diagnostic tests were not performed on all uninfested animals. Of 19 infested bobcats, 18 had a positive skin scraping (95%) and 11 had a positive fecal flotation (58%). Of 39 uninfested bobcats, 19 of 19 bobcats for which a skin scraping was available had a negative skin scraping (100%), while 34 of 34 bobcats for which fecal flotation was available had a negative fecal flotation (100%). On fecal PCR, 10 of 19 infested bobcats were positive for a test sensitivity of 53%. All 39 uninfested bobcats were fecal PCR-negative (100% specificity). The positive predictive value of the fecal PCR was 100% and the negative predictive value was 81%. The kappa statistic for test agreement between fecal flotation and fecal PCR was 0.59, indicating a moderately strong association.

### Analytic sensitivity and specificity

Serial dilution from a fecal sample with 300 mites per gram of feces showed a detectable positive band for all dilutions down to 1:16, corresponding to the ability to detect 1.88 mites in a 200-mg sample of feces. This was confirmed by applying PCR testing to DNA isolated from only two mites, yielding a positive band on PCR. In silico analysis of the primers using a BLAST search (National Center for Biotechnology Information, 2012) revealed that the forward primer was universal across the subclass Acari while the reverse primer had a 100% match to *N. cati*. The next most-closely related organisms had a homology of 86% or less. The *N. cati* fecal PCR assay showed no detectible bands for all other mite species tested (*Sarcoptes scabiei*, *Psoroptes cuniculi*, and *Notoedres centrifera*), but all showed detectible bands when PCR was performed using less-specific sarcoptiform primers (Zahler et al., 1999), which confirmed the presence of mite DNA in the sample.

### Excluded bobcats

Two bobcats were excluded from the main analysis, although both represent cases that highlight the value of the fecal PCR test. The first bobcat had very mild clinical signs of mange, including crusting only around the ears, but had a negative skin scraping and fecal flotation, leading to his exclusion as an infested case. Nevertheless, he was weakly positive on fecal

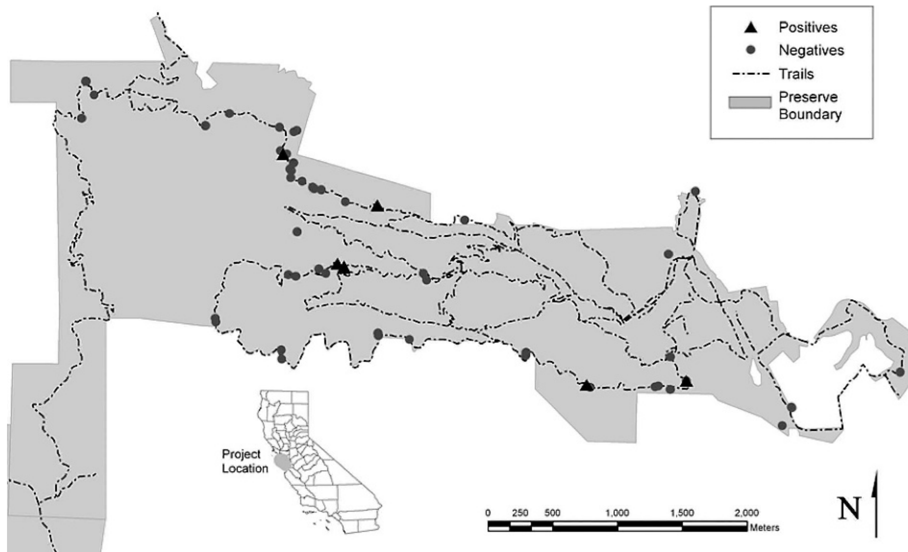


FIGURE 1. Spatial distribution of fecal samples positive and negative by PCR for the mite, *Notoedres cati,* collected in Rancho San Antonio County Park and Open Space Preserve, Santa Clara County, California, United States, December 2010–July 2011.

PCR. The second bobcat had no apparent clinical signs of mange and was negative on fecal flotation (no skin scraping was performed) but also showed a weak positive band on the fecal PCR. This bobcat died 6 mo after the time of sample collection with severe mange, leading to his exclusion; therefore, we suspect this bobcat may have had occult mange at the time of initial evaluation.

### Free-ranging bobcat findings

Sixty-five confirmed bobcat fecal samples were collected from RSA, although it was not confirmed that these represented different animals and likely represented multiple samples from the same animals. Of these 65 samples, 8 (12%; 95% CI: 0.06, 0.23) were positive for *N. cati* DNA on the fecal PCR assay. When adjusted for the calculated test sensitivity of 53%, the corrected proportion of fecal samples containing *N. cati* was 23% (95% CI: 0.14, 0.36). Three apparent spatial clusters of infested fecal samples were observed when locations of negative and positive fecal samples were plotted on a map of RSA (Fig. 1).

## DISCUSSION

We developed a novel, noninvasive PCR assay to detect notoedric mange in bobcats. Although not evaluated for this project, this assay may also be applied to skin samples and has the potential to be useful for diagnosis in other felids as well. When applied to feces, the assay had perfect specificity and a moderate sensitivity of 53%, which is likely due to several factors. Fecal flotation indicates that the number of mites passed in feces is variable; we hypothesize that this may be associated with inconsistent grooming by the animals, often when they are suffering from severe mange. This hypothesis is supported by the fact that the kappa statistic for test agreement between fecal flotation and PCR showed a moderately strong association between tests. Additionally, many PCR tests for feces lack sensitivity due to inhibitors of *Taq* DNA polymerase that remain after extraction of the DNA (Wilde et al., 1990; Stacy-Phipps et al., 1995; Monteiro et al., 2001). DNA from stool is often degraded; this can also decrease PCR sensitivity although we used

modified kits specifically designed for extraction of DNA from feces. This moderate sensitivity and the possibility of false-negative tests would imply that caution is required when attempting to rule out mange in a single individual. The assay can detect as few as two mites and detected mite infestation in two bobcats with very mild signs that were both negative on fecal flotation and from a skin scraping for one bobcat for which it was available. Although we could not rule out that these were false positives, this is unlikely as we detected no false positives in the cases that met the defined inclusion criteria. This finding may indicate that the sensitivity of the fecal PCR test, relative to the other diagnostic methods in this study, is higher during the early, mild stages of infection. The fecal PCR assay is also specific for *N. cati*. We targeted the internal transcribed spacer region of nuclear ribosomal DNA (rDNA) for the development of this PCR because of its good coverage for arthropods in the GenBank database and because this is a hypervariable region which allowed for good specificity of the test, with no cross-reaction with other mite species including the very closely related species *N. centrifera*.

When applied at a population level as done for this study, the PCR assay's use allowed us to confirm the presence of notoedric mange and define at least a minimum geographic range of cases at one preserve undergoing an apparent outbreak of mange in northern California. From among the 65 bobcat fecal samples collected from Rancho San Antonio County Park and Open Space Preserve, eight were PCR positive which, when adjusted for sensitivity, implies that approximately 16 samples truly did contain mite DNA. This proof-of-concept example also highlights that data collected in this way may present a problem of duplicate sampling of bobcats unless some additional information allows for the identification of individual bobcats. To ensure individual bobcats are not represented more than once in a fecal-based prevalence estimate, there are several possible solutions. Genetic analysis of fecal samples to identify individual animals would allow for an estimate of prevalence without having to capture and handle animals (Ruell and Crooks, 2007; Ruell et al., 2009). An additional benefit would be the ability to determine gender of the bobcat, which can help in stratification of risk assessment as well as assessment of basic ecology such as deposition of feces in scrapes or particular substrates or over differing areas of space by males versus females. Another option might be collection of feces at camera stations if individual bobcats can be identified visually. Alternatively, if home ranges can be identified (e.g., by radio-telemetry), fecal samples could be collected from inside discrete, nonoverlapping home ranges.

Confirming the presence of mange in an area is an important first step toward understanding the ecology and impact of this disease in bobcat populations. Given the severity of cases for individual bobcats and the relatively high number of cases observed in both southern California and RSA, mange might be an important contributor to bobcat population declines (Serieys, unpubl. data). The RSA proportion of positive fecal samples of nearly 25% is alarming and suggests that further monitoring of this population is critically important. In general, the population impact of mange has not been assessed for felids, although notoedric mange caused by a mite from the same genus (*N. centrifera*) was reported to contribute to large die-offs of the threatened western gray squirrels (*Sciurus griseus*) in Washington State (Cornish et al., 2001).

Notoedric mange has been reported in bobcats in California as early as the 1950s (Lavoipierre, 1964) but it may be increasing in prevalence in southern California and emerging in northern California. The recent discovery of cases in northern California supports the premise that notoedric mange might be an emerging

disease of conservation concern for California's wild felid populations. This disease has the potential to cause population declines in bobcat populations already impacted by other stressors including habitat fragmentation, urbanization, and anticoagulant exposure (Riley et al., 2007). There could be spillover from infected feral cats or other animal reservoirs, likely exacerbated by human development and increased cat-bobcat contact as well as an increase in susceptibility to mange resulting from other stressors or disease.

Much more work is needed to understand fully the impact of notoedric mange on bobcat populations in California. We describe a novel, noninvasive test that is able to detect mange in a bobcat population in which mange-caused mortalities were reported and without the need to trap and handle animals. This novel PCR assay will be an important tool to assess the distribution and spread of notoedric mange in bobcats. It may also be useful to detect mange-free bobcat populations, which would allow us to focus population and disease management efforts on at-risk populations. The assay is likely to be useful for detection of notoedric mange in mountain lions as well as domestic cats, particularly feral cats, which may also suffer from mange and could represent an important contributor to mange in peri-urban bobcat populations.

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## LITERATURE CITED

Bowman DD, Hendrix CM, Lindsay DS, Barr SC. 2002. *Notoedres cati*. In: Feline clinical parasitology. Iowa State University Press, Ames, Iowa, pp. 394–399.

Cornish TE, Linders MJ, Little SE, Vander Haegen WM. 2001. Notoedric mange in western gray squirrels from Washington. *J Wildl Dis* 37:630–633.

Foley RH. 1991. A notoedric mange epizootic in an island's cat population. *Feline Pract* 19:8–10.

Gordis L. 2009. *Epidemiology*. Elsevier/Saunders, Philadelphia, Pennsylvania, 308 pp.

Gross TL. 2005. Feline notoedric acariasis. In: *Skin diseases of the dog and cat: Clinical and histopathologic diagnosis*. Blackwell Science, Ames, Iowa, pp. 219–220.

Guaguere E, Prelaud P, Craig M, Scott DW. 1999. Notoedric mange. In: *A practical guide to feline dermatology*. Merial, Iselin, New Jersey, pp. 3.1–3.2.

Klompen JSH. 1992. *Phylogenetic relationships in the mite family Sarcoptidae (Acari : Astigmata)*. The University of Michigan Museum of Zoology, Ann Arbor, Michigan, 154 pp.

Lavoipierre MMJ. 1964. Mange mites of the genus *Notoedres* (Acari: Sarcoptidae) with descriptions of two new species and remarks on notoedric mange in the squirrel and the vole. *J Med Entomol* 1:5–17.

Leone F. 2007. Canine notoedric mange: A case report. *Vet Dermatol* 18:127–129.

Maehr D, Greiner E, Lanier J, Murphy D. 1995. Notoedric mange in the Florida panther (*Felis concolor coryi*). *J Wildl Dis* 31:251–254.

Monteiro L, Gras N, Vidal R, Cabrita J, Megraud F. 2001. Detection of *Helicobacter pylori* DNA in human feces by PCR: DNA stability and removal of inhibitors. *J Microbiol Methods* 45:89–94.

National Center for Biotechnology Information. 2012. *Primer-BLAST*. http://www.ncbi.nlm.nih.gov/tools/primer-blast/. Accessed June 2011.

Nowell K, Jackson P. 1996. *Wild cats: Status survey and conservation action plan*. International Union for Conservation of Nature and Natural Resources, Gland, Switzerland, 335 pp.

Paterson S. 2008. Notoedric mange (feline scabies). In: *Manual of skin diseases of the dog and cat*. Blackwell Pub., Oxford, UK, pp. 115–135.

Pence D, Matthews FD III, Windberg LA. 1982. Notoedric mange in the bobcat, *Felis rufus*, from south Texas. *J Wildl Dis* 18:47–50.

Pence D, Tewes M, Shindle D, Dunn D. 1995. Notoedric mange in an ocelot (*Felis pardalis*)

from southern Texas. *J Wildl Dis* 31: 558–561.

R-Development Core Team. 2012. Comprehensive R Archive Network (CRAN). http://cran.r-project.org/. Accessed December 2012.

Riley SPD, Bromley C, Poppenga RH, Uzal FA, Whited L, Sauvajot RM. 2007. Anticoagulant exposure and notoedric mange in bobcats and mountain lions in urban southern California. *J Wildl Manage* 71:1874–1884.

Rogan WJ, Gladen B. 1978. Estimating prevalence from the results of a screening test. *Am J Epidemiol* 107:71–76.

Ruell EW, Crooks KR. 2007. Evaluation of noninvasive genetic sampling methods for felid and canid populations. *J Wildl Manage* 71:1690–1694.

Ruell EW, Riley SPD, Douglas MR, Pollinger JP, Crooks KR. 2009. Estimating bobcat population sizes and densities in a fragmented urban landscape using noninvasive capture-recapture sampling. *J Mammal* 90:129–135.

Scott DW, Horn RT Jr. 1987. Zoonotic dermatoses of dogs and cats. *Vet Clin North Am Small Anim Pract* 17:117–144.

Scott DW, Miller WH, Griffin CE. 2001. Feline scabies. In: *Muller & Kirk's small animal dermatology*. W.B. Saunders, Philadelphia, Pennsylvania, pp. 483–484.

Stacy-Phipps S, Mecca JJ, Weiss JB. 1995. Multiplex PCR assay and simple preparation method for stool specimens detect enterotoxigenic *Escherichia coli* DNA during course of infection. *J Clin Microbiol* 33:1054–1059.

Thompson M. 2012. *Notoedric mange*, www.petplace.com/cats/notoedric-mange/page1.aspx. Accessed March 2012.

Uzal FA, Houston RS, Riley SPD, Poppenga R, Odani J, Boyce W. 2007. Notoedric mange in two free-ranging mountain lions (*Puma concolor*). *J Wildl Dis* 43:274–278.

Verde M. 2005. Zoonotic dermatoses in cats. In: *Small animal and exotics. Proceedings of the North American Veterinary Conference, Volume 19, Orlando, Florida, USA, 8–12 January, 2005*. Eastern States Veterinary Association, Gainesville, Florida, pp. 292–294.

Wengert GM, Gabriel MW, Foley JE, Kun T, Sacks BN. 2012. Field and molecular techniques for identifying intraguild predators of fishers and other North American small carnivores. *Wildl Soc Bull:* In press.

Wilde J, Eiden J, Yolken R. 1990. Removal of inhibitory substances from human fecal specimens for detection of group A rotaviruses by reverse transcriptase and polymerase chain reactions. *J Clin Microbiol* 28:1300–1307.

Woodward M. 2005. *Epidemiology: Study design and data analysis*. Chapman & Hall/CRC, Boca Raton, Florida, 699 pp.

Zahler M, Essig A, Gothe R, Rinder H. 1999. Molecular analyses suggest monospecificity of the genus *Sarcoptes* (Acari: Sarcoptidae) 1. *Int J Parasitol* 29:759–766.

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