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Frequent detection of anticoagulant rodenticides in raptors sampled in Taiwan reflects government rodent control policy

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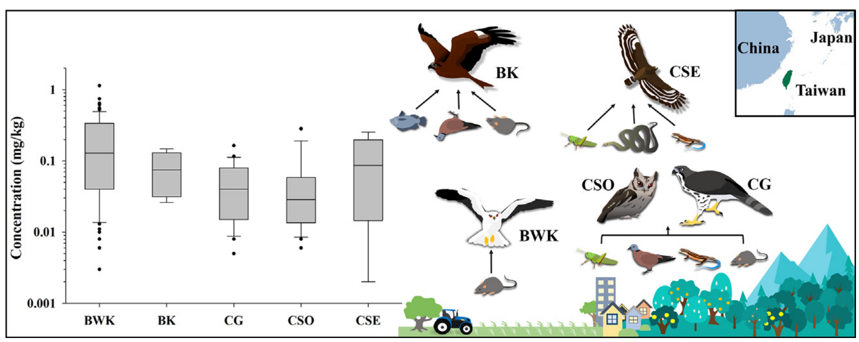
HIGHLIGHTS

- This was the first screening study of rodenticide residues of raptors in Asia.
- Most raptor species in this study have never been tested for rodenticide exposure elsewhere.
- Rodent-eating, scavenging, and snake-eating species were at higher risk of exposure.
- Seasonal trends were consistent with timing of government anti-rodent campaigns.

GRAPHICAL ABSTRACT



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ABSTRACT

Anticoagulant rodenticides (ARs) are known to cause extensive secondary exposure in top predators in Europe and North America, but there remains a paucity of data in Asia. In this study, we collected 221 liver samples from 21 raptor species in Taiwan between 2010 and 2018. Most birds were recovered from rescue organizations, but some free-ranging individuals were obtained from bird-strike prevention measures at airports. ARs were detected in 10 species and more than half of the total samples. Common rodent-eating Black-winged Kites (*Elanus caeruleus*) had the highest prevalence (89.2%) and highest average sum concentration (0.211 ± 0.219 mg/kg), which was similar between free-ranging birds at airports and injured birds from rescue organizations. Scavenging Black Kites (*Milvus migrans*) and snake-eating Crested Serpent-eagles (*Spilornis cheela*) had the second highest prevalence or sum concentration, respectively. Seven different AR compounds were detected, of which brodifacoum was the most common and had the highest average concentration, followed by flocoumafen and bromadiolone. The frequency of occurrence in the three most numerous species (Black-winged Kite, Crested Goshawk [*Accipiter trivirgatus*], and Collared Scops-owl [*Otus lettia*]) was significantly higher in autumn than summer, which is consistent with the timing of the Taiwanese government's supply of free ARs to farmers. Regional differences in the detection of individual compounds also tended to reflect differences in human population density and use patterns (in agriculture or urban-dominated environments). Clinical poisoning was confirmed in Black Kites with sum concentrations as low as 0.026 mg/kg; however, further study of interspecific differences in AR sensitivity and potential population effects are needed. In addition, continued monitoring remains important given the Taiwanese government has modified their farmland rodent control policy to gradually reduce free AR supplies since 2015.
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1. Introduction

Since the 1950s, first generation anticoagulant rodenticides (FGARs) such as warfarin started to enter the pest control market and rapidly became the dominant method to control rodents worldwide. The mechanism of all ARs were similar: they blocked the vitamin K cycle, resulting in the intoxication and potential death by internal bleeding often over several days (Murray, 2018). After extensive use, rodents showed resistance to these FGARs within a decade. Therefore, multiple new and more toxic second generation anticoagulant rodenticides (SGARs) were developed in the 1980s (Rattner et al., 2014; Elliott et al., 2016).

Although rodenticides are almost indispensable in current agricultural production and urban developments, they have also been shown to harm or kill non-target wildlife through direct consumption of baits (primary exposure, Vyas, 2017; Shore and Coeurdassier, 2018) or preying or scavenging on exposed animals (secondary exposure, Rattner et al., 2014; López-Perea and Mateo, 2018). Many top avian predators have experienced widespread exposure in Europe and North America (Thomas et al., 2011; Jacquot et al., 2013; Rattner et al., 2014; Elliott et al., 2016). The extent of AR exposure in these predators may be influenced by multiple factors, including their dietary composition, habitat use, and the local use and application patterns of ARs (Sánchez-Barbudo et al., 2012; Hughes et al., 2013; Ruiz-Suárez et al., 2014; Geduhn et al., 2016; Elmeros et al., 2018; Koivisto et al., 2018; Lohr, 2018). Moreover, AR sensitivity can vary among species and even individuals (Murray, 2011; Thomas et al., 2011). As a result, there is a need for more data from different predator species and different regions to evaluate exposure and potential poisoning of avian predators globally (Berny, 2007; Rattner et al., 2014; van den Brink et al., 2018).

Many Asian countries have serious rodent problems and rely heavily on both FGARs and SGARs (Singleton, 2003; Singleton et al., 2010), and the rodenticide market in Asia Pacific is expected to grow rapidly in the near future due to increasing human population and fewer government regulations (Zion Market Research, 2016). However, according to a review by López-Perea and Mateo (2018), the majority of wildlife rodenticide exposure studies to date have been conducted in seven countries: Canada, United States, United Kingdom, Denmark, France, Spain, and New Zealand. The extent of secondary exposure of predators by ARs in Asia remains poorly understood. In addition, many Asian raptor species belong to Indo-Malayan ecoregion (Olson et al., 2001), which differ from European and North American species. However, to date, only a few AR related studies of Barn Owls (*Tyto alba*) have been conducted in Malaysia (Naim et al., 2010; Salim et al., 2014; Salim et al., 2016).

In 2013, we confirmed the first Black Kite (*Milvus migrans*) rodenticide secondary poisoning in Taiwan (Hong et al., 2018). Here, we conducted a comprehensive screening for 14 rodenticides in the liver of 21 raptor species that represent diverse diets and habitats across the country and over multiple seasons and years.

2. Materials and methods

2.1. Study area

Taiwan is a 36,000 km^2 island country in Eastern Asia with a population size of 23 million. Starting in the 1950s, warfarin was widely used in agriculture and for urban rodent control. Since 1980, the Taiwanese government started an annual anti-rodent campaign and provided up to 900 t of SGARs (mainly brodifacoum, flocoumafen and bromadiolone, active ingredient 0.005%, recommended usage was 1 kg/ha farmland) to farmers and residents for free each year (Lu et al., 2003). The anti-rodent campaign has been held simultaneously in all counties over a one week period in late autumn. In total, 7000 km^2 of farmland (nearly 74% of the plains, i.e., below 100 m above sea level, a.s.l.) and 3.31 million houses, both in urban and rural areas, had rodenticides distributed per annum. During the 1980s, approximately 7 million rodent tails were collected in a single annual campaign (Lu et al., 2003). In addition, brodifacoum (SGAR) was also heavily used to control squirrel (mainly *Callosciurus erythraeus*) damage in planted mountain forests in the 1980s (Kuo et al., 1985). The government has continued supplying SGARs for free, although the amount has been reduced gradually to 400–600 t per year (Hong et al., 2018). These estimates do not account for the private purchases by farmers and urban residents.

For this study, we divided Taiwan into five regions (North, Central, Southwest, South, and East), where each region contained several counties. Regions were divided based on our knowledge of population and agricultural land cover characteristics. The human population density is highest in the North (1967/km^2) and lowest in the East (98/km^2) (Dept. of Household, 2018). The Central, Southwest, and Southern regions are mainly agricultural (population densities are between 556 and 629/km^2). The majority of the plains and low-elevation hills (i.e., below 500 m a.s.l.) in Taiwan have been converted into urban or agricultural developments except for a part of the hills in the East region. Mountainous areas with elevations above 500 m a.s.l. are mostly natural or planted forest.

2.2. Sample collection

Liver samples ($n = 221$) were mainly collected from wildlife rescue organizations, including the Wildlife Rescue and Research Center in Endemic Species Research Institute, Taichung Wildlife Rescue Group, Pingtung Wildlife Rescue Center, and Wild Bird Societies in each county. Samples were collected between 2010 and 2018, but 95% were from 2013 to 2016. For the Black-winged Kite (*Elanus caeruleus*), 60 of the samples came from four airports located in the Central, Southwest, and South regions. Bird-strike prevention measures in these airports used bird nets and shotguns and the Black-winged Kite was the most common raptor captured or shot.

Birds found dead or injured were sent to the rescue organizations by the public, animal protection organizations, or from airport staff (only Black-winged Kite). Most birds were labelled with the administrative district (township-level) where they were found, but without a precise location. Liver samples (2 g) were taken if birds were euthanized or had died, and were frozen at −20 °C until analysis. Necropsy was performed within two days after death. Clinical symptoms were recorded by the veterinary staff in rescue organizations. It is difficult to diagnose AR poisoning merely according to clinical symptoms (Murray, 2017, 2018). Therefore, only birds that met three requirements were confirmed as AR poisoning: 1) diagnosis of AR poisoning via necropsy by a pathologist, 2) AR residues detected in livers, and 3) showing no evidence of poisoning by other pesticides (310 pesticide compounds screened, Y.-H. Sun, unpublished data).

A total of 21 raptor species were collected and subsequently classified as specialist or generalist according to their dietary composition (see complete species list and diet categories in Table S1). Dietary specialists included rodent-eating species (e.g., Black-winged Kite), scavenger (Black Kite), bird-eating species (e.g., Besra [*Accipiter virgatus*]), snake-eating species (Crested Serpent-eagle [*Spilornis cheela*]), and insect-eating species (e.g., Oriental Honey-buzzard [*Pernis ptilorhynchus*]). Generalist predators included Crested Goshawk (*Accipiter trivirgatus*) and Collared Scops-owl (*Otus lettia*). Most species are resident and found in urban or agricultural environments, but a few species (e.g., Mountain Hawk-Eagle [*Nisaetus nipalensis*]) are found in remote mountainous areas.

2.3. Rodenticide analysis

Rodenticide analysis of each sample was conducted by one of the two officially accredited laboratories (Taiwan Agricultural Chemicals and Toxic Substances Research Institute, abbreviation TACTRI, $n = 98$; and ABM International Lab Inc., $n = 123$) in Taiwan. Both labs tested 13 rodenticides, 12 of them were the same, including eight FGARs (warfarin, coumachlor, coumafuryl, coumatetralyl, chlorophacinone, diphacinone, pindone, and valone) and four SGARs (brodifacoum, bromadiolone, difenacoum, and flocoumafen). The 13th rodenticide tested in these two labs was either difethialone (tested by TACTRI) or vacor (tested by ABM), although neither of these compounds were detected in any samples. Because limits of quantification (LOQs) of some rodenticides in these two labs were not the same, we adopted the higher limit of two labs for each rodenticide. According to this standard, the LOQ of brodifacoum was 0.010 mg/kg, warfarin, coumachlor, coumafuryl, coumatetralyl, difenacoum, flocoumafen, and vacor were 0.002 mg/kg, and the rest were set at 0.005 mg/kg (reported as wet weight concentrations). For the three species with the most numerous samples (Black-winged Kite, Crested Goshawk, and Collared Scops-owl, respectively), we did not find any differences between the two labs in the sum concentration, number of ARs detected in one sample, nor detection frequency by species. See Table S2 for interlab comparison.

Rodenticide analyses were all performed by Liquid Chromatograph Tandem Mass Spectrometry (LC/MS/MS), but the preparation and extraction of samples in the two labs were different. In the TACTRI lab, a liquid-liquid microextraction method was used. Liver samples (1 g) were homogenized before extraction with acetonitrile to which sodium chloride was then added. The solution was centrifuged, and the resulting supernatant was cleaned up with acetonitrile saturated n-hexane. The final extract was analyzed by a Waters Xevo TQ MS spectrometer and an Acquity UPLC C18 100 × 2.1 mm (1.6 μm) column. A quality control test at two concentrations (0.02 and 0.1 mg/kg, each $n = 3$) showed the recovery rates for each AR were between 66 and 102%, the relative standard deviations were <20%, and the LOQs varied between 0.002 and 0.005 mg/kg. In the ABM lab, the samples were prepared by a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method as described by Anastassiades et al. (2003) and modified according to Vudathala et al. (2010). Liver samples (1 g) were homogenized, with 10 ml acetonitrile and extraction salt (4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogencitrate sesquihydrate), and then shaken vigorously. The solution was centrifuged, and the supernatant was cleaned up with addition of 400 mg primary secondary amine (PSA), 400 mg C18 end-cap, and 1200 mg anhydrous magnesium sulfate using solid phase extraction. The extract was centrifuged again, and the supernatant was blown dry by nitrogen. Extracts were reconstituted in acetonitrile and then analyzed by a Waters Xevo TQ MS spectrometer and an Acquity UPLC T3 100 × 2.1 mm (1.8 μm) column. One concentration quality control test (0.02 mg/kg, $n = 5$) was conducted. The recovery rates for each AR were between 68.5 and 124.9%, most of the relative standard deviations were <20% (except vacor was 26.9%), and the LOQs also varied between 0.002 and 0.005 mg/kg, except brodifacoum was 0.010 mg/kg. The recoveries of two labs in this study all met the requirements of Taiwanese official regulation of laboratory quality control (except vacor in ABM lab), and were therefore, not recovery corrected.

2.4. Data analysis

We detected multiple rodenticides in individual samples. Therefore, given the similar mechanism of action, the sum of concentrations of each AR (based on assumptions of concentration addition) was used to estimate cumulative toxicity risk (Thomas et al., 2011; Rattner et al., 2014; López-Perea and Mateo, 2018). The individual and sum AR concentration data were presented both on arithmetic and geometric mean in order to compare with previous studies. A sum concentration between 0.1 and 0.2 mg/kg has often been considered as a threshold of AR poisoning, although these values were based on studies of Barn Owls (Newton et al., 1998) and lack toxicological testing in other species. We used the same thresholds to allow for comparison of results with published studies.

We evaluated the effect of different sample sources (from rescue centers or airports) for Black-winged Kites. The summed concentration of ARs was compared between sources via Mann-Whitney U tests, and the number of ARs detected in one sample and the detection frequency (%, detection of either AR) were evaluated via Chi-square test. For samples below detection, we used the median semi-variance (SemiV) method to apply random values to the left-censored data to permit statistical analyses (Zoffoli et al., 2013).

We then built five mixed effects models to test the effects of species, season, and region (fixed factors) and lab (random effect) on the ARs detected in raptor tissues. One linear mixed effects model was used to assess factors affecting sum AR concentrations. Four generalized linear mixed effects models were used to assess factors affecting the detection frequency (%) of total (sum) ARs and three individual ARs (brodifacoum, flocoumafen, and bromadiolone). Since the sample sizes for some species were small (Table S1), for modeling we combined data for the three most numerous species. Season was categorized as spring (March–May), summer (June–August), autumn (September–November), and winter (December–February). Sum concentrations were $\log_{10}$ - transformed to improve normality. Models were performed using the package lme4 (Bates et al., 2014) and lmerTest (Kuznetsova et al., 2015) in R version 3.5.1 (R Core Team, 2018). Results were considered significant if $p < 0.05$.

Apart from presenting an overall mean for the three most numerous species, we used basic statistics to compare detection frequency and sum concentration among seasons for each of these species. Since the sample sizes for each species and season did not meet the requirements of the chi-square test, pairwise comparisons in detection frequency were evaluated using Fisher's exact tests. Sum concentrations among seasons were compared using one-way ANOVA and post-hoc pairwise comparisons. The detection frequencies were plotted on a map for the main AR compounds in each region of Taiwan, with data shown for the ten species with detectable ARs (see Table 1).

3. Results

3.1. Detection and concentration of anticoagulant rodenticides

ARs were detected in 10 species and in 61.5% of the total 221 raptor samples analyzed. Seven different AR compounds were detected (Table 1); brodifacoum was the most common AR and had the highest average and maximum concentration. Among detected samples, 60.3% contained more than one AR, with a maximum of six ARs in a Crested Goshawk (Fig. 1a). One Crested Goshawk contained a low concentration of valone, which was never registered in Taiwan (Table S2).

For species with a sample size above five, total frequency of detection was highest in Black-winged Kite (89.2%), followed by Black Kite (75%) (Table 1). The average sum concentration was also highest in Black-winged Kite (mean. = 0.211 mg/kg), followed by Crested Serpent-eagle (mean = 0.102 mg/kg) (Fig. 1b). A total of 22.6% and 14.9% of the samples exceeded the proposed toxicity thresholds of 0.1 and 0.2 mg/kg, most of which were Black-winged Kites (Table 1). The two sample sources (rescue centers, $n = 14$, or airports, $n = 60$) of Black-winged Kite were not significantly different in sun concentration or detection frequency, suggesting sick or injured birds were not more likely to be exposed than wild caught individuals (all $p > 0.05$).

3.2. Difference among species, season and regions

In the three most numerous species, sum concentrations in Black-winged Kite were significantly higher than Crested Goshawk and Collared Scops-owl (both $p < 0.001$, Table S3). Detection frequency for all ARs was not different among the three species ($p > 0.05$). The detection of brodifacoum in Crested Goshawk and Collared Scops-owl were significantly lower than Black-winged Kite (both $p < 0.01$, Table S3). The detection of flocoumafen in Collared Scops-owl was lower than Black-winged Kite ($p < 0.05$, Table S3).

Table 1
Frequency of detection (%) and concentration (mg/kg) of total and single anticoagulant rodenticide (AR) exposure, and percentage of individuals with >0.1 and >0.2 mg/kg total AR, in 21 raptor species in Taiwan. The majority of samples (95%) were collected between 2013 and 2016. Values reflect only those samples with detectable ARs.

Species	Total							Brodifacoum		Flocoumafen		Bromadiolone		Difenacoum		Diphacinone		Coumatetralyl		Valone	
	n	n+[a]	Freq.	A-Mean[b]	G-Mean[c]	>0.1	>0.2	Freq.	G-Mean	Freq.	G-Mean	Freq.	G-Mean	Freq.	G-Mean	Freq.	G-Mean	Freq.	G-Mean	Freq.	G-Mean
Black-winged Kite	74	66	89.2	0.211	0.107	48.6	37.8	82.4	0.073	54.1	0.022	33.8	0.050	10.8	0.021						
Crested Goshawk	46	27	58.7	0.052	0.035	10.9	0	39.1	0.028	28.3	0.012	19.6	0.016	6.5	0.009	15.2	0.015			2.2	0.012
Collared Scops-owl	42	24	57.1	0.055	0031	7.1	4.8	40.5	0.034	26.2	0.009	11.9	0.009	4.8	0.006	7.1	0.010				
Crested Serpent-eagle	12	5	41.7	0.102	0.044	16.7	8.3	33.3	0.018	41.7	0.026			8.3	0.004						
Black Kite	8	6	75.0	0.080	0.066	25.0	0	75.0	0.043	12.5	0.095	50.0	0.009								
Oriental Honey-buzzard	6	2	33.3	0.006	0.004	0	0			16.7	0.002					16.7	0.009				
Short-eared Owl	6	2	33.3	0.032	0.023	0	0	16.7	0.010			16.7	0.054								
Eastern Grass-owl	3	2	66.7	0.248	0.135	33.3	33.3	66.7	0.111	33.3	0.015	66.7	0.020								
Eurasian Kestrel	3	1	33.3	0.483	0.483	33.3	33.3	33.3	0.483												
Common Buzzard	1	1	100.0	0.020	0.020	0	0					100.0	0.014					100.0	0.006		
Other 11 species[d]	20	0	0																		
Total	221	136	61.5	0.138	0.061	22.6	14.9	50.2	0.052	32.9	0.017	21.5	0.027	6.4	0.013	5.0	0.013	0.5	0.006	0.5	0.012

[a] n+: Number with rodenticide exposure.
[b] A-Mean: Arithmetic mean.
[c] G-Mean: Geometric mean.
[d] Complete species list and diet categories available in Table S1.

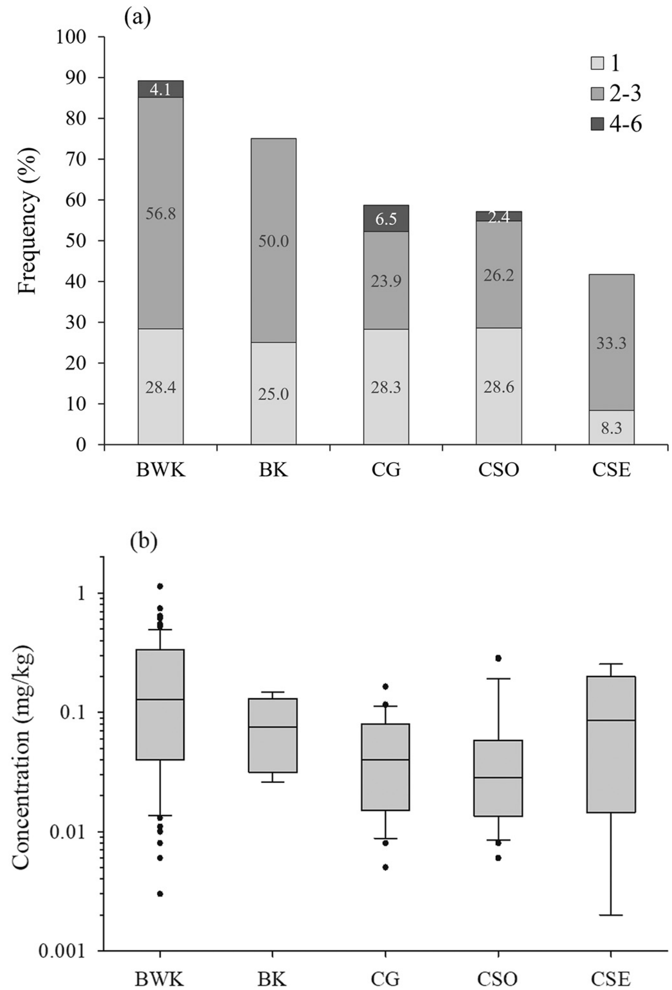

Fig. 1. Species differences in (a) the total AR detection frequency (%), and the proportion of samples with one or more (1, 2 to 3, or 4 to 6) compounds detected, and (b) the sum concentration (mg/kg). Central lines of boxplots show the median, boxes represent the interquartile range, whiskers show the minimum and maximum values within 1.5× from the inter-quartile range, and dots represent outliers. The values included only detectable samples. Species abbreviations: Black-winged Kite (BWK), Black Kite (BK), Crested Goshawk (CG), Collared Scops-owl (CSO), Crested Serpent-eagle (CSE).

Sum concentration and detection frequency of ARs in the three most numerous species in autumn were both significantly higher than summer (both $p < 0.05$, Table S3). Detection of individual ARs was not significantly affected by season (Table S3). In a single species, the Crested Goshawk, detection frequency of ARs in winter was significantly higher than summer ($p = 0.022$), and detection frequency of ARs in Collared Scops-owl in both autumn and winter tended to be higher than summer (nearly significant, both $p = 0.057$) (Fig. 2a). Detection frequency of ARs in Black-winged Kite was not significantly different among seasons (all $p > 0.05$); however, sum concentration in Black-winged Kite was different among seasons ($p = 0.048$, Fig. 2b). Pairwise comparisons showed that the sum concentration in winter was higher than in summer ($p = 0.020$) and autumn ($p = 0.025$). In the other two species, sum concentration was not different among seasons (all $p > 0.05$).

Model analysis of sum concentration and detection frequency of the three most numerous species showed similar geographical patterns (Table S3). Brodifacoum, flocoumafen, and bromadiolone were commonly found across all regions of Taiwan (Fig. 3). The detection of flocoumafen was significantly higher in the Central region than in the South ($p < 0.05$, Table S3). In the map of Taiwan showing detection frequency of multiple ARs by region, difenacoum was only detected in the North, Central, and Southwest regions, and diphacinone was only detected in the Southwest, South, and Eastern regions (Fig. 3).

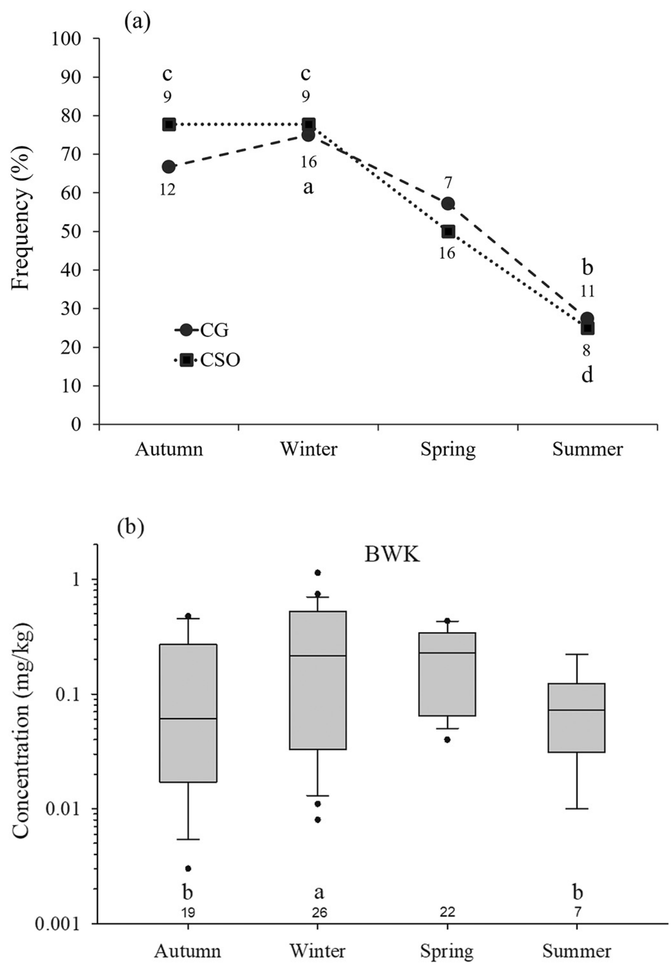

Fig. 2. Seasonal trends of (a) total AR detection frequency (%) in Crested Goshawk (CG) and Collared Scops-owl (CSO), and (b) sum concentration (mg/kg) of Black-winged Kite (BWK). The numbers in figures represent sample sizes for each season. Central lines of boxplots show the median, boxes represent the interquartile range, whiskers show the minimum and maximum values within 1.5× from the inter-quartile range, and dots represent outliers. Letters a and b represent a significant difference ($p < 0.05$) between two seasons, and letters c and d represent a nearly significant difference ($p = 0.057$).

Coumatetralyl and valone were both only detected once in the Southwest region.

3.3. Cases of suspected rodenticide poisoning

Four birds were necropsied due to suspected rodenticide poisoning. Three Black Kites were all found dead or dying without evidence of trauma. One appeared to have a stomach hemorrhage (detectable brodifacoum at 0.033 mg/kg). The other two Black Kites appeared to have severe bleeding in the mouth and internal organs (one bird with detectable concentrations of 0.026 mg/kg of two SGARs and the other with 0.124 mg/kg of three SGARs). One Black-winged Kite was rescued because of a clavicular fracture, but multiple hemorrhages of several organs were found after death (detectable concentrations of 0.476 mg/kg of two SGARs).

4. Discussion

This is the first large scale monitoring study of AR secondary exposure of raptors in Asia, and most of the raptor species reported here have never been tested anywhere else in the world. We found ARs have entered the diverse food chain of most resident raptor species in the plains and low-elevation hills of Taiwan. The rodent-eating species (Black-winged Kite), the scavenger (Black Kite), and snake-eating species (Crested Serpent-eagle) were at higher risk of exposure. The Crested Serpent-eagle was also one of a few snake-eating species having AR exposures previously reported (Sánchez-Barbudo et al., 2012; López-Perea and Mateo, 2018). Most detected rodenticides were SGARs, largely brodifacoum, and the seasonal trend in prevalence and peak concentrations were consistent with the timing of the anti-rodent campaigns held by the government annually in late autumn.

4.1. The AR secondary exposure risk by species

Specialized rodent predation, scavenging habits, and use of anthropogenic urban and agricultural environments are three main factors that increase the risk of AR secondary exposure (López-Perea and Mateo, 2018). The Black-winged Kite occupies open plains habitats and is highly adapted to agricultural environments in Taiwan (Severinghaus et al., 2012). The proportion of rodents in the diet was up to 91.9% in Black-winged Kites, and their main prey (*Rattus losea*, *Bandicota indica*, and *Apodemus agrarius*) are common targets of rodent control by farmers (Severinghaus and Hsu, 2015). Therefore, the Black-winged Kite appears to be the main species at risk of secondary rodenticide exposure in Taiwan. Compared to other AR screening studies, the prevalence of detection (89.2%) in the Black-winged Kite in Taiwan was very high, and over one third of the samples (37.8%) exceeded the threshold of 0.2 mg/kg (Newton et al., 1998; Newton et al., 1999), which is higher than most reported raptor species (see a review by Lohr, 2018). Another rodent-eating species in Taiwan, the Eastern Grass-owl (*Tyto longimembris*) is critically endangered. Given that their diet contains 98% small mammals (by mass), AR exposure could be one factor contributing to their population decline (Lin et al., 2007; Severinghaus et al., 2012).

The Black-winged Kite's population has increased and expanded its range dramatically since this species first appeared and was observed breeding in Taiwan in 2001 (Weng, 2004; Severinghaus et al., 2012). Since there is a lack of resident raptor species in the plains environments of Taiwan (Severinghaus et al., 2012), the Black-winged Kite rapidly occupied this niche and is now frequently captured in airports. In less than two decades, their distribution expanded from the Southwest region to almost all the plains regions of Taiwan (eBird, 2018). Similarly, this species has also expanded its range into southern Europe (Balbontín et al., 2008; Karakaş, 2012), the Middle East (Vosoghi et al., 2012), and China (Lin et al., 2004). Range expansion in this species has mainly been attributed to climate change, land-use changes (Balbontín et al., 2008), and high annual productivity (Lin et al., 2004; Abed and Salim, 2018). We found no difference in AR contamination of free-ranging Black-winged Kites captured at airports compared to injured birds sent to rescue organizations. The population of Black-winged Kite, although largely exposed to ARs, is nevertheless increasing. This warrants further studies on differential toxicity of SGARs among Asian raptor species.

In contrast, Black Kites had the second highest AR detection frequency (75%) in Taiwan probably due to its scavenging habit and utilization of anthropogenic environments. This scavenger was abundant in Taiwan before the mid-1970s (Swinhoe, 1863; Chen and Yen, 1973); however, their population declined dramatically in the 1980s. According to several recently confirmed poisoning cases, their endangered status has been proposed to be related to extensive rodent and avian pest control with SGARs and the highly toxic insecticide carbofuran since the 1980s (Hong et al., 2018). In this study, three Black Kites appeared to have signs of internal hemorrhage even with liver sum concentrations as low as 0.026 and 0.033 mg/kg (both brodifacoum). Similar lethal brodifacoum concentrations have also been reported in a Great Horned Owl (*Bubo virginianus*, 0.01 mg/kg), Golden eagle (*Aquila chrysaetos*, 0.03 mg/kg) (Stone et al., 1999), and Red-tailed Hawk (*Buteo jamaicensis*, 0.012 mg/kg) (Murray, 2011).

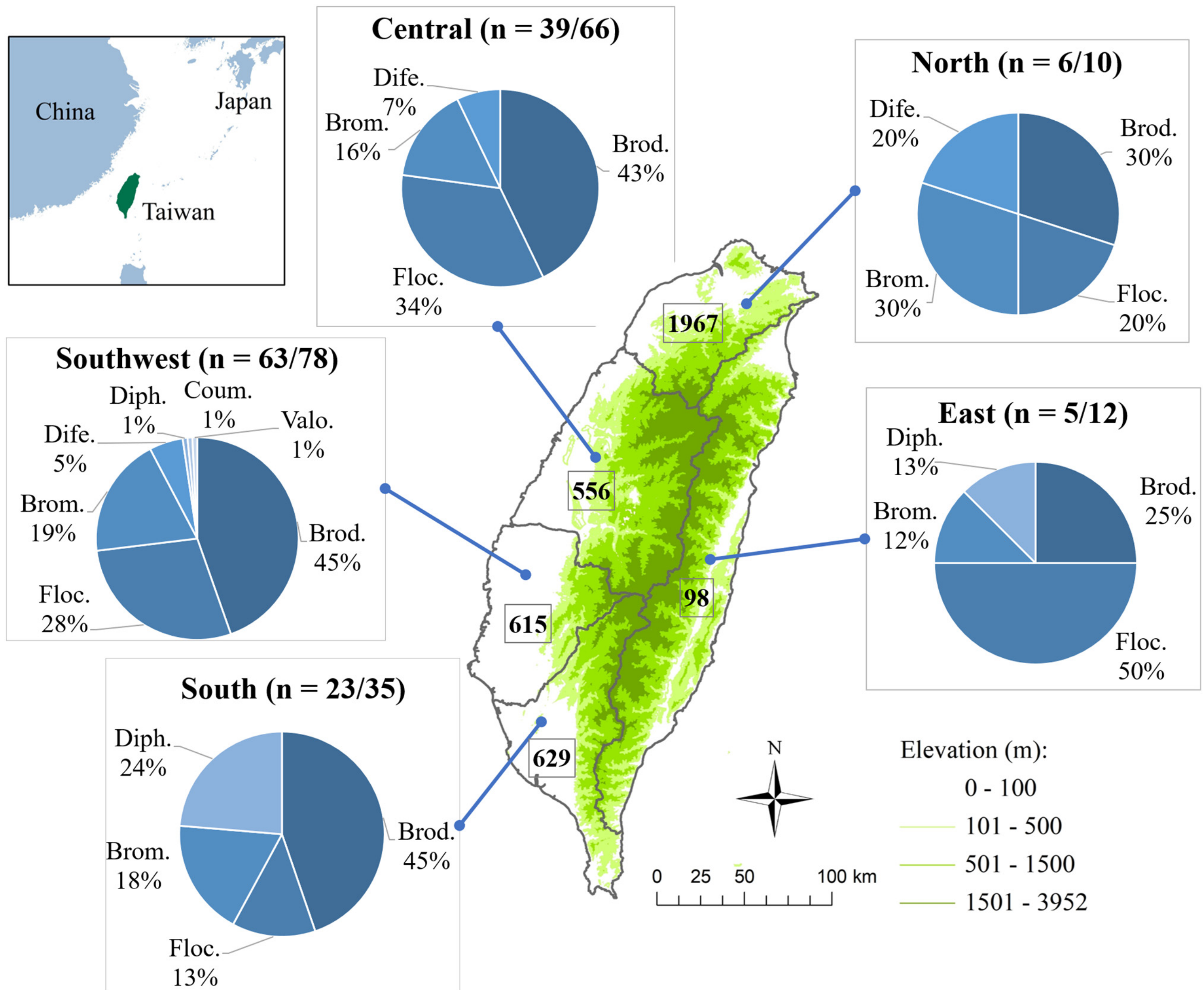

Fig. 3. Map showing frequency of detection (%) of multiple ARs in five regions of Taiwan for ten raptor species (species with detectable AR, see Table 1) combined. Sample sizes (number of samples with detectable AR/number tested) for each region are shown in brackets. Human population densities (individuals/km^2) of each region are shown on map with frames. AR abbreviations: Brodicafoum (Brod), Bromadialone (Brom), Coumatetralyl (Coum), Difenacoum (Dife), Diphacinone (Diph), Flocoumafen (Floc), Valone (Valo).

This suspected AR sensitivity and high prevalence of exposure could make the Black Kite more susceptible to AR secondary poisoning.

The second highest AR concentrations were found in the Crested Serpent-eagle, which confirmed the previous speculation that reptile prey could be an important potential exposure pathway in the food chain (Hoare and Hare, 2006; López-Perea and Mateo, 2018; Lohr and Davis, 2018). Their dietary composition (by number) are mainly snakes (72.2%) and lizards (15.5%), and only a few rodents (4.5%), in northern region of Taiwan (Lin, 2005). The Crested Serpent-eagle inhabits forest habitat consisting of low-elevation hills and mountains (Severinghaus et al., 2012), which may reduce their exposure. Nevertheless, the high concentrations in this snake-eating species may suggest that snakes are also at significant risk of secondary exposure to ARs.

The AR prevalence and sum concentrations were similar in two generalist species. The Crested Goshawk and Collared Scops-owl inhabit analogous habitat with Crested Serpent-eagle, but started to colonize urban woody parks from the 2000s (Severinghaus et al., 2012; Lin et al., 2015). The dietary composition (by number) of these two species were also similar: 52.1–67.7% birds and 19.2–25.8% small mammals (Huang et al., 2006; Yao et al., 2016). The AR prevalence was lower than the Black-winged Kite which may be expected since rodents are not their main prey. Small birds are known to feed on AR baits directly or prey on AR-contaminated invertebrates and then become prey to bird-eating species (Hughes et al., 2013; Masuda et al., 2014; Vyas, 2017; Alomar et al., 2018). Two specialized bird-eating species sampled in this study (see Table S1) did not have detectable AR exposure, but the sample size ($n = 6$) was small. Likewise, insect-eating species have a potential risk of AR exposure, but only one insect-eating species, the Oriental Honey-buzzard (*Pernis ptilorhynchus*), had detectable ARs in tissues. The diet of this species is mainly wasps (*Polistes* spp., 78% in frequency) and occasionally snakes (14.3%) (Yao et al., 2016). Given the levels found in the Crested Serpent-eagle, we suspect that the AR exposure in Honey buzzards could also result from consuming contaminated snakes.

None of the raptor species inhabiting forest habitats in mountainous areas had any detectable ARs; however, the sample size was limited ($n = 4$). The Taiwan Forestry Bureau was known to use brodifacoum to control widespread squirrel damage in planted forests in the 1980s, and some anecdotes from hunters indicated that the wild mammal population declined notably after rodenticide application (Kuo et al., 1985). This use stopped in the 1990s because the role of the Forestry Bureau transformed its mandate from for-profit forestry to forest protection and recreation (Lee and Hsu, 2010). Some migratory species had detectable ARs, but it remains difficult to distinguish where and how they were exposed (Christensen et al., 2012). For future AR screening studies, we recommend prioritizing resident over migratory species.

4.2. Seasonal and regional use patterns

The AR detection frequency or sum concentration was significantly different among seasons in our three most numerous species, and this seasonal trend was consistent with the timing that the government provided large quantities of rodenticides for free each autumn. The annual anti-rodent campaign was usually held in November, which is not the peak period of rodent damage, but due to dry weather and lack of food in farmland, the rodenticides generally have higher efficacy (Lu et al., 2003; TACTRI, 2012). We also found the late-autumn and following winter appear to be the most frequent period of AR secondary exposure. However, rodent populations can recover within three months after AR application (Lu et al., 2003), and some farmers will use rodenticides again in spring prior to planting. This pattern seems may explain the higher AR concentrations of Black-winged Kite in spring. The late-spring and summer are during the Taiwanese wet season, so the ARs may not persist long in the field and consequently pose a lower risk for predators.

The most frequently detected ARs were brodifacoum, flocoumafen, and bromadiolone, the three SGARs registered for agricultural pesticide use (see Table S2). Brodifacoum and flocoumafen were the two ARs the government provided in recent years, but bromadiolone has not been provided by the government since 1995 because it was ineffective for certain rodent species (e.g., *Bandicota indica*, *Mus musculus*, and *Apodemus agrarius*) (Lu et al., 2003). However, all AR products registered as agricultural pesticides can be readily purchased privately, which suggests the high prevalence of bromadiolone in raptors is likely from the public buying their own supplies. In Taiwan, almost all rodenticide products sold on the market were SGARs, and they were sold without any restrictions (i.e., bait boxes not required). According to an investigation of farmers' behavior (Y.-H. Sun, unpublished data), users preferred powder, granular, or liquid type of ARs more than wax-rice mixed bait (i.e., a cookie, provided by the government).

Difenacoum and coumatetralyl have only been registered for use in urbanized locations of Taiwan. These rodenticides were used in residential areas, campus grounds, factories, and city streets to protect public health and are not allowed for use in farmlands. However, as agricultural pesticides, the public can buy difenacoum and coumatetralyl at certain stores without restrictions. The highest frequency of difenacoum was detected in the Northern region, which is the most densely populated area. Urban areas have frequently been associated with higher AR exposure of non-target predators in other studies (Hindmarch et al., 2017; Koivisto et al., 2018; Lohr, 2018; López-Perea et al., 2019).

Coumatetralyl is one of the two registered FGARs but was only detected once at a low concentration. This may be because it is uncommon on the market or because it has a relatively short half-life compared with the SGARs (Horak et al., 2018; Koivisto et al., 2018). Warfarin is the other registered FGAR, but we are not aware of any commercial products available in the Taiwanese market in recent years. There was another FGAR detected in birds from this study, diphacinone; however, no registered product was available after 2013. Similarly, valone was detected in one resident species but this compound has never be registered in Taiwan. The detection of diphacinone and valone suggest that some illegal rodenticide products may be available.

4.3. Conclusions

This study documented extensive secondary AR exposure of raptors in Taiwan, an Asian country where traditionally the use of ARs has been supported by the government and have almost no restrictions on their application. Wildlife species occupying the plains and low-elevation hills in Taiwan were often believed to be threatened by habitat loss, but until recently have rarely been considered affected by pesticides or other environmental contaminants (Hong et al., 2018). Given the abundant population size, widespread distribution, high rodent diet and adaptation to agricultural environments, we suggest the Black-winged Kite could be a good indicator species for monitoring ARs in Taiwan and across Asia. To evaluate sub-lethal effects and toxicity of free-ranging Black-winged Kites and other species, blood clotting assays could be used in future studies (Hindmarch et al., 2019). In addition, further research on understanding farmers' behavior, the extent of AR primary exposure and exposure pathways are also needed.

Previous research revealed the Black Kite is susceptible to AR poisoning, and since 2015, the government has reduced the free supply of rodenticides (Hong et al., 2018). However, the annual anti-rodent campaign which has been in place for almost 40 years, requires education of the public who often believe that rodent control is the responsibility of the government. Education and campaigns to increase awareness about the consequence of rodenticide applications remains a priority in Taiwan, and some physical and biological control methods (e.g., raptor perches and owl boxes) have been recently promoted. Ultimately, sales and use restrictions of SGAR products also need to be considered to protect avian top predators.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.07.076.

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