Intentional Carbofuran Poisoning in 7 dogs

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Abstract

Background: Carbofuran was a widely used board-spectrum pesticide, which despite his strict regulation and bans for more than one decade is still encountered in cases of intentional poisoning in dogs and wildlife. The objective of the study was to provide a complete and detailed description of the pathological, histological and toxicological findings of the 7 cases of intentional carbofuran poisoning in dogs.

Results: In this retrospective study 7 cases of carbofuran intoxication recorded from July 2015 to June 2017 were analyzed. Following complete history recording, all cases were examined by complete necropsy and histopathology. The carbofuran intoxication was confirmed in all cases by gas-chromatography. The postmortem examination revealed extensive haemorrhages and congestion located mainly within the respiratory, nervous and cardiovascular systems, accompanied by degeneration and necrosis within the lung, heart, and kidneys.

Conclusions: Although banned in the European Union, carbamate poisoning is still frequently encountered, especially in wild animals. This paper will contribute to a better understanding of occurrence and pathogenesis of acute carbofuran exposure in dogs, and add some peculiar pathological features of this poisoning to current literature.

Background

Carbofuran (2,3-dihydro-2,2-dimethyl-7 benzo furanyl methy carbamate) was one of the most frequently encountered carbamate. It is used in the agriculture from the 1970`s (31) till it was banned since 31.12.2009 in the USA (41), and since 05.12.2011 in Romania, by the Law of Chemical Substances nr 254.

Furadan or otherwise can still be sourced from several countries as Tanzania and Uganda. Indeed, a recent survey indicated that carbofuran was readily available in about 80% of the sampled agro vet stores in Uganda (27).

The most commonly marketed carbofuran were Furadan, Bay 70143, Curater, D 1221, Yaltox, Furacarb, ENT 27164 (39,4). Carbofuran is available in granular, liquid and powder formulations (42, 39) it is used in agriculture and forestry as a board-spectrum systemic insecticide, nematocide and acaricide (19, 27).

Although this substance was banned almost ten years ago, our cases show that it is still encountered in cases of intentional poisoning.

The main objective of this manuscript is to present the gross and histopathological findings in a serial case of carbofuran poisoning in dogs in combination with the results of toxicological screening by GS-MS/MS of samples collected from the deceased animals, and also to point the important contribution of such analysis to the criminal investigation.

Exposure to carbamates can occur by oral ingestion, inhalation or dermal absorption. Given the high toxicity of carbamates any usage errors, regarding mixing or storage of this substance can lead to intoxication.

The oral LD50 is about 3-19 mg/kg of body weight in different animal species (40). The oral LD50 for dogs is 19 mg/kg and the LC50 for inhalation is 52 mg/kg (38). Younger animals can be intoxicated by a smaller dose than adults, because of their underdeveloped enzymatic system (29). Repeated exposure like frequent spraying can also cause intoxication. Animals can be intoxicated only by licking the empty pack.

Carbofuran is metabolized in 3-hidroxycarbofuran and 3-cetocarbofuran, two highly toxic metabolites. Metabolism of carbamates in the organism is fast, and a great part of metabolic activation and detoxification is

taking place in the liver. The excretion of the metabolites is urinary and digestive; residues can be detected in feces, saliva and milk (18). Vomit and/or diarrheic contents may be found near the animal, in addition to the remains of the poisoned food (15, 45).

In animals, necropsies are performed by veterinary pathologists or *veterinary general practitioners*, which should be made aware of the possible circumstances that lead to the carbofuran-related death of the animals, and follow established protocols, both for the necropsy and sampling for toxicology. A toxicologist should always be consulted about the appropriate matrices according to the pathologist's suspicions, as well as the best methods for handling the samples, in order to obtain consistent and reliable results (6, 8, 12, 14, 25, 43).

The majority of the animals fatally poisoned by carbamates usually present a set of non-specific gross and histopathological findings, such as systemic congestion and multiple areas of haemorrhage (17, 28, 44).

Results

The toxicological examination established the diagnosis by gas chromatography; carbofuran was detected in all of the cases.

The pathological findings for each case are presented separately in Table 1, and we described below the most relevant gross and histopathological findings following carbofuran intoxication.

The external examination of the body revealed dried saliva around the oral cavity and a pink color around the mouth (Fig. 1.-B), and on some parts of the body, usually on the lateral parts where the animal could have touched his fur with the mouth (Fig. 1.-C). At four dogs we found epistaxis.

At eye level, we noticed multifocal 3-rd eyelid haemorrhages, associated with diffuse uveal congestion and hyphema (unilateral or bilateral). (Fig. 1.-D, E)

At three dogs subcutaneous and muscular haemorrhages were present.

Generally, the gastric content was composed of an unknown pink-colored foreign substance mixed with bread or meat, with chicken parts, even feathers. In the pharynx and esophagus pink coloration of the mucosa was noticed. The small intestine and its content were colored in pink too. Also, micro haemorrhages of the colon were found.

Histology revealed that the stomach mucosa had desquamated to catarrhal inflammatory lesions, mainly involving the superficial epithelium and congestion in the deep part of lamina propria and submucosa.

One dog presented a diffuse, acute, minimal hepatic congestion and another one had diffuse splenic congestion.

Necropsy revealed in the cardiovascular system haemorrhagic pericardial content and in some case subendocardial congestions (Fig.2.-A) and haemorrhages. In the myocardium diffuse and congestions (Fig.2.-B) and haemorrhage were found. In the myocardium of an individual, the myocardial fibers were replaced by fibrous tissue, with some atrophied myofibers sequestrated in the scar tissue, which could be the consequence of a chronic myocardial infarct.

In the upper respiratory tract, the larynx and trachea had diffuse congestion and the lungs in most of the cases had shown acute, severe, diffuse, bilateral pulmonary congestion and edema (Fig.3.–A), with multifocal petechial

and ecchymosis.

Histologically in the lungs, severe vascular changes were detected, i.e. septal congestion (Fig.3.-C) associated with the presence of numerous siderocytes, and diffuse edema in the alveoli, bronchioles and bronchi (Fig.3.-B).

In the brain, the main findings were bilateral meningeal and cerebral acute congestion (Fig.4.-A) with occasional petechial. Histologically, the main changes detected in the brain were represented by cerebral congestion and gliosis, including the presence of glial nodules and discrete vascular cuffing (margination) mainly with lymphocytes. Additionally, some neurons presented in the cytoplasm a dark brown material (most likely lipofuscin). The lepto-meningeal blood vessels underwent congestion associated with local edema (Fig. 4.-B).

Bilateral, diffuse renal congestion accompanied by tubular degeneration and necrosis was recorded in all cases. Histologically, cortical-medullar congestion (Fig.4.-C), vacuolar degeneration of the epithelium of the renal cortical tubules (Fig.4.-D), congestion of the glomerular tuft and Bowman's capsule thickening, associated with the presence of a proteinaceous (hyaline) material in the urinary space were found.

Discussion

Residential and industrial use of carbamate and organophosphate pesticides was widespread in the United States. According to the 1997 findings of the US Environmental Protection Agency, over 40 organophosphate pesticides and 22 carbamate pesticides were listed among 900 pesticides which pose the highest risks to human health were registered for use in the United States (3). Both organophosphate and carbamate pesticides primarily target of action in the nervous system of insects. Exhibiting many structural similarities with naturally occurring compounds, organophosphate and carbamate interfere with the conduction of signals and cholinergic reactions in the nervous system of insects via inhibiting the release of the enzyme acetyl cholinesterase at the synaptic junction. Eserine, parathion, and malathion are further examples of cholinesterase inhibitors responsible for the hydrolysis of body choline esters, including acetylcholine at cholinergic synapses (3, 18).

Basically, organophosphates and carbamates are neurotoxicants, but directly or indirectly several vital organs are affected, as these chemicals produce a variety of toxicological effects on the central nervous system, peripheral nervous system, cardiovascular, pulmonary, ocular, neurobehavioral, immunological, reproductive, placental, cutaneous, and other body systems. In addition, these insecticides cause neurodegeneration, oxidative stress, endocrine disruption, and many other toxic effects (3).

Carbofuran (Furadan) after almost a decade of banning is still causing intoxications amongst animals (10). Illegal poisoning of wildlife and domestic animals is a worldwide issue (28).There are high numbers of carbofuran poisonings in birds, Novotny et al (28) found sporadic cases of small carnivore intoxication, although martens and foxes are thought to be the main object of poisoners. Also, domesticated animals such as pets, mainly dogs, and livestock are at risk of being poisoned with carbofuran (40). The clinical signs in accidental or intentional carbamates poisoning are unspecific reflecting a combination of muscarinic and nicotinic hyperstimulation.(18)

Carbamates are reversible acetylcholinesterase inhibitors, derived from carbamic acid. Carbamate causes inhibition of the activity of acetylcholinesterase, which is an enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine in two separated components: choline and acetic acid (16, 21). This results in an excess of acetylcholine in the synaptic cleft and prolonged binding to the post-synaptic receptors (32). AChE inhibition is causing hyperstimulation of cholinergic receptors, followed by muscarinic, nicotinic and central nervous signs. AChE inhibitors may also impair the endothelial function, due to their toxicity to endothelial cells (20, 46) and

to the vascular wall (46). The overstimulation of the somatic nervous system usually results in tremors, muscle twitches, and piloerection, as well as ataxia and paresis. Cholinergic tracts are also important to both the parasympathetic and sympathetic autonomic nervous systems, but especially to the former. They conduct impulses from the neural ganglia to a multitude of organs such as the heart, the endocrine glands, and the digestive system (27).

Systemic effects may occur in less than 30-60 minutes, generally after 6 hours, and rare after 12 hours. Muscarinic symptoms are associated usually with SLUDGE symptoms (salivation, lacrimation, urination, diarrhea, gastroenteritis) in addition to bradycardia, dyspnea, and miosis. Local effects usually occur because of direct contact with the product. Symptoms can be observed after a few minutes or can be delayed several days, in the case of cutaneous exposure (30).

Intoxication with a cholinesterase inhibitor may lid too apparently opposite clinical signs like individuals who may show either constriction or dilation of the pupils, or a speeding up or slowing down of the heartbeat.

The autonomic nervous system is a constant subject of adjustment through feedback mechanisms, and because of this, each individual may react differently to various levels of cholinergic stimulation. Death usually occurs due to respiratory failure and cardiac arrest (19).

The7 cases described here are a clear example of carbofuran used for intentional poisoning in dogs. In most of these confirmed-cases, the results were further used by authorities for legal investigations. However, based on the information of the police reports, there are common reasons to kill both dogs and cats, and these can be related to domestic or social violence (1, 2, 11).

The investigation in cases of intentional animal poisoning is as serious as those in human cases (23, 26), yet it is a very challenging and difficult activity (8).

The investigation of an incident that involves the death of wildlife specimens generally consists of a field inquiry, a postmortem examination and when necessary, chemical analysis to determine whether a poison might be responsible (5). For pesticide detection, multistage mass spectrometry (MS/MS) is considered a very useful tool to detect low levels of an analyte when coupled with chromatographic techniques (22). For our toxicological analysis, we used a triple quadrupole mass spectrometry analyzer, operated in the selective reaction monitoring mode which improved significantly both the sensitivity and selectivity of the analytical determination, as the Luzardo et al (22) developed method for the identification of 117 Pesticides. The differences consist in the type (they used liver) and the weight (2 g sample) of the sample and there are some differences referring to the dilutions and to the quantity of the solvent. As a similarity the use of sonication should be mentioned, which improves the extraction efficiency and improve the recoveries of certain key pesticides, such as carbofuran. Therefore Luzardo et al (22) added a 5-min sonication to the extraction protocol, which in our cases lasted 15-min. Another method using homogenized 2 g liver samples is based on a new analytical multiclass method named QuEChERS technique (35), developed by Sell et al. and validated according to the requirements of SANCO/12571/2013 (34).

In our study, the pathological exam revealed predominant pulmonary lesions. Thus, the carbofuran poisoning induced respiratory and cardiac depression, which led to the death of the dogs. The hyperstimulation affects the vascular tone and cell permeability and also the tissue perfusion (9) that could cause interstitial blood pooling (congestions) and edema. Like Motas-Gusman et al (24) we found acute pulmonary congestion, pulmonary edema, and emphysema, but without constrictions and bronchial rupture. Pulmonary haemorrhage is typically described especially in acute intoxication cases (18), lesions which were present in our study too. Novotny et al (28)

revealed dried saliva around the oral cavity, congestion of the organs and haemorrhagic necrosis of the small gut. In our study we found only one dog with foamy salivation, four presented epistaxis and four were stained around the oral cavity with pink colored foreign substance (interpreted as being the consumed carbofuran).In 6 cases, we observed ocular changes consisting of conjunctival haemorrhages or congestion and unilateral or bilateral hyphema)(table 1).

Conclusions

Nowadays, although banned in the European Union, carbamate poisoning is still encountered, especially in wild animals. In this study, we describe the gross and histopathological changes present in 7 dogs acutely poisoned with carbamates. The most frequently encountered changes were located within the respiratory system and were represented by diffuse tracheal congestions, pulmonary congestion, haemorrhages, and edema. Also meningeal cerebral congestion and haemorrhage, along with the renal congestion, diffuse hepatic and splenic congestion were frequently observed. In some cases a pink foreign substance was found on the muzzle and on the fur. Correlated with the bait examination and toxicological results, this was interpreted as being the carbofuran-containing poison. This observation could be an indication for furadan intoxication for clinicians especially if it is associated with suggestive clinical signs or sudden death of the animals.

Methods

The seven cases (Tab.2.) of intentional carbofuran poisoning included in this study were identified in the archives of the Pathology Department of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. All cases were submitted for pathological diagnosis between 2015 and 2017, also all of these cases have a registered number from the Pathology Department of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, where all of the data can be found. In four cases the local authority requested detailed investigations, and in the rest of the three cases, the animals were submitted for necropsy by the owners.

This study was approved by our institutional ethics committee, named “Comisia de Bioetica” of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

In all cases, the clinical history, the pathological and toxicological findings (including the examination of the bite) were reviewed.

The baits were found near the animals, or in the gastric content presenting a pink colored mixture of bread or different kind of meat (sheep or chicken meat or viscera, including feathers).

A complete postmortem necropsy and histopathology examination were undertaken in all these cases less than 24 h after death.

For the histological examination, samples were fixed in 10% buffered neutral formalin, routinely embedded in paraffin, and 4 micrometers sections were realized, and stained in Hematoxylin-Eosin.

During the necropsy, samples of the gastric content and from the baits were collected and submitted for toxicological examination.

For this study, the toxicological exam was realized by gas chromatography coupled with triple quadrupole mass spectrometry (GC-MS/MS) using solid (baits) and liquid samples (gastric content) (34). The analysis was

conducted by the national reference laboratory of the National Sanitary Veterinary and Food Safety Agency of Romania in Cluj-Napoca, using the standard Romanian methods, and in all the cases the samples indicated carbofuran intoxication.

For the toxicological exam, the 5 g samples were extracted by acetone, homogenized after adding the methylene chloride and ethyl acetate and centrifuged. The whole organic extract was taken up and evaporated to dryness with nitrogen evaporator, then quantitatively passed into the vial of the gel-permeation purification equipment, by adding a mixture of cyclohexane and ethyl acetate. The purified samples thus evaporate to dryness at the nitrogen evaporator. The residue was taken up in hexane, stirred, sonicated, centrifuged and then injected into the GC-MS/MS equipment (36, 37).

List Of Abbreviations

AChe – acetyl cholinesterase

GC-MS/MS – gas chromatography –multistage mass spectrometry

H&E - Hematoxylin-Eosin;

QuEChERS –Quick, Easy, Cheap, Rugged and Safe

Declarations

- **Ethics approval and consent to participate**: For this study the authors have the approval of the ethics committee with 188 reference number, approved by the “Comisia de Bioetica” of the University of Agricultural Sciences and Veterinary Medicine and the owner’s approval.
- **Consent for publication**: Not applicable
- **Availability of data and materials**: The data that support the findings of this study are available at the Department of Veterinary Pathology, University of Agricultural Sciences and Veterinary Medicine, where the cases have registered number. Data are however available from the authors, upon reasonable request and with the permission of the Department of Veterinary Pathology, University of Agricultural Sciences and Veterinary Medicine.
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- **Authors' contributions:** DP drafted the manuscript and coordinated the interpretations. ALN helped to design the manuscript and completed the data analysis and interpretation. FT carried out the post mortem examination and helped to draft the necropsy analysis. AG was involved the histopathological data analysis. AO performed the toxicological analysis and revised the manuscript. CM helped to complete the revised article. All authors read and approved the final manuscript.
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Tables

Tab.1. The postmortem necropsy findings

Case Nr	External	Musculo-scheletal	Gastrointestinal and liver	Cardiovascular	Respiratory	Urinary	Central nervous System
I	Pink colored foreign substance (abundant) staying the fur around the mouth and muzzle	No significant findings	Pink colored foreign substance (abundant) staying the GI content (mainly bread) and mucosa of the upper GI Diffuse, acute, minimal hepatic congestion	No significant findings	Congestion and acute pulmonary edema (diffuse, acute)	Renal severe congestion, cortical-tubular necrosis (bilateral, diffuse, severe)	Meningeal cerebral congestion (diffuse, mild)
II	Pink-coloring the fur around the muzzle and on parts of the fur with a pink substance Conjunctiva hemorrhage of the third eyelid, acute, severe bilateral hyphema	Thoracic-cervical muscular and subcutaneous hemorrhages, petechial and ecchymosis (associated with hemorrhagic lymph nodes) Hemorrhagic joint fluid	Pink colored foreign substance admixed with chicken meat, feathers and corn within the pharynx, esophagus and stomach Diffuse gastric and small intestinal congestion	Hemorrhagic pericardial content	Trachea: diffuse congestion (severe) Parietal sub pleural hemorrhages, Lung: Bilateral, multifocal-coalescing (ecchymosis) pulmonary acute hemorrhages, with diffuse congestion and edema (severe);	Renal Congestion; Urinary bladder: sub mucosal ecchymosis	Meningeal cerebral congestion and hemorrhage, diffuse, bilateral, acute, severe
III	Unilateral bulbar conjunctiva congestion	No significant findings	Pharynx diffuse congestion and edema Oral, esophageal and gastric contents with pink chicken carcass residue Focal small intestinal hemorrhages (trans mural)	Hemorrhagic pericardial content Aortic valvular diffuse edema	Larynx and trachea: diffuse congestion (severe) Acute, severe, bilateral pulmonary congestion and edema of the bronchi and trachea	Multifocal perivascular cortico-medullary hemorrhages	Meningeal cerebral congestion
IV	Pink coloring of the fur Severe, unilateral hyphema, Epistaxis	No significant findings	Pharyngeal, Esophageal and gastric contents of chicken carcass debris soaked in a pink substance Externally expressed	No significant findings	Pulmonary edema	Sub mucosal hemorrhages (paint brush) Congestion	Congestion

			lower digestive (colon) hemorrhages				
V	Pink bright substance on the fur and muzzle Severe, acute, bilateral hyphema Epistaxis	No significant findings	Gastric pink food contenting the oral cavity on the esophageal level Gastro intestinal and splenic diffuse congestion	Pericardial hemorrhagic content	Acute, severe, bilateral pulmonary hemorrhages, confluent suffusions, edema in the main bronchi and trachea	Sub mucosal multifocal congestion (minimal)	Acute, bilateral, diffuse meningeal cerebral hemorrhage Diffuse brain edema
VI	Foamy salivation, epistaxis, oral cavity hemorrhages, diffuse uveal congestion and hyphema	Acute muscular hemorrhages, axillary area	Pink colored foreign substance (abundant) staying the gastric and duodenal content (bread) and mucosa	Subepicardial congestion, and sub endocardia hemorrhages (suffusions) (RV and A); aortic valvular diffuse edema	Trachea: diffuse congestion (severe) Lung: Multifocal-coalescing (ecchymosis) pulmonary acute hemorrhages, with diffuse congestion and edema (severe); diffuse alveolar emphysema	Congestion	Meningeal congestion (diffuse, mild)
VII	Epistaxis, diffuse uveal congestion and hyphema	Acute muscular hemorrhages thoracic-abdominal and lumbar (massive)	Gastric mucosa congestion (diffuse); gastric content-plastic (possible carbofuran container) admixed with meat (presumably bite), hair, grass and blood	Subepicardial hemorrhages (ecchymosis); Hemorrhagic pericardial content	Trachea: diffuse congestion (severe) Lung: Multifocal (ecchymosis) pulmonary acute hemorrhages diffuse congestion and edema (severe); focal emphysema	-	Meningeal congestion and edema (diffuse, severe)

Tab.2. Case history and clinical findings

Case	Age	Sex	Race	Clinical history	Baits Presence	Coloured fur
I	6	F	Common breed	Vomiting, muscle tremors, death in 1 hour	No	Yes
II	~	M	Common breed	Acute death	Yes	Yes
III	~	M	German shephard	Acute death	Yes	No
IV	~	F	German shephard	Acute death	Yes	Yes
V	~	M	Common breed	Acute death	Yes	Yes
VI	4	M	Common breed	Found dead with foamy saliva	Yes	No
VII	1	M	Common breed	Acute death	Yes	No

Figures

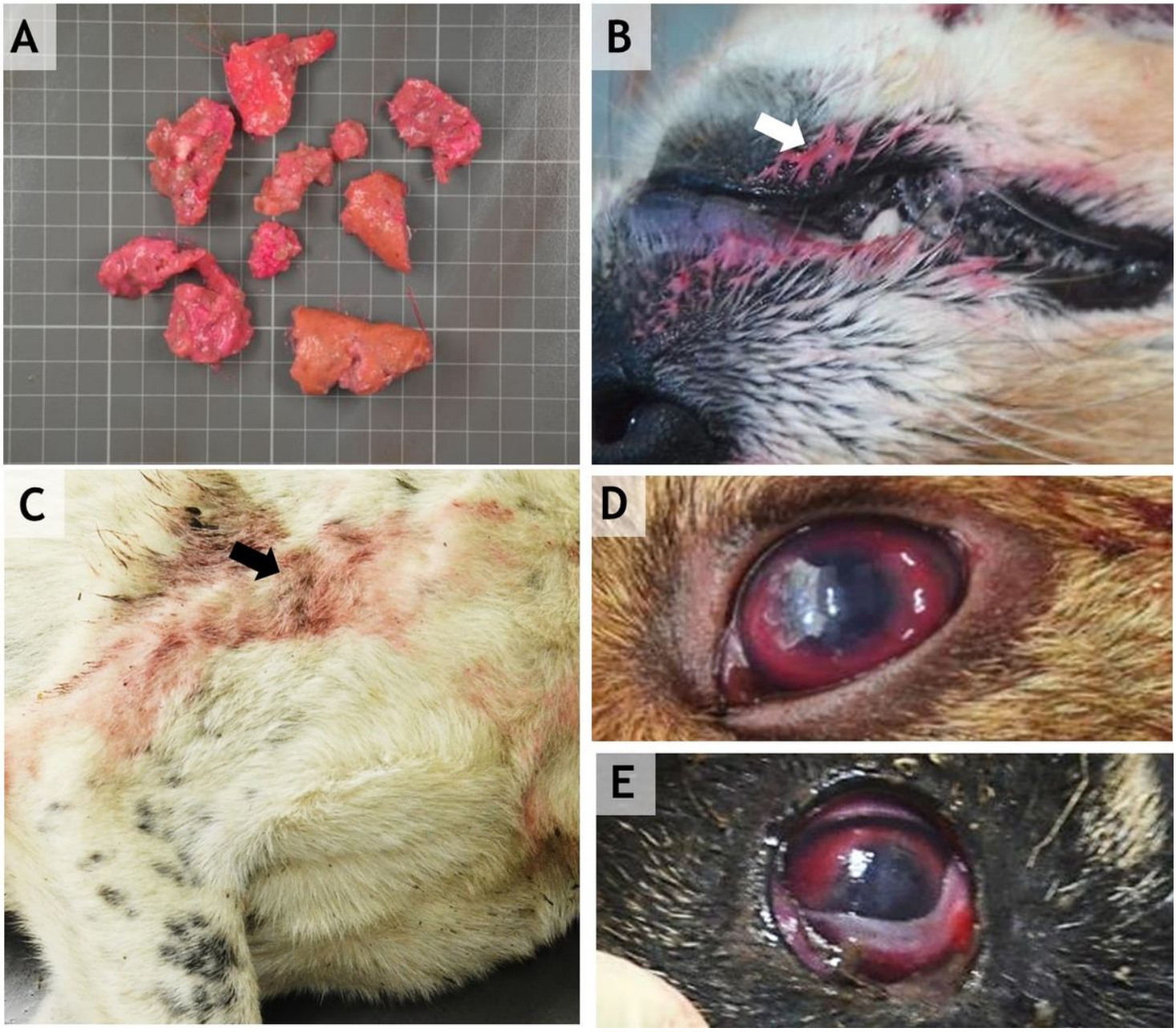

Figure 1

A: The bait, containing an abundant-pink liquid B and C: A pink liquid (presumptively furadan) staining the fur around the mouth and thorax D: Diffuse uveal congestion and hyphema E: Multifocal 3-rd eyelid haemorrhages

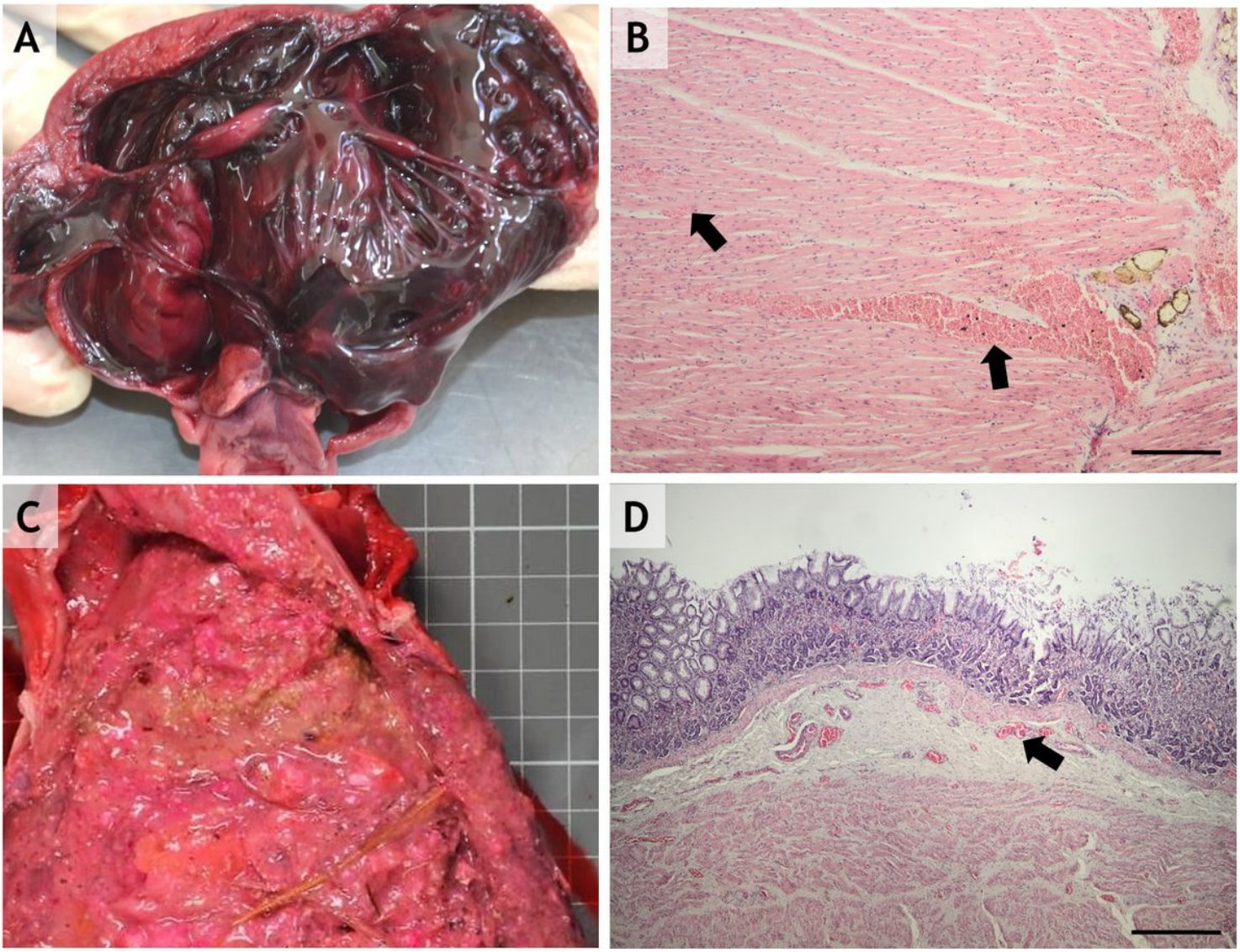

Figure 2

A: Diffuse Subendocardia hemorrhages B: Myocardial congestion and hemorrhage H&E stain, ob x 10 C: Diffuse gastric congestion and gastric content mixed with a pink foreign substance D: Diffuse gastric congestion within the lamina propria and submucosa; H&E stain, ob x 10; scale bar=200µm

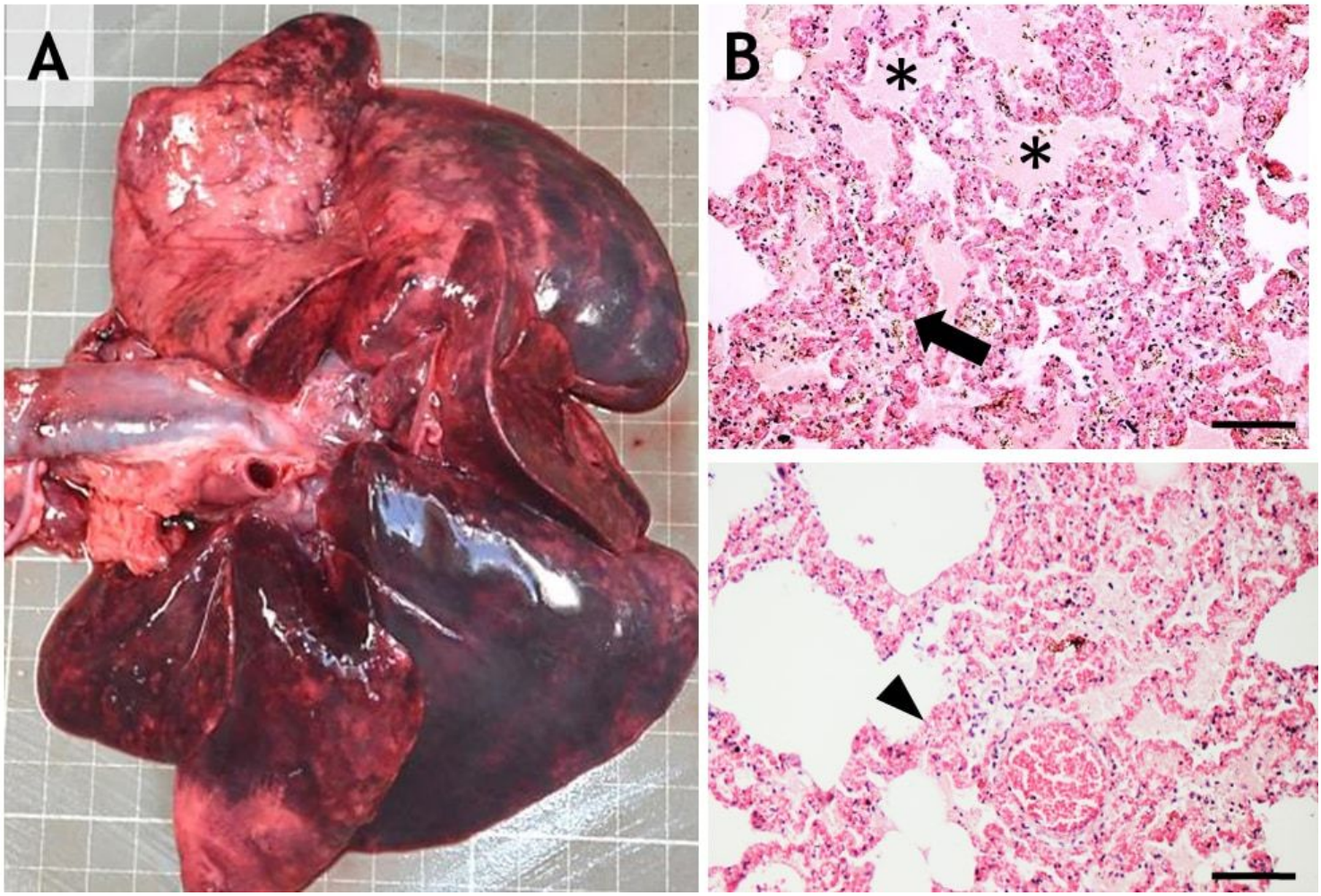

Figure 3

A: Acute, extensive and bilateral pulmonary congestionn, edema, and multifocal haemorrhages B and C: Alveolar-septal congestion (arrow), edema (asterisks) and haemorrhage (arrowhead); H&E stain, ob x 20; scale bar=100µm

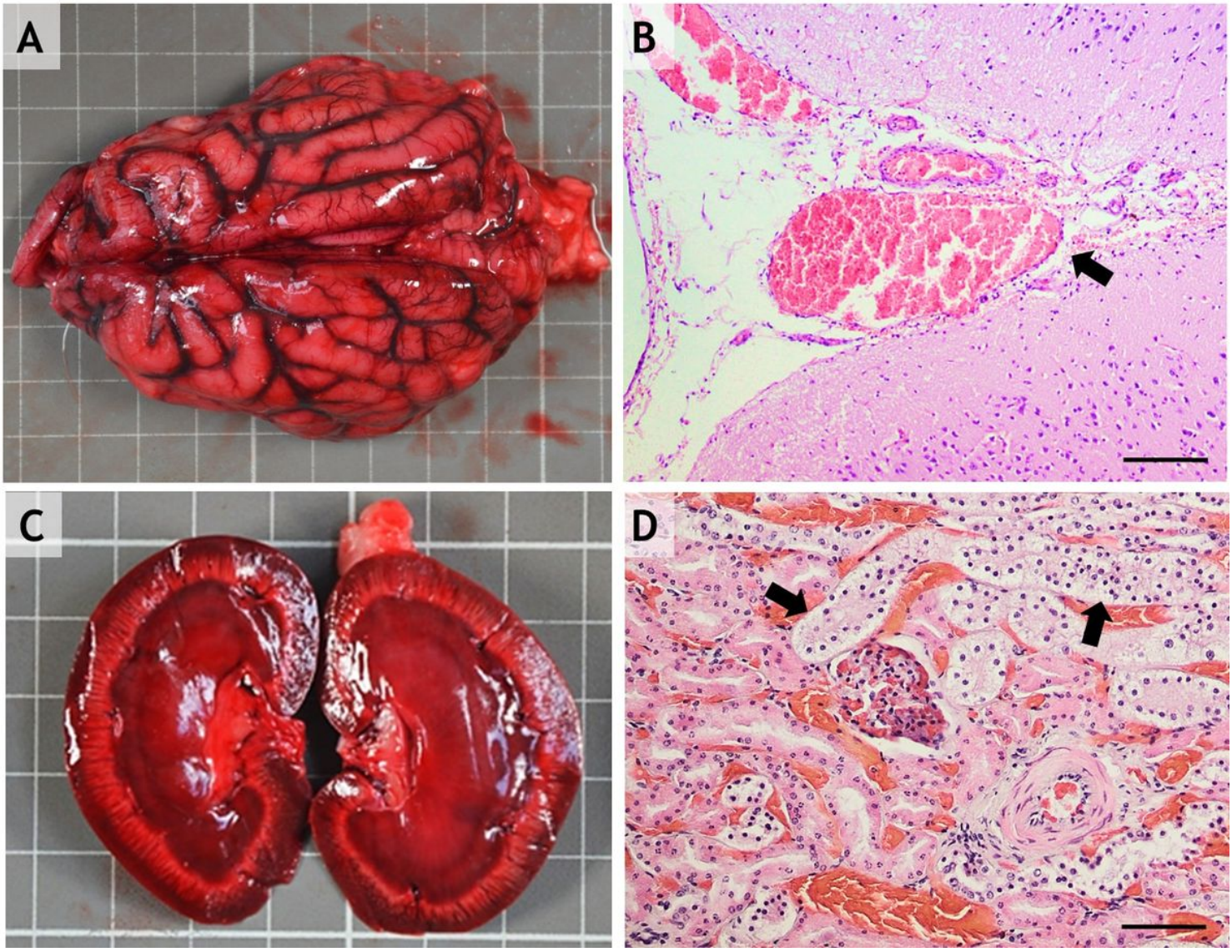

Figure 4

A: Diffuse Sub-endocardia hemorrhages B: Myocardial congestion and haemorrhage H&E stain, ob x 10 C: Diffuse gastric congestion and gastric content mixed with a pink foreign substance D: Diffuse gastric congestion within the lamina propria and submucosa; H&E stain, ob x 10; scale bar=200µm