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A Retrospective Study of Cases of Acetyl Cholinesterase Inhibitor Poisoning in the Coyote (*Canis latrans*) and the Bald Eagle (*Haliaeetus leucocephalus*) in the Canadian Prairies

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Abstract

Objective: Wildlife death from organophosphate and carbamate pesticide exposure has been documented previously in Canada. Wildlife exposure to these agents can occur through primary toxicity (i.e., inhalation), ingestion of contaminated water or food, or secondary toxicity through scavenging on toxic carrion. This paper describes epidemiologic information pertaining to confirmed acetyl cholinesterase inhibitor pesticide lethality in the coyote and bald eagle over a 16-year period in the Canadian Prairies.

Methods: Epidemiologic case information from the diagnostic records of Prairie Diagnostic Services confirmed lethal acetyl cholinesterase inhibitor poisoning in 58 coyotes (*Canis latrans*) and 60 bald eagles (*Haliaeetus leucocephalus)* from 1998 to 2013. Brain acetyl cholinesterase enzyme activity suppressed to 50% or greater was indicative of toxicity and death.

Results: Coyote case submissions varied both annually ($p<0.0001$) and temporally ($p<0.0001$). Submissions were highest in the years of 2000, 200, and 2002 (collectively 46.6%). The months of most frequent submission were May and April (36.2%). Bald eagle cases were also influenced annually ($p<0.0001$) and temporally ($p<0.0001$). Confirmed poisoning in bald eagle carcasses was most frequent during two seasonal periods: May through April and December through January. Years 2000, 200, and 2004 comprised 43.3% of the bald eagle poisonings during the investigational period. Annual and temporal distribution of coyote and bald eagle cases were comparable but were not significantly correlated. Brain acetyl cholinesterase activities within 20% of the mean in unaffected cases were considered background. These activities were 3.44 ± 1.52 µmol/min/g in the coyote and 15.18 ± 3.37 µmol/g/min in the bald eagle.

Conclusion: Poisoning in wildlife with acetyl cholinesterase inhibitor pesticides continues to be a regular occurrence in the Canadian prairies. Increased surveillance and monitoring of pesticide use should be considered to mitigate future poisonings.

Keywords: Acetyl cholinesterase inhibition; Bald eagle; Coyote; Pesticide; Poisoning; Retrospective

Introduction

Acetyl cholinesterase inhibitor insecticides have a longstanding history of use in Canadian agriculture. Following the nation-wide ban of the organochlorine class of insecticides in the early 1970s, organophosphate and carbamate insecticides were introduced as alternatives for pest management [1-2]. The major advantage associated with the introduction of these insecticides was low environmental persistence coupled with efficacious insect control.

The major disadvantage associated with use of acetyl cholinesterase inhibitor pesticides is the non-target poisoning in domestic and wildlife animal species. The acetyl cholinesterase inhibitor pesticides act to inhibit the acetyl cholinesterase enzyme. The nervous system of vertebrates is highly conserved throughout evolution and thus renders any animal species vulnerable to poisoning. Phosphorylation (by organophosphate agents) or carbamylation (by carbamate agents) of acetyl cholinesterase prevents the enzyme's ability to hydrolyse acetylcholine [3-4]. Consequently, uncontrolled binding of acetylcholine to post-synaptic neurons will occur. Stimulation of nicotinic acetylcholine receptors and muscarinic acetylcholine receptors at the postsynaptic neurons results in the rapid onset of classical symptomatology [3].

Clinical signs of acetyl cholinesterase inhibitor exposure may be divided into three categories: muscarinic, nicotinic, and central [4]. Predominant symptomatology includes miosis, salivation, lacrimation, urination, defecation, involuntary muscle fasciculation, dyspnea, bronchoconstriction, and death from respiratory failure [3-4].

Extreme toxicity (e.g. rat oral LD50 of carbofuran=6.0-7.8 mg/kg body weight) and extensive availability of acetyl cholinesterase inhibitor insecticides enables the accidental and intentional animal poisoning with these agents [5]. Exposure to animals can occur through multiple routes: inhalation of sprayed agricultural pesticides (i.e., primary toxicity), consumption of contaminated water or food,

dermal contact for insect control in fur (depending on the agent or formulation used), and secondary toxicity from the ingestion of animals previously poisoned with acetyl cholinesterase inhibitor pesticides [1-6].

Targeted poisoning of coyotes (*Canis latrans*) with organophosphate and carbamate pesticides has been identified in Canada and the United States [1-8]. The continued economic losses from coyote-related livestock kills have deemed the animal a nuisance for livestock producers. Coyotes will aggressively prey upon livestock, especially young, old, or unthrifty animals [9]. In Canada, legislation is in place to control coyote populations and reduce livestock attacks through various methods of hunting, trapping, and poisoning. The latter means of coyote control is controversial and highly regulated. Registered lethal poisons for coyote control include sodium cyanide gas injection cartridges (Alberta only), sodium fluoroacetate tablets (Alberta and Saskatchewan only), and toxic neck collars [9,10]. Despite the availability and potency of approved pesticides for coyote control, occurrence of illegal coyote poisoning with acetyl cholinesterase inhibitor pesticides remains a regular occurrence [2].

A consequence of off-label pesticide use may be the secondary poisoning of scavenger species [2]. Scavengers, including eagles, magpies, ravens, foxes, and skunks, may opportunistically feed on animal carcasses. Scavengers can become poisoned through ingestion of either (1) toxic bait placed with intent to poison or (2) toxic carrion of animals that died from primary poisoning. In these instances, the poisoning event can be referred to as secondary toxicity, as the scavenger species were not the intended target of the toxicant [2]. This is also referred to as "relay toxicity". Multiple scavengers can become poisoned from a single intoxication event due to relay toxicity [1].

Multiple factors influence poisoning occurrence with these agents. The ingested pesticides remain toxic in the muscle and organs of a poisoned animal post-mortem [7]. In addition, the rate of biotransformation in the environment and tissue half-life of these pesticides are partially dependent on the weather conditions. Breakdown of these agents is more expedient in warmer climates (i.e., May through August in Canada) than in colder conditions (i.e., September through April in Canada) [11]. The biology of the scavenging species (i.e., time of year associated with scavenging) is important to consider as well. These factors affect the period in which an animal can suffer secondary toxicity.

A top predator that employs scavenging as a feeding strategy is the bald eagle (*Haliaeetus leucocephalus*) [7,8,12]. The association between deaths in coyotes and secondary toxicity bald eagles from acetyl cholinesterase inhibitor poisoning has been identified previously in both Canada and the United States [7,8,12]. Coyotes are perceived as pests to livestock producers, leading to the baiting of materials to poison coyotes. Bald eagles may scavenge on the toxic coyote carcass and become poisoned. The predominance of agriculture in Canada (most notably the prairie provinces of Alberta, Saskatchewan, and Manitoba) and the United States has led to a continual reliance on acetyl cholinesterase inhibitor pesticides in agriculture.

The primary objective of this paper is to describe epidemiological information on the occurrence of poisoning cases with anticholinesterase pesticides in coyotes and bald eagles in Western Canada. A secondary objective is to ascertain whether there is an association between these occurrences. Information presented in this study may aid regulatory agencies responsible for the management of pesticides in the refinement of anticholinergic pesticide approval and renewal strategies.

Materials and Methods

Epidemiologic case information from confirmed lethal anticholinesterase poisoning in coyotes and bald eagles across the Canadian prairies was obtained from the records of Prairie Diagnostic Services (PDS) located in the Western College of Veterinary Medicine (WCVM) in Saskatoon, Saskatchewan. Toxicological testing was conducted in all of the cases upon submission from 1998 to 2013. Submissions were only encountered from the Canadian provinces of Alberta, Saskatchewan, and Manitoba. A modification of the Ellman method enabled the determination of acetyl cholinesterase enzyme activity in brain specimens [13]. Units of enzyme activity were reported as micromoles of substrate hydrolyzed per minute per gram of brain tissue, wet weight (µmol/min/g). Acetyl cholinesterase enzyme suppression was compared to previously established reference values in the species of interest to designate toxicity and/or exposure to anticholinergic agents (Hill and Fleming; Blakley and Yole 2002). Normal brain acetyl cholinesterase values were considered to be the mean ± 20% [1,14]. Brain acetyl cholinesterase suppression of 50% or greater was considered to be consistent with acute intoxication [1,14]. Annual and temporal (monthly) case incidence from 1998 to 2013 in both species was examined. Cases of poisoning in both species in close proximity to one another were identified; the co-occurrence of poisoning events in both species was compared using correlation coefficients. Statistical differences in case occurrence by year and month in each species were determined by chi square analysis ($p<0.01$).

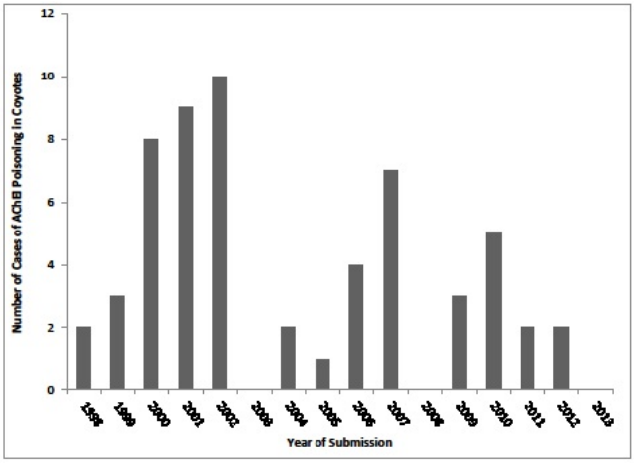

Figure 1: Incidence of confirmed anticholinesterase pesticide poisoning in the coyote (*Canis latrans*) over a 16 year period in Western Canada (n=58). The probability of no year effect determined by Chi Square Goodness of Fit is $p<0.0001$.

Results

Prairie Diagnostic Services identified cases associated with clinical manifestations and death due to acetyl cholinesterase inhibitor pesticide exposure in 58 coyotes and 60 bald eagles in the Canadian prairies over a 16 year period.

Coyotes poisoned with acetyl cholinesterase inhibitor pesticides were observed in 13 of the 16 years. Case submissions to PDS were absent in 2003, 2008, and 2013. Coyote case distribution was influenced by year ($p<0.0001$) (Figure 1). Years 2000, 2001 and 2002 had the highest occurrence of coyote submission and represented 46.6% of coyote cases. Poisoned coyotes were most frequently submitted in the springtime months of April and May ($p<0.0001$) (Figure 2), which accounted for 53.5% of the confirmed coyote cases during the investigational period.

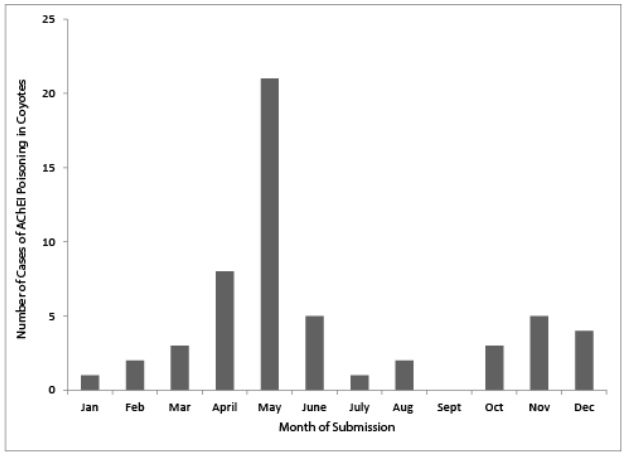

Figure 2: Seasonal incidence of anticholinesterase pesticide poisoning in the coyote (*Canis latrans*) in Western Canada (n=58). The probability of no seasonal effect determined by Chi Square Goodness of Fit is $p<0.0001$.

Bald eagle poisoning varied by year ($p<0.0001$) and season ($p<0.0001$). Poisoned bald eagle cases were highest in the years of 2000, 200, and 2004 (Figure 3). These years comprised 43.3% of the cases submitted. Submissions were absent in 2003 and 2008. The occurrence of eagle poisoning with acetyl cholinesterase inhibitor agents occurred most often during two time periods: the spring (May and April) and mid-winter (December and January) (Figure 4). These time periods accounted for 33.3% and 35% of poisoned bald eagle cases respectively.

Annual and temporal trends in the poisoning in coyotes and bald eagles showed some similarity (Figures 1-4). The correlation coefficients of these trends were 0.48 and 0.45, respectively. Annual incidence of coyote and bald eagle poisonings tended to show correlation but was not statistically significant ($p=0.0599$). The correlation of temporal incidence of coyote and bald eagle poisonings with Acetyl cholinesterase inhibitor agents was not significant ($p=0.1421$).

Species	Mean ± SD (n)
Bald Eagle	15.18 ± 3.37 (52)
Coyote	3.44 ± 1.52 (13)

Table 1: Reference brain cholinesterase values in the bald eagle (*Haliaeetus leucocephalus*) and the coyote (*Canis latrans*). Enzyme activity levels are reported as units of micromoles of acetylthiocholine idodide hydrolyzed per minute per gram of brain tissue (wet weight) at 25°C.

Mean normal reference brain acetyl cholinesterase activities were determined to be 3.44 ± 1.52 µmol/min/g in the coyote and 15.18 ± 3.37 µmol/g/min in the bald eagle (Table 1).

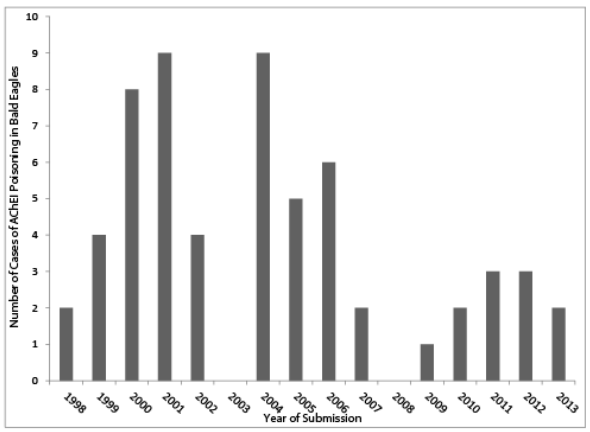

Figure 3: Incidence of anticholinesterase pesticide poisoning in the bald eagle (*Haliaeetus leucocephalus*) over a 16 year period in Western Canada (n=60). The probability of no year effect determined by Chi Square Goodness of Fit is $p<0.0001$.

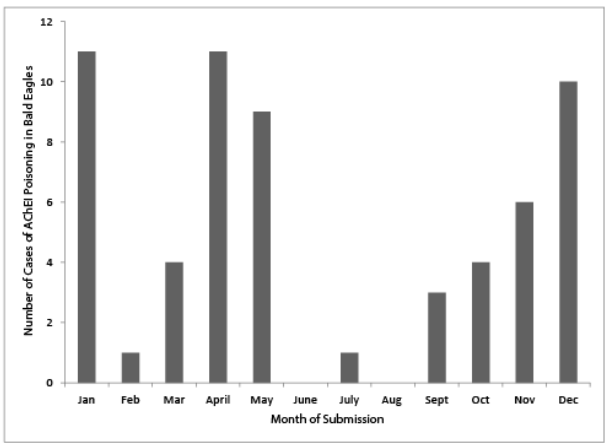

Figure 4: Seasonal occurrence of anticholinesterase pesticide poisoning in the bald eagle (*Haliaeetus leucocephalus*) from 1998 to 2013 in Western Canada (n=60). The probability of no seasonal effect determined by Chi Square Goodness of Fit is $p<0.0001$.

Discussion

Acetyl cholinesterase inhibitor poisoning of non-target wildlife species is typically an annual occurrence in the Canadian prairies. The diagnostic toxicology laboratory Prairie Diagnostic Services confirmed 58 cases of poisoning in coyotes and 60 cases in bald eagles with anticholinesterase pesticides through postmortem testing over the 16-year period of 1998 through 2013.

Reference normal brain acetyl cholinesterase concentrations in non-poisoned animals are the basis for the determination of toxicity in cases of suspected poisoning [1,14]. Enzyme suppression is estimated by dividing the activity of the sample by the reference value for that species. Suppression of 50% or greater is consistent with lethal exposure and death. The brain tissue from 52 and 13 non-poisoned bald eagles and coyotes submitted during the investigational period were determined to have reference acetyl cholinesterase activities of 15.18 ± 3.37 μmol/min/g and 3.44 ± 1.52 μmol/min/g, respectively. These values are comparable to previously published values in the two species [14]. Substantial inter- and intraspecies variation is evident.

Coyotes with acetyl cholinesterase inhibitor poisoning were submitted for diagnostic testing most frequently during the months of April and May. One explanation for the increase in case number at this time of year is calving in livestock operations. It is reasonable to speculate that the presence of calves increase the likelihood of livestock coyote attacks. Coyote exposure to pesticide spray drift alone is unlikely since many of the recorded deaths were observed during the spring before the spraying season. Illegal use of pesticides may represent a method of coyote control to prevent livestock deaths. Previous studies reported livestock carcasses laced with anticholinesterase pesticides were used as coyote bait [1]. A study from Kansas, USA reported that carbamate-laced sheep carcasses were found in the same field as the carcasses of three bald eagles, two coyotes, and a red-tailed hawk (*Buteo jamaicensis*) [1]. These animals were determined to have died of toxic carbofuran exposure. Individuals admitted that the poisoned sheep carcasses were placed to kill coyotes [1]. Given the perceived severity of the coyote problem, legal means of control may seem insubstantial when faced with the potential losses. Strict limits on the quantities approved coyote-control poisons and reporting requirements to government authorities encourage non-compliance.

Submissions of bald eagles with acetyl cholinesterase activity suppression indicative of toxicity occurred most frequently in spring and mid-winter. Similar temporal patterns in the coyote and bald eagle in the springtime are suggestive of secondary toxicity and may be related to scavenging on poisoned baits placed for coyotes during calving season. Bald eagles scavenge carrion and prey upon moribund animals in addition to other feeding opportunities. Due to the rapid hydrolysis of organophosphate and carbamate pesticides at warm ambient temperatures, the proximity of dead eagles (and other wildlife species) to dead coyotes or poisoned livestock carcasses is often useful to identifying secondary toxicity. Proximity of carcasses and evidence of predation on coyotes by bald eagles has been noted previously in Western Canada [1-11].

The second temporal peak for bald eagle poisoning is the months of December and January (mid-Winter). Winter-time poisoning is contrary to registered use of acetyl cholinesterase inhibitor pesticides. This observation is suggestive of secondary toxicity. Lack of readily available food sources during the winter may influence bald eagles to scavenge carrion more extensively than in warmer months when prey is easy to access. However, it is not clear why coyote submissions were not increased during these months, as coyotes are suspected to be the primary target of poisoned baiting. In addition, migratory bald eagles overwinter in the United States and are not expected to be present in high numbers on the Canadian prairies in the wintertime. It is important to note that the timeframe for poisoning is greater during the winter months: animal corpses and associated insecticides in the tissues are preserved while frozen. It can be speculated that any remaining bald eagles during the winter would be more at risk to acetyl cholinesterase inhibitor pesticide exposure.

The seasonal distribution of bald eagle deaths found in this study are comparable to those reported in a study of the occurrence of organophosphate or carbamate pesticide poisoning in the bald eagle and the golden eagle (*Aquila chrysaetos*) in Western Canada [2]. The researchers found that eagle poisonings occurred most often during the months of April (n=14) and December (n=9). The authors attributed the decline in cases between the months of June and September to decreased baiting, increased carcass decomposition in warmer weather, and eagle migratory nesting patterns. These factors cannot be ruled out in the present study.

Raptor species, including the bald eagle, appear to be one of the most at-risk groups for secondary toxicity from acetyl cholinesterase inhibitor agents. In a study conducted from 1980 to 2000, the death of 158 bald eagles was attributed to exposure to Acetyl cholinesterase inhibitor pesticides [7]. A study of raptor poisoning in the United Kingdom, United States, and Canada found that ingestion of the gastrointestinal contents or surface materials of poisoned vertebrates was largely responsible for secondary toxicity in avian species [7]. Poisoning cases in coyotes and bald eagles were observed in most years from 1998 to 2013, with the exception of 2003 and 2008 for both species and 2013 for coyotes. It is compelling, yet unclear, why there was a lapse in cases from both species in 2003 and 2008. However, cases of poisoning in both species appear to have declined over time. Two possibilities may aid in the explanation of case decline over time. First is the ban of the common carbamate pesticide carbofuran in Canada in 2009 [15]. With the phase-out, it is expected that carbofuran misuse would decline over time. Second is the implementation of a pilot project for coyote control in Saskatchewan. Between November 2009 and March 2010, more than 71 000 coyotes were exterminated in the province [16]. A bounty was paid as an incentive for participation in the project. Decreased acetyl cholinesterase inhibitor poisoning occurrence in recent years may reflect success of both the carbofuran ban and the Saskatchewan coyote control project. Similar projects were not enacted in Alberta and Manitoba, thus the influence of the Saskatchewan bounty program in these provinces is unknown.

The occurrence of poisoning on an annual basis is indicative of a multifactorial problem in Western Canada. Incidences of coyotes and bald eagles poisoned with anticholinesterase pesticides highlights the need for continued monitoring, submission, and testing of wildlife carcasses in Western Canada. Further, increased surveillance on the use of acetyl cholinesterase inhibitor pesticides is required on the Canadian prairies. Diagnostic toxicological testing for acetyl cholinesterase suppression should be routinely considered for dead wildlife in areas of intense agricultural operations.

Conclusion

Caudal neostigmine can be used safely as a model of fast–track anesthesia for congenital heart surgery; also it provides effective analgesia with opioid sparing effects. This allows faster recovery of patients, and helps to provide an efficient and cost-effective service. However, better control of postoperative vomiting by antiemetic prophylaxis after caudal neostigmine is required.

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