BMC Veterinary Research

RESEARCH

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First report on the occurrence of anticoagulant rodenticides toxicosis in nontarget animals in Thailand

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Abstract

Background Anticoagulant rodenticides (ARs) are widely used worldwide to control rodent populations, yet their toxicity to nontarget animal species, such as dogs and cats, raises significant concerns. Until now, there has been no information about the occurrence status of ARs toxicosis in Thailand. This study presents occurrence data on ARs poisoning in animal specimens analysed at the Department of Veterinary Pharmacology, Faculty of Veterinary Science, Chulalongkorn University, Thailand. Data from January 1, 2018, to December 31, 2023, was collected retrospectively, focusing on confirmed ARs intoxication cases identified through chemical analysis using thin-layer chromatography (TLC) and spectrophotometry methods. Detailed information on animal species, ages, sex, and types of animal specimens analysed was included.

Results During the study period, 35 cases (63.6%) out of 55 tested positive for ARs. Dogs accounted for 77.1% of the ARs-positive cases. Notably, specimens from wild animals and exotic pets, including a turkey, a wild boar, a goose, and three Patagonian mara, were also tested positive for ARs poisoning. Both liver and stomach content specimens showed high agreement in ARs detection, suggesting the potential utility of stomach content analysis alongside liver specimens, which has not been previously reported.

Conclusions This retrospective study underscores the risk of ARs toxicosis in nontarget species. TLC and spectrophotometry methods serve as reliable screening tools for confirming ARs intoxication diagnosis. This study provided a reference for future research on the epidemiology on ARs toxicosis among nontarget species.

Keywords Animal, Nontarget species, Occurrence, Rodenticide, Toxicosis

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Background

Anticoagulant rodenticides (ARs) have been used worldwide since the mid - twentieth century as a means of controlling rodent populations [1]. These compounds are classified into two generations: the first generation, including warfarin and coumatetralyl, and the second generation, comprising brodifacoum, bromadiolone, and difenacoum. The first generation of ARs has been employed for rodent control since 1950. However, due to the emergence of resistance in the target species over time, these compounds are now being replaced by the second generation of ARs, which were introduced in the 1970s. Modifications, such as the addition of a lipophillic side chain to the 4 - hydroxycoumarin skeleton, has increased their affinity for hepatic tissue, resulting in enhanced potency and longer half - lives compared to the first generation of ARs [2]. However, these changes also elevate the risk of severe morbidity and mortality in both target species such as rodents and nontarget species including pet animals and wildlife that are exposed to the second generation of ARs [3].

Both generations of ARs share a similar mechanism of action, disrupting the vitamin K cycle in the liver by inhibiting vitamin K epoxide reductase activity. This inhibition leads to a progressive reduction in the pool of vitamin K necessary for activating coagulation factors II, VII, IX, and X, resulting in their depletion and an increased risk of bleeding [4]. As a consequence, blood vessels lose their elasticity, leading to ruptures of large blood vessels, manifested clinically by extensive hemorrhages and hematomas [5, 6]. Animals affected by ARs intoxication typically exhibit signs of generalised bleeding, with hemorrhages reported in various locations such as body cavities, the gastrointestinal tract, the uterus, the upper airways, the pericardium, joints, and the eyes [7, 8].

The primary purposes of using rodenticides are to protect buildings, installations, crops, stored human food and animal feed, as well as to prevent the spread of rodent-borne diseases. However, ARs poisoning in nontarget species is a significant global concern [9]. Studies have identified ARs as one of the most common substances responsible for pet poisonings [10]. Data from animal poison centers indicate that rodenticides are the top toxins to which pets are exposed, accounting for 3.8% of cases in Europe and 7% in the USA [11, 12]. Exposure to ARs in pet animals typically occurs through accidental contact or ingestion. Given that the vitamin K pathway is conserved across humans and terrestrial vertebrates, the clinical effects of ARs poisoning are similar across species, although susceptibility may vary greatly. While certain species, such as rodents, hares and rabbits, swine, canids (dog, fox), mustelids (stoats), and birds of prey, are highly susceptible, many herbivores (e.g., ruminants, horses) exhibit lower susceptibility [13]. Moreover, previous studies have shown that susceptibility varies both within and between species and across different AR compounds [14, 15].

Despite the lack of precise estimates regarding the number of rodents and non-rodent animals affected by ARs, the widespread availability and global usage of these compounds suggest a substantial impact on animal populations worldwide [1]. In Thailand, agriculturists frequently use ARs to control rodent populations in agricultural areas, primary targeting black rats (*Rattus rattus*) and brown rats (*Rattus norvegicus*). Recently, a report documented ARs-positive cases of exotic pets (Patagonian mara) submitted for ARs analysis in Thailand [7]. However, information on the occurrence of ARs toxicosis in others animal species in Thailand remains limited. Therefore, studying and documenting the occurrence of AR intoxication is crucial to raising awareness and mitigating the risks to nontarget animal species.

In this retrospective study, we investigated the occurrence of AR poisoning in animal specimens analysed at the Department of Veterinary Pharmacology, Faculty of Veterinary Science, Chulalongkorn University (DVPCU), between 2018 and 2023. This study aims to provide a reference dataset for future research on the epidemiology of AR toxicosis in nontarget species.

Materials and methods

Ethical approval and informed consent

This retrospective study does not contain any studies with human participants or animals performed by any of the authors.

Study period and location

DVPCU, established in 1990, serves as a toxicology center providing analysis for various toxicants including ARs, insecticides, herbicides, and mycotoxins, to animal hospitals, veterinarians, general practitioners, and the public in Thailand. This retrospective study encompasses data collected from January 1, 2018 to December 31, 2023, focusing on cases confirmed to have AR intoxication through chemical analysis. Suspected cases were identified based on history, common clinical signs preceding death, and gross pathological findings. Detailed information on animal species (e.g. dog, cat, or others), ages, sex, and types of animal specimens analysed were included in the study.

Sample analysis for AR screening

For the chemical analysis of ARs, all animal specimens, comprised liver tissue, intestinal tissue, stomach contents, blood and urine underwent analysis using thin layer chromatography (TLC) and spectral analysis via derivative spectrophotometry [16]. Briefly, the animal specimens were ground and subjected to extraction

using chloroform under vapor conditions. The resulting extracts were filtered, and any residual material underwent further extraction and filtration. The final residue was then reconstituted in 1 ml of chloroform. TLC separation was conducted using silica gel G plates (Merck, USA) as the stationary phase and a mobile phase consisting of methyl ethyl ketone: benzene (6:120, v/v). Standard solutions and control extracts of the animal samples were also prepared for comparative analysis. Quantification was achieved by spiking the extracts of animal samples with anticoagulant standards (SigmaAldrich, USA) and running them under the standard TLC protocol. Spot detection of ARs was facilitated by overspraying the TLC plates with hydrogen peroxide followed by ferric chloride. A case was considered AR-positive when both methods yielded positive results.

Data analysis

All statistical analyses were conducted using IBM SPSS Statistics for Windows (version 29 Amonk, NY: IBM corp). For descriptive purposes, continuous variables were presented as either mean ± SD or median with interquartile range (Q_1 and Q_3), depending on the normality of the data, which was assessed using the Shapiro – Wilk test. Categorical variables were expressed as percentages of the total. The demographic characteristics of dogs were compared between the AR-positive and AR-negative groups, considering the appropriate sample size. Differences in age between AR-positive and AR-negative dogs were assessed using either Student's t-test or the Mann-Whitney U test, depending on the data distribution. The distribution of sex between the two groups was compared using Pearson's Chi-square test. Agreement between the type of specimen and AR analysis results obtained from liver and stomach content was evaluated using percentage agreement and Cohen's kappa. A Cohen's kappa value between 0.41 and 0.6 indicated moderate agreement, values between 0.61 and 0.8 indicated substantial agreement, and values between 0.81 and 1.0 indicated almost perfect agreement [17]. A p value < 0.05 was considered statistically significant.

Results

During the study period, a total of 55 animal cases were submitted to DVPCU for AR detection, comprising 43 dogs, 6 cats, 3 Patagonian maras, one goose, one turkey, and one wild boar. Among these cases, 35 (63.6%) tested positive for AR poisoning using both TLC and spectrophotometry methods (Fig. 1). Within the AR-positive group of 35 cases, dogs accounted for 27 cases (77.1%), followed by two cats (5.7%), one goose (2.9%), one turkey (2.9%), one wild boar (2.4%) and 3 Patagonian maras (8.6%) (Tables 1 and 2).

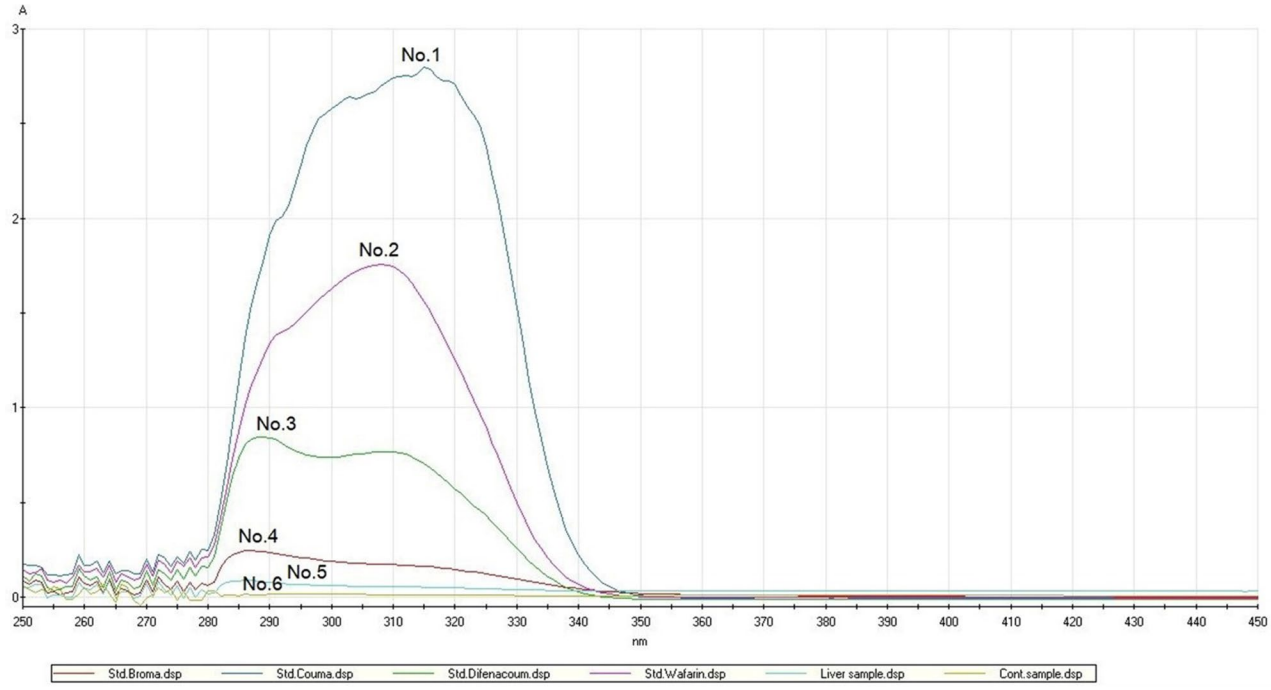

Fig. 1 Spectrophotometry data of standard ARs and the liver specimen of a dog. Peak points of AR standards and sample specimens; standard coumatetralyl (No.1), standard warfarin (No.2), standard difenacoum (No.3), standard bromadiolone (No.4), liver sample (No.5) and stomach content sample (No.6). X - axis: optical density (OD). Y - axis: wavelength (nanometers)

Table 1 Description of dogs and cats with AR-positive results

Year	Dog			Cat		
	Male	Female	ND*	Male	Female	ND*
2018	1	4	-	-	-	-
2019	2	1	-	-	-	-
2020	2	-	-	-	-	-
2021	4	3	2	1	-	-
2022	2	1	2	-	-	-
2023	-	1	1	-	1	-
Total	11	10	5	1	1	

* ND = no data

Table 2 Description of wild animals and exotic pets with AR-positive results

Species	Year	Number	Lifestyle
Wild boar (*Sus scrofa*)	2020	1	outdoor
Goose (*Anser cynoides*)	2021	1	indoor/outdoor
Patagonian mara (*Dolichotis patagonum*)	2023	3	indoor/outdoor
Turkey (*Meleagris gallopavo*)	2023	1	indoor/outdoor

Companion animals

The median age of dogs in the AR-positive group (2 years: $Q_1 = 1.0$, $Q_3 = 5.0$ years) did not differ significantly from that of dogs in the AR-negative group (2 years: $Q_1 = 2.0$, $Q_3 = 5.0$ years) (p-value = 0.708). Additionally, there was no significant difference in the proportion of males and females between the AR-positive group (61.1%) and the AR-negative group (66.7%) (p-value = 0.741) (Table 1). Among the dogs, various breeds were represented, including 7 breeds: Beagle (1 negative), Cavalier King Charles Spaniel (1 positive), Doberman Pinscher (1 positive), German Shepherd (1 positive), Golden Retriever (1 negative), Pomeranian (1 positive), Siberian Husky (1 positive and 1 negative), and mixed breed (17 positive and 6 negative). In the case of cats, mixed breed was the predominant breed, with one male and one female cat testing positive for ARs.

Wild animals and exotic pets

All submitted samples of suspected AR exposure in wild animals and exotic pets tested positive for ARs. Sex information was available for the wild boar and turkey specimens, both of which were female.

Agreement between submitted sample types

The total of 29 liver samples, 47 stomach content samples, and a single sample each for urine, blood, and intestinal tissue from specimens were submitted for AR analysis (Table 3). Among the 22 animals for which both liver and stomach content samples were submitted, 16 samples (72.7%) tested positive for ARs in both liver and stomach content, while 4 samples tested positive only in the liver, and 2 samples tested positive only in the stomach content. The Cohen's kappa value was 0.421, indicating moderate agreement.

Gross pathological lesion

The most prevalent lesion observed in all submitted specimens was hemorrhage in the liver (Fig. 2).

Discussion

AR poisoning is a major issue in veterinary clinical toxicology and a significant concern for veterinarians. The impacts of ARs on nontarget animals have been well documented worldwide. ARs are highly toxic to nontarget species (e.g. dogs, cats, exotic pets, and wildlife), even after a single exposure [18]. This retrospective study presents data on the prevalence of AR poisoning in nontarget species analysed at DVPCU from 2018 to 2023. At DVPCU, TLC and spectrophotometry are used to analyse ARs in animal specimens. AR screening methods such as TLC are valuable tools for confirming AR intoxication in cases where ingestion is not directly observed [7]. However, the limitations of TLC technique still remain, including its inability to identify specific types of ARs in affected specimens. More advanced techniques, such as high performance liquid chromatography (HPLC) and gas chromatography mass spectroscopy (GCMS), can quantify AR residues in samples. These methods are

Table 3 Type and number of submitted specimens with AR-positive results

Specimens	2018	2019	2020	2021	2022	2023
Liver (*n*=29)	4	3	3	3	2	4
Stomach content (*n*=47)	2	0	2	6	3	3
Others* (*n*=3)	0	0	0	0	0	0

*Each for urine, blood, and intestine tissue

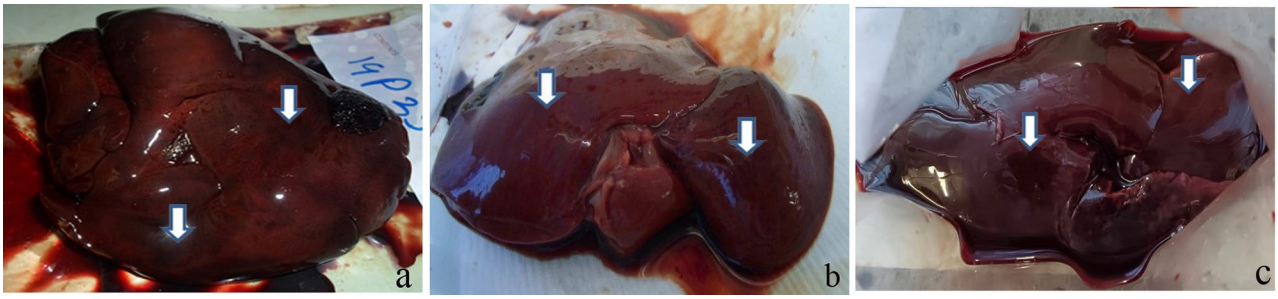

Fig. 2 Gross pathology of liver specimens. Hemorrhagic lesions (arrows) in a dog (**a**); a cat (**b**) and a Patagonian mara (**c**)

more specific and sensitive, making them better suited for identifying different types of ARs [19]. Nevertheless, TLC and spectrophotometry remain reliable screening tests for AR identification [16]. To enhance diagnostic accuracy, more precise techniques like HPLC or GCMS should be employed for identifying specific AR compounds ingested by animals.

From 2018 to 2023, DVPCU received 55 cases submitted for AR testing, with 35 cases (63.6%) testing positive using both TLC and spectrophotometry methods. However, it is noteworthy that 20 cases (36.4%) suspected of AR intoxication yielded negative results. This discrepancy may arise from challenges in identifying clinical signs that resemble AR poisoning, as other diseases or conditions (e.g. neoplasia, liver failure, autoimmune thrombocytopenia, and hereditary coagulation disorders) can cause similar coagulopathies [20]. Screening tests for ARs provide valuable insight into whether coagulopathy results from AR toxicosis, helping to prevent misinterpretation. Since there are no acceptable concentrations of ARs in animals, a positive result confirms that the animal has been exposed to some quantity of ARs [7], regardless of whether the owner suspects exposure [20].

Dogs constituted the majority of affected species, comprising 77.1% of AR-positive cases, consistent with previous reports [18, 19]. Most of the dogs and cats in this study lived indoors, potentially exposing them to ARs through the ingestion of poisoned bait. Dogs could be poisoned by ingesting bait used indoors, improperly storage, or misuse of ARs. The average age of AR-positive dogs was approximately 2 years, suggesting that younger dogs may be more susceptible due to their curious behavior. Additionally, there was no significant difference in the occurrence of AR poisoning between female and male dogs in this study. However, the sample size was too small to assess sex distribution among cats and other species in the present study.

Interestingly, wild animals and exotic pets, including a turkey, a wild boar, a goose, and three Patagonian mara, were also diagnosed with AR poisoning. These animals, living both indoors and outdoors, are probably exposed to ARs through the ingestion of poisoned bait or by consuming poisoned rodents [7]. These findings highlight the unintentional poisoning of nontarget animals due to AR use, consistent with recent reports on the environmental transfer of AR residues leading to mortality or morbidity in wildlife [7, 21].

In terms of specimen types, liver samples have proven to be the most appropriate for detecting ARs due to their high accumulation of these substance [22]. On the other hand, three specimens each of intestinal tissue, urine, and blood were submitted by veterinarians to DVPCU for AR analysis. All of these specimens tested negative. However, it cannot be definitively concluded whether these were true negative or if the specimens were simply not suitable for AR detection using our laboratory methods. The detection of ARs in the liver serves as a key diagnostic tool for identifying AR poisoning, particularly in animals presenting with coagulopathies. In this study, both liver specimens and stomach contents showed moderate agreement in AR detection, suggesting that stomach content analysis may be a useful complementary tool alongside liver testing, which has not been previously reported. One possible explanation for the high agreement between these two sample types is that animals ingested a high dose of ARs and experienced sudden death, leaving AR residues still present in the stomach. Regarding specimen submission to DVPCU, we recommend collecting whole livers or at least 100 g of liver for AR evaluation. However, stomach content can also be submitted alongside liver specimens in certain cases. According to our data, liver specimens consistently yield positive results, reinforcing their reliability for AR detection.

Conclusions

Our retrospective study highlights the risk of AR poisoning in domestic animals, wildlife, and exotic pets, which are nontarget species exposed either directly or through the ingestion of poisoned rodents. Screening tests for ARs, such as TLC and spectrophotometry, serve as reliable tools for confirming AR intoxication, especially in cases where ingestion is not directly observed. According to our data, positive results were consistently found in liver specimens. Additionally, stomach content analysis

also demonstrated potential utility for detectingARs in cases of sudden death. As ARs remain the preferred rodenticides for managing rodent populations, it is crucial toclosely monitor their use and implement appropriate handling measures to prevent unexpected harm to nontarget species and the environment.

Acknowledgements
The authors would like to thank Assistant professor Simon Jeremy Peter Wright, Faculty of Arts, Chulalongkorn University for English language editing.

Author contributions
P.C. contributed and designed the experiments; collected animal data, analyzed the data and wrote the manuscript. V.H. statistically analyzed the data and wrote the manuscript. S.T. contributed to pathological study and manuscript preparation. All authors have read and approved the final manuscript.

Funding
Not applicable.

Data availability
Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Was obtained from the owners.

Competing interests
The authors declare no competing interests.

Received: 6 August 2024 / Accepted: 24 April 2025
Published online: 11 May 2025

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