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## SERUM CHEMISTRY, HEMATOLOGIC, AND POST-MORTEM FINDINGS IN FREE-RANGING BOBCATS (*LYNX RUFUS*) WITH NOTOEDRIC MANGE

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**ABSTRACT:** Notoedric mange was responsible for a population decline of bobcats (*Lynx rufus*) in 2 Southern California counties from 2002–2006 and is now reported to affect bobcats in Northern and Southern California. With this study we document clinical laboratory and necropsy findings for bobcats with mange. Bobcats in this study included free-ranging bobcats with mange (n = 34), a control group of free-ranging bobcats without mange (n = 11), and a captive control group of bobcats without mange (n = 19). We used 2 control groups to evaluate potential anomalies due to capture stress or diet. Free-ranging healthy and mange-infected bobcats were trapped or salvaged. Animals were tested by serum biochemistry, complete blood count, urine protein and creatinine, body weight, necropsy, and assessment for anticoagulant rodenticide residues in liver tissue. Bobcats with severe mange were emaciated, dehydrated, and anemic with low serum creatinine, hyperphosphatemia, hypoglycemia, hypernatremia, and hyperchloremia, and sometimes septicemic when compared to control groups. Liver enzymes and leukocyte counts were elevated in free-ranging, recently captured bobcats whether or not they were infested with mange, suggesting capture stress. Bobcats with mange had lower levels of serum cholesterol, albumin, globulin, and total protein due to protein loss likely secondary to severe dermatopathy. Renal insufficiency was unlikely in most cases, as urine protein:creatinine ratios were within normal limits. A primary gastrointestinal loss of protein or blood was possible in a few cases, as evidenced by elevated blood urea nitrogen, anemia, intestinal parasitism, colitis, gastric hemorrhage, and melena. The prevalence of exposure to anticoagulant rodenticides was 100% (n = 15) in bobcats with mange. These findings paint a picture of debilitating, multisystemic disease with infectious and toxic contributing factors that can progress to death in individuals and potential decline in populations.

Until recently, notoedric mange caused by the mite *Notoedres cati* has only been reported in isolated cases in free-ranging felids (Penner and Parke, 1954; Pence et al., 1982; Maehr et al., 1995; Pence et al., 1995; Ryser-Degiorgis et al., 2002). Severe mange and declining bobcat (*Lynx rufus*) populations were first observed in 2002 by the National Park Service (NPS) in Santa Monica Mountains National Recreation Area (SMMNRA), which straddles Ventura and Los Angeles counties (Riley et al., 2007). Coincident with the mange epizootic in 2002 in SMMNRA, there was a precipitous decline in the annual survival rate for radio-collared bobcats. Survival rates declined from a high of 0.847 in 1999 (5-yr average, 0.770) to 0.280 by 2003 (Riley et al., 2007). Since 2002, notoedric mange cases in bobcats have been documented in at least 6 other Southern and Northern California counties including Orange, San Diego, Santa Barbara, Santa Clara, Contra Costa, and Santa Cruz (Riley et al., 2007) (Boydston et al., unpubl. data; Serieys et al., unpubl. data).

Severe mange in free-ranging and domestic animals is often associated with immunocompromise (Pence and Ueckermann, 2002). Humans are more likely to suffer severe disease due to infestation with a related mite, *Sarcoptes scabiei*, if they are

concurrently immunocompromised (Walton et al., 2004; Roberts et al., 2005). In Southern California a statistical association between mange susceptibility in bobcats and anticoagulant rodenticide (AR) exposure has been reported in which 39 bobcats with advanced mange in SMMNRA tested positive for ARs in liver samples (Riley et al., 2007); similar results have been found in other California counties as well (Serieys et al., unpubl. data). Domestic cats are reported to be generally tolerant of low-grade AR exposure (Erickson and Urban, 2004), although it is not known whether this tolerance is experienced by other felids as well. Sublethal toxicosis associated with chronic, low-grade exposure to ARs has been hypothesized to predispose bobcats to other medical conditions such as an inability to mount strong, anti-mite immunity (Riley et al., 2007). However, the physiologic mechanism by which AR exposure could potentially increase bobcat susceptibility to notoedric mange is unknown. Other predisposing conditions that could exacerbate the susceptibility of some free-ranging felid species to mange could include nutritional and environmental stress, coinfection with other disease organisms, and genetic inbreeding or deleterious genetic conditions.

Assessing the physiological parameters of bobcats with mange could prove valuable to understanding the progression of disease in individual bobcats. In the present study, we report clinical serum biochemistry and complete blood count (CBC) values for opportunistically sampled bobcats with severe mange in Southern California. We compared serum biochemistry and CBC values of bobcats with mange to data from a healthy control group of captive bobcats and free-ranging bobcats in Southern California with no evidence of mange and little evidence of chronic AR exposure. We evaluated the data for potential associations between CBC and serum chemistry values and AR exposure. We compare the percent body weight difference for 12 bobcats with healthy weights paired with mange-infested body weights. We also present the results of necropsy examinations of 18 bobcats with clinical mange. Finally, we compare urinalysis results from a subset of free-ranging, apparently healthy bobcats

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and mange-infested bobcats, presenting the first reported urine values for free-ranging captured bobcats.

## MATERIALS AND METHODS

### Bobcats

Bobcats in this study included free-ranging bobcats with severe, clinical mange ( $n = 16$ ), a control group of free-ranging bobcats without mange and minimal AR exposure ( $n = 11$ ), and a captive control group without mange ( $n = 19$ ). We used 2 control groups to evaluate potential anomalies due to capture stress or diet. Prospective sampling occurred in California in Los Angeles, Ventura, Orange, Contra Costa, and San Diego counties during field studies conducted by National Park Service (NPS) or United States Geological Survey (USGS) biologists. In Orange County, samples came from mange-infested bobcats that were found opportunistically during a USGS field study spanning 2005–2010. In Los Angeles and Ventura counties samples were collected from 2008–2011 during an ongoing bobcat ecology study begun in 1996 in SMMNRA. SMMNRA is 40 km from downtown Los Angeles and comprises 153,075 acres. SMMNRA encompasses large regions consisting of continuous habitat with minimal urban development as well as fragmented areas with low-to-high density residential development, golf courses, office buildings, and agricultural areas. Notoedric mange has been observed throughout the park in bobcats except in the most continuous, natural-core habitat in the western-most region of SMMNRA.

Bobcats with severe clinical mange were trapped or found in SMMNRA ( $n = 5$ ) and Orange County ( $n = 9$ ), provided by a local resident in San Diego County ( $n = 1$ ), or provided by Animal Control in Contra Costa County ( $n = 1$ ). Three bobcats in SMMNRA were caught in cage traps (Tru-catch traps, Belle Fourche, South Dakota) checked every 12 hr as part of ongoing fieldwork. All other mange bobcats were found moribund and captured by residents using no capture devices or captured by animal control officers using catchpoles. All bobcats with mange, with the exception of the individual from San Diego County, were transported to a veterinary clinic for sampling. Individuals, with the exception of the San Diego County case, were anesthetized with a combination of approximately 6–10 mg/kg ketamine HCl and 0.05 mg/kg medetomidine prior to sample collection. The San Diego County individual died during transport to a clinic and was sampled within 5 min of death via cardiocentesis.

Captive bobcats were sampled at 2 rehabilitation facilities near the study area in Ventura and San Diego counties. These bobcats had diets of rodents, poultry, beef, and commercial carnivore diet, and notoedric mange was not observed in any individuals in the collections. Individuals in this group were anesthetized with approximately 5 mg/kg ketamine HCl and 0.1 mg/kg medetomidine by blow dart or jabstick, sampled, and then returned to the collection.

Bobcats in the free-ranging control group ( $n = 11$ ) were sampled during ongoing fieldwork by NPS biologists in a protected state park encompassing the most continuous and natural habitat in SMMNRA. From 2008–2011, NPS biologists sampled bobcats in this region as part of a radio-collaring study. Notoedric mange has not been reported for this population. Animals were captured in cage traps (Tru-catch traps, Belle Fourche, South Dakota) checked every 12 hr. Animals were anesthetized with ketamine HCl 5 mg/kg and medetomidine 0.1 mg/kg and samples were collected in the field. Adult individuals in the free-ranging healthy control group were radio-collared as part of the NPS study and had been documented to utilize only the western natural-core region of SMMNRA. Individuals whose radio-collar locations were not observed outside the boundaries of the protected state park areas during the course of the NPS study (Riley et al., unpubl. data) were used as free-ranging controls because they were assumed to be at minimal risk for AR exposure.

An additional 18 free-ranging bobcat carcasses with mange from Los Angeles, Ventura, San Diego, and Santa Barbara counties were available for necropsy within 48 hr following death. One was euthanized shortly after being admitted to a wildlife rehabilitation center due to severe mange, 1 was hit by a car, 1 had trauma suggestive of predation, and all others had severe multisystemic disease and mange.

Body weights of 12 bobcats were obtained as part of the NPS long-term bobcat study in Los Angeles and Ventura counties when the individuals were in an apparently healthy state with no mange evident.

These body weights were compared with weights from the same bobcats at a time when they were severely infested with mange.

### Sample collection

From captive, free-ranging healthy and from free-ranging, mange-infested bobcats 1 ml of blood was collected via venipuncture ( $n = 45$ ) or cardiocentesis ( $n = 1$ ) into serum separating tubes and centrifuged within 1 hr of collection and the serum removed. One-half a milliliter of blood was also collected into EDTA tubes. If available, urine was collected via cystocentesis of the urinary bladder. For 11 of 16 mange-infested bobcats that were moribund and died, 2 grams of liver were collected post-mortem and frozen at  $-20\text{ C}$  for AR analysis. Bobcat necropsies were performed on fresh or previously frozen carcasses and were conducted by veterinary pathologists. For histopathology, tissues were fixed in 10% buffered formalin, embedded in paraffin, thinly sectioned, and stained with hematoxylin and eosin.

### Notoedric mange evaluation

Skin scrapings were performed, at the time of capture for live animals or at the time of necropsy, on the head and just in front of the ears (sites reported to have the highest density of mites in infested animals) (Foley, 1991; Guaguère et al., 1999; Scott et al., 2001; Gross et al., 2005). Skin scrapings were performed as in Riley et al. (2007) and Stephenson et al. (2013). Briefly, tissue was scraped with a sterile surgical blade into mineral oil and then examined microscopically under  $\times 100$  magnification. Mites were identified as *N. cati* based on morphological 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). In 2 cases mites were reported as “consistent with *Sarcoptes*” although they were more likely to have been *Notoedres*. Notoedric mange is the most common mite associated with feline mange (Malik et al., 2006) and sarcoptic and notoedric mites have few distinguishing characteristics. Further, previously published reports utilizing samples from Southern California have detected only notoedric mites (Riley et al., 2007; Stephenson et al., 2013).

### Laboratory assays

Serum chemistry and CBCs were performed at a commercial laboratory (IDEXX, Irvine, California; Antech Diagnostics, Irvine, California; University of California Veterinary Medicine Teaching Hospital [VMTH], Davis, California). Urine evaluation was performed in the Chemistry Laboratory of the VMTH. All pathogen testing reported here for necropsied bobcats was done in laboratories at California Animal Health and Food Safety. Culture, serological, or molecular testing was ordered by the pathologist if gross or histopathologic lesions suggested particular infectious causes.

Residues of warfarin, coumachlor, bromadiolone, brodifacoum, diphacinone, chlorophacinone, and difethialone were assessed in bobcat livers by high performance liquid chromatography (HPLC) and LC-MS/MS, as previously described (Riley et al., 2007; Ruder et al., 2011). Limits of quantitation for these anticoagulants vary according to their sensitivity to UV or fluorescence detection during LC-MS/MS. In tissue these are 0.01 ppm for brodifacoum, 0.05 ppm for bromadiolone, warfarin, and coumachlor, and 0.25 ppm for chlorophacinone, diphacinone, and difethialone. ARs that were determined to be positive by LC-MS/MS, but were below the limit of quantitation by HPLC, were defined as “positive.”

### Data analyses

Data analyses were performed using R statistical software (R Development Core Team, 2010) and  $P$ -values of  $P \leq 0.05$  were considered significant. For multiple statistical tests we used Bonferroni-corrected  $P$ -values. Summary statistics including the mean, standard deviation, and median were calculated for each group of bobcats for each chemistry and hematological parameter. All data were tested for normality using the Shapiro-Wilk test for normality. Non-normally distributed values were compared among bobcat groups using a Kruskal-Wallis test; normally distributed values were compared using ANOVA. For those blood chemistry parameters found to be statistically different using an ANOVA or Kruskal-Wallis test, we performed either a Mann-Whitney or a Welch's  $t$ -test, respectively, for comparisons of each of the 3 groups. Within each

TABLE I. Mean, standard deviation (SD), and median for serum chemistry and hematological parameters for each bobcat group. \*Indicates  $P \leq 0.01$  and † indicates  $P \leq 0.001$ .

Parameter	Mange			Free-ranging healthy			Captive			ISIS‡	
	n	Mean (SD)	Median	n	Mean (SD)	Median	n	Mean (SD)	Median	n	Mean (SD)
Alkaline phosphatase (U/L)	15	11.9 (10.8)	8	11	16.3 (9.0)	15.5	19	35.1 (37.2)	13	130	19.0 (22.0)
Alanine aminotransferase (U/L)*	15	52.5 (54.0)	47	11	71.0 (35.1)	59	19	26.0 (6.0)	25	129	42.0 (20.0)
Aspartate aminotransferase (U/L)†	13	112.3 (95.1)	71	11	140.4 (81.8)	137.5	19	33.1 (10.5)	31	110	41.0 (22.0)
Albumin (g/dl)†	15	2.0 (0.8)	2	11	3.4 (0.3)	3.4	19	3.8 (0.2)	3.8	132	3.4 (0.7)
Total protein (g/dl)†	15	5.2 (1.9)	5	11	7.3 (0.3)	7.3	19	7.2 (0.5)	7.2	134	7.0 (0.7)
Globulin (g/dl)	15	3.2 (1.2)	3	9	3.9 (0.3)	3.9	19	3.4 (0.4)	3.4	132	3.7 (0.7)
Total bilirubin (mg/dl)	15	0.2 (0.1)	0.1	11	0.3 (0.1)	0.3	19	0.2 (0.1)	0.2	136	0.3 (0.2)
Blood urea nitrogen (mg/dl)†	16	84.0 (42.0)	89	11	39.0 (10.1)	40.5	19	35.1 (8.1)	38	143	32.0 (11.0)
Creatinine (mg/dl)†	14	0.7 (0.3)	0.7	11	2.0 (1.1)	1.5	19	1.8 (0.5)	1.8	140	2.0 (0.6)
Cholesterol (mg/dl)†	16	61.5 (43.1)	52	11	114.7 (42.4)	95	19	143.6 (30.0)	145	125	132.0 (36.0)
Glucose (mg/dl)†	15	82.0 (130.3)	42	11	212.4 (82.6)	211.5	19	167.8 (26.7)	167	132	171.0 (61.0)
Calcium (mg/dl)†	15	7.5 (1.8)	8	11	9.3 (0.5)	9.2	19	10.1 (0.5)	10.1	139	9.8 (0.7)
Phosphorus (mg/dl)†	15	8.0 (3.2)	7.3	11	5.0 (1.1)	4.9	19	5.1 (1.5)	4.5	128	5.4 (1.4)
Chloride (mmol/L)†	16	129.9 (10.0)	134	11	120.1 (4.8)	120.2	19	119.5 (2.0)	120	140	120.0 (5.0)
Potassium (mmol/L)	16	4.3 (1.3)	4	11	4.2 (0.4)	4.1	19	4.1 (0.4)	4.1	137	4.5 (0.6)
Sodium (mmol/L)	16	156.0 (14.3)	159	11	153.1 (4.8)	153	19	152.5 (1.3)	153	137	152.0 (4.0)
Leukocytes (K/ $\mu$ l)*	9	19.3 (12.9)	22.9	11	13.9 (4.0)	15.5	10	7.3 (2.7)	6.3	135	6.9 (5.1)
Neutrophils (K/ $\mu$ l)*	9	15.4 (11.0)	17.6	11	11.6 (4.3)	11.6	10	4.8 (2.6)	3.8	123	4.2 (3.9)
Lymphocytes (K/ $\mu$ l)	9	1.4 (0.8)	1.4	11	1.5 (0.8)	1.2	10	1.8 (0.5)	1.7	129	2.1 (1.8)
Monocytes (K/ $\mu$ l)*	9	0.7 (0.4)	0.7	11	0.4 (0.2)	0.3	10	0.2 (0.1)	0.2	113	0.2 (0.3)
Eosinophils (K/ $\mu$ l)*	9	1.7 (1.6)	1.9	11	0.4 (0.2)	0.3	10	0.4 (0.2)	0.4	117	0.5 (0.4)
Basophils (K/ $\mu$ l)	9	0.0 (0.1)	0	11	0.0 (0.0)	0	10	0.0 (0.0)	0	23	0.3 (0.7)
Hematocrit (%)†	9	23.8 (11.2)	18.7	11	40.4 (3.7)	40.6	10	34.3 (7.2)	36.3	148	36.7 (6.1)
Platelets (K/ $\mu$ l)	9	485.3 (144.7)	503	11	402.8 (74.0)	404	10	384.0 (138.0)	409	73	407.0 (143.0)

‡ 2011, International Species Information System (ISIS), Apple Valley, Minnesota 55124-8151, USA.

group we also tested for differences between males and females for each blood parameter using a Mann-Whitney or a Welch's *t*-test. Differences between healthy and clinically mange-infested bobcats' urine protein and creatinine levels were assessed with 2-tailed Student's *t*-tests as were differences in body weights for bobcats while healthy and while infested with mange. Differences between anticoagulant concentrations in necropsied bobcats with evidence of gastrointestinal hemorrhage, compared with those without hemorrhage, were performed using a Mann-Whitney test. Finally, for animals for which we had anticoagulant residue results paired with hematological parameters, we tested for correlations between AR concentrations and blood chemistry and hematocrit values using Spearman's test.

## RESULTS

Of the 15 animals examined at a veterinary hospital, 10 were in moribund condition and 11 died within 24 hr of sample collection despite treatment that included administration of fluids, vitamin B, antibiotics, and acaricides. In each case, a veterinarian or veterinary pathologist attributed the cause of mortality to mange-related complications.

### Clinical serum chemistry

Serum chemistry values for captive healthy, free-ranging healthy, and free-ranging mange-infested bobcats were compared by group (Table I). Serum total protein, blood urea nitrogen (BUN), phosphorus, and chloride were normally distributed and thus compared by ANOVA; the other analytes had non-normal distributions for at least 1 bobcat group and so were analyzed by Kruskal-Wallis test. For serum alkaline phosphatase, globulin,

total bilirubin, and potassium, mean or median values did not differ from VMTH-established normal reference intervals for domestic cats or International Species Information System (ISIS) values for bobcats (Flesness, 2003). Correlations were not observed between blood chemistry parameters and anticoagulant residue concentrations.

Although alanine aminotransferase (ALT) values tended to be within the normal reference interval for all 3 bobcat groups, the healthy captive bobcats had significantly ( $P \leq 0.01$ ) lower mean ALT levels compared with both the mange-infested and free-ranging healthy bobcats (Table I). Captive bobcats did not have elevations in aspartate aminotransferase (AST) while both healthy and mange-infested free-ranging bobcats had significantly ( $P \leq 0.001$ ) elevated values that were more pronounced in the free-ranging healthy bobcats. Serum albumin, total protein, creatinine, cholesterol, and calcium were significantly ( $P \leq 0.001$ ) decreased in bobcats with mange compared to both free-ranging healthy and captive healthy bobcats, and the values for these infested bobcats were lower than the normal reference interval. All 3 bobcat groups had mean BUN levels that exceeded reference intervals, and this was significantly ( $P \leq 0.001$ ) elevated in mange-infested bobcats. Glucose values were particularly difficult to characterize: both free-ranging and captive healthy bobcats tended to have values above the normal reference interval while mange-infested bobcats had a mean glucose of 82 mg/dl, which was significantly ( $P \leq 0.001$ ) lower than that for the healthy bobcat groups. Serum phosphorus and sodium were within normal limits in both captive and free-ranging healthy

bobcats but were significantly ( $P \leq 0.001$ ) elevated in bobcats with mange.

### Complete blood counts

Complete blood counts were performed on samples from 30 of 46 bobcats in the same free-ranging mange-infested, free-ranging healthy, and captive healthy control groups (Table I). Statistically significant differences among groups were detected in hematocrits and in leukocyte, neutrophil, monocyte, and eosinophil counts. Mange-infested bobcats had a leukocytosis with a mean leukocyte count of 19,300/ $\mu\text{l}$  (range 2,800–39,600/ $\mu\text{l}$ ) compared with 13,900/ $\mu\text{l}$  (2,800–20,100/ $\mu\text{l}$ ) in free-ranging healthy and 7,300/ $\mu\text{l}$  (4,000–11,700/ $\mu\text{l}$ ) in captive healthy bobcats. Similarly neutrophil, monocyte, and eosinophil counts were elevated in mange-infested bobcats compared with free-ranging healthy and captive healthy bobcats. Although the trend for these white blood cell counts was higher in mange-infested bobcats, their values ranged widely (neutrophils 700–31,000/ $\mu\text{l}$ , monocytes 200–1,200/ $\mu\text{l}$ , eosinophils 50–4,300/ $\mu\text{l}$ ) compared with free-ranging healthy (neutrophils 3,420–18,090/ $\mu\text{l}$ , monocytes 161–820/ $\mu\text{l}$ , eosinophils 160–640/ $\mu\text{l}$ ) and captive control bobcats (neutrophils 2,000–9,360/ $\mu\text{l}$ , monocytes 80–585/ $\mu\text{l}$ , eosinophils 126–918/ $\mu\text{l}$ ). Significant differences between groups after correction for multiple tests were observed between the captive healthy and mange-infested groups for monocytes and eosinophils ( $P = 0.003$  for each test). Free-ranging healthy bobcats also had significantly higher leukocytes ( $P < 0.001$ ), neutrophils ( $P < 0.001$ ), and monocytes ( $P = 0.01$ ) compared with captive healthy bobcats. Hematocrits in mange-infested bobcats ranged from 10.9–40.0%, with a mean of 23.8%, compared with means of 40.4% (range 36.4–42.9%) in free-ranging healthy and 34.3% (range 24.0–45.0%) in captive healthy bobcats. Hematocrits did not correlate with AR residue concentrations.

### Urine values

Urine specific gravities ranged from 1.027 to  $>1.040$ , confirming renal sufficiency. Urine protein:creatinine ratios were assessed in 9 bobcats in order to determine whether renal protein loss was occurring. Four of these samples came from bobcats with mange, and the mean and standard deviations were 45.0 mg/dl ( $\pm 15.9$ ) for protein values and 312.4 ( $\pm 277.3$ ) for creatinine. Protein values in the 5 healthy bobcats were 84.4 ( $\pm 125.6$ ) and creatinine values were 207.8 ( $\pm 125.6$ ). These differences were not statistically significant. Only 1 protein:creatinine ratio, in a clinically healthy bobcat, exceeded 1 and that urine sample was frankly blood-contaminated.

### Necropsy

Necropsies were performed on 18 bobcats from 4 counties in California. Eleven of these bobcats were male, 6 were female, and for 1 bobcat the sex was not recorded. There were 11 adults, 5 juveniles, and 4 for which the age group was not recorded. All bobcats that were necropsied ranged from thin to emaciated and all had moderate to severe mange confirmed by microscopic examination of skin scraping. In addition, bobcats were infested with various endoparasites and ectoparasites as shown in Table II. All bobcats had a severe dermatitis which consistently affected the head, ears, and neck and, in severe cases, was generalized.

Affected skin was alopecic with adherent crusts, excoriations, and lichenification in chronic cases. Microscopically, the dermatitis was characterized by acanthosis, hyperkeratosis, and parakeratosis with serocellular crusts and subcorneal pustules; mites were numerous and embedded in the epidermis. Two individuals had a concurrent *Malassezia* dermatitis. In 4 bobcats gastrointestinal contents resembled hemorrhage. Other findings in bobcats included splenic or lymph node lymphoid depletion, histiocytosis, or both, bacteremia due to *Staphylococcus xylosum*, *Escherichia coli*, or coagulase-negative *Staphylococcus* sp., degenerative changes in the adrenal gland, chronic leptospirosis associated with *Leptospira pomona*, pneumonia, respiratory nematodes, or enterocolitis (Table II). Three bobcats were tested for *Toxoplasma gondii* antibodies by latex agglutination and 2 were positive. Of 6 that were tested for FeLV and FIV by ELISA, all were negative (Table III). Six bobcats were also tested for rabies virus by fluorescent antibody testing and all were negative.

### Anticoagulant residue analysis

Liver samples from 27 mange-infested bobcats were tested for AR exposure (Table IV) and all 27 were exposed to anticoagulants. Four different ARs were detected in bobcats: brodifacoum, bromadiolone, diphacinone, and difethialone, and 21 of the 27 exposed bobcats had residues of 2 or more compounds. Bobcats with AR exposure were from Orange, San Diego, Los Angeles, Ventura, Santa Clara, San Diego, and Santa Barbara counties. Anticoagulant residue concentrations were not observed to correlate with gastrointestinal hemorrhage for necropsied individuals, although our sample size was small. Two bobcats without mange from the free-ranging healthy control group used for this study were also positive for AR in liver tissue, both at levels below the minimum level for quantification. These were the only liver samples available for testing from the free-ranging healthy bobcat group. However, clotting times measured by partial thromboplastin time and proteins invoked in Vitamin K absence for all free-ranging healthy bobcats were within normal limits at the time of their capture and blood collection for use in this study (Riley et al., unpubl. data; Serieys et al., unpubl. data).

### Body weight

Paired healthy and mange-infested body weights were available for 11 adult bobcats (4 male, 7 female) and 1 juvenile male. Weight loss was attributed to severe mange-infestation and the mean body weight difference across males and females was 39%. The mean weight loss was the same for both males and females and ranged from 30–56% ( $P = 0.002$  for males and  $P < 0.001$  for females).

## DISCUSSION

Notoedric mange is a potentially fatal and emerging disease of concern in bobcat populations in California. Ongoing ecological studies suggest that mange may contribute to bobcat population regulation and decline. However, mange per se often is not considered fatal, and thus we sought to understand what medical factors might underlie and then contribute to the progression of such severe disease. In this study, the majority of bobcats with mange were emaciated and dehydrated, had severe panhypoproteinemia, probably due to a protein-losing dermatopathy, and

TABLE II. Summary of necropsy data from 18 bobcats with notoedric mange. \* Indicates histopathology performed. C: cestodes, A: ascarids, N: nematodes, WNL: within normal limits, LN: lymph nodes, N/A: not examined.

ID	Endoparasites	Ectoparasites	Gastrointestinal	Lymphopoietic	Hepatic	Urogenital	Pulmonary	Other
D22*	C, A	M	colitis	WNL	hemosiderosis	WNL	WNL	degenerative myopathy
D03*	C	M, T	WNL	LN erythrophagocytosis	WNL	interstitial nephritis	WNL	none
D45*	C	M	WNL	lymphoid depletion	hepatocellular vacuolation	renal tubular vacuolation	WNL	keratitis
D27*	C, A	M	WNL	lymphoid depletion	WNL	interstitial nephritis	WNL	none
S40	A	M	WNL	WNL	hepatocellular degeneration and necrosis	WNL	N/A	none
D16*	A	M	melena	grossly enlarged LNs	WNL	WNL	WNL	none
D66*	N/A	M	WNL	reactive LN with erythrophagocytosis	WNL	WNL	Interstitial pneumonia	Presumptive hypoproteinemia, serous atrophy of fat, myocytic protozoa, thyroid follicular collapse
B66*	C	M	unidentified intraenterocyte structures	lymphadenopathy	congestion, hemosiderosis, lipoidosis	glomerulosclerosis	WNL	none
D36*	C, A	M, T, F	WNL	WNL	WNL	WNL	pulmonary edema	Salmonella D1, fat atrophy, yeast hemorrhage, meningoencephalitis, yeast
D13*	C	M	WNL	prescap LN enlarged	WNL	interstitial nephritis, lymphocytic	WNL	hemorrhage, meningoencephalitis, yeast
D96*	C, A	M	WNL	lymphoid depletion	enlarged	WNL	hemorrhage (iatrogenic)	ethanized
D42*	A	M, T	WNL	lymphoid depletion	WNL, <i>Escherichia coli</i> isolated	WNL	<i>E. coli</i> pneumonia	severe thyroid atrophy
				lymphofollicular hyperplasia, lymphadenopathy				
B71*	N	M	WNL	lymphadenopathy	WNL	pale with lymphoplasmacytic infiltrates	WNL	trauma—HBC
B18*	C, A	M, F	gastric hemorrhage	N/A	N/A	renal foci of unknown cause, chronic multifocal	white nodules on lung	none
B72	N	M, F	WNL	white pulp depletion	WNL	WNL	WNL	ocular N
B60	N	T	enterocolonic hemorrhage	WNL	WNL	adhesion	N	none
B68	N, C	M	small intestinal hemorrhage	LN's blackened	WNL	WNL	WNL	none
B19	N, C	T	hemorrhagic gastritis, colitis	WNL	nematode migration	WNL	WNL	trauma—predation

TABLE III. Results of diagnostic tests performed on bobcats as part of necropsy examination. Y: yes. Pos: positive. Neg: negative. —: test not performed.

ID	AR	Toxoplasmosis	Leptospirosis	Felv-FIV	Rabies	FIP
D22	Y	pos	neg	neg	—	—
D03	Y	—	pos	neg	—	—
D45	Y	neg	neg	neg	—	neg
D27	Y	—	pos	neg	—	—
S40	—	—	—	—	—	—
D16	Y	—	—	—	—	—
D66	—	—	—	—	—	—
B66	Y	pos	—	neg	neg	—
D36	Y	—	—	—	—	—
D13	Y	—	—	—	neg	—
D96	Y	—	—	—	neg	—
D42	Y	—	—	—	—	—
B71	Y	—	—	neg	—	—
B18	Y	—	—	—	—	—
B72	—	—	—	—	—	—
B60	Y	—	—	—	—	—
B68	Y	—	—	—	—	—
B19	Y	—	—	—	—	—

were exposed to ARs. Overall these findings paint a picture of a severe multisystemic disease that may result from infectious and toxic injury but leads to death in individuals and a decline in populations.

CBC and serum chemistry findings can be important sources of information in ecological studies of bobcats and can serve as indicators of nutritional status, disease, trauma, habitat quality, and other environmental stressors (Fuller et al., 1985; Kocan et al., 1985; Heidt et al., 1988; Knick et al., 1993). By compiling chemistry, hematological, necropsy, and AR exposure results, we identified particular systems that appear to be targeted during the development of severe mange. There were multiple indicators consistent with chronic disease, emaciation, and dehydration: these include anemia (due to blood loss or chronic disease), low creatinine (emaciation), and hyperphosphatemia (dehydration). The leukocytosis observed in many of the bobcats with mange is consistent with, although not diagnostic for, an inflammatory process. Similar to our findings, eosinophilia was observed in humans infested with sarcoptic mange (Falk and Matre, 1982) while neutrophilia was observed in laboratory rabbits infected with sarcoptic mange (Arlan et al., 1988). The abnormal leukocyte counts observed in bobcats with mange suggest pathological conditions resulting from severe infestation.

Liver enzymes ALT and AST, neutrophils, and monocytes were elevated in free-ranging, recently captured bobcats whether or not they were infested with mange, suggesting that these elevations are due to capture stress. Previous research has indicated that capture stress can influence hematological parameters, particu-

larly leukocyte counts (Kocan et al., 1985; Marco and Lavin, 1999). However, in mange-infested bobcats several liver function indicators were abnormal including albumin, glucose, and cholesterol. Although necropsy did not indicate widespread liver pathology in mange-infested bobcats, we believe that emaciation, protein loss, bleeding, or sepsis (or any combination) can account for these abnormal values.

Bobcats with mange had low levels of cholesterol, albumin (which accounts for the low serum calcium), globulin, and total protein. Given the lack of significant liver pathology, it is likely that protein production was normal and there was no evidence of renal protein loss. Rather, the protein loss is most likely secondary to severe dermatopathy. In fact, the severity of the dermatopathy was similar to crusted scabies in people, an extreme form of mange characterized by large numbers of mites, high IgG and IgE levels, and the development of hyperkeratotic skin crusts over extensive regions of the body (Walton et al., 2004). In these cases, skin lesions often become secondarily infected and regional lymphadenopathy is common. Interestingly, three of the bobcats had adrenal enlargement, which can occur secondary to chronic stress or immune stimulation and also produce immunosuppressive glucocorticoids which could exacerbate mange.

While the protein loss in the skin of these bobcats was considerable, it is also important to determine whether there were other sources of protein loss; however, there was no evidence of third space protein loss on necropsy. Renal disease was not supported on necropsy except for a few cases such as those of leptospirosis. Moreover, there was no evidence for protein-losing nephropathy based on urine protein:creatinine ratios. Some of the bobcats did have evidence of pre-renal azotemia secondary to dehydration (azotemia, hyperchloremia, hypernatremia, high urine specific gravity, and hyperphosphatemia).

We are concerned about a primary gastrointestinal loss of protein or blood in some of the bobcats. Gastrointestinal bleeding is consistent with the observed anemia and increased BUN in the absence of increased serum creatinine, but the elevated BUN could also be due to a high protein diet or emaciation. We could

TABLE IV. Summary of results of anticoagulant rodenticide testing in livers of bobcats with notoedric mange.

Anticoagulant	Brodifacoum	Bromadiolone	Diphacinone
Number of positive bobcats	19	19	5
Maximum residue level (ppm)	0.68	1.4	0.75
Mean residue level (ppm)	0.23	0.62	0.49

not use fecal occult blood tests to test for gastrointestinal bleeding in this species because bobcats are strict carnivores (Fedriani et al., 2000; Riley et al., 2010). The cause of GI bleeding could be related to the AR exposure and could represent an important problem that has previously been overlooked.

Exposure to ARs was widespread in this case series of bobcats from Northern and Southern California. Bromadiolone and brodifacoum were detected most frequently, which may reflect regional use patterns as well as the fact that these compounds have the longest half-lives, approximately more than 120 days, for some species (Parmar et al., 1987; U.S. EPA, 1998; Vandembroucke et al., 2008). Previous work showed that AR exposure is extremely widespread in Southern California, with liver testing in SMMNRA revealing exposure in 87% of mountain lions, 83% of coyotes, and 93% of bobcats (Riley et al., 2007; Beier et al., 2010; Gehrt and Riley, 2010). More broadly, across six southern California counties, 88% of bobcats (n = 168) tested positive for ARs with 77% exposed to multiple AR compounds (Serieys et al., unpubl. data), supporting previous work in Southern California (Riley et al., 2007). In the present dataset 100% of liver samples from bobcats with mange were positive, often to multiple compounds. In Southern California bobcats' favored woodrats, gophers, and ground squirrels (Fedriani et al., 2000; Riley et al., 2010), none of which are labeled targets for AR use but all of which could likely succumb if AR is ingested.

Bobcats are widely distributed, common carnivores that coexist with people in peri-urban locations and experience some of the same hazards as people and domestic animals (Crooks, 2002; Riley et al., 2003). Our study identified free-ranging bobcats with severe, generalized dermatologic infectious disease concurrent with widespread exposure to ARs. The clinical and pathological data on bobcats with mange suggest a multifactorial process, possibly involving immunosuppression or other factors, leading to the progression of severe dermatopathology. The link between mange and AR exposure is unclear but could involve chronic debilitation or other causal routes. Nevertheless, anthropogenic stressors on these bobcats, the severity and suffering attributable to severe mange, and the distribution of cases across the state of California imply that adopting land and natural resource management strategies to mitigate against mange and ARs may be important in the conservation of this and other vulnerable species.

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