Research in Veterinary Science 102 (2015) 142–149

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/yrvsc

Poisoning of cats and dogs by the carbamate pesticides aldicarb and carbofuran

Adriana de Siqueira [a,*], Fernanda Auciello Salvagni [a], Alberto Soiti Yoshida [c], Vagner Gonçalves-Junior [b], Atilio Sersun Calefi [a], André Rinaldi Fukushima [a,b], Helenice de Souza Spinosa [a,b], Paulo César Maiorka [a]

[a] Department of Pathology, School of Veterinary Medicine and Animal Sciences, University of São Paulo, São Paulo, Brazil
[b] Laboratory of Toxicology, Department of Pathology, School of Veterinary Medicine and Animal Sciences, University of São Paulo, São Paulo, Brazil
[c] Institute of Criminal Sciences, Sao Paulo State, Brazil

ARTICLE INFO

Article history:
Received 13 August 2014
Received in revised form 12 June 2015
Accepted 6 August 2015

Keywords:
Aldicarb
Carbofuran
Carbamate pesticides
Cats/dogs
Forensic veterinary medicine
Animal cruelty

ABSTRACT

The intentional and accidental poisoning of animals and people is a threat to public health and safety worldwide. Necropsies and histopathological examinations of 26 cats and 10 dogs poisoned by the carbamates aldicarb and carbofuran, confirmed by thin layer chromatography (TLC) and high performance liquid chromatography with diode-array detector (HPLC-DAD) were analysed, with variable *post mortem* interval and conservation of the carcass. Biological matrices were collected for toxicological and histopathological analyses. High performance liquid chromatography with diode-array detector (HPLC-DAD) was utilized to detect aldicarb and its metabolites, aldicarb sulphoxide and aldicarb sulphone, and carbofuran. The variable *post mortem* interval and the method of conservation of the carcass may be harmful to toxicological, necroscopic and histopathological analyses, that should be performed in order to provide reliable evidences to investigate possible poisoning of animals, which is cruel crime, and are usually linked to domestic or social conflict.

1. Introduction

The intentional and accidental poisoning of animals and people by carbamates aldicarb and carbofuran is a threat to public health and public safety worldwide (Grendon et al., 1994; MacFarlane et al., 2011; Otieno et al., 2010; Proença et al., 2004). Since October 2012, the Brazilian Health Surveillance Agency (ANVISA) has prohibited the utilisation of aldicarb in Brazil, known in the criminal scenario as "chumbinho", however this action has not yet stopped or even decreased the cases of poisoning by this substance. Aldicarb and carbofuran are known for their action as reversible inhibitors of the acetylcholinesterase enzyme (Baron and Merriam, 1988; Risher et al., 1987). They are commonly and illegally used for killing animals and people (Motas-Guzmán et al., 2003; Novotný et al., 2011; Rebelo et al., 2011; Xavier et al., 2007c); used as a suicide/homicide method (Proença et al., 2004; Rizos et al., 2004) or misused as illegal rodenticide (Campelo and Caldas, 2010; Centers for Disease and Prevention, 1997; Lima and Reis, 1995; Nelson et al., 2001; Rebelo et al., 2011). These substances are easily obtained and readily consumed by the target species because they can be easily mixed with canned pet food, meat, fish and put inside sausage, which is frequently utilized as bait, generally in a single lethal dose (Frazier et al., 1999; Motas-Guzmán et al., 2003; Xavier et al., 2007b,c). The owner usually finds the animal already dead or presenting severe clinical signs common in the cholinergic crisis such as dyspnoea, diarrhoea or seizure (Khan, 2012; Risher et al., 1987; Rizos et al., 2004), which requires immediate veterinary intervention. Vomit and/or diarrheic contents may be found near the animal, in addition to the remains of the poisoned food (Frazier et al., 1999; Motas-Guzmán et al., 2003; Xavier et al., 2007c).

Owners of cats and dogs have sought police enforcement in order to report such deaths under suspicious circumstances, and a police report is produced, which may culminate in a criminal investigation. The Brazilian Federal Environmental Law number 9605 from February 12, 1998 considers animal cruelty as a crime. However, recent pending changes in the Brazilian Penal Code seek to consider it a crime also under civil and criminal instances, instead of only environmental, so malicious acts that culminate in the suffering and death of an animal, such as intentional poisoning, would have an increased penalty. This situation reinforces the importance of the research and the use of a forensic approach to analyse crimes against animals, as shown in recent studies (Byard and Boardman, 2011; Cooper and Cooper, 2008; Gerdin and McDonough, 2013; Munro, 1998; Munro and Munro,

Abbreviations: AChE, acetylcholinesterase; ACh, acetylcholine; ACN, acetonitrile; ASN, aldicarb sulphone; ASX, aldicarb sulphoxide; HPLC-DAD, high performance liquid chromatography with diode-array detector; TLC, thin layer chromatography.

* Corresponding author at: Department of Pathology, School of Veterinary Medicine, University of São Paulo, Av. Prof. Dr. Orlando Marques de Paiva, 87, 05508 270, Cidade Universitária, São Paulo/SP, Brazil.
E-mail address: siqueira.adriana.vp@gmail.com (A. de Siqueira).

http://dx.doi.org/10.1016/j.rvsc.2015.08.006
0034-5288/

2013; Newbery and Munro, 2011). In humans, the autopsies of cases where the death has occurred under suspicions, violent or unknown circumstances are performed by forensic pathologists in medicolegal laboratories (Fierro, 2005; Law et al., 2012; Molina et al., 2007; Randall et al., 1998). In animals, necropsies are performed by veterinary pathologists or veterinarians, which should be made aware of the possible circumstances that lead to the death of the animals, and follow established protocols, both for the necropsy and samples collection for toxicology and histopathology. A toxicologist should always be consulted about the appropriate matrices according to the pathologist's suspicions, as well as the best methods of handling of the samples, in order to obtain consistent and reliable results (Byard and Boardman, 2011; Cooper and Cooper, 2008; Drummer et al., 2013; Flanagan et al., 2005; Munro, 1998; Wyman, 2012).

The majority of the animals fatally poisoned by carbamates usually presents a set of non-specific gross and histopathological findings, such as systemic congestion and multiple areas of haemorrhage (Grendon et al., 1994; Novotný et al., 2011; Xavier et al., 2007b). Although gross and histopathological analyses are commonly impaired by the conservation of the body, such as freezing, or autolysis and putrefaction due to the exposure to environmental conditions, a thorough necropsy and a histopathological analysis are crucial for investigating diseases or other potential cause of death (Fierro, 2005; Munro, 1998; Chatelain et al., 2012).

A recurring problem is that the crime scene in the intentional poisoning of animals is rarely preserved or examined thoroughly, what impairs the criminal investigation (Merck, 2012; Sinclair et al., 2006). Because of the lack of an expert examination of a crime scene involving animals, the chain of custody can only be initiated and secured once the animal is brought to the necropsy and receives a registration number and can be maintained and tracked along with the registration of samples to be sent to the toxicology laboratory (Flanagan et al., 2005; Wyman, 2012).

The toxicological screening plays a crucial role in medicolegal cases. The accurate identification of possible toxic agents may take a long time. Because of the legal potential of the case, to ensure the reliability of the results, samples should be maintained under a rigorous chain of custody (de Zeeuw, 2004; Drummer et al., 2013; Skopp, 2010; Wyman, 2012). Toxicological analysis is necessary for the confirmation of substances such as aldicarb and carbofuran and their metabolites because of their similar gross appearance and necropsy findings. High performance liquid chromatography with diode-array detector (HPLC-DAD) has been applied for the purpose of identification of different metabolites (Harper et al., 1998; Otieno et al., 2010). For example, the metabolites aldicarb sulphone (ASN) and aldicarb sulphoxide (ASX) have higher anticholinesterase action than aldicarb; ASX is 23 times more effective than aldicarb and 60 times more effective than ASN (Montesissa et al., 1994).

The aim of this study is to present the main gross and histopathological findings of carbamate-poisoned cats and dogs, in combination with the results of toxicological screening by TLC and HPLC-DAD of samples collected from the deceased animals, and to highlight the important contribution of such analyse to the criminal investigation.

2. Materials and methods

We analysed the necropsies and histopathological findings of 26 cats and 10 dogs with carbamate poisoning confirmed by thin layer chromatography (TLC) screen, followed by high performance liquid chromatography with diode-array detector (HPLC-DAD). A control group of 26 cats and 10 dogs with similar lesions, but tested negative to the carbamates was also evaluated. These necropsies were performed in the Animal Pathology Service of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo (FMVZ-USP), Brazil, between August 2011 and August 2013. The gross changes were photographed, and tissue samples and other biological matrices, such as peripheral blood and vitreous humour, were collected for histopathological and HPLC toxicological analysis. Gastric content was screened by TLC before the HPLC analysis. Thirty six samples were positive for aldicarb, and one animal had no sufficient gastric content due to vomiting, and few black granules were localized on its esophageal mucosa.

The animal species, age, gender, breed, body conservation, estimated time elapsed between death and necropsy, carcass conservation, necropsy requester and the owner's information about the possible circumstances of death were also recorded. Whenever available, police reports were also analysed to obtain additional information, such as possible suspects and their motivations to kill the animal and the findings at the crime scene. A database was developed using the software Excel®.

2.1. Statistical analysis

Comparisons between the poisoned animals and control groups' gross and histopathological findings were performed using two-tailed Fisher's exact test. Only values of $p < 0.05$ were considered significant for all of the analyses. The statistical tests were performed with GraphPad Prism software, version 6 (Analytical Software, San Diego, California, USA).

2.2. Necropsy and histopathological analysis

We made a complete photographic documentation from the necropsy using a graduated ruler with the animal identification in each picture. All the external changes, including *post mortem* alterations, such as the evidence of autolysis and putrefaction and presence of maggots were recorded. During internal examination, all the viscera were evaluated and photographed. Whenever possible, we collected up to four fragments measuring approximately 1 cm^3 of the lung, heart, stomach, liver, kidney and pancreas. The brain was divided in 4 parts: cerebrum, cerebellum, pons and medulla, and one of each was collected. Tissue samples were fixed in a 10% buffered formalin solution and processed for histology. Histological slides were stained with haematoxylin and eosin, and evaluated under a light microscope by two veterinary pathologists.

2.3. Toxicological analysis

2.3.1. Collection of material

At necropsy, whenever possible, we collected and individually packed gastric content, peripheral blood and samples of liver, vitreous humour and lungs. All samples were identified and sealed in a single plastic bag and transfered to the Laboratory of Toxicology, where the samples were registered and maintained at −20 ° C until the analysis, which did not exceed 10 days.

2.3.2. Thin layer chromatography (TLC) screening

Gastric contents were screened by TLC. The technical pattern of aldicarb used in the chemical analysis showed 99.7% purity. The following reagents were utilized: dichloromethane, acetone, petroleum ether, sodium chloride, anhydrous sodium sulphate, platinum chloride and potassium iodide. All analytical procedures were performed in a laminar flow hood. TLC was performed using solid phase (adsorbent) chromatoplates made from silica gel 60, without a fluorescent indicator, with the dimensions of 10 x 20 cm. Activation of the plate was performed in an oven (100 ° C) for at least two hours before use. The aldicarb was extracted from the matrix employing a solution of dichloromethane:acetone:petroleum ether (1:1:1) and it was analysed using technical standards, using as eluent solvent system consisting of n-hexane -acetone (4:1).

Samples were considered positive when discolouration of the chromogenic reagent (iodine platinum) was observed to form small