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PREVALENCE OF ANTICOAGULANT RODENTICIDES IN FECES OF WILD RED FOXES (*VULPES VULPES*) IN NORWAY

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ABSTRACT: High occurrence of anticoagulant rodenticides (ARs) in wildlife is a rising concern, with numerous reports of secondary exposure through predation. Because of widespread distribution of the red fox (*Vulpes vulpes*), they may act as sentinels for small mammal-hunting predators in rural, suburban, and urban areas. No AR surveillance in wild mammals with analyses of residues in feces has been conducted throughout a single country. We collected 163 fecal samples from presumed healthy red foxes from 18 out of 19 counties in Norway. The foxes were shot during regular hunting between January and December 2016 and samples collected directly after death. Fecal samples were analyzed for six ARs: brodifacoum, bromadiolone, coumatetralyl, difenacoum, difethialone, and flocoumafen. We detected ARs in 54% (75/139) of the animals. Brodifacoum was most frequently detected (46%; 64/139), followed by coumatetralyl (17%; 23/139), bromadiolone (16%; 22/139), difenacoum (5%; 7/139), difethialone (1%; 2/139), and flocoumafen (1%; 2/139). More than one substance was detected in 40% (30/75) of the positive foxes, and 7% (5/75) of these animals were exposed to four different ARs. There were no statistically significant seasonal, age, or sex differences in foxes after exposure to one AR compound. We found a significant difference in occurrence of brodifacoum and coumatetralyl in foxes from different geographical areas. These findings demonstrate fecal analyses as a valuable method of detecting AR exposure in red foxes. We suggest using direct fecal sampling with analyses as a method to evaluate the occurrence of ARs in live endangered wildlife in connection with radio tagging or collaring operations.

Key words: Carnivores, fecal analyses, nontarget animal, predators, rat poison, secondary exposure, wildlife.

INTRODUCTION

Use of anticoagulant rodenticides (ARs) for urban and agricultural rodent control has been extensive the past 60 yr. These rodenticides inhibit vitamin K epoxide reductase and are designed to induce lethal hemorrhage (Watt et al. 2005). First-generation anticoagulant rodenticides (FGARs), including warfarin, diphacinone, coumatetralyl, and chlorophacinone, were developed in the 1950s. Extensive use of FGARs led to resistance against these rodenticides in both brown rats (*Rattus norvegicus*) and house mice (*Mus musculus*), resulting in their acquired and inherited tolerance and cross-resistance between compounds

(Rowe and Redfern 1965; Greaves and Renison 1973; Hadler and Shadbolt 1975). This prompted the development of second-generation anticoagulant rodenticides (SGARs), such as brodifacoum, bromadiolone, difenacoum, difethialone, and flocoumafen. Compared to FGARs, SGARs have higher toxicity and prolonged liver half-life and are effective after a single exposure (Watt et al. 2005). The SGARs can cause mortality after several days, allowing animals to ingest multiple doses and accumulate high concentrations in their body (Daniels 2013).

Predators can accumulate ARs through ingesting bait (primary exposure), by consuming poisoned prey (secondary exposure), or by

ingesting prey secondarily exposed to ARs (tertiary exposure; Daniels 2013; Gabriel et al. 2018). Wildlife studies in Europe and North America have shown 23–100% AR occurrence in liver samples from predators such as American mink (*Neovison vison*; Ruiz-Suárez et al. 2016), bobcats (*Lynx rufus*; Riley et al. 2007; Serieys et al. 2013), stoats (*Mustela erminea*) and weasels (*Mustela nivalis*; McDonald et al. 1998; Elmeros et al. 2011), red foxes (*Vulpes vulpes*; Tosh et al. 2011; Tjus 2014), polecats (*Mustela putorius*; Shore et al. 2003), and stone martens (*Martes foina*; Elmeros et al. 2018). In Norway SGARs have been detected in raptors found dead in the wild, such as the golden eagle (*Aquila chrysaetos*) and eagle owl (*Bubo bubo*; Langford et al. 2013). To our knowledge, no publications have investigated AR occurrence in wild mammals in Norway.

Large amounts of ARs may cause bleeding and death in animals. Even small amounts of rodenticides in the liver are suspected to cause a variety of sublethal effects. Residues of AR affect reproduction by reducing sperm motility, increasing embryonic mortality, and causing teratogenic effects and neonatal death (Greaves 1993; Munday and Thompson 2003; Robinson et al. 2005). Vidal et al. (2009) suggested an association between chlorophacinone residues in voles (*Microtus arvalis*) and increased susceptibility to the bacterium *Francisella tularensis*. Additionally, a correlation between increased parasite load and AR residues was found in bobcats and fishers (*Martes pennant*), suggesting a chronic weakening of the animal (Gabriel et al. 2012; Serieys et al. 2013). Furthermore, sublethal AR exposure is suggested to increase mortality when the animals are subjected to environmental stressors (Jaques 1962). Finally, rodenticides can reduce body condition of poisoned animals (Elmeros et al. 2011), impairing hunting ability and making them more susceptible to accident, injury, and predation.

The ARs have an enterohepatic circulation and accumulate in the liver (Huckle et al. 1988; Watt et al. 2005). Nontarget animal exposure to ARs is usually measured by

analyses of residues in the liver. The major elimination route is through bile and feces (Huckle et al. 1988; WHO 1995). An experiment in foxes demonstrated prolonged excretion of bromadiolone in feces for 2–19 d after no AR residues could be detected in plasma. Fecal residues were still detectable at the conclusion of the experiment (Sage et al. 2010). Because of long fecal elimination of ARs, we suggest fecal analysis as a suitable method to investigate this unintended exposure.

The aim of our study was to estimate the occurrence of ARs in feces of presumed healthy red foxes throughout a country. In addition, AR exposures were compared between age groups, seasons, and geographical regions with different human population densities.

MATERIALS AND METHODS

Population and study area

We collected 163 fecal samples from red foxes shot by experienced hunters in 2016 (January throughout December) in a project monitoring the parasite *Echinococcus multilocularis* commissioned by the Norwegian Food Safety Authority (Madslie et al. 2017). The samples were collected from 56 municipalities (ranging in size from 7,000 to 310,600 ha), representing 18 out of 19 counties in Norway and including areas surrounding three major cities in Norway (Oslo, Bergen, and Trondheim). The municipalities were divided in groups based on human population density. Population density per square kilometer for each municipality in 2016 was obtained from Statistics Norway (Statistics Norway 2018).

Sample collection

The hunter removed feces directly from the rectum immediately after death and submitted fresh samples to the Norwegian Veterinary Institute within 2 d. In the statistical analyses, 24 of the 163 samples consisted of mostly hair and were omitted. The foxes were shot during the licensed hunting season from January to mid-April and mid-July to late December and grouped according to sampling season: winter ($n=66$) from January to February and December, spring ($n=30$) from March to May, summer ($n=20$) from June to August, and autumn ($n=23$) from September to November. Most samples were collected during the winter, due to preferred tracking

conditions in the snow. The hunters provided information on sex (male or female) and estimated age (juvenile, <1 yr old, or adult), together with the municipality and date when the fox was killed. The hunters estimated age according to foxes' size and the presence of deciduous teeth and determined the sex based on presence or absence of a penis. Of the 139 foxes analyzed, 65 were male, 64 female, and the sex of 10 was not determined. The samples were immediately frozen at -80°C upon arrival at the Norwegian Veterinary Institute and kept frozen at this temperature for 3 d, before being stored at -20°C until preparation. One sample per fox was analyzed.

Sample analysis

The samples were lyophilized to dryness before analyses at the laboratory at the Department of Forensic Sciences at Oslo University Hospital. We have previously described and validated procedures for fecal extraction and AR analysis (Seljetun et al. 2018). In brief, ARs were extracted from feces by liquid-liquid extraction with acetonitrile and dichloromethane followed by separation using a Waters Acquity ultra performance liquid chromatography (UPLC) BEH C18 column (Waters Corporation, Milford, Massachusetts, USA) with a mobile phase consisting of 5 mM ammonium formate buffer (pH 10.2) and methanol. Positive electrospray ionization tandem mass spectrometry detection was performed on a triple quadrupole mass spectrometer (Waters), using two multiple reaction monitoring transitions. Limits of quantification were set at the level of the lowest calibrators: brodifacoum 2.6 ng/g, coumatetralyl 1.5 ng/g, bromadiolone 2.6 ng/g, difenacoum 2.2 ng/g, difethialone 2.7 ng/g, and flocoumafen 2.7 ng/g. Criteria of signal-to-noise ratios were above 10 as well as precision and accuracy within $\pm 20\%$. The extraction recovery ranged from 18% to 69%. Concentrations of ARs above limits of quantification were classified as positive, while detectable AR concentrations below quantitation limits were labeled as trace concentrations. The ARs analyzed in this study were brodifacoum, bromadiolone, coumatetralyl, difenacoum, difethialone, and flocoumafen, which are all registered for use in Norway.

Statistical analysis

After rejecting the 24 of 163 fecal samples that were mostly hair, the 139 remaining samples were grouped according to age, sex, season, and human population density. Data from cases where information on age or sex was lacking were excluded in the corresponding proportion estimates. To test the sensitivity of the specific categorization of rural, suburban, and urban from

human population density, we included variants of population measures. Municipalities with fewer than 10 inhabitants per km^2 were first categorized as rural, 11–200 inhabitants as suburban, and more than 200 inhabitants as urban. We then reduced the definition of rural municipalities to less than five inhabitants per km^2 and altered suburban municipalities to 6–200 inhabitants. Finally, we categorized municipalities based on population only with rural area (1,000–10,000), suburban area (10,000–50,000), and urban area (50,000–180,000).

Estimated prevalence of foxes positive for ARs was calculated for the total of all samples ($n=139$) and within groups. Differences between prevalence of AR substances were tested using the McNemar χ^2 test, whereas significant differences in AR exposure between groups were tested using the Pearson χ^2 tests. *P* values of the Pearson χ^2 test were obtained with Monte Carlo simulations using 10,000 replicates. Single AR exposure was classified as a sample being positive for one AR compound, and multiple AR exposure was specified as samples being positive for at least two AR compounds.

The relationship between AR exposure and the covariates age, sex, and seasons were investigated by multiple logistic regression analyses. The full model included age, sex, and season. However, results from simple regressions were reported if one or the two other covariates did not improve the model according to the Akaike information criterion value. To emphasize possible confounding effects, potential dependency between samples from the same county was tested for by including a random effect of county (variance of random effect=0); however, the inclusion of a random effect did not influence the results. All analyses were performed using R (version 3.5.0, R Development Core Team 2016). Results were considered significant when *P* values were below 0.05.

RESULTS

Prevalence of ARs

At least one AR compound analyzed was detected in 54% (75/139) fecal samples (Table 1). Brodifacoum was most frequent and was identified in 46% (64/139) of the foxes, significantly more than coumatetralyl (17%, 23/139; $\chi^2=30.56$, $P<0.0001$, $\text{df}=1$) and bromadiolone (16%, 22/139; $\chi^2=33.92$, $P<0.0001$, $\text{df}=1$; Fig. 1). In contrast, difenacoum was found in only seven foxes (5%) and difethialone and flocoumafen in two samples each

TABLE 1. Fecal samples from 139 wild red foxes (*Vulpes vulpes*) collected in Norway in 2016 for analysis of anticoagulant rodenticides (ARs), by sex, age, location, and the occurrence of ARs within each group. Anticoagulant rodenticides were found in 54% (75/139) of the samples.

Fox classifications	No.	% Positive
Sex		
Female	64	59
Male	65	49
Unknown	10	50
Age		
Juvenile	50	48
Adult	78	58
Unknown	11	55
Location		
Rural	44	48
Suburban	64	61
Urban	31	48

(1%). Among the AR-positive fecal samples, most samples (60%; 45/75) contained a single AR, but multiple substances were detected in 40% (30/75), with two (27%; 20/75), three (7%; 5/75), and four (7%; 5/75) compounds, respectively.

Seasonal variance

Exposure of foxes varied by season with 61% (14/23) foxes positive for ARs in the autumn, 53% (35/66) in the winter, 57% (17/30) in the spring, and 45% (9/20) in the summer (Fig. 2). There were no significant seasonal differences in exposure to a single

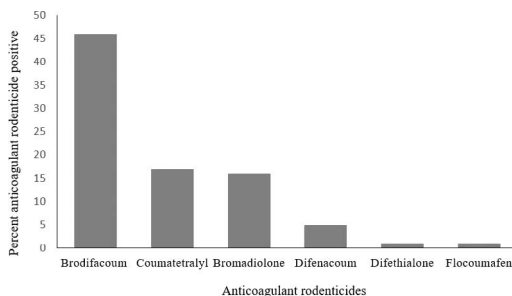


FIGURE 1. Occurrence of different anticoagulant rodenticide compounds in 139 fecal samples collected from presumed healthy wild red foxes (*Vulpes vulpes*) in Norway in 2016.

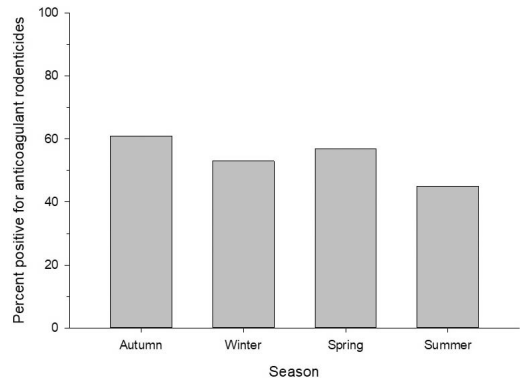


FIGURE 2. Seasonal occurrence of anticoagulant rodenticide compounds in 139 fecal samples from red foxes (*Vulpes vulpes*) in Norway in 2016. Exposure varied by season with 61% (14/23) foxes positive for anticoagulant rodenticides in the autumn (September–November), 53% (35/66) in the winter (January–February and December), 57% (17/30) in the spring (March–May), and 45% (9/20) in the summer (June–August).

AR ($\chi^2=1.20$, $P=0.759$). In exposure to multiple ARs, season tended to be significant ($\chi^2=7.17$, $P=0.065$); exposures to more than one AR was slightly more common in the autumn compared to spring (Wald test, $P=0.037$) and winter (Wald test, $P=0.031$).

Sex and age differences

Of the 139 foxes analyzed, 65 were male, 64 female, and the sex of 10 was not determined (Table 1). Fecal residues of at least one AR were detected in 49% (32/65) males, 59% (38/64) females, and 50% (5/10) of those of unknown sex. There was no significantly different in AR exposure between sexes ($\chi^2=1.34$, $P=0.299$). Exposure to ARs between ages ranged from 58% (45/78) adults, 48% (24/50) juveniles, and 55% (6/11) of unknown sex. Positive findings were not significantly different between ages for either single or multiple AR exposure ($P>0.437$). Logistic regression indicated a tendency of positively association between sex and exposure to ARs when combined with age. In adult female foxes, 68% (23/34) were positive to ARs, compared to 49% (46/93) in a combined group of juveniles and adult male foxes ($P=0.066$).

TABLE 2. The percent (number) of fecal samples from wild red foxes (*Vulpes vulpes*) containing different anticoagulant rodenticides (ARs) by geographical population areas in Norway in 2016. The location where the foxes were shot in Norway and the fecal samples collected were defined in terms of human population as rural (1,000–10,000), suburban area (10,000–50,000), and urban area (50,000–180,000).

Population	Samples	Fecal samples with ARs						
		Any	Brodifacoum	Coumatetralyl	Bromadiolone	Difenacoum	Difethialone	Flocoumafen
Rural	44	48 (21)	41 (18)	11 (5)	11 (5)	5 (2)	0	2 (1)
Suburban	64	61 (39)	58 (37)	12 (8)	19 (12)	6 (4)	3 (2)	0
Urban	31	48 (15)	29 (9)	32 (10)	16 (5)	3 (1)	0	3 (1)
Total	139	54 (75)	46 (64)	17 (23)	16 (22)	5 (7)	1 (2)	1 (2)

Prevalence of ARs in foxes correlated to human population densities

Foxes in suburban areas had an AR occurrence of 61% (39/64), compared to rural (48%; 21/44) and urban (48%; 15/31) foxes (Table 2). However, this difference in AR exposure was not statistically significant ($\chi^2=2.55$, $P=0.285$). To determine if a change in classification of human population density might influence the results, we repeated the analyses with the alternative measures of rural, suburban, and urban category. There was no significant difference between different human population densities in the total exposure; individual compounds differed significantly between population areas. Coumatetralyl was increased in urban compared to rural areas ($P=0.032$), while brodifacoum was increased in suburban compared to urban areas ($P=0.010$). Significant differences were also independent of the specific choices of urban, suburban, and rural population densities.

DISCUSSION

Sources of AR exposure

The high prevalence of 54% foxes exposed to ARs in our study was most likely due to ingestion of rodents. Rodents dominate their diet, with 26–47% of consumed food volume (Contesse et al. 2004; Kidawa and Kowalczyk 2011). In Norway, season and rodent cycles influence the quantity of rodents that foxes ingest (Jensen and Sequeira 1978; Panzacchi et al. 2008). Another factor contributing to increased rodent ingestion and, hence, roden-

ticide exposure is the clinical signs of AR-poisoned animals displaying slow movements and abnormal activity (Cox and Smith 1992; Brakes and Smith 2005). Predators will selectively hunt such vulnerable prey, thus increasing the risk of secondary poisoning. Additional important food items for foxes are mammals such as cervids, mountain hares (*Lepus timidus*), and carnivores and wild birds (Kidawa and Kowalczyk 2011). Carnivores secondary exposure to ARs could have contributed to the high occurrence of residues found in red foxes. Furthermore, foxes as facultative carnivores consume plants, berries, and invertebrates depending on season (Larivière and Pasitschniak-Arts 1996; Panzacchi et al. 2008). Invertebrates constitute a minor percentage of food volume in foxes, but ARs have also been detected in cockroaches, beetles, and gastropods (Howald 1997; Craddock 2003; Alomar et al. 2018). Thus, rodenticide exposure through invertebrates is possible.

Previous studies in red foxes demonstrated ARs in 60–95% of liver samples (Tosh et al. 2011; Daniels 2013; Geduhn et al. 2015), which is higher than our findings. One reason for this difference is probably due to high lipid solubility and affinity binding sites for ARs in the liver that results in its being the organ with highest tissue concentration (Huckle et al. 1988; WHO 1995). In addition, ARs are not homogenously dispersed in feces, lowering the recovery compared to liver analysis. A low-dose study of flocoumafen in rats demonstrated a mean fecal elimination of 28% (Huckle et al. 1988). Differences between countries in

the availability of ARs may also be a factor. Furthermore, these previous studies were multiyear studies, compared to our single-year study. This could affect the results, because rodent population and AR use can vary between years. Last, collection of material in some of the previous studies were restricted to roadkill, sick, or dead foxes discovered in the field, in contrast to our presumed healthy foxes. Sometimes, ARs can decrease fitness and cause abnormal behavior of exposed animals (Erickson and Urban 2004; Elmeros et al. 2011), which may predispose them to vehicular strikes. In addition, AR exposure is a possible cause of illness and mortality; this will increase the likelihood of positive findings in samples from sick or dead animals. Excluding possibly unexposed healthy animals in studies may introduce a bias that leads to an overestimate of the AR prevalence in wildlife.

We detected brodifacoum more frequently (46%) than other ARs, significantly higher than coumatetralyl and bromadiolone. Langford et al. (2013) presented similar findings in raptors in Norway with brodifacoum and bromadiolone occurring most frequently. However, coumatetralyl was not analyzed in that study. In Sweden and Finland, bromadiolone and coumatetralyl were the most common residues found in foxes (Tjus 2014; Koivisto et al. 2016). We suspect the difference between the countries in occurrence of these ARs is caused by higher sale of brodifacoum in Norway compared to other Scandinavian countries. The Norwegian Environment Agency has currently no data of sales volume or use of ARs in Norway, making these comparisons difficult. Since 2014, Norway's regulatory framework restricts AR use for both public and licensed professionals (Lovdata 2018). Tamper-proof bait stations are mandatory for both FGARs and SGARs, and the public is restricted to indoor use only. However, our results demonstrated continued exposure to nontarget wildlife despite these legislative measures.

More than one AR were detected in 40% of the positive foxes. Only one commercial product contains a combination of two ARs

(bromadiolone and difenacoum) out of 46 government-approved AR products in Norway, which does not fully explain the occurrence of multiple compounds in the foxes. Another possible explanation could be migratory birds and wildlife that come to Norway are exposed to combination products in other countries. However, products with combinations of ARs are not commercially sold in other European countries (López-Perea et al. 2015). We believe that accumulation of ARs in wildlife is more likely due to multiple exposures to contaminated prey over time.

Seasonal variance

We did not find a significant difference in seasonal variance of AR residues in foxes, consistent with a previous study in Northern Ireland and Great Britain (Tosh et al. 2011). In contrast, Elmeros et al. (2011) found the highest AR occurrences throughout winter in weasels and stoats in Denmark. In France a higher occurrence of AR poisoning in European mink (*Mustela lutreola*) was identified during autumn and late winter (Fournier-Chambrillon et al. 2004). Differences in diet and climatic conditions are probable explanations of this variation. In addition, winter food hoarding has been documented in foxes, making seasonal comparisons of AR exposure in this species difficult (Sklepkovych and Montevecchi 1996). Furthermore, SGARs have long persistence in the body. For compounds like brodifacoum, with an estimated liver half-life of 282–350 d (European Commission 2010), detection of possible seasonal variances is of limited value.

Sex and age differences

We did not find association between AR exposure and sex, which is in accordance with previous studies in red foxes (Tosh et al. 2011) and other wild predators (Shore et al. 2003; Elmeros et al. 2011; Ruiz-Suárez et al. 2016). However, sex differences in the extent of territory usage, with single male foxes having a larger home range than females, have been observed (Larivière and Pasitschniak-Arts 1996). This could have influenced our study

results, as male foxes may have preyed on rodents from different geographical areas, which would not necessarily reflect the human population density of the municipality where they died.

We found no correlations between AR exposure and age groups in our study. A similar lack of associations was observed in other carnivores, such as bobcats, weasels, and stoats (McDonald et al. 1998; Serieys et al. 2015). However, a correlation between AR exposure and increased age was found in American mink (Ruiz-Suárez et al. 2016) and European polecats (*Mustela putorius*; Sainsbury et al. 2018).

Habitat influence

The red fox is widely distributed, living in both rural habitats and in proximity to residential areas (Adkins and Stott 1998). Different population densities can influence AR exposure in nontarget animals due to varying rodenticide use and differences in the foxes' diets. Wildlife in urban areas is considered to be at greater risk of exposure to ARs, due to frequent rodent control in residential areas. However, a higher consumption of rodents in agricultural landscapes is suggested by Kidawa and Kowalczyk (2011). We did not find a significant relation between prevalence of ARs in foxes and human population density. This is in accordance with a study in Finland with no significant relationship between overall AR concentration and environmental variables such as farm density and industrial surroundings (Koivisto et al. 2018). In contrast, San Joaquin kit fox (*Vulpes macrotis mutica*) demonstrated the highest AR exposure in low-density development areas (Nogueira et al. 2015). These regions generally included single-family housing units, which is similar to our suburban areas. Our AR findings with correlation to human population density are in contrast to previous studies in bobcats (Serieys et al. 2015), hedgehogs, and birds of prey (López-Perea et al. 2015, 2019; Lohr 2018), but variation in species' consumption of rodents and diversity of AR use between countries

could explain the differences. A more precise landscape analysis with geographical situation of each sample would have improved our study, as building density, landscape elements, agricultural lands, and livestock density affect rodent population and AR use. This was, however, not possible with our data.

Fecal analysis

Fecal analysis is a valuable method of monitoring AR residues in the body, because fecal excretion persists after residues are no longer detectable in plasma (Sage et al. 2010). Fox feces is inhomogeneous and contains plant material and hair, which influences the extraction recovery and AR concentration. Nevertheless, our fecal analyses demonstrated a high occurrence of AR residues in the presumed healthy foxes. Prat-Mairet et al. (2017) observed a decline in AR concentration when feces were exposed to natural decomposition outdoors, indicating the necessity to collect feces within 5 d to produce reliable results. However, fecal samples in our study were collected from the fox immediately after death, reducing natural degradation in the feces. Sampling scats from the ground lead to a risk of species misclassification, and studies report 18–25% erroneous identification of presumed fox feces according to DNA analysis of the scats (Jacquot et al. 2013; Fourel et al. 2018). In addition, the direct fecal sampling method assures that only one sample is collected from each individual animal. A previous study of the fecal analysis in a poisoned dog demonstrated transference to other live AR-exposed animals (Seljetun et al. 2018).

Our study demonstrated that more than half of the wild red fox population in Norway is exposed to ARs. Because of widespread distribution of the red fox, they may act as sentinels for other mammal-hunting predators, including endangered species such as arctic fox (*Vulpes lagopus*), gray wolf (*Canis lupus*), and Eurasian lynx (*Lynx lynx*), since they feed on some of the same resources as the red fox (Shirley et al. 2009; Wikenros et al. 2017).

Government radio tagging under sedation is performed in surveillance of free-ranging gray wolves, wolverines, brown bears (*Ursus arctos*), and Eurasian lynx in Norway (Arnemo et al. 2017). Using our method and sampling feces directly from animals during these radio tagging or collaring operations will enable authorities to monitor the occurrence of ARs in live endangered wildlife.

In conclusion, our fecal analyses revealed widespread AR exposure in presumed healthy red foxes throughout Norway. Red foxes were susceptible to AR exposure both as scavengers in urban areas and as opportunistic predators with a diet of rodents, birds, small carnivores, and invertebrates potentially exposed to ARs. Despite government restrictions implemented in 2014, our results demonstrated that ARs are a continuing hazard in nontarget wildlife. Monitoring AR residues in wildlife is challenging. Studies are often based on liver analyses from necropsied animals found opportunistically, which may overestimate the prevalence in wildlife as healthy unexposed animals are not included in the sampling. Our study showed fecal analyses to be a valuable method for evaluating AR exposure in wildlife, which could be a useful method of AR assessment in other wildlife studies.

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LITERATURE CITED

- Adkins CA, Stott P. 1998. Home ranges, movements and habitat associations of red foxes *Vulpes vulpes* in suburban Toronto, Ontario, Canada. *J Zool* 244:335–346.
- Alomar H, Chabert A, Coeurdassier M, Vey D, Berny P. 2018. Accumulation of anticoagulant rodenticides (chlorophacinone, bromadiolone and brodifacoum) in a non-target invertebrate, the slug, *Deroceras reticulatum*. *Sci Total Environ* 610:576–582.
- Arnemo JM, Evans AL. 2017. *Biomedical protocols for free-ranging brown bears, wolves, wolverines and lynx*. Inland Norway University of Applied Sciences, Evenstad, Norway, 16 pp.
- Brakes CR, Smith RH. 2005. Exposure of non-target small mammals to rodenticides: Short-term effects, recovery and implications for secondary poisoning. *J Appl Ecol* 42:118–128.
- Contesse P, Hegglin D, Gloor S, Bontadina F, Deplazes P. 2004. The diet of urban foxes (*Vulpes vulpes*) and the availability of anthropogenic food in the city of Zurich, Switzerland. *Mamm Biol* 69:81–95.
- Cox P, Smith RH. 1992. Rodenticide ecotoxicology: Pre-lethal effects of anticoagulants on rat behaviour. In: *Proceedings of the 15th vertebrate pest conference*, Vertebrate Pest Council, Newport Beach, California, 3–5 March, pp. 165–170.
- Craddock P. 2003. *Aspects of the ecology of forest invertebrates and the use of brodifacoum*. PhD Dissertation, University of Auckland, Auckland, New Zealand, 237 pp.
- Daniels D. 2013. *Second generation anticoagulant rodenticide assessment*. Department of Pesticide Regulation, Sacramento, California, 53 pp.
- Elmeros M, Christensen TK, Lassen P. 2011. Concentrations of anticoagulant rodenticides in stoats *Mustela erminea* and weasels *Mustela nivalis* from Denmark. *Sci Total Environ* 409:2373–2378.
- Elmeros M, Lassen P, Bossi R, Topping CJ. 2018. Exposure of stone marten (*Martes foina*) and polecat (*Mustela putorius*) to anticoagulant rodenticides: Effects of regulatory restrictions of rodenticide use. *Sci Total Environ* 612:1358–1364.
- Erickson WA, Urban DJ. 2004. *Potential risks of nine rodenticides to birds and nontarget mammals: A comparative approach*. US Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Washington, DC, 230 pp.
- European Commission. Communication and Information Resource Centre for Administrations, Businesses and Citizens. 2010. Directive 98/8/EC concerning the placing of biocidal products on the market. *Assessment Report. Brodifacoum. Product-type 14 (Rodenticide)*. Office for Official Publications of the European Communities, Brussels, Belgium, 136 pp.
- Fourel I, Sage M, Benoit E, Lattard V. 2018. Liver and fecal samples suggest differential exposure of red fox (*Vulpes vulpes*) to trans- and cis-bromadiolone in areas from France treated with plant protection products. *Sci Total Environ* 622:924–929.
- Fournier-Chambrillon C, Berny PJ, Coiffier O, Barbedienne P, Dassé B, Delas G, Galineau H, Mazet A, Pouzenc P, Rosoux R, et al. 2004. Evidence of secondary poisoning of free-ranging riparian mustelids by anticoagulant rodenticides in France: Implications for conservation of European mink (*Mustela lutreola*). *J Wildl Dis* 40:688–695.

- Gabriel MW, Diller LV, Dumbacher JP, Wengert GM, Higley JM, Poppenga RH, Mendia S. 2018. Exposure to rodenticides in Northern Spotted and Barred Owls on remote forest lands in northwestern California: Evidence of food web contamination. *Avian Conserv Ecol* 13:2.
- Gabriel MW, Woods LW, Poppenga RH, Sweitzer RA, Thompson C, Matthews SM, Higley JM, Keller SM, Purcell K, Barrett RH, et al. 2012. Anticoagulant rodenticides on our public and community lands: Spatial distribution of exposure and poisoning of a rare forest carnivore. *PLoS One* 7:e40163.
- Geduhn A, Jacob J, Schenke D, Keller B, Kleinschmidt S, Esther A. 2015. Relation between intensity of biocide practice and residues of anticoagulant rodenticides in red foxes (*Vulpes vulpes*). *PLoS One* 10:e0139191.
- Greaves JH, Rennison BD. 1973. Population aspects of warfarin resistance in the brown rat, *Rattus norvegicus*. *Mamm Rev* 3:27–29.
- Greaves M. 1993. Anticoagulants in pregnancy. *Pharmacol Therapeut* 59:311–327.
- Hadler MR, Shadbolt RS. 1975. Novel 4-hydroxycoumarin anticoagulants active against resistant rats. *Nature* 253:275–277.
- Howald GR. 1997. *The risk of non-target species poisoning from brodifacoum used to eradicate rats from Langara Island, British Columbia, Canada*. Master of Science Thesis, University of British Columbia, Canada, 175 pp.
- Huckle KR, Hutson DH, Warburton PA. 1988. Elimination and accumulation of the rodenticide flocoumafen in rats following repeated oral administration. *Xenobiotica* 18:1465–1479.
- Jaquet M, Coeurdassier M, Sage M, Fourel I, Dinkel A, Parmentier AL, Dervaux A, Rieffel D, Prat-Mairey Y, Raoul F, et al. 2013. Linking predator exposure and patterns of treatments with anticoagulant rodenticides by using faeces. In: *Proceedings of the 9th European vertebrate pest management conference*, Finnish Forest Research Institute, Turku, Finland, 22–27 September, p. 30.
- Jaques LB. 1962. Spontaneous hemorrhage with anticoagulants. *Circulation* 25:130–139.
- Jensen B, Sequeira DM. 1978. The diet of the red fox (*Vulpes vulpes* L.) in Denmark. *Dan Rev Game Biol* 10:1–18.
- Kidawa D, Kowalczyk R. 2011. The effects of sex, age, season and habitat on diet of the red fox *Vulpes vulpes* in northeastern Poland. *Acta Theriol* 56:209–218.
- Koivisto E, Koivisto P, Hanski IK, Korkolainen T, Vuorisalo T, Karhilahti A, Välttilä V, Loivamaa I, Koivisto S. 2016. *Prevalence of anticoagulant rodenticides in non-target predators and scavengers in Finland*. Report of the Finnish Safety and Chemicals Agency (Tukes), Helsinki, Finland, 40 pp.
- Koivisto E, Santangeli A, Koivisto P, Korkolainen T, Vuorisalo T, Hanski IK, Loivamaa I, Koivisto S. 2018. The prevalence and correlates of anticoagulant rodenticide exposure in non-target predators and scavengers in Finland. *Sci Total Environ* 642:701–707.
- Langford KH, Reid M, Thomas KV. 2013. The occurrence of second generation anticoagulant rodenticides in non-target raptor species in Norway. *Sci Total Environ* 450:205–208.
- Larivière S, Pasitschniak-Arts M. 1996. *Vulpes vulpes*. *Mamm Species* 537:1–11.
- Lohr MT. 2018. Anticoagulant rodenticide exposure in an Australian predatory bird increases with proximity to developed habitat. *Sci Total Environ* 643:134–144.
- López-Perea JJ, Camarero PR, Molina-López RA, Parpal L, Obón E, Solá J, Mateo R. 2015. Interspecific and geographical differences in anticoagulant rodenticide residues of predatory wildlife from the Mediterranean region of Spain. *Sci Total Environ* 511:259–267.
- López-Perea JJ, Camarero PR, Sánchez-Barbudo IS, Mateo R. 2019. Urbanization and cattle density are determinants in the exposure to anticoagulant rodenticides of non-target wildlife. *Environ Pollut* 244:801–808.
- Lovdata. 2018. *Forskrift om biocider (biocidforskriften)*. <https://lovdata.no/dokument/SF/forskrift/2017-04-18-480>. Accessed August 2018.
- Madslie K, Albin-Amiot C, Jonsson ME, Henriksen K, Hamnes IS, Urdahl AM, Heier BT, Enemark HL. 2017. The surveillance programme for *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) in Norway 2016. Norwegian Veterinary Institute, Oslo, Norway, 7 pp.
- McDonald RA, Harris S, Turnbull G, Brown P, Fletcher M. 1998. Anticoagulant rodenticides in stoats (*Mustela erminea*) and weasels (*Mustela nivalis*) in England. *Environ Pollut* 103:17–23.
- Munday JS, Thompson LJ. 2003. Brodifacoum toxicosis in two neonatal puppies. *Vet Pathol* 40:216–219.
- Nogreire TM, Lawler JJ, Schumaker NH, Cypher BL, Phillips SE. 2015. Land use as a driver of patterns of rodenticide exposure in modeled kit fox populations. *PLoS One* 10:e0133351.
- Panzacchi M, Linnell JDC, Serrao G, Eie S, Odden M, Odden J, Andersen R. 2008. Evaluation of the importance of roe deer fawns in the spring–summer diet of red foxes in southeastern Norway. *Ecol Res* 23: 889–896.
- Prat-Mairet Y, Fourel I, Barrat J, Sage M, Giraudoux P, Coeurdassier M. 2017. Non-invasive monitoring of red fox exposure to rodenticides from scats. *Ecol Indic* 72:777–783.
- R Development Core Team. 2016. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.rstudio.com/> Accessed July 2018.
- 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.
- Robinson MH, Twigg LE, Wheeler SH, Martin GR. 2005. Effect of the anticoagulant, pindone, on the breeding

- performance and survival of merino sheep, *Ovis aries*. *Comp Biochem Physiol B Biochem Mol Biol* 140:465–473.
- Rowe FP, Redfern R. 1965. Toxicity tests on suspected warfarin resistant house mice (*Mus musculus* L.). *Epidemiol Infect* 63:417–425.
- Ruiz-Suárez N, Melero Y, Giel A, Henríquez-Hernández LA, Sharp E, Boada LD, Taylor MJ, Camacho M, Lambin X, Luzardo OP, et al. 2016. Rate of exposure of a sentinel species, invasive American mink (*Neovison vison*) in Scotland, to anticoagulant rodenticides. *Sci Total Environ* 569:1013–1021.
- Sage M, Fourel I, Coeurdassier M, Barrat J, Berny P, Giraudoux P. 2010. Determination of bromadiolone residues in fox faeces by LC/ESI-MS in relationship with toxicological data and clinical signs after repeated exposure. *Environ Res* 110:664–674.
- Sainsbury KA, Shore RF, Schofield H, Croose E, Pereira MG, Sleep D, Kitchener AC, Hantke G, McDonald RA. 2018. Long-term increase in secondary exposure to anticoagulant rodenticides in European polecats *Mustela putorius* in Great Britain. *Environ Pollut* 236:689–698.
- Seljetun KO, Eliassen E, Karinen R, Moe L, Vindenes V. 2018. Quantitative method for analysis of six anticoagulant rodenticides in faeces, applied in a case with repeated samples from a dog. *Acta Vet Scand* 60:3.
- Serieys LEK, Armenta TC, Moriarty JG, Boydston EE, Lyren LM, Poppenga RH, Crooks KR, Wayne RK, Riley SP. 2015. Anticoagulant rodenticides in urban bobcats: Exposure, risk factors and potential effects based on a 16-year study. *Ecotoxicology* 24:844–862.
- Serieys LEK, Foley J, Owens S, Woods LW, Boydston EE, Lyren LM, Poppenga RH, Clifford DL, Stephenson N, Rudd J, et al. 2013. Serum chemistry, hematologic, and post-mortem findings in free-ranging bobcats (*Lynx rufus*) with notoedric mange. *J Parasitol* 99:989–996.
- Shirley MDF, Elmhagen B, Lurz PWW, Rushton SP, Angerbjörn A. 2009. Modelling the spatial population dynamics of arctic foxes: The effects of red foxes and microtine cycles. *Can J Zool* 87:1170–1183.
- Shore RF, Birks JDS, Afsar A, Wienburg CL, Kitchener AC. 2003. Spatial and temporal analysis of second-generation anticoagulant rodenticide residues in polecats (*Mustela putorius*) from throughout their range in Britain, 1992–1999. *Environ Pollut* 122:183–193.
- Sklepkevych BO, Montevocchi WA. 1996. Food availability and food hoarding behaviour by red and arctic foxes. *Arctic* 49:228–234.
- Statistics Norway. 2018. *Table 11342: Population and area (M) 2007–2018*. <http://www.ssb.no/en/statbank/table/11342>. Accessed January 2018.
- Tjus SE. 2014. *Biocider's spread in the environment and their health and environmental risks: Screening in 2000–2013: A knowledge overview*. Naturvårdsverket Rapport 6634, Stockholm, Sweden, 381 pp. [In Swedish.]
- Tosh DG, McDonald RA, Bearhop S, Llewellyn NR, Fee S, Sharp EA, Barnett EA, Shore RF. 2011. Does small mammal prey guild affect the exposure of predators to anticoagulant rodenticides? *Environ Pollut* 159:3106–3112.
- Vidal D, Alzaga V, Luque-Larena JJ, Mateo R, Arroyo L, Viñuela J. 2009. Possible interaction between a rodenticide treatment and a pathogen in common vole (*Microtus arvalis*) during a population peak. *Sci Total Environ* 408:267–271.
- Watt BE, Proudfoot AT, Bradberry SM, Vale JA. 2005. Anticoagulant rodenticides. *Toxicol Rev* 24:259–269.
- WHO (World Health Organization). 1995. *Anticoagulant rodenticides-environmental health criteria* 175. International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, 50 pp.
- Wikenros C, Aronsson M, Liberg O, Jarnemo A, Hansson J, Wallgren M, Sand H, Bergström R. 2017. Fear or food—abundance of red fox in relation to occurrence of lynx and wolf. *Sci Rep* 7:9059.

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