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Prevalence of First and Second-generation Anticoagulant Rodenticide Exposure in California Mountain Lions (*Puma concolor*)

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ABSTRACT: In 2016, the Wildlife Investigations Lab initiated a statewide mountain lion health surveillance study to understand population health and anticoagulant rodenticide (AR) exposure. Exposure to first-generation (FGARs) and second-generation (SGARs) anticoagulant rodenticides are common in predators such as raptors, wild canids (i.e., foxes and coyotes) and bobcats. However, statewide data regarding rodenticide exposure in these species, including mountain lions, have been limited. Our objectives were to determine the statewide prevalence and geographic distribution of AR exposure in necropsied mountain lions. We used liquid chromatography/mass spectroscopy to detect rodenticides in liver samples from 111 (77 male: 34 female) mountain lion carcasses from 37 counties that died between Feb 2016 and Feb 2017. Necropsied carcasses were lions taken on depredation permits, vehicular strike, public safety, or other reasons. Overall, we detected ARs from the liver tissue of mountain lions from 35 counties with 105 of the 111 (94.5%) lions having exposure. We detected FGARs in 81 individuals (73%) from 33 counties and SGARs in 102 individuals (92%) from 35 counties. Seventy-eight individuals (70%) were exposed to both SGARs and FGARs while 6 (5%) individuals had no detectable AR concentration. Of the FGARs detected, diphacinone was the most common and was observed in 67% of sampled individuals. Brodifacoum was the most common SGAR, detected in 90% of sampled individuals. Exposure to FGARs was correlated with exposure to SGARs (χ^2 = 5.8, p = 0.01). Exposure to ARs was not associated with lower body condition score. Although our study represents only one year of data, we demonstrate that exposure to both FGARs and SGARs is widespread in California's mountain lions. We recommend continued AR screening of livers from mountain lion carcasses to further enhance our understanding about the relative contributions they may have on population health. Continued monitoring would also measure the effectiveness of regulatory changes intended to reduce non-target wildlife exposure to rodenticides.

KEY WORDS: exposure, first-generation anticoagulant rodenticide, mountain lion, non-target hazard, *Puma concolor*, second-generation anticoagulant rodenticide

INTRODUCTION

Anticoagulant rodenticide (AR) baits are commonly used to control rodent pests in urban, suburban, and agricultural areas (Litovitz et al. 1998, Maroni et al. 2000) and have been used for introduced rodent eradication from islands (Howald et al. 2010, Martin and Richardson 2017). There are two general classifications for ARs; first-generation (FGAR, e.g., chlorophacinone, diphacinone, warfarin), which are considered less hazardous as they require multiple feedings over several days to be lethal and are rapidly eliminated from the liver after absorption, and second-generation (SGARs, e.g., bromadiolone, brodifacoum, difenacoum, and difethialone) which are more potent, require fewer feedings for lethality, and are retained in the hepatic tissue for longer periods of time (Fisher et al. 2003). Both FGARs and SGARs have the potential of creating primary and secondary poisoning risks to non-target wildlife, especially for predatory mammals and birds as they consume targeted prey that has ingested ARs (Hosea 2000, Riley et al. 2007, McMillin et al. 2008, Gabriel et al. 2012, Serieys et al. 2015). However, a much lower number of FGAR exposures have been detected in non-target wildlife in comparison to SGARs (Erickson and Urban 2004). Given the longevity of tissue retention in animals and the exposure prevalence of SGARs in non-target wildlife, California placed new regulations on these materials in 2014, restricting their use

to certified pesticide applicators. However, FGARs remain available for purchase by the public for commensal rodent control.

The California mountain lion (*Puma concolor*) is a specially protected mammal and is distributed statewide. Despite large-scale habitat alteration and fragmentation, this adaptable apex predator persists even in fragmented habitats surrounding and within urbanized regions of Los Angeles and the San Francisco Bay Area. Previously, AR-related deaths of two mountain lions and a high prevalence of SGAR exposure were documented prior to the 2014 regulatory change (Riley et al. 2007), raising concerns about conservation impacts of AR exposure on the species.

The aims of this study were to determine the prevalence and geographic distribution of AR exposure post-regulatory change in mountain lions. We sampled individuals that were intentionally killed or had died between 18 and 30 months after the regulation change to allow adequate time for residues to clear from prior exposure. We utilized mountain lion carcasses collected by the California Department of Fish Wildlife (CDFW) as part of a broader statewide mountain lion health surveillance program led by the Department's Wildlife Investigations Laboratory (WIL). The CDFW is mandated by Fish and Game Code §§ 4807(b) to perform necropsies on all depredation mountain lion carcasses for the state. Additionally, the CDFW responds to other incidents
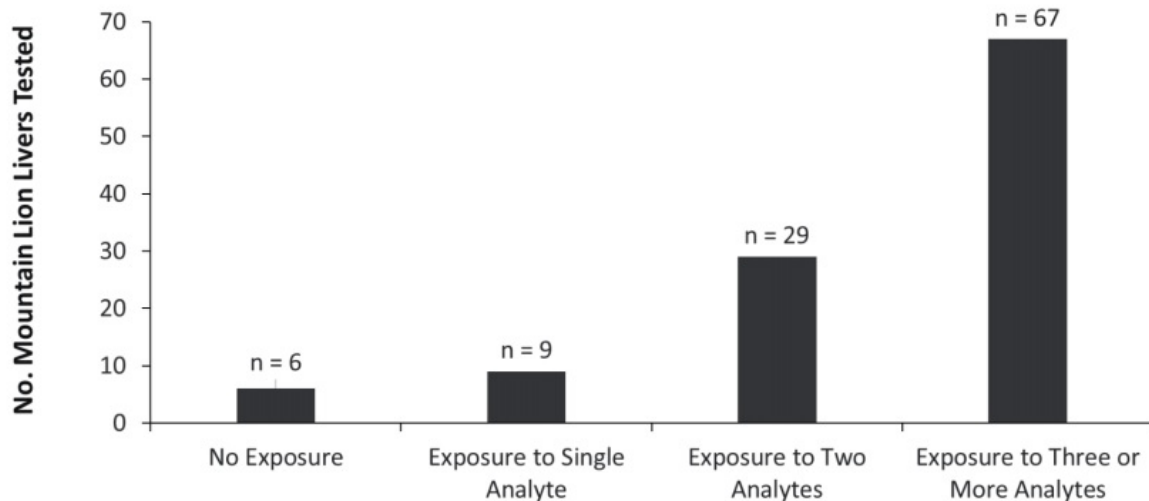
Figure 1. Cumulative number of rodenticide analytes detected in 111 mountain lion livers sampled from 37 California counties between January 2016 and February 2017.

resulting in the recovery of carcasses such as fatal traumatic injury from roadside mortalities or poaching, public safety, found dead, or humane euthanasia due to illness.

METHODS

We tested liver tissue collected from 111 mountain lions across 37 counties from January 2016 to February 2017. Carcasses were frozen in -25C° freezers until they could be necropsied by the WIL. The sex, age class, body condition and, when possible, the cause of death were determined. AR intoxication was determined to be a cause of death or morbidity if one or more AR was detected in the liver and coagulopathy with no other cause (e.g., trauma) was identified. Body condition was assessed on a 1-5 scoring system (BCS): 1 (emaciated), 2 (thin), 3 (average/normal), 4 (heavy), and 5 (obese). BCS of 3 or higher was considered good, while lower than 3 was not. Age class was determined based on dentition, coat pattern and coloration, mass, and body length. Tissue samples were collected and archived in -80C° freezers post-necropsy.

Liver samples were analyzed for warfarin, coumachlor, bromadiolone, brodifacoum, diphacinone, chlorophacinone, and difethialone using a previously published method modified for tissue analysis (Palazoglu et al. 1998). Briefly, samples were initially screened for the presence of ARs by liquid chromatography-tandem mass spectrometry (LC-MS/MS). According to this method, any positive anticoagulant sample was then quantitated by high-performance liquid chromatography (HPLC) using either ultraviolet diode array detection (diphacinone, chlorophacinone, and difethialone) or fluorescence detection (warfarin, coumachlor, bromadiolone, and brodifacoum). Limits of quantitation (LOQs) for these anticoagulants varied according to their sensitivity to ultraviolet or fluorescence detection. In tissue, LOQs 0.01 ppm for brodifacoum; 0.05 ppm for bromadiolone, warfarin, and coumachlor; and 0.25 ppm for

chlorophacinone, diphacinone, and difethialone. Anticoagulants that were determined to be positive by LC-MS/MS, but were below the reporting limit by HPLC, were defined as trace.

Mountain lion necropsy data and rodenticide results were archived in Excel (Microsoft, Redmond, WA) and statistical tests were performed using R (R Core Team 2017). We used chi-square goodness of fit tests and Fisher Exact tests to evaluate if, sex, age class, body condition score, or exposure to one class of rodenticides was a function of exposure. Only p-values of ≤0.05 were considered significant.

RESULTS

The 111 mountain lions sampled represented all age classes and included 14 cubs (≤9 months), 11 yearlings (10-12 months), 32 sub-adults (13-23 months), and 54 adults (≥2 years). Thirty-four animals were female and 77 were male. Depredation permit mortalities accounted for 72% of carcass submissions (n = 79) while vehicle strike (n = 13), non-depredation related gunshot (n = 7), and other causes (n = 12) accounted for the remaining submissions.

Necropsied mountain lions originated from 37 of the 58 counties in California. Anticoagulant rodenticide exposure was detected across all but two counties in 105 (94.5%) of the 111 animals tested. Eighty-one (73%) individuals were exposed to FGARs and 102 (92%) were exposed to SGARs while 78 (70%) were exposed to both FGARs and SGARs. Sixty-four percent (n = 67) of lions tested were exposed to three or more analytes (Figure 1). Detectable concentrations of ARs in the liver ranged from trace to 1200 parts per billion (Table 1). Diphacinone was the most common FGAR detected and was detected in 67% of sampled individuals, while brodifacoum was the most common SGAR, detected in 90% of sampled individuals. Exposure to FGARs was positively correlated with exposure to SGARs ($\chi^2 = 5.8$, p = 0.01).

Table 1. Anticoagulant rodenticide detection in 111 mountain lion livers in parts per billion (ppb). Trace is the lowest routinely quantified concentration of an analyte in a sample - the analyte may be detected, but not quantified, at concentrations below the reporting limit.

Anticoagulant Generation	Analyte	No. of Mountain Lions with Analyte Exposure	Detection Range (ppb)	Reporting Limit (ppb)
First	Chlorophacinone	25	None, Trace - 520	20
	Warfarin	7	None, Trace	20
	Diphacinone	74	None, Trace - 800	50
Second	Brodifacoum	100	None, Trace - 390	50
	Bromadialone	84	None, Trace - 1200	50
	Difenacoum	3	None, Trace - 20	20
	Difethialone	22	None, Trace - 170	50

Rodenticide intoxication was not a cause of mortality in our sample. Exposure to AR was not associated with lower BCS; 85 (76.5%) of necropsied mountain lions were in good body condition (BCS $\geq$3). Mountain lions younger than nine months of age were 4 times less likely to be exposed to SGARs (p = 0.002) when compared to other age classes, and males were 2.5 times more likely to have FGAR exposure than females (p = 0.03).

DISCUSSION

Our 2016 statewide assessment of non-target AR exposure in necropsied mountain lions demonstrated a very high prevalence of exposure (94%) distributed statewide. Second generation ARs were more commonly detected than FGARs, despite the 2014 regulatory change restricting SGAR use to certified pesticide applicators. In addition, the majority of lions in our sample were exposed to multiple ARs; brodifacoum and bromadiolone were the most commonly detected analytes. Exposures were detected in all but two sampled counties (San Benito and Santa Barbara) indicating that exposure is not confined to a particular region of the state.

In contrast to previous work documenting AR-related coagulopathy in two mountain lions in southern California (Riley et al. 2007), AR-related coagulopathy was not observed during necropsies of any mountain lions in this study. Possible explanations could be that the severity of trauma in the lions we sampled obscured our ability to detect coagulopathy, or perhaps there was a brief increase in rodenticide use during the southern California study between 2000-2004. Felids have a higher tolerance to ARs than do canids such as coyotes (*Canis latrans*) and foxes (*Vulpes* sp and *Urocyon cinereoargenteus*), and predatory birds (Petterino and Paolo 2001, van Beusekom 2015). Thus, differences in AR sensitivity could be one explanation as to why mountain lions could have such varying concentrations of exposure to one or multiple analytes and yet remain unaffected. However, the exposure prevalence could have implications for less tolerant, co-occurring species with overlapping prey base. Furthermore, the consequences of AR exposure in mammalian predators is still mostly unknown and even for sensitive species, the relationship between concentrations of ARs in the liver and mortality is unclear. Recent studies investigating a fatal notoedric mange outbreak in free-ranging bobcats from Los Angeles have suggested that chronic AR exposure down-regulates the immune system thus increasing a bobcat's susceptibility to the mite

Notoedres cati, an ectoparasite which was not previously known to cause disease epizootics in wild felids (Serieys et al. 2015). Our study noted no consistent occurrence of a disease process compatible with immunosuppression.

Rodenticide use is positively correlated to proximity to human development and exposure prevalence increases with urbanization (Cypher 2010, Cypher et al. 2014, Serieys et al. 2015). However, mountain lions maintain large home ranges, both near and far from human-development and California is home to a diverse set of biomes thus making the source of exposure difficult to interpret. Furthermore, SGARs can be retained in hepatic tissue for nearly one year, making it difficult to ascertain the timeframe and seasonality in which the exposure occurred. Male lions disperse farther than females when establishing a territory and have larger home ranges (Hopkins 1981, Grigione et al. 2002), which may explain why males are more likely to be exposed to FGARs than females. Mountain lion cubs were less likely to be exposed to SGARs than any other age class possibly because they have had less time to accumulate SGARs in their hepatic tissue than older age classes.

We feel our sample prevalence of AR exposure is a reasonable representative point estimate for the free-ranging population. The accurate assessment of AR exposure in free-ranging wildlife is often difficult because the majority of testing relies on post-mortem sampling usually collected from carcasses opportunistically. This can introduce sampling bias towards certain causes of death. However, carcasses have been the primary source of information for epidemiological studies and information about exposure to pathogens or toxicants can be obtained by examining and sampling specific tissues that are otherwise difficult to acquire from live animals (Hall et al. 2010). Although not random, our sample of mountain lions had wide geographic representation (37 of 58 California counties), encompassed multiple causes of mortality (e.g., not just sick individuals) and included all age and sex categories.

The high prevalence of SGARs in our sample two years post-regulatory change was unexpected. Therefore, continued AR monitoring is warranted, and future studies are needed to identify the source(s) of exposure in mountain lions.

ACKNOWLEDGEMENTS

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