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Evaluating sublethal anticoagulant rodenticide exposure in deceased predatory birds of South-East Queensland, Australia

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Abstract
The use of anticoagulant rodenticides (ARs) to manage rodent populations can result in unintentional lethal or sublethal poisoning of non-target wildlife, particularly predatory birds. In south-east Queensland, Australia, rodent infestations fluctuate due to favourable environmental conditions, leading to increased AR use and incidences of secondary poisoning. Globally, lethal and sublethal AR exposure has been documented in predatory birds. However, in Australian predatory birds, both the lethal exposure limits and the impacts of sublethal exposure are poorly understood. This study examines AR exposure in 23 raptors and 1 nightjar. Postmortem liver samples were analysed using liquid chromatography mass spectrometry (LC–MS). Traumatic injuries were observed in 15 birds, and rodent remains were found in the gizzards of 9 individuals. LC–MS revealed that 13 birds had sublethal exposure to ARs, with warfarin, a first-generation AR (n = 11) being the most common, followed by second-generation ARs brodifacoum (n = 3), difethialone (n = 1), and flocoumafen (n = 1). Only six of the thirteen AR-positive birds had rodent remains in their gastrointestinal tracts, highlighting the potential of AR bioaccumulation and associated impacts over time. The contribution of sublethal AR exposure to the death of these predatory birds remains unproven and underscores the need for ongoing research into AR exposure in native predatory birds, especially in areas where threatened avifauna inhabit human-dominated landscapes.

Keywords Raptors · Rodenticide · Secondary poisoning · Anticoagulant · South-east Queensland · Liquid chromatography mass spectrometry · Toxicology · Birds of prey

1 Introduction

Mice infestations have posed significant challenges for Australian farmers for centuries and are particularly common in south-east Australia, where large areas of land are used for intensive cropping [1]. Favourable environmental conditions lead to rapid population growth from less than 1 mouse/hectare to over 1000 mice/hectare within a span of 1 to 1.5 years [2]. Notably, heavy rainfall over 1 to 2 years following a series of dry years, significantly elevates the risk of mice infestations [2]. This pattern is caused by the cyclic climatic conditions of the El Niño Southern Oscillation and has recently been observed in south-east Australia.

To manage sizable rodent populations, farmers typically deploy anticoagulant rodenticides (ARs), which are compounds that act by impeding blood clotting, resulting in internal bleeding and eventual death in affected animals [3].

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Both first-generation anticoagulant rodenticides (FGARs) and second-generation anticoagulant rodenticides (SGARs) are deployed, with SGARs exhibiting higher toxicity and requiring fewer exposure events to induce fatality when compared to FGARs [3]. Recent Australian studies have documented secondary poisoning incidents in Powerful Owls (*Ninox strenua)* in Victoria [4] and Southern Boobook Owls *(Ninox boobook)* in Western Australia [5]. This problem is reported globally in diurnal raptors, although the extent of impact this has on mortality remains unclear [6–11].

A critical consideration in assessing the effects of ARs on predatory birds is the morphological overlap between traumatic injuries and coagulopathy. In cases where internal bleeding is observed, distinguishing between injuries resulting from external trauma and those attributable to coagulopathy can be challenging. Preservation of specimens may influence the integrity of the coelomic environment, potentially resulting in disintegration of properly formed clots, while underlying conditions unrelated to AR exposure, such as liver disease, could also contribute to coagulopathy [12]. Consequently, it is difficult to differentiate the effects of traumatic injuries from changes induced by ARs and other non-AR underlying conditions. To investigate this issue, the present study conducted comprehensive pathological examinations and liquid chromatography mass spectrometry (LC–MS) testing to quantify AR levels in liver tissues of predatory birds.

2 Materials and methods

2.1 Specimen collection and data collection

A total of 24 predatory bird cadavers representing various species were opportunistically acquired and submitted for postmortem examinations at The University of Queensland (UQ). These specimens were either donated by UQ's Veterinary Teaching Hospital (UQ VTH) after being euthanised or found as roadkill. Specimens were also opportunistically collected while driving around south-east Queensland and northern New South Wales for other unrelated research studies between June 2022 and November 2023; a period coinciding with regional mice infestations. During these drives, cadavers found as roadkill were collected and enrolled into the study. All specimens included in the study were deceased prior to their involvement in the research project and none were euthanised for research purposes. Cadavers were excluded from the study if they were not predatory species or if their bodies were deemed excessively damaged due to secondary trauma, postmortem consumption by scavengers or decomposition. Excluded birds were not tallied as many of the specimens were originally submitted for undergraduate educational purposes, thus efforts to count them were beyond the scope of the study. Specimens were collected under the Department of Environment and Science, Scientific Purposes Permit WA0041428 as well as UQ and University of Southern Queensland (UniSQ) Animal Ethics Committees (AECs), Permits ANRFA 2022/AE000719 and 21REA001 respectively.

2.2 Gross pathological examination

Cadavers were either examined immediately upon their delivery to the laboratory or frozen at −20 °C for up to 4–5 days before being thawed at 4 °C for autopsy. Subsequently, comprehensive and systematic autopsies were performed following internal UQ protocols [13]. Species identification was confirmed by ornithologists (co-author L.B. and pers. comm. S. Debus) and sex was determined during autopsy by direct visualisation of gonads. In raptorial birds, age is typically determined through plumage characteristics, with immature birds of most species showing feathers that are differently patterned or coloured to adult plumage. Depending on the species, birds older than 2–3 years have adult plumage. Eye colour was also used as an aging indicator, as immature birds tend to have lighter irises in comparison to the more intense colours displayed by adult eyes [14]. During gross examination, findings recorded included lesions in organs or skeleton, presence of any coelomic fluid and the subjective grading of preservation, hydration, and nutritional status. Preservation status was assessed as poor, fair, or good based on the degree of organ discolouration, tissue friability and presence of fly larvae. Hydration status was determined as mild, moderate, or severe and assessed by the degree of muscle tissue tackiness and the retraction of the ocular globes into the orbit. Nutritional status was determined from body condition based on the quantity of pectoral musculature and cavitary or visceral fat stores. Birds were allocated a body condition score of 1 to 5 (1; severely underconditioned, 3; optimal body condition, 5; severely over conditioned). Gizzard contents were examined in every case to determine the potential presence of digested rodent remains.

2.3 Histopathology

Tissues collected for histopathology varied on a case-by-case basis, however, representative sections of brain, heart, trachea and/or larynx, air sacs, thyroid glands, lungs, liver, oesophagus, crop, proventriculus, ventriculus, pancreas, intestines, spleen, kidneys, gonads, and skeletal muscle were often included. Tissues were fixed in 10% neutral buffered formalin for 24–72 h and processed routinely to produce 4 µm-thick hematoxylin and eosin (H&E)-stained slides.

2.4 Liver tissue sample preparation

Liver tissues were sampled from each cadaver before being transported to CSIRO Ecosciences Precinct, Dutton Park, Brisbane, Australia for LC–MS analysis. To account for the differences in water composition during decay, all liver tissues were homogenised and freeze dried. Freeze dried tissues were then sampled (ca. 2.0 g) into a clean 50.0 mL falcon tube. A 2.0 mL aliquot of reverse osmosis (RO) water (18Ωm) and a ceramic homogenisation stone was added to each tube and manually shaken for 2 min. A further 2.0 mL aliquot of 5% formic acid (RO water) was added to each sample and mixed for a further 60 s in a thermomixer incubator (1,000 rpm at 4˚C). Cold acetonitrile (10 mL, with 2% formic acid) was then added, and the samples shaken vigorously for 2 min by hand and further incubated at 4˚C (1,000 rpm) for 15 min [15]. Sample tubes were then centrifuged at 5000 rpm for 5 min, and 3 mL of the supernatant filtered through Agilent Captiva EMR—Lipid cartridge (3 mL, 300 mg; part number 5190–1003) to remove any liver-associated lipids and fine particulates. The EMR cartridges were washed with 600 µL acentrorile:water solution (80:20, v/v) after the sample supernatant was passed through. The filtered supernatant was then dried under a stream of high purity nitrogen and reconstituted in 500 µL methanol:water (80:20, v/v). Solvent calibration standards for the standard curve were prepared at 0.01, 0.1, 0.5, 1, 5, 10, 20, 50, 100, 250, 500, 750 and 1,000 ppb. The internal standard mixture, which included flocoumafen-d4 was spiked at the level of 50 ng/g for selective calibration of the corresponding analytes. Pre-spiked chicken liver QC samples were fortified by spiking the appropriate standard working solution into homogenized chicken liver samples with at various low (0.10, 0.25, and 0.50 ppb), mid 1.5, 2.0, and 5.0 ppb), and high (20.0 ppb) levels. The data were processed with Agilent MassHunter quantification software (v10.2). Calibration curves gave R^2 values between 0.9683 and 0.9971 for the rodenticides using linear regression fit.

2.5 LC–MS analytical standards

All solvents were sourced from Supelco (hyper grade for LC–MS, LiChrosolv). Neat analytical standards of warfarin, difenacoum, bromadiolone, difethialone, brodifacoum, coumatetralyl, flocoumafen, and pindone were sourced from Sigma Aldrich and Novachem. Labelled flocoumafen-d4 (Novachem) was used as a surrogate in all extractions.

Sample extracts were separated on an Agilent InfinityLab Poroshell 120 EC-C18 (3.0 × 100 mm, 2.7 µm; part number 695975–302), and analysed on an Agilent 6546 Liquid Chromatography Time-of-Flight Mass Spectrometer (LC-QToF) with an Agilent Jet Stream source coupled with an Agilent Infinity II Flex UHPLC system. Mobile phase A comprised 2 mM ammonium acetate in RO water, while mobile phase B comprised 100% methanol. The drying gas flow was 10 L/minute, with a nebulizer set at 40 psi. The drying gas temperature was 450˚C. The capillary voltage was 2500 V for both positive and negative ionisation modes. instrument was operated in a data independent mode using a quadrupole-resolved all ions (Q-RAI) analysis method utilising target compound precursors, retention time and collision energies (0, 10, 20, and 40 V) to quantify selected rodenticides [16, 17].

2.6 Statistical analysis

Fisher's exact test was used for assessing significance between two categorical variables, Pearson's chi-square test was applied when comparing continuous variables and ANOVA testing was applied when comparing a categorical and a continuous variable. All tests were conducted with a significance level of 95%.

3 Results

A total of 24 cadavers from 11 predatory bird species (10 raptors and 1 nightjar) were examined (Table 1), comprising 14 adults and 10 immature birds. Among the 24 birds in the study, 13 were male, 10 were female, and 1 could not be determined. Eastern Barn Owls (*Tyto javanica)* were the most represented species (n = 10), followed by Australian

Boobook Owls (*Ninox boobook boobook*; n = 3). Also examined were two Wedge-tailed Eagles (*Aquila audax)*, two Black-shouldered Kites (*Elanus axillaris*), a Peregrine Falcon (*Falco peregrinus*), a Powerful Owl (*Ninox strenua*), a Grey Goshawk (*Accipiter novaehollandiae*), a Brown Goshawk (*Accipiter fasciatus*), a Nankeen Kestrel (*Falco cenchroides*), a Black Kite (*Milvus migrans*), and a Tawny Frogmouth (*Podargus strigoides*).

In total, 13 liver samples tested positive for rodenticides registered for use in Australia. The most frequently detected rodenticide was warfarin, a FGAR, found in 11 birds. The other remaining ARs detected in the liver samples were all SGARs. Brodifacoum was found in 3 samples, while difethialone and flocoumafen were each found in 1 sample. Among the 13 birds that tested positive for AR exposure, 3 were found to have been exposed to multiple ARs; all three had warfarin detected alongside one of the SGARs (brodifacoum, difethialone or flocoumafen).

The sample distribution in this study shows a notable predominance of Strigiformes, particularly Barn Owls, over other raptor orders such as Accipitriformes and Falconiformes. Fourteen of the twenty-four of the specimens analysed were owls, despite the region's high raptor diversity. Notably, the proportion of individuals testing positive for ARs was comparable between Strigiformes (7/14) and non-Strigiformes (6/10). However, the relationship between nocturnal and diurnal predatory birds and the occurrence of AR was not significant ($p = 0.92$).

The relationship between age group (adult and immature) and each of the ARs present was tested. Results revealed a significant positive correlation between age group and brodifacoum, with an f-value of 5.15 ($p = 0.03$; 95% CI [−0.0013, 0.0113]), indicating immature birds were significantly more likely to have higher concentrations of brodifacoum when compared to adult birds.

A summary of postmortem examination findings is found in Table 1. Manifestations such as bruising, extensive internal and external bleeding characterised by non-clotted blood were common, as were findings compatible with rodent remains in the digestive tract. Evidence of trauma-associated injuries was observed in 15 birds, including fractures, organ lacerations, bruising, and free blood accumulated in the coelomic cavity, denominated haemocoelom (Fig. 1). Of the birds with traumatic injuries, 12 were found on the roadside while the remaining 3 were included into the study after being brought into the UQ VTH. In contrast, 5 of the birds not found to have traumatic injuries were brought into the UQ VTH while the remaining 4 were found on the roadside. Figure 2 presents a summary of the locations from which cadavers were obtained for this study.

Haemocoelom without evidence of clotted blood was a common finding, observed in 10 birds, 6 of which were also found to have rodent remains in their gastrointestinal tracts. Six of the specimens that showed haemocoelom without clotted blood did not have any other grossly obvious signs of trauma e.g. external lacerations or fractures (Table 1). Three birds had evidence of avian malaria infection, with presence of intra-erythrocytic apicomplexan parasites in peripheral circulation or within endothelial cells (Australian Boobook Owl, Barn Owl, Black-shouldered Kite). Two birds (Barn Owl, Black-shouldered Kite) had a mild to moderate chronic enteritis, as well as sporulated coccidian oocysts within enterocytes (possibly *Goussia* sp.) [18].

Of the 13 birds that tested positive for ARs, 4 had evidence of haemocoelom without clotted blood—a finding possibly compatible with a coagulopathy—on postmortem examinations. A comparison between finding evidence of a coagulopathy on postmortem examination with the occurrence of AR presence on LC–MS was not significant ($p = 0.41$).

Among the 13 birds that tested positive for ARs, 6 were found to have digested rodent contents within their gastrointestinal tract at the time of death. No rodent contents were found in 6 and the presence of digested rodents was undetermined in 1 raptor. A comparison between positive AR test results on LC–MS and the presence of digested rodent remains during postmortem examinations was not significant ($p = 0.41$). Rodents were also found in 3 of the 11 birds that did not test positive for ARs.

4 Discussion

4.1 LC–MS analysis

SGARs such as brodifacoum and difethialone pose a high secondary risk to birds when ingested due to being 'single dose' anticoagulants. SGARs have an increased potency and prolonged persistence in tissues, resulting from their high lipid solubility and resistance to metabolic breakdown compared to FGARs [3]. However, brodifacoum in the Barn Owl liver (n = 2) and Wedge-tailed Eagle (n = 1) was present in sublethal quantities as the brodifacoum LD50 for owls and other predatory bird species is 0.15–9.68 mg/kg [19]. Difethialone was measured in the Tawny Frogmouth liver sample at a concentration of 0.1 mg/kg, which was also below the difethialone LD50 for birds (0.26–23.4 mg/kg; for Bobwhite,

Table 1 Summary of the signalment, diagnosis and presence of digested rodents of the 24 predatory birds as determined via postmortem examination and Liquid Chromatography Mass Spectroscopy (LC–MS)

Common and Scientific Name	Age	Sex	Postmortem Diagnosis	Digested Rodents	LC–MS results (mg/kg)			
					Warfarin	Difethialone	Brodifacoum	Flocoumafen
Eastern Barn Owl *(Tyto javanica)*	Immature	M	Blunt force trauma, with haemocoelom and suspected myocarditis	Yes	n.d	n.d	0.03 ± 0.01	n.d
	Immature	M	Blunt force trauma, with visceral and muscular haemorrhages	Yes	n.d	n.d	0.05 ± 0.01	n.d
	Adult	M	Blunt force trauma, with haemocoelom, and chronic myocarditis/endocarditis and acute renal tubular necrosis	Yes	n.d	n.d	n.d	n.d
	Immature	M	Blunt force trauma, with haemocoelom; underlying minimal/mild multi-organ inflammation	Yes	n.d	n.d	n.d	n.d
	Adult	M	Open, cardiopulmonary arrest, cause undetermined	No	< 0.01 ± 0.01	n.d	n.d	n.d
	Adult	M	Blunt force trauma, with unilateral radioulnar fracture	No	0.19 ± 0.05	n.d	n.d	< 0.01 ± 0.01
	Adult	F	Blunt force trauma, with haemocoelom	No	n.d	n.d	n.d	n.d
	Adult	M	Blunt force trauma, with right wing fracture and left stifle dislocation	No	n.d	n.d	n.d	n.d
	Adult	Unk	Open, cardiopulmonary arrest, cause undetermined	No	n.d	n.d	n.d	n.d
	Immature	M	Blunt force trauma, with haemocoelom and intestinal coccidiosis	No	n.d	n.d	n.d	n.d
Australian Boobook Owl *(Ninox boobook boobook)*	Immature	F	Blunt force trauma, with muscular and visceral haemorrhages	Yes	0.21 ± 0.05	n.d	n.d	n.d
	Immature	M	Open, muscular haemorrhages and suspected focal liver infarction	Yes	0.34 ± 0.15	n.d	n.d	n.d
	Immature	F	Open, favouring acute process leading to anorexia/hyporexia	No	0.47 ± 0.17	n.d	n.d	n.d
Wedge-tailed Eagle *(Aquila audax)*	Immature	F	Appendicular open fracture with callous formation with minimal cardiomyocytic necrosis	Yes	0.64 ± 0.23	n.d	0.05 ± 0.01	n.d
	Adult	M	Blunt force trauma, with focal cranial fracture, extensive cranial/cervical subcutaneous haemorrhages and unilateral globe rupture	No	n.d	n.d	n.d	n.d
Black-shouldered Kite *(Elanus axillaris)*	Adult	F	Blunt force trauma, with hepatic laceration and haemorrhages in lung, kidney, trachea, maxillary sinus; haemocoelom	No	n.d	n.d	n.d	n.d
	Adult	F	Tick paralysis	No	0.16 ± 0.05	n.d	n.d	n.d
Peregrine Falcon *(Falco peregrinus)*	Adult	F	Necrotizing (medullary) nephropathy	No	< 0.01 ± 0.01	n.d	n.d	n.d
Powerful Owl *(Ninox strenua)*	Adult	M	Blunt force trauma, with visceral rupture and coelomitis	Yes	n.d	n.d	n.d	n.d
Grey Goshawk *(Accipiter novaehollandiae)*	Immature	Unk	Blunt force trauma	Unk	0.25 ± 0.08	n.d	n.d	n.d
Brown Groshawk *(Accipiter fasciatus)*	Immature	F	Penetrating trauma	Unk	n.d	n.d	n.d	n.d

Table 1 (continued)

Common and Scientific Name	Age	Sex	Postmortem Diagnosis	Digested Rodents	LC–MS results (mg/kg)			
					Warfarin	Difethialone	Brodifacoum	Flocoumafen
Nankeen Kestrel *(Falco cenchroides)*	Adult	F	Coelomic soft tissue tumour with secondary haemorrhages and anaemia	No	n.d	n.d	n.d	n.d
Black Kite *(Milvus migrans)*	Adult	F	Blunt force trauma, with massive haemorrhages and hypovolaemic shock	Yes	0.10 ± 0.03	n.d	n.d	n.d
Tawny Frogmouth *(Podargus strigoides)*	Adult	F	Open, cardiopulmonary arrest, cause undetermined	No	0.29 ± 0.06	0.10 ± 0.05	n.d	n.d

n.d. = not detected; M = Male; F = Female; Unk = Unknown

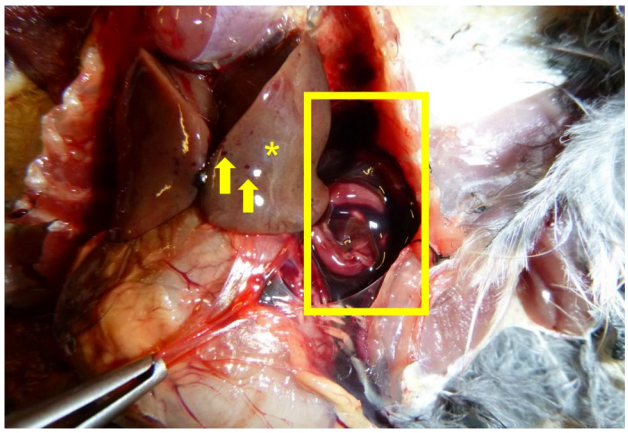

Fig. 1 Autopsy of a Boobook Owl. The liver (asterisk) is extremely pale brown due to blood loss with evidence of red, pin-point haemorrhages (petechia; arrows). The coelom is filled with uncoagulated blood (rectangle)

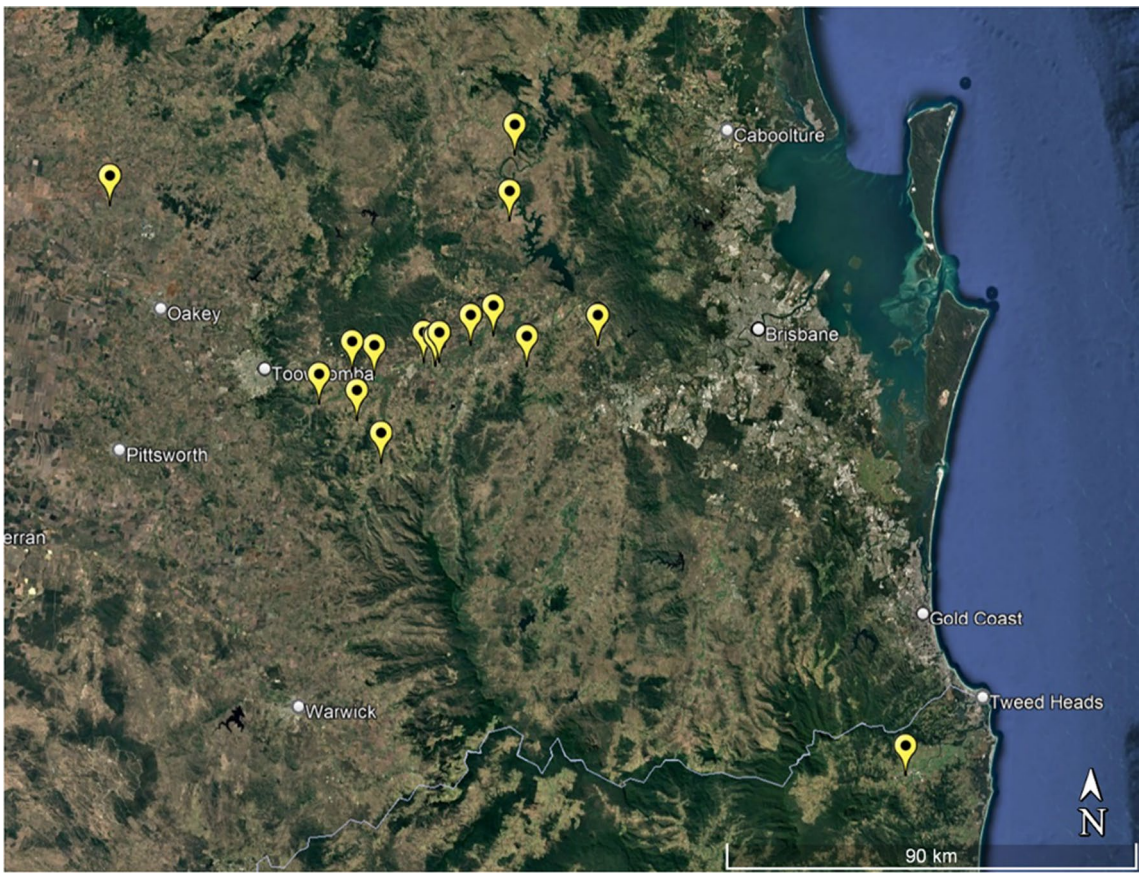

Fig. 2 Map of South-east Queensland and Northern New South Wales, Australia, showing the locations where avian cadavers were obtained from for the study in 2022 and 2023

Japanese Quail, and Mallard) [19]. Brodifacoum has been shown to cause persistent coagulopathies in birds for up to 11 days, even at sublethal doses, due to its prolonged half-life [20]. As such, it is thought that SGARs, especially brodifacoum and difethialone, pose the greatest risk to predatory birds.

The small sample size of predatory birds in this study limits the interpretation of certain findings. For example, the moderately negative, but significant relationship between bird age and the concentration of brodifacoum detected on LC–MS suggests that, as birds age, the concentration of brodifacoum in their tissues decreases. Although purely speculative, this trend could indicate that older birds may have better detoxification mechanisms or have longer intervals between exposures, allowing them more time to metabolize or eliminate the compound. Alternatively, younger birds may be more susceptible to brodifacoum exposure as a lack of hunting experience might lead them to go after weaker

prey such as those poisoned by ARs. It is also possible that birds heavily affected by brodifacoum do not survive long enough to reach adulthoods, or that sublethal accumulation of brodifacoum in younger birds could cause growth abnormalities or reduce their rate of growth, contributing to the observed trend [20]. However, this result must be interpreted with caution as a small sample size might confound the data and the 95% CI of −0.0013–0.0113 might suggest results are not fully representative.

The negative, population-level effects of ARs extend beyond primary poisoning in predatory or carnivorous species; thus, it becomes biologically relevant to investigate the effects of sublethal exposures which are arguably more common than estimated. In wild birds, sublethal SGAR toxicosis has been shown to cause symptoms such as lethargy, reduced agility, and wing droop [19]. These effects result in decreased fitness, impairing the birds' ability to hunt and navigate their environment, increasing their risk of vehicular accidents. Thus, it would be a fair assumption that the birds collected as roadkill could have been negatively influenced by a sublethal SGAR toxicosis. However, in this study there was a greater occurrence of sublethal FGAR toxicosis detected.

FGARs, such as warfarin, present a lower secondary mortality risk to predatory birds compared to SGARs [21]. However, all ARs, regardless of generation, act by attaching to the enzyme vitamin K 2,3-epoxide reductase and stops the cell from recycling vitamin K. As a hydroquinone, vitamin K is needed for the synthesis of prothrombin and other factors that assist with coagulation [22]. Thus, sublethal FGAR toxicosis may present similar symptoms to SGAR. The concentration of warfarin documented in this study was below the LD50 that has been determined in Bobwhites, Japanese Quails and Mallards (525–2150 mg/kg; [19]), but could be high enough to present symptoms of sublethal toxicosis. Birds affected by warfarin may remain debilitated yet capable of hunting, increasing their susceptibility to vehicular collisions as peak coagulopathy typically occurs 1–4 days after a single lethal ingestion. In cases of sublethal ingestion, coagulopathies may take a longer time to manifest [19].

Coagulopathies are often identified as the cause of mortality in autopsies of lethally poisoned animals. This has led to the common perception that the Vitamin K deficiency mechanism is the categorical impact of AR toxicosis. However, this presentation does not adequately encompass the varied clinical signs seen in cases of sublethal toxicoses. Research conducted in other regions has documented the sublethal effects of anticoagulant exposure on the life history of predatory birds. For instance, studies on vultures in the Pacific Northwest have suggested that sublethal doses of SGARs can lead to impaired foraging success, decreased body conditions and reduced reproductive outcomes, ultimately impacting population dynamics [23]. These findings suggest that the sublethal impacts of both warfarin and SGARs are relevant not only within our study area but also in broader ecological contexts. However, it is essential to consider the high variability in sensitivity to anticoagulants among different avian species [19], as established LD50 values may not adequately reflect the risk to all predatory birds. Therefore, focusing on the sublethal effects and their implications for the fitness and behaviour of predatory birds could enhance our understanding of how anticoagulant exposure affects avian populations, particularly in relation to road mortality. Further studies should look to establishing LD50 values for a representative selection of Australian predatory bird species.

4.2 Association between postmortem and LC–MS results

Traumatic injuries were evident in 15 of the examined cadavers, consistent with common lesions observed in wildlife affected by vehicle collisions. The identification of haemocoelom without clotted blood, evidence of visceral/muscular hemorrhages or lacerations, fractures and absence of other chronic significant limiting lesions was observed in 10 birds, of which 4 were also positive to ARs. It is important to note that there can be overlaps between the morphology of traumatic injuries and coagulopathies. Additionally, brain damage caused by AR poisoning can lead to additional traumatic injuries during seizures and possibly an increase in the frequency of traffic accidents. However, it can be difficult to differentiate the primary cause of the traumatic injuries seen during postmortem examinations. Altogether, these postmortem findings could suggest an underlying coagulopathy [24], consistent with patterns observed in cases of AR poisoning events [3]. Furthermore, other causes of coagulopathy unrelated to AR toxicosis may also be present, necessitating a comprehensive evaluation to identify potential underlying health issues.

In addition to rodenticide toxicoses, several other factors contribute to coagulopathies in predatory birds, such as heavy metal toxicoses. While heavy metal toxicities are more commonly observed in captive birds, these have also been documented in wild predatory birds [25]. Infectious diseases such as avian malaria, are another important factor that can lead to reduced stamina, coagulopathies and anaemia due to haemolysis [26, 27]. In the present study, 3 birds had evidence of low burden apicomplexan infections compatible with avian malaria, either within circulating erythrocytes or endothelial cells. With the potential overlap with some postmortem findings with an

acute, severe avian malaria infection (massive haemolysis, pulmonary oedema), and the lack of clinical history and clinical pathology data (e.g. evidence of regenerative anaemia), it is challenging to interpret the clinical significance these infections had. Only one Barn Owl exhibited mild chronic myocarditis, potentially linked to avian malaria infection. This condition is clinically significant due to its effects on contractility and electrical signal conduction, which are particularly detrimental for animals with high metabolic rates and oxygen demands, such as birds.

Although there was no evidence of septicaemia or severe bacterial infections in this study, coagulopathies can also be induced by other infectious diseases such as *Salmonella*, *Escherichia coli* or Aspergillosis [24, 28]. Furthermore, parasitic infections, including helminths and coccidia (e.g. those resembling *Goussia* sp.) which were observed in presumably low burdens in multiple birds in this study, may contribute to coagulopathies through indirect mechanisms, such as immune system modulation or organ dysfunction [29].

Rodent remains were discovered in the gastrointestinal tract of 9 of the examined birds, aligning with previous literature documenting the dietary preferences of predatory birds [30, 31]. Over half of the birds (6/10) exhibiting haemocoelom were also found to have rodent remains within their gastrointestinal tract. From these, fewer of the birds that tested positive for ARs were also found to have digested rodent remains (6/13), but there was no statistically significant association between positive AR results detected on LC–MS and the presence of digested rodents. This suggests various potential exposure pathways for ARs, including environmental contamination or bioaccumulation in liver tissues from previously ingested poisoned rodents. This also suggests the possibility of other routes of AR intake aside from the gastrointestinal tract, and as LC–MS samples were only taken from the liver, the presence of AR toxicants in other organs cannot be ruled out.

Interpreting these results is subject to several limitations. Although rodent presence in the gastrointestinal tract supports dietary data on predatory birds, it does not necessarily clarify the role of rodent ingestion in AR accumulation within these birds. For ARs, bioaccumulation may occur through repeated exposures rather than solely through recent ingestion of a poisoned rodent, making the significance of a single gastrointestinal finding uncertain in terms of toxicant accumulation or adverse health impacts. Furthermore, we did not evaluate the rodent residues themselves for AR presence, which limits our ability to definitively attribute AR exposure in these birds to the ingestion of contaminated rodents.

Additionally, there is considerable variation in the dietary habits of the predatory bird species studied. Some, such as goshawks, are bird specialists rather than rodent predators, whereas others, including Barn Owls and Black-shouldered Kites, are rodent specialists. Such dietary distinctions may influence AR exposure risk, as rodent-specialist birds are theoretically at greater risk of exposure to poisoned rodents than are bird-specialists. The absence of specific dietary differentiation in this study represents a notable limitation. Future investigations should examine species-specific feeding behaviours, test rodent residues for AR presence, and consider long-term AR bioaccumulation to improve understanding of AR exposure risks across diverse predatory bird taxa.

4.3 Taxa-specific discussions

Owls exhibit a notable overrepresentation in this study, comprising 14 of the recovered cadavers. This observation may be explained by their heightened susceptibility to bioaccumulation and secondary toxicosis with ARs, attributed to their comparatively limited hepatic detoxification capacity relative to other avian species [32]. However, the AR detection rate in owls (50%) is comparable to that in non-owl birds examined (60%), this suggests a higher chance of mortality for birds in the order Strigiformes but there is no evidence that the increase in mortality is from AR poisoning. Other factors likely contributing to their overrepresentation in the present study relate to their hunting habits. Owls are nocturnal raptors actively hunting at night, when rodents are more active [33–35]. Additionally, it has been hypothesised that owls often use headlights from vehicles to hunt, increasing their risk of vehicular accidents [36].

The dietary and physiological characteristics of Tawny Frogmouths (*Podargus strigoides*) may render them particularly vulnerable to secondary poisoning by SGARs. As lipid-storing birds, they accumulate fat reserves for winter [37], potentially facilitating the bioaccumulation of lipophilic compounds such as SGARs. Frequent exposure to SGARs in Tawny Frogmouths has also recently been documented in Victoria [38], emphasising the need for further research into the ecological impacts on this species.

4.4 Conservation impact

Although all bird species examined in this study are classified as Least Concern (LC) by the International Union for the Conservation of Nature (IUCN), this study has illustrated that sublethal toxicoses could potentially have broader, population-level implications for conservation. In fact, it could be argued that such sublethal effects could result in overt detrimental outcomes, due to reduced fitness. It is also important to note that certain species can still be globally listed as LC, such as the Powerful Owl, with population estimates of only 2,200 to 2,800 mature individuals worldwide, raising concerns about the population-level impact of potential localised extinctions. Similarly, the Grey Goshawk is estimated by the IUCN to have fewer than 10,000 individuals, with a decreasing population trend and classified as Endangered (EN) in several Australian states, making its population status equally questionable. Australian raptor populations, in general, are not thoroughly studied, complicating accurate assessments of their conservation status and susceptibility to threats like secondary AR poisoning.

Current guidelines advocate the use of FGARs, such as warfarin or coumatetralyl to control rodent populations over SGARs, because FGARs are known to break down quicker in rodents in comparison to SGARs, decreasing the chances of unintended or secondary poisoning. Our findings indicate sublethal exposures to warfarin are common in south-east Queensland carnivorous birds, potentially warranting a revision of current legislation and/or public education to advocate for deployment of other FGARs considered safer for predatory birds, such as coumatetralyl [5, 39].

Coumatetralyl is regarded as a safer alternative, particularly given the reported instances of warfarin resistance in rodents globally [40], including in Australia [5, 41]. While resistance to coumatetralyl has been identified in some rodent populations [42], there have been no documented cases of resistance in Australia. Mammals, including rodents, are generally more sensitive to coumatetralyl than birds [39], allowing for the use of lower doses to achieve the desired effect in rodent control. This reduced dosage further minimises the risk of unintentional secondary poisoning in non-target wildlife, including predatory birds.

5 Conclusions

This study provides insights into the potential ecological risks posed by ARs to predatory birds in south-east Queensland, Australia. Exposures to both FGARs such as warfarin and SGARs were prevalent in the predatory bird species examined. While the detected AR concentrations were below acute toxicosis thresholds reported for other bird species, sublethal exposure may be an indirect contributor to a substantially increased risk of mortality. Potential indirect impacts on fitness and overall survival include increased susceptibility to vehicular accidents, impairments in hunting ability, and long-term physiological effects. Some of the known physiological effects include coagulopathies and anaemia, highlighting the need of ongoing population monitoring including blood work when possible and autopsies of deceased birds. The sublethal impacts of AR on predatory birds remains unquantified and largely unconsidered in the recommended use of FGAR for rodent control.

The findings of this study highlight the importance of considering alternative rodenticides, such as coumatetralyl, with lower secondary poisoning risks. It also emphasizes the need for further investigation into the complex interactions between rodent management practices and native predatory bird populations. Future research focused on these areas would be beneficial and should include further physiology studies of predatory birds exposed to secondary ARs, rodent toxicity studies to determine resistance to FGARs, and surveys of AR users to assess the type, quantity and frequency of AR applications. Understanding the prevalence and toxicological impacts of ARs on predatory birds is crucial for developing effective conservation strategies that mitigate the ecological risks associated with rodent control in agricultural landscapes.

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Declarations

Competing interests The authors declare no competing interests.

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