Risk of Anticoagulant Rodenticide Exposure for Mammals and Birds in Parc National des Pyrénées, France

Marie Lestrade, Jérôme Lafitte, Timothée Vergne, Claire Guinat, Philippe Berny, Corinne Novella, Guillaume Le Loc'H

Risk of Anticoagulant Rodenticide Exposure for Mammals and Birds in Parc National des Pyrénées, France

Authors: Lestrade, Marie, Vergne, Timothée, Guinat, Claire, Berny, Philippe, Lafitte, Jérôme, et al.

Source: Journal of Wildlife Diseases, 57(3) : 637-642

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/JWD-D-20-00125

DOI: 10.7589/JWD-D-20-00125

Journal of Wildlife Diseases, 57(3), 2021, pp. 637–642

Risk of Anticoagulant Rodenticide Exposure for Mammals and Birds in Parc National des Pyrénées, France

Marie Lestrade,[1] **Timothée Vergne,**[1,5] **Claire Guinat,**[1,5] **Philippe Berny,**[2] **Jérôme Lafitte,**[3] **Corinne Novella,**[4] **and Guillaume Le Loc'h**[1,6] [1]Interaction Hôtes-Agents Pathogènes, Université de Toulouse, Institut National de Recherche pour l'Agriculture et l'Environnement, École Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles, 31000 Toulouse, France; [2]Laboratoire de toxicologie, Vetagro Sup, 1 Avenue Bourgelat, 69280 Marcy-l'Étoile, France; [3]Parc National des Pyrénées, 2 Rue du IV Septembre, 65 000 Tarbes, France; [4]Laboratoires des Pyrénées et des Landes, Rue des Écoles, 64150 Lagor, France; [5]These authors contributed equally to this article; [6]Corresponding author (email: guillaume.leloch@envt.fr)

ABSTRACT: The extensive use of anticoagulant rodenticides (ARs) to control rodent populations poses intoxication risks for wildlife: persistence of ARs in rodents can cause secondary exposure and poisoning of predators or scavengers. We investigated risk factors for wildlife exposure to ARs in the Parc National des Pyrénées (PNP), France, using a multivariable logistic regression analysis. A total of 157 liver samples were collected from carcasses of 10 mammal and three bird species found in the PNP between 2010 and 2018 and screened for presence of AR residues. First- and second-generation ARs were detected in more than 60% of red fox (*Vulpes vulpes*) and stone marten (*Martes foina*) samples and in around 40% of wild cat (*Felis silvestris*), European pine marten (*Martes martes*), American mink (*Neovison vison*), and Eurasian Buzzard (*Buteo buteo*) samples. Wildlife exposure to ARs was significantly associated with species having a regular consumption of small mammals (odds ratio [OR]: 2.5, 95% confidence interval [CI]: 1.1–5.8) being collected in the Ossau valley (OR: 2.5, 95% CI: 1.1–6.1) and between 2013 and 2015 (OR: 4.8, 95% CI: 2.0–11.7). We identified wild species that could be targeted for risk-based surveillance program for AR secondary exposure and determined high risk areas in which alternative measures should be applied for rodent control.

Key words: Anticoagulant rodenticides exposure, Parc National des Pyrénées, risk factors, toxicology, wildlife.

Anticoagulants rodenticides (ARs), which are primarily used to control rodent populations in urban and agricultural areas, cause important hemorrhage by inhibiting blood clotting in mammals and birds (Berny et al. 1997). The persistence of ARs in rodents (mainly in the liver) poses intoxication risks for nontarget species, such as predators or scavengers, by repeated ingestion of contaminated rodents (Grolleau et al. 1989). This is especially true for second-generation ARs, which are more potent and persistent in liver (Vandenbroucke et al. 2008).

The Parc National des Pyrénées (PNP), France, is a protected mountain area involved in environmental protection and sustainable land management that hosts up to 240 different vertebrate species. Despite possible substantial impacts on nontarget wildlife, ARs are still commonly used for rodent control within the PNP area. Our objective was to assess secondary exposure of wildlife to ARs and to identify associated risk factors in the PNP.

A total of 157 liver samples were collected from carcasses of 10 mammal and three bird species between 2010 and 2018 in the PNP area (Fig. 1). Carcasses were collected by PNP rangers, mostly along the road axes. Samples were screened for ARs presence by the veterinary and toxicology laboratory of VetAgro Sup (Lyon, France) using high performance liquid chromatography (HPLC) using both fluorescence and diode array detection covering full ultraviolet-spectrum (Meiser 2005) or liquid chromatography coupled with tandem mass spectrometry (LC-MSMS; Fourel et al. 2010). The presence of ARs in a sample was defined by identifying at least one of eight ARs compounds: flocoumafen, difethialone, difenacoum, coumatetralyl, warfarin, chlorophacinone, bromadiolone, and brodifacoum. Those eight ARs are marketed in France as biocides (control of commensal rodents around and within buildings) and bromadiolone also as a plant protection product (control of voles for crop protection). The limit of detection was be-

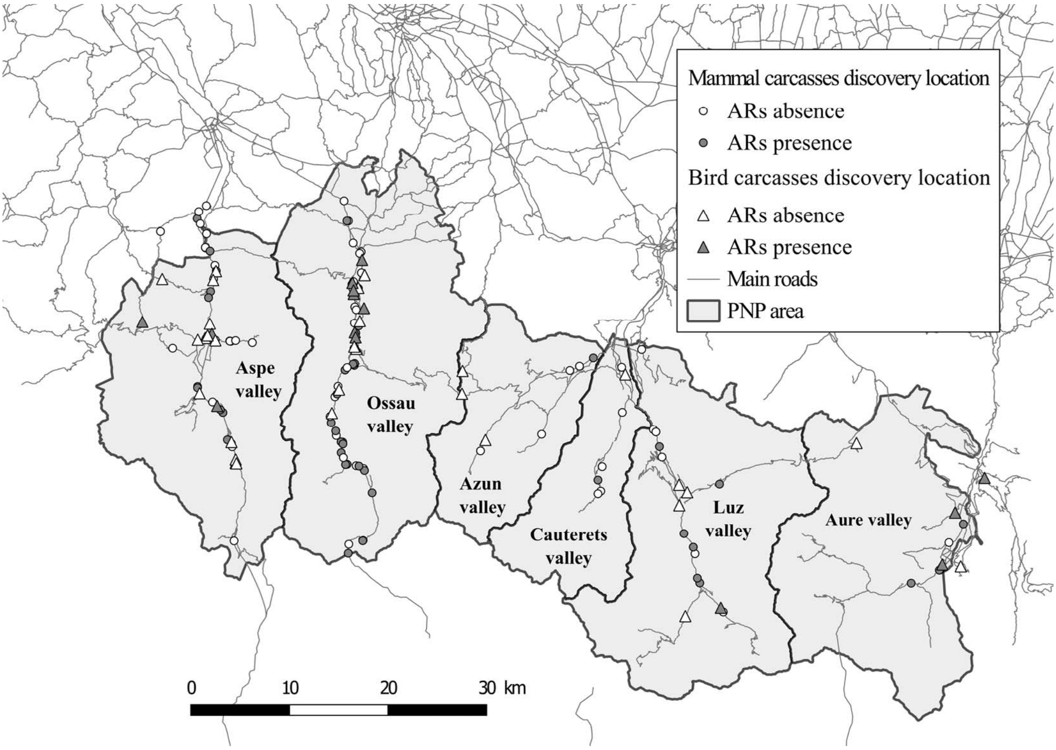

FIGURE 1. Spatial distribution of 157 liver samples collected from carcasses of 10 mammal and three bird species between 2010 and 2018 in Parc National des Pyrénées (PNP) in France and analyzed for anticoagulant rodenticides (AR). Shaded zone represents the PNP area. Circles represent mammal carcass and triangles represent bird carcasses. Gray shapes represent carcasses in which detectable levels of AR were found and white shapes represent carcasses without detectable levels of AR.

tween 0.01 and 0.1 μg/g for HPLC, and from 0.001 to 0.015 μg/g for LC-MSMS, depending on AR tested.

Eight explanatory variables were investigated as potential risk factors for AR exposure in wildlife: percentage of urban and agricultural areas in the home range of the dead animal, taxonomy class (mammal or bird), diet (occasional or regular consumption of small mammals such as rodents), presence of signs of vehicle collision, 3-yr time periods (2010–12, 2013–15, or 2016–18), season, and PNP valley (Aspe, Ossau, Azun-Cauterets, Luz, Aure). The percentage of urban and agricultural areas around the location of a carcass that was sampled was calculated by merging the shapefiles from the French governmental CORINE Land Cover database (European Environment Agency 2018) corresponding to the species home range area (Austin et al. 1996; Ruys and Bernard 2014) in urban and agricultural areas centered on the spatial location of each sampled individual using QGIS software (QGIS Development Team 2019). Because vole populations, one of the main targets of the use of ARs, present cyclical and multiyear fluctuations, 3-yr time periods and the location (PNP valley) of carcass discovery were also included as explanatory variables to take into account potential temporal and spatial autocorrelations in the observations.

The binary outcome variable in the logistic regression was defined as the presence or absence of measurable AR residues in liver samples. Following a univariable analysis, explanatory variables with a $P<0.2$ were included in a multivariable logistic regression. Pair-wise correlation tests were performed for the selected explanatory variables using Ken-

TABLE 1. Exposure to anticoagulant rodenticides (ARs) in 157 liver samples collected in 2010–18 from carcasses of 10 mammal and three bird species in the Parc National des Pyrénées (PNP) in France. The overall prevalence of ARs was 41.4 (44/111) in mammals and 34.5 (16/46) in birds. The overall prevalence in both mammals and birds was 38.0 (60/157).

Common name	Scientific name	Prevalence of ARs (*n* positive/*n* tested)[a]	Years	Consumption of small mammals	Home range[b]
Mammals					
Red fox	*Vulpes vulpes*	62 (21/34)	2010–18	Regular	115 ha
Wild cat	*Felis silvestris*	42 (5/12)	2010–18	Regular	Male: 387 ha; female: 200 ha
Common genet	*Genetta genetta*	0 (0/4)	2010–17	Regular	770 ha
European polecat	*Mustela putorius*	14 (1/7)	2010–17	Regular	Male: 46 ha; female: 41 ha
European pine marten	*Martes martes*	41 (7/17)	2010–18	Regular	Male: 800 ha; female: 350 ha
Stone marten	*Martes foina*	67 (4/6)	2010–17	Regular	210 ha
European weasel	*Mustela nivalis*	50 (1/2)	2014	Regular	Male: 17 ha; female: 4 ha
Eurasian badger	*Meles meles*	16 (3/8)	2010–18	Occasional	275 ha
Eurasian otter	*Lutra lutra*	0 (0/5)	2011–17	Occasional	Male: 40 km (watercourse); female: 20 km (watercourse)
American mink	*Neovison vison*	40 (2/5)	2010–17	Regular	Male: 6 km (watercourse); female: 3 km (watercourse)
Birds					
Eurasian Buzzard	*Buteo buteo*	42 (5/12)	2010–15	Regular	314 ha
Red Kite	*Milvus milvus*	29 (2/7)	2011–18	Regular	700 ha
Eurasian Griffon	*Gyps fulvus*	33 (9/27)	2010–18	Occasional	Considered larger than the PNP area

[a] From 2010 to 2016, ARs analyses were made using high performance liquid chromatography. Liquid chromatography coupled with tandem mass spectrometry was used from 2017.

[b] Home range used for statistical analyses (Austin et al. 1996; Ruys and Bernard 2014).

dall rank correlation, chi-square, Fisher's exact, Kruskal-Wallis one-way analysis of variance, and Mann-Whitney *U*-tests, depending of the variable type. Variable selection in the multivariable analysis was based on a backward stepwise procedure according to the Akaike's information criterion, the best model being the one with the smallest Akaike's information criterion. Odds ratio (OR) with 95% confidence interval (CI) were retrieved from the best model. The adequacy of the model was assessed with the Hosmer Lemeshow test. Statistical analyses were conducted with R software (R Core Team 2011).

We detected ARs in 38.2% (60/157) of all liver samples (Table 1). The most frequently found AR was bromadiolone (62%, 37/60), followed by difethialone (13%, 8/60), chlorophacinone (12%, 7/60), difenacoum (8%, 5/60), brodifacoum (3%, 2/60), and flocoumafen (2%, 1/60). Red fox (*Vulpes vulpes*) and stone marten (*Martes foina*) were the most exposed species to ARs, with 62% (21/34) and 67% (4/6) of positive samples, respectively. More than 40% of positive samples for ARs were found in four other species: wild cat (*Felis silvestris*), European pine marten (*Martes martes*), American mink (*Neovison vison*), and Eurasian Buzzard (*Buteo buteo*). Two other species, the Red Kite (*Milvus milvus*) and the Eurasian Griffon (*Gyps fulvus*), showed approximately 30% positivity (Table 1).

TABLE 2. Risk factors significantly associated with the outcome variable, presence of anticoagulant rodenticide residues in liver samples, in the multivariable analysis ($P<0.05$) of 157 liver samples collected in 2010–18 from carcasses of 10 mammal and three bird species in the Parc National des Pyrénées in France.

Explanatory variable	Categories	Odds ratio	95% Confidence interval	*P*
Three-year time period	2010–12	Reference		
	2013–15	4.8	2.0–11.7	0.001
	2016–18	1.8	0.7–4.5	0.222
Valley	Aspe	Reference		
	Ossau	2.5	1.1–6.1	0.036
	Azun-Cauterets	0.4	0.1–1.9	0.287
	Luz	1.6	0.5–5.3	0.424
	Aure	3.2	0.7–16.2	0.148
Consumption of small mammals (diet)	Occasional	Reference		
	Regular	2.5	1.1–5.8	0.027

Among the eight explanatory variables tested in the univariable analysis, four (diet, percentage of agricultural areas, 3-yr time period, and PNP valley) were selected for inclusion in the multivariable logistic regression. No pair-wise correlation was found among these variables. Three explanatory variables (diet, 3-yr time period, and PNP valley) were retained in the final model ($P<0.05$; Table 2). Exposure to ARs was significantly associated with species having a regular consumption of small mammals (OR: 2.5, 95% CI: 1.1–5.8) being collected in the Ossau valley (OR: 2.5, 95% CI: 1.1–6.1) and between 2013 and 2015 (OR: 4.8, 95% CI: 2.0–11.7). The Hosmer Lemeshow test indicated a good logistic regression model fit ($P=0.256$).

Despite a mountainous terrain, a wildlife conservation policy, and low human activities, a number of wildlife species was exposed to ARs in the PNP, with risk of exposure matching more-inhabited areas. As previously described (Grolleau et al. 1989; Lefebvre et al. 2017), our results show that species that regularly consume small mammals were more exposed to ARs, supporting exposure of nontarget wildlife secondary to the ingestion of poisoned rodents (Sage et al. 2008; López-Perea and Mateo 2018). Among those species, the highest risk of AR exposure was found in red fox (62%, 21/34). This prevalence was consistent with previous studies in which 60% to 85% of red foxes were AR positive in the Doubs region of France (Fourel et al. 2018), Germany (Geduhn et al. 2015), and Spain (Lopez-Perea et al. 2019). Exposures of Eurasian Buzzards and Red Kite (42%, 5/12 and 29%, 2/7, respectively) are lower than those identified in Spain (64% and 88%, respectively; Sánchez-Barbudo et al. 2012; López-Perea et al. 2015).

We highlighted relevant species that could be used as risk-based sentinels for monitoring exposure to ARs in the PNP. These included red fox and mustelids, such as European pine marten and stone marten, due to their wide distribution in agricultural and urban areas and their high risk of exposure. Despite a low sample size, our results suggested that surveillance should also focus on the Eurasian Buzzard due to its wide distribution and its regular consumption of small mammals. Previous studies also showed high levels of exposure in Common Barn Owl (*Tyto alba*) and Tawny Owl (*Strix aluco*; Sánchez-Barbudo et al. 2012; López-Perea et al. 2015), suggesting that nocturnal birds of prey species could also be relevant for ARs surveillance. In our study, Eurasian Griffon was identified as highly exposed to ARs, (33% positive), although it consumes dead rodents only very occasionally. However, this species would not represent a relevant sentinel species for AR exposure in the PNP, as their home range far exceeds the PNP area.

Other risk factors for ARs exposure were identified in this study, such as in which of the PNP valleys the animals lived. Samples from the Ossau valley showed a higher risk of exposure than those from other valleys. This could be explained by greater agricultural and dairy farming activities in Ossau as compared to other valleys. Indeed, forage crops represent suitable areas for the development of grassland rodents and are thus at higher risk of AR exposure due to the implementation of rodent control measures. Samples collected between 2013 and 2015 were also found to be more exposed to ARs. Despite the absence of reliable data on the volume of AR used, it can be hypothesized that the succession of several flood events in many valleys of the PNP from 2012 to 2014 resulted in the increase in rodent populations and thus the increased use of ARs. No association was found between AR exposure and presence of signs of vehicle collision, which indicated that there was no relation between AR exposure and the onset of neurologic symptoms that could have led to a collision. Moreover, no association was found between AR exposure and percentage of urban and agricultural areas in the home ranges. Ecologic data could be used to improve the definition of the home range for each species, in particular by taking into account the altitude and the distribution of ecosystem types.

A limitation of our study was that carcasses were collected along the main roads of the PNP. Future risk factor analyses conducted in the PNP could be improved by applying more systematic and homogeneous collecting methods throughout the study area. Due to limited available data, we did not consider the specific biochemistries of the ARs in our study, although their specific uses and toxicities may be different (Vandenbroucke et al. 2008). Because of the time span of our study, analytical techniques changed dramatically from HPLC to LC-MSMS with increasing sensitivity and specificity. Recent evolutions of the analytical techniques reduced the risk of false positive or false negative results. Finally, gaining more accurate information on additional variables, such as home ranges, would benefit future research studies in the PNP.

LITERATURE CITED

Austin GE, Thomas CJ, Houston DC, Thompson DB. 1996. Predicting the spatial distribution of buzzard *Buteo buteo* nesting areas using a geographical information system and remote sensing. *J Appl Ecol* 33:1541–1550.

Berny PJ, Buronfosse T, Buronfosse F, Lamarque F, Lorgue G. 1997. Field evidence of secondary poisoning of foxes (*Vulpes vulpes*) and buzzards (*Buteo buteo*) by bromadiolone, a 4-year survey. *Chemosphere* 35:1817–1829.

European Environment Agency. 2018. *CORINE land cover*. https://land.copernicus.eu/pan-european/corine-land-cover/clc2018. Accessed June 2019.

Fourel I, Hugnet C, Goy-Thollot I, Berny P. 2010. Validation of a new liquid chromatography–tandem mass spectrometry ion-trap technique for the simultaneous determination of thirteen anticoagulant rodenticides, drugs, or natural products. *J Anal Toxicol* 34:95–102.

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.

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.

Grolleau G, Lorgue G, Nahas K. 1989. Toxicité secondaire, en laboratoire, ďun rodenticide anticoagulant (bromadiolone) pour des prédateurs de rongeurs champêtres: buse variable (*Buteo buteo*) et hermine (*Mustela erminea*). *EPPO Bull* 19:633–648.

Lefebvre L, Fourel I, Queffélec S, Vodovar D, Megarbane B, Benoit E, Siguret V, Lattard V. 2017. Poisoning by anticoagulant rodenticides in humans and animals: Causes and consequences. In: *Poisoning—from specific toxic agents to novel rapid and simplified techniques for analysis*, Malangu N, editor. *InTechOpen*. https://hal.archives-ouvertes.fr/hal-01668708. Accessed June 2020.

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, Sanchez-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.

López-Perea JJ, Mateo R. 2018. Secondary exposure to anticoagulant rodenticides and effects on predators.

In: *Anticoagulant rodenticides and wildlife*. Springer, Cham, Switzerland, pp. 159–193.

Meiser H. 2005. Detection of anticoagulant residues by a new HPLC method in specimens of poisoned animals and a poison control case study. *J Anal Toxicol* 29: 556–563.

QGIS Development Team. 2019. *QGIS geographic information system*. Open Source Geospatial Foundation, Beaverton, Oregon. http://qgis.org. Accessed June 2019.

R Core Team. 2011. *The R project for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org. Accessed April 2017.

Ruys T, Bernard Y. 2014. *Atlas des Mammifères sauvages d'Aquitaine*. Cistude Nature & LPO, Aquitaine, France, 157 pp.

Sage M, Cœurdassier M, Defaut R, Gimbert F, Berny P, Giraudoux P. 2008. Kinetics of bromadiolone in rodent populations and implications for predators after field control of the water vole, *Arvicola terrestris*. *Sci Total Environ* 407:211–222.

Sánchez-Barbudo IS, Camarero PR, Mateo R. 2012. Primary and secondary poisoning by anticoagulant rodenticides of non-target animals in Spain. *Sci Total Environ* 420:280–288.

Vandenbroucke V, Bousquet-Melou A, De Backer P, Croubels S. 2008. Pharmacokinetics of eight anticoagulant rodenticides in mice after single oral administration. *J Vet Pharmacol Ther* 31:437–445.

Submitted for publication 8 July 2020.
Accepted 8 December 2020.