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 case 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

Nowadays in the European Union, carbamate poisoning is a rarely reported entity. Although banned in the EU, carbofuran is still used in Romania for crop protection and poisoning cases are encountered in animals, and it can be purchased. This paper will contribute to a better understanding of occurrence and pathogenesis in cases of acute carbofuran exposure in dogs and add some necropsy and histopathology data 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 (16) till it was banned since 31.12.2009 in the USA (21), and since 05.12.2011 in Romania, by the Law of Chemical Substances nr 254.

Furadan or otherwise, can still be sourced from nearby Tanzania and Uganda. Indeed, a recent survey indicated that carbofuran was readily available in about 80% of the sampled agro vet stores in Uganda (13).

The most commonly marketed carbofuran were Furadan, Bay 70143, Curater, D 1221, Yaltox, Furacarb, ENT 27164 (19,1). Carbofuran is available in granular, liquid and powder formulations (22, 19) it is used in agriculture and forestry as a board-spectrum systemic insecticide, nematocide and acaricide (7), meaning that when it is applied it enters into a plant, is transported by the sap, and when insects or other pests feed on other parts of the plant, they become poisoned (13).

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

The main objective of this manuscript is to present the gross and microscopically finding in a serial case of carbofuran poisoning in dogs.

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 led to intoxication.

The oral LD50 oral is about 3-19 mg/kg of body weight in different animal species (20). The oral LD50 for dogs is 19 mg/kg and the LC50 for inhalation is 52 mg/kg (18). Younger animals can be intoxicated by smaller dose then adult ones, because of their underdeveloped enzymatic system (15). Repeated exposure like frequent spraying can also cause intoxication. Animals can be intoxicated only by licking the empty pack.

Carbofuran is metabolized in 3-hidroxicarbofuran 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 (6).

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 lesions for carbofuran intoxication along with necropsy and histology pictures.

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 were the animal could have touch 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 sub mucosa.

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

Necropsy revealed in the cardiovascular level haemorrhagic pericardial content, and in some case subendocardial congestions (Fig. 2.-A) and haemorrhages.

In the myocardium diffuse sub endocardia haemorrhages and myocardial 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 lung had acute, severe, diffuse, bilateral pulmonary congestion and edema (Fig. 3. –A), with multifocal petechial and ecchymosis present in the majority of the cases.

In the lung, severe vascular changes were detected, i.e. septal congestion (Fig. 3.-C) associated with the presence of numerous siderocytes, and diffuse edema in the lung airways (Fig. 3.-B).

In the brain the predominant feature was the meningeal and bilateral cerebral acute congestion (Fig. 4.-A) with some multifocal petechial haemorrhage.

From a histologic pathological point of view in the brain, the main changes detected were represented by cerebral congestion and gliosis, including the presence of glial nodules and discrete vascular cuffing (margination) mainly with lymphocytes and glial cells. 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.

Regarding the kidney, the lesions occurred were represented by corticomedulary congestion (Fig. 4.-C), vacuolar degeneration of the epithelium from the cortical tubules of the nephrons (Fig. 4.-D), congestion of the glomerular tuft of the renal corpuscles associated with the presence of a proteinaceous (hyaline) material in the urinary space, and Bowman's capsule thickening.

Discussion

Carbofuran (Furadan) after almost a decade of banning is still causing intoxications amongst animals (4). Illegal poisoning of wildlife and domestic animals is a worldwide issue (14).

Compared to the high number of carbofuran intoxicated birds, Novotny and editors (14) found sporadic cases of small carnivore intoxication (except otters) although martens and foxes are thought to be the main object of poisoners. Also domesticated animals such us pets, mainly dogs, and livestock are at risk of being poisoned with carbofuran (20). The clinical signs in accidental or intentional carbamates poisoning are unspecific, a combination between muscarinic and nicotinic signs, but can be an indication of this intoxication.

Carbamates are reversible acetyl cholinesterase inhibitors, derived from carbamic acid. Carbamate cause inhibition of the activity of acetyl cholinesterase, which is an enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine in two separated components: choline and acetic acid (5, 9). This results in an excess of ACh in the synaptic cleft and continued binding to the post-synaptic receptors (17). Acetyl cholinesterase inhibition is causing hyper stimulation of cholinergic receptors, followed by muscarinic, nicotinic and central nervous signs. AChE inhibitors may also impair the endothelial function, because they are toxic to endothelial cells (8, 23) and to the vascular wall (23).The overstimulation of the somatic nervous system usually results in tremors, muscle twitches and pilo erection, as well as paresis, resulting ataxia. 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 (13).

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 (salivation, lacrimation, urination, diarrhea, gastroenteritis) symptom and include 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 case of cutaneous exposure.

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 constant subject of adjustment through feedback mechanisms, and because of this, each individual may react different to different levels of cholinergic stimulation, and different doses. Death usually occurs due to respiratory failure and cardiac arrest.

These 7 cases of carbofuran-poisoning described here are clear cases were carbofuran was used for intentional poisoning. In most of these confirmed-cases, the results were further used by authorities for legal investigations.

The investigation in cases of intentional animal poisoning are us serious us those in human cases (10.12), yet it is a very challenging and difficult activity (2).

In our study the pathological exam revealed predominant pulmonary lesions. Thus it induced respiratory and cardiac depression, which led to the death of the dogs. The hyper stimulation affects the tone and cell permeability and also the tissue perfusion (3) that could cause interstitial blood pooling (congestions) and edema. Like Motas-Guzman (11) we found acute pulmonary congestion, pulmonary edema and emphysema, but without constrictions and bronchial rupture. In carbamate intoxication pulmonary haemorrhage and petechial can be found (especially in convulsion cases) described by Gupta RC in 2006 (6), lesions which were present in our study too, at dogs with acute carbofuran intoxications.

Conclusions

Nowadays in the European Union, carbamate poisoning is a rarely reported entity. Although banned in the EU, it can be purchased, and it still can cause problems for veterinarians if it is used inadequate for crop protection or intentional for poisoning animals. In the seven dogs with carbofuran intoxication at the external exam the pink foreign substance found on the muzzle and on the fur could be an indication for furadan intoxication for clinician veterinarians especially if it is associated with the sudden death of the animal. This pink strange color can be usually found from the oral cavity too the stomach and small intestine. We described the gross and histopathologigal lesions showing that in carbamates intoxication cases, morphopathological analysis reveals significant lesions at the respiratory system like diffuse tracheal congestions, pulmonary congestion and edema and unspecific necropsy findings like the meningeal cerebral congestion and haemorrhage, along with the renal congestion, diffuse hepatic and splenic congestion.

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 data can be found. In four cases the local authority requested the detailed investigations, and in the rest of three cases the animal owner did.

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 this 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.

Toxicological exam was realized by GC-MS (gas chromatography – mass spectroscopy) from the gastric content and baits. The samples indicated carbofuran intoxication in all the cases.

Abbreviations

Ach – acetyl cholinesterase

GC-MS – gas chromatography – mass spectroscopy

H&E - Hematoxylin-Eosin;

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.
- **Competing interests:** The authors declare that they have no competing interests.
- **Funding:** Not applicable
- **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. 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

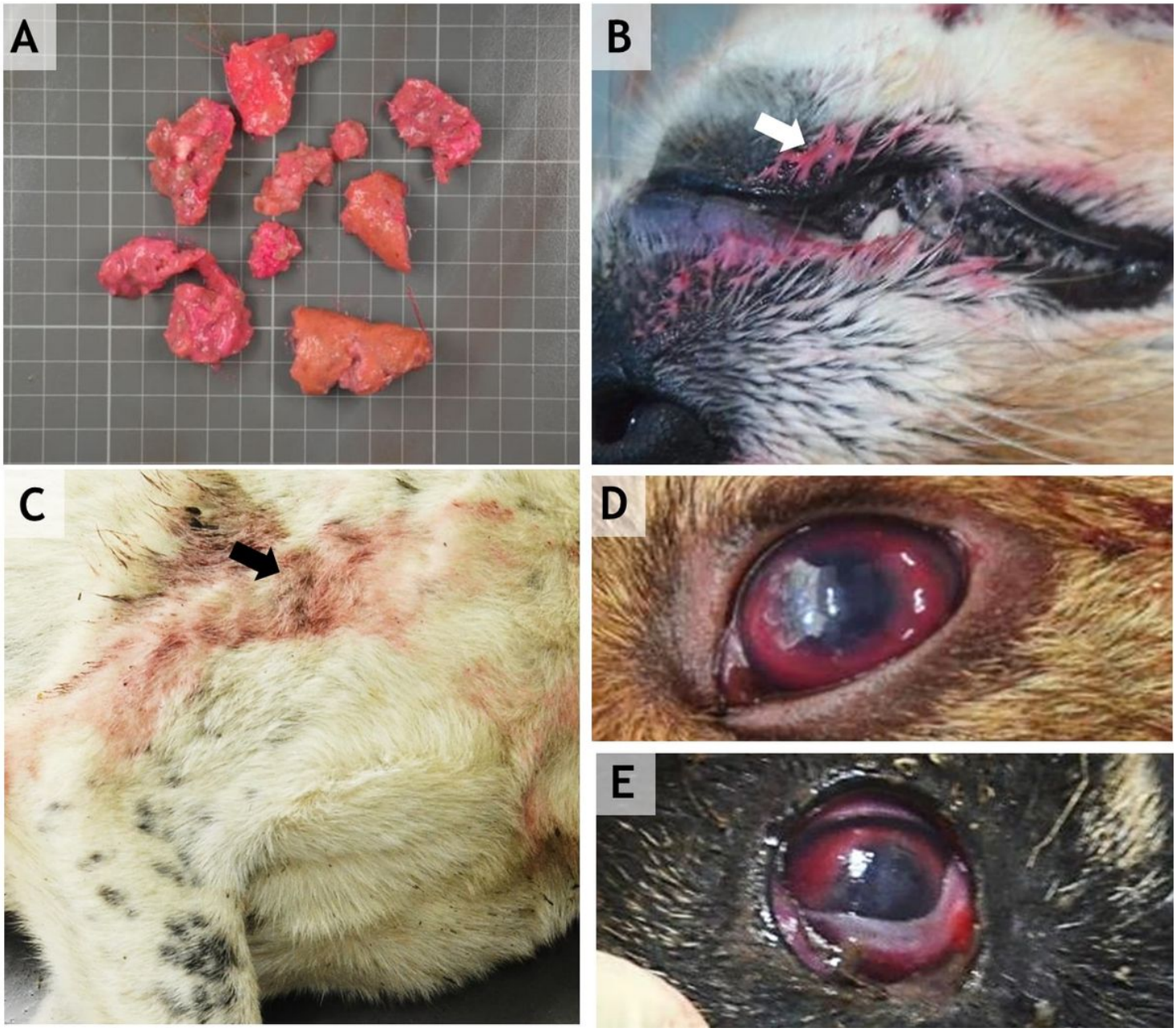

Figure 1

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

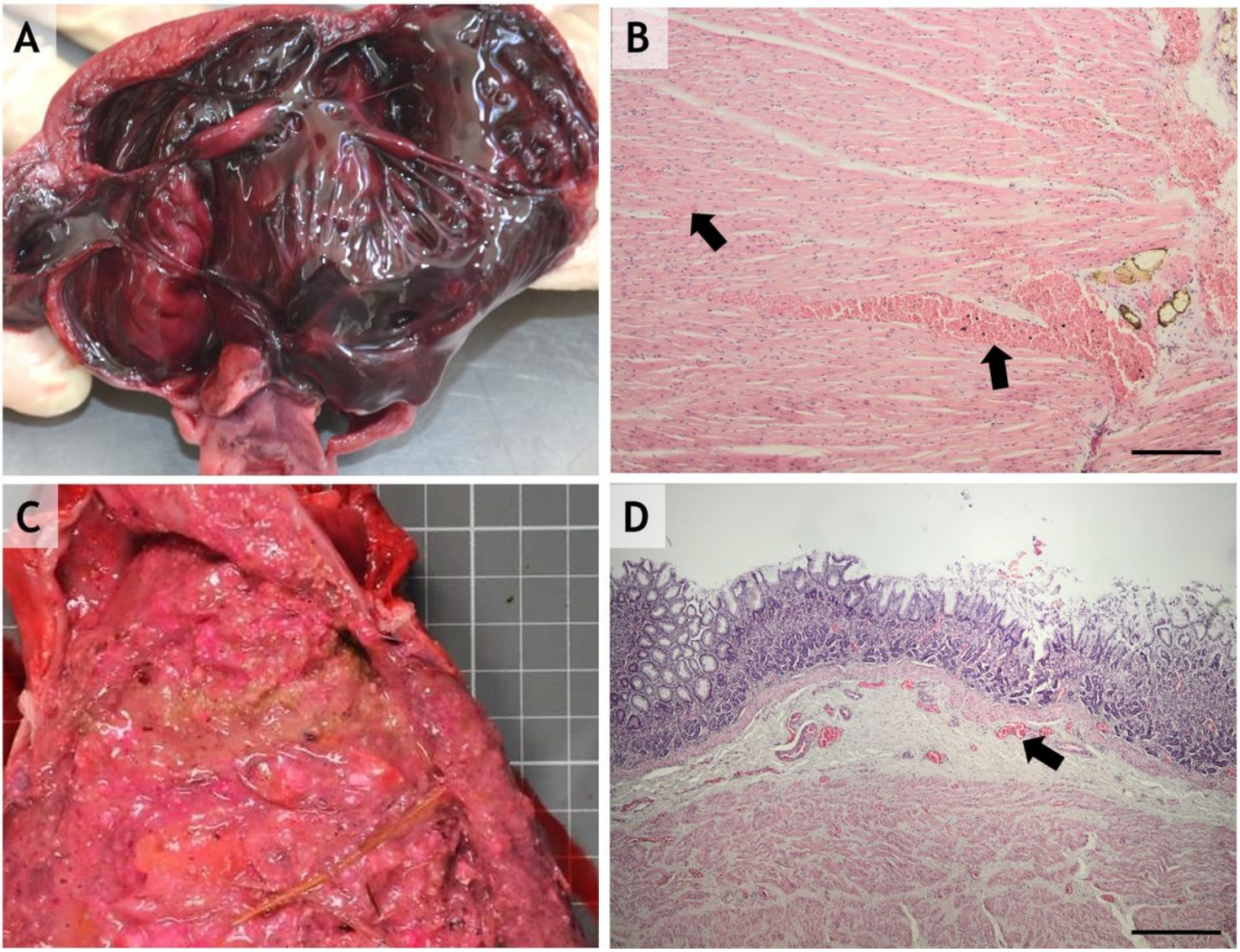

Figure 2

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

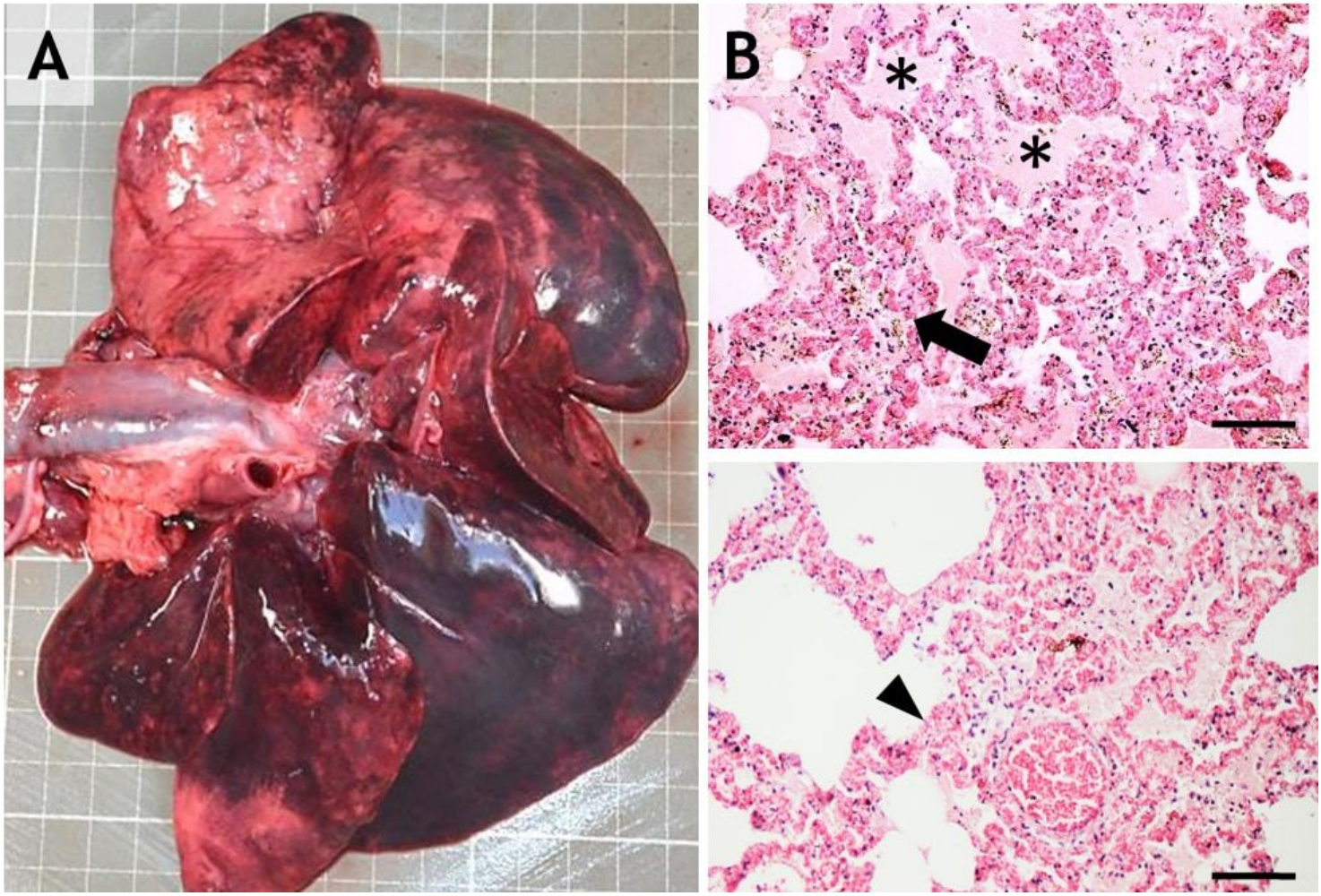

Figure 3

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

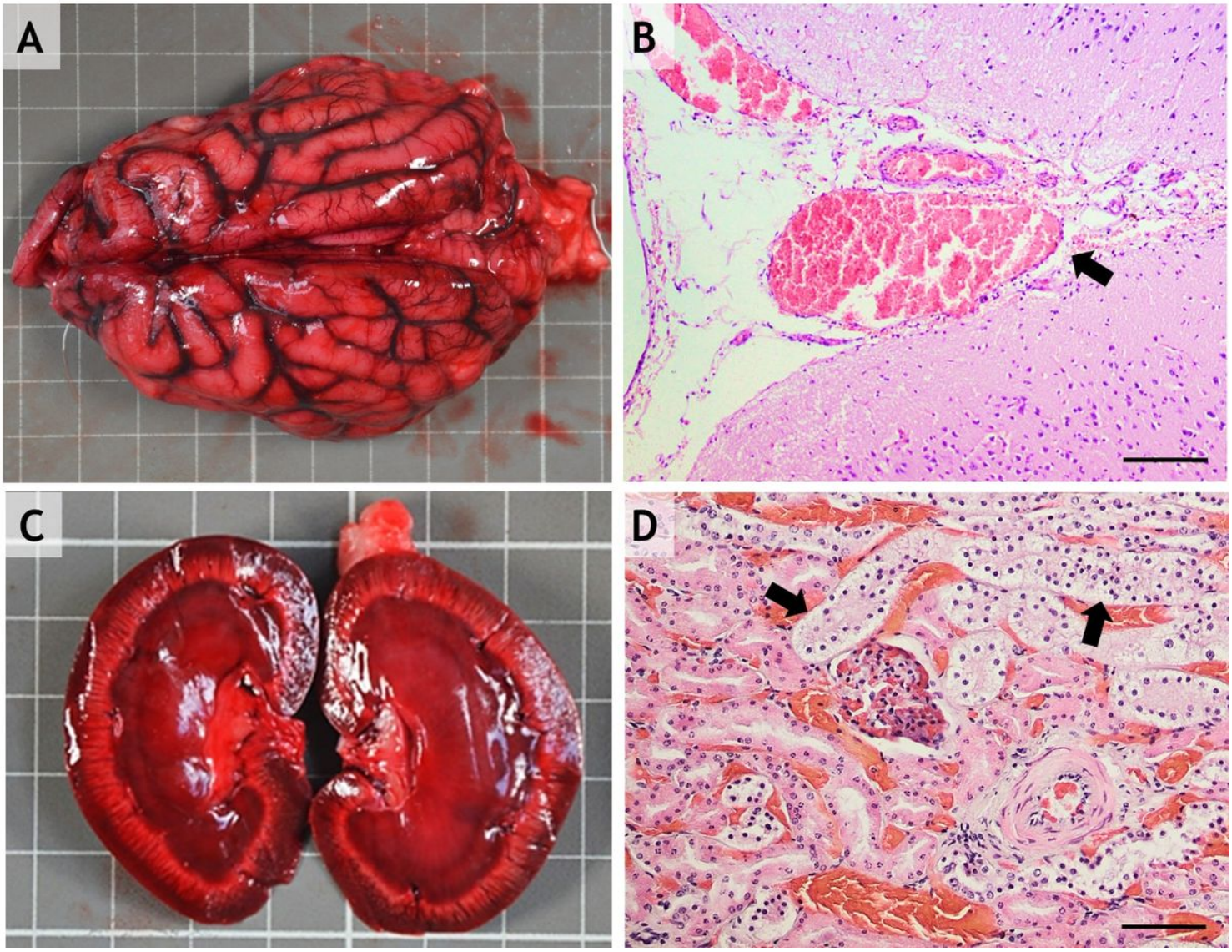

Figure 4

. 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