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MACROSCOPIC AND MICROSCOPIC ASPECTS IN SOME CASES OF ETHYLENE GLYCOL POISONING IN DOGS AND CATS

ASPECTE MACROSCOPICE ȘI MICROSCOPICE ÎN UNELE CAZURI DE INTOXICAȚIE CU ETILENGLICOL LA CÂINI ȘI PISICI

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ABSTRACT | REZUMAT

Intoxication by ethylene glycol is the second most common cause of animal mortality, according to the American Association of Poison Control Centers. Ethylene glycol is a product with low acute toxicity when acting through skin contact or if inhaled. But its toxicity is fully manifested when it is ingested; it is indicated that the fatal dose of ethylene glycol is 4.4 ml/kg for dogs, 1.4 ml/kg for cats, 7-8 ml/kg for birds, and 2–10 ml/kg for bovine, with younger animals being more susceptible to such intoxications. From our studies, we observed that this toxic substance has been identified in particular as the main toxic agent in cats and dogs, having physical properties favourable to ingestion (slightly sweet taste, non-existent smell) and high toxicity. Ethylene glycol (antifreeze) intoxications, although they are not new, have recently started to be more intentional than accidental, requiring the performance of medico-legal veterinary laboratory expertise. As a result, the basis of this work is a number of 56 medico-legal laboratory veterinary examinations, respectively 34 dogs and 22 cats, in which we will present the macroscopic and microscopic aspects induced by this toxic product in animals.

Keywords: ethylene glycol, animals, dogs, cats

Conform Asociației Americane a Centrelor de Control al Otrăvirii, intoxicația cu etilenglicol este a doua cea mai frecventă cauză a mortalității animalelor. Etilenglicolul este un produs cu toxicitate scăzută atunci când acționează prin contact cu pielea sau prin inhalare. Dar toxicitatea sa se manifestă pe deplin atunci când este ingerată, fiind indicat că doza letală de etilenglicol este de 4,4 ml/kg la câini, 1,4 ml/kg la pisici, 7-8 ml/kg la păsări și 2-10 ml/kg. pentru bovine. Animalele mai tinere fiind mai susceptibile la astfel de intoxicații. Din studiile noastre, am observat că această substanță toxică a fost identificată în special ca principalul agent toxic la pisici și câini, având proprietăți fizice favorabile ingestiei (gust ușor dulceag, miros inexistent) și toxicitate ridicată. Intoxicațiile cu etilenglicol (antigel), deși nu sunt noi, recent au început să fie mai mult intenționate decât accidentale, necesitând efectuarea unor expertize medico-legale de laborator veterinar. La baza acestei lucrări se află un număr de 56 de expertize medico-legale de laborator veterinar, respectiv 34 de câini și 22 de pisici, în care se vor prezenta aspectele macroscopice și microscopice induse de acest produs toxic la animale.

Cuvinte cheie: etilenglicol, animale, câini, pisici

Injuries caused by various toxic chemicals represent a particularly vast field of study for veterinary forensic medicine. Poisoning situations in domestic and wild animals can be induced accidentally or can be the result of an intentional action aimed at the destruction of some harmful species and more. Considering the danger of human contamination through contact with dead animals or products derived from them, or the danger and risk that, as a result of some environmental pollution, the existence of some protected species will be endangered, the current legislation sanctions the persons responsible for accidental or deliberate pollution of water, soil, or atmosphere with waste or dangerous substances or electrical means for killing wild animals and fish (7, 8, 9, 10). Also, the increased frequency of cases of poisoning, including with ethylene glycol, was observed in a very large number of domestic pets (cats and dogs) as a result of the intentional act of killing, which is why it was considered necessary to establish the Service in Romania for Animal Protection (S.P.A.) within the Ministry of Internal Affairs (M.A.I.) - County Police Inspectorates (I.P.J.) and respectively the General Directorate of Police of the Municipality of Bucharest (D.G.P.M.B.).

Following the ingestion of baits with ethylene glycol, or the substance as such, it is absorbed at the level of the digestive mucosa (gastric mucosa in the first phase), and the metabolism takes place at the liver and kidney levels into glycolaldehyde by alcohol dehydrogenase (4). Under the action of aldehyde-dehydrogenase, ethylene glycol is then converted to glycolic acid (3). The following is the production of a serious acidosis. The main risk is that of severe metabolic acidosis with depression of the central nervous system, cardiopulmonary failure, and renal failure (1). 4–12 hours after ingestion, symptoms of cerebral depression and severe metabolic acidosis begin to appear. In later stages, there is severe metabolic acidosis, arterial hypertension, pulmonary insufficiency, decreased diuresis, and acute renal insufficiency (2, 3). Calcium oxalate crystallises in blood and other tissues, whereas oxalates crystallise in urine (5). Calcium oxalate precipitation in the renal cortex causes re-

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duced glomerular filtration and renal failure (5). The renal circulation consumes calcium, and hypocalcemia might ensue (5). Due to the fact that ethanol is the main substrate of alcohol dehydrogenase, its presence can block the metabolism of ethylene-glycol (5). Next, glucolate is metabolised into glyoxylic acid. At this point, the molecules can be converted into oxalates, highly toxic substances, or through the less dangerous pathway of glutamate or alpha-ketoadipic acid (9). A small amount of ethylene glycol and its metabolites are excreted in the urine. Oxalic acid in the urine can give rise to oxalate dihydrate or monohydrate, which crystallises, following which these crystals become visible through specific histopathological staining methods for tissues such as renal parenchyma, lung parenchyma, or even central nervous tissue (Fig. 8). To establish the exact cause of death as ethylene glycol poisoning, the toxicological results are edifying and accurate.

MATERIALS AND METHODS

The study, carried out over a period of 2 years (2021-2022) analysed the macroscopic and microscopic anatomopathological picture of 56 pets (dogs and cats) that had as the cause of death ethylene glycol poisoning with or without the involvement of another toxic, of which 34 dogs and 22 cats, observing how the incidence was higher in the case of dogs than in that of cats and knowing that there is a major difference in behaviour between the two species.

The corpses were received at the Institute of Diagnostic and Animal Health in two forms, respectively, as an integral part of the medico-legal veterinary laboratory expertise or only with suspicion of ethylene glycol poisoning, a fact that led to a different approach to sampling management. In both situations, for the toxicological examination, in order to detect the toxic principle represented by ethylene glycol, the stomach contents (when possible), fragments or whole kidneys, a fragment of the lung, and the central nervous system (preferably the cerebral hemispheres) were taken from the corpses. These samples were processed separately and simultaneously to perform two complementary tests that complete each other, namely the toxicological examination through a screening test for the identification of ethylene glycol through liquid chromatography coupled with mass spectrometry from pathological material and the histological examination through specific methods of staining to highlight the cytohistopathological changes that can be attributed to this toxin.

Methods

For the cases investigated in the National Reference Laboratory for Veterinary Forensic Medicine within the Institute of Diagnosis and Animal Health, we approached the diagnostic conduct used in veterinary medico-legal expertise to establish the cause of death in animals, which was then confirmed by highlighting the toxic principle through different methods taken in the study. The basic suspicion supported by the antemortem clinical picture is that of intoxication with different toxic principles.

A first stage in conducting a forensic veterinary laboratory examination is represented by the identification of the animals, the description of the external examination of the corpse, and the thorough notation of all distinctive signs that can strengthen the suspicion of intoxication, such as leaks of any kind at the level of the animal's hair, natural openings, and artificial openings [e.g., bloody discharges from the oral cavity, nasal cavities, and anus, the presence of stomach contents (vomit) in the oral cavity or on the hair around it]. Next, the corpse is opened using classic necropsy techniques or, as the case may be, a technique adapted to the type of injuries present. Sampling of samples from cadavers suspected of death due to ethylene glycol (antifreeze) poisoning consists of the sampling of gastric contents (when present in the stomach) for toxicological examination by liquid chromatography coupled with mass spectrometry and various tissue fragments such as kidney, lung, and brain for histological examination. The results of the toxicological examination are qualitative and not quantitative in the case of antifreeze detection, so the identification of traces of ethylene glycol is justifiable for a positive result and to draw the conclusion of intoxication with this toxic principle. For the histological examination, small fragments of organs are taken from the corpses (especially kidneys, liver, lungs, heart, pancreas, and CNS), which will be further subjected to classic histopathological techniques (fixation, refixation, shaping, embedding in paraffin, microtome cutting, spreading, staining, and reading the slides). These techniques are specific to the anatomical-pathological examinations that open the suspicion of ethylene glycol poisoning, naturally following the toxicological examination of the samples taken from the corpse.

Several techniques are used for the detection of ethylene glycol, of which the most frequently used and most practical is the screening test for the identification of ethylene glycol by liquid chromatography coupled with mass spectrometry from pathological material. This method presents the following principle: the sample to be analysed is homogenized, and diluted in a ratio of 1:9 with water. Ethylene glycol is derivatized with benzoyl chloride in the basic medium. The derivatization reaction is stopped with glycine, and the resulting compound is extracted with pentane. The organic phase is separated by centrifugation, and a part is evaporated to dryness in a stream of nitrogen. The residue is taken up in buffered acetonitrile, and the compound is determined by liquid chromatography coupled with mass spectrometry. In the analysis report, "present" is entered if ethylene glycol was identified in the sample or "undetectable" if this compound was not identified, along with limited detection (LD = 1 g/kg). If ethylene glycol was identified in the analysed sample, in the analysis report, at the final conclusion, it is written: "The compound identified in the analysed sample is toxic for animals/birds".

RESULTS AND DISCUSSIONS

As a result of the pathological examinations carried out on the corpses received over the two years of the study, several common aspects of ethylene glycol poisoning in animals could be observed, but also many specific aspects in each individual case, especially due to the fact that the two species, canids and felines, although they are both classified as carnivores, present specific physiology and pathophysiology. On the other hand, the anatomopathological picture differs significantly due to the amount of toxic ingested, the physiological state of the animal, the specifics of breed, age, and even sex. The substance is metabolised in the liver, enters the systemic circulation, and depending on

several factors such as the degree of filling of the stomach during ingestion, the amount of substance ingested, and the temperature of the environment. It ends up forming crystals specifically at the level of different organs such as the kidneys, brain, or lung. The pathological changes observed in all animals, regardless of the mentioned variables, are the following: accentuated vascular ectasia at the level of subcutaneous and intradermal vessels (Figs. 1 and 4); microhaemorrhages and congestion at the level of the gingival mucosa at the base of the teeth; when the cavities are opened, there is a lack of effusions of any kind (a fact frequently encountered in poisoning with anticoagulant raticides and less frequently in poisoning with carbofuran) (Figs. 2 and 5); when sectioning the blood vessels and expressing it, non-coagulated, red-black, flowing blood is observed; pulmonary emphysema at the level of the apical and cardiac lobes and pulmonary congestion in the diaphragmatic lobes, lack of foamy effusions at the level of the tracheal lumen or from the bronchial tree; accentuated ectasia of the right and left coronary arteries, red microinfarcts on their collaterals; renal congestion in the corticomedullary against a degenerative background, haemorrhage in the basin (Figs. 3 and 6), ectasia of the renal subcapsular vessels; cloudy urine, little in quantity; haemorrhagic gastritis, wrinkled gastric mucosa, generally very little gastric content represented by haemorrhagic gastric juice; hepatic congestion, increase in volume of the hepatic parenchyma with the stretching of the Glisson capsule, on sectioning, non-oxygenated blood is expressed in a large amount; cerebral congestion, ectasia of vessels on the surface of the cerebral cortex, meningeal congestion, haemorrhage at the base of the skull and in the Turkish saddle.

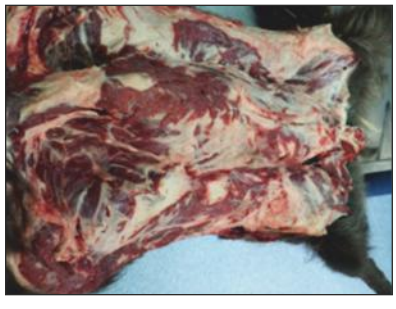

Fig. 1. Subcutaneous conjunctive tissue, relative normal aspect of the muscular and adipose tissue (dog, 3 years old, half-breed)

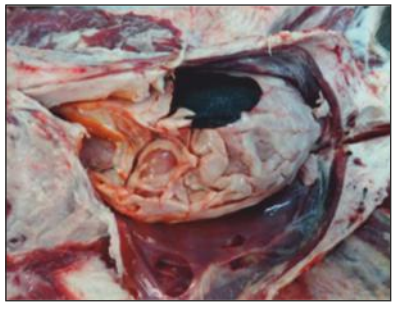

Fig. 2. Abdominal cavity, peritoneum slightly infiltrated with sulphur-meth-haemoglobin, spleen enlarged in volume, slightly congested; without sero-haemorrhagic effusions (dog, 3 years old, half-breed)

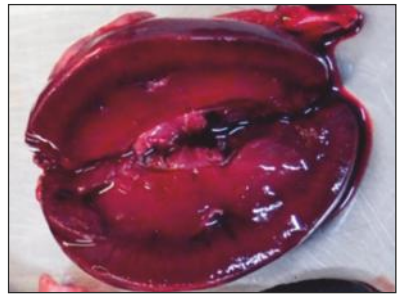

Fig. 3. Renal congestion in the cortical and medullary, haemorrhage at the level of the renal pelvis, a large amount of blood is leaking on the longitudinal section (dog, 3 years old, half-breed)

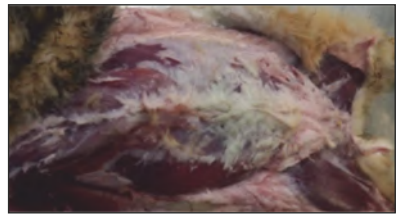

Fig. 4. Subcutaneous connective tissue with relatively normal appearance; mild vascular ectasia (cat, ~1 year, half-breed)

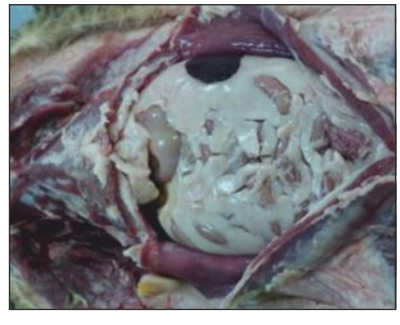

Fig. 5. Abdominal cavity with peritoneum infiltrated by adipose tissue; spleen slightly increased in volume; without sero-haemorrhagic effusions (cat, ~1 year, half-breed)

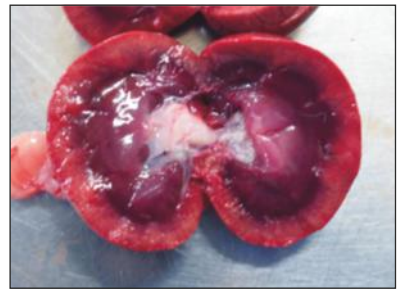

Fig. 6. Kidney on longitudinal section, congestion especially in the medulla, degeneration of the parenchyma of the renal cortex (cat, ~1 year, half-breed)

Less frequently, the following anatomical-pathological changes have been observed: pinkish-reddish serosanguineous discharges from the oral cavity and nasal cavities; sero-haemorrhagic effusions in the abdominal and thoracic cavities; hemopericardium; accentuated pallor of the con-

junctival mucosa; focal haemorrhages at the level of the pancreas; thickened bladder walls.

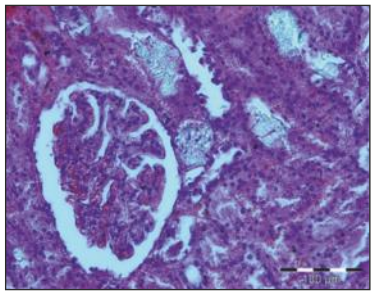

Fig. 7. Numerous oxalate crystals, birefringent, rosette-grouped in the lumen of proximal convoluted tubules in a cat intoxicated with ethylene glycol (kidney, HE, x100)

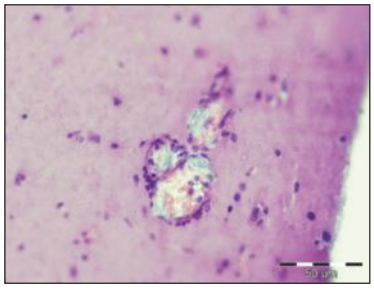

Fig. 8. Oxalate crystals grouped in the vascular lumen in a dog intoxicated with ethylene glycol (brain, HE, x100)

The histopathological examination of various organs revealed specific aspects of ethylene glycol poisoning in animals as well as microscopic aspects highlighted in general in the case of poisoning. Among the general histopathological changes specific to ethylene glycol poisoning, we mention: at the level of the heart, hyalinization and fragmentation of cardiomyocytes are evident, accompanied by phenomena of necrobiosis, oedema and interfibrillar microhaemorrhages; in the lung: haemorrhagic infiltrations with obliteration of the alveolar pattern, bronchiectasis, and hyperaemia in the interseptal capillaries; the presence of oxalate crystals, birefringent, mostly unique (Fig. 9), much less frequent than in the case of the kidney; in the liver: hepatocytic granulovacuolar degeneration accompanied by microhaemorrhages and venocentrolobular hyperaemia, oedema in the spaces of Disse; in the brain (Fig. 8): microhaemorrhages, gliosis, and the rare presence of oxalate crystals in the cerebral arterioles, a fact primarily due to the existence of the blood-brain barrier, but worrying because it demonstrates the fact that these microscopic changes in the case of ethylene glycol poisoning are profound and important. The most representative histopathological picture, however, is based in the kidneys, where hyperaemia and intense degenerative-necrotic processes could be observed at the proximal and distal convoluted tubules accompanied by severe renoepitheliocytosis.

The presence of numerous oxalate crystals, birefringent, single or multiform, with a clustered or rosette-like arrangement, in the lumen of the proximal convoluted tubules and in the renoepithelia of the renal tubules, indicate changes specific to ethylene glycol poisoning (Fig. 7). Following the toxicological laboratory examination, the presence of ethylene glycol in the gastric content was identified, through specific techniques, and the results were interpreted by the presence of ethylene glycol in the sample to be analysed, a fact that represents a qualitative and not a quantitative result. So, if even traces of this to-

Table 1

Differentiated aspects between the macroscopic and microscopic picture in dogs and cats

	C.N.S. (macroscopic)	**C.N.S.** (microscopic)	**Heart** (macroscopic)	**Heart** (microscopic)	**Kidneys** (macroscopic)	**Kidneys** (microscopic)	**Lungs** (macroscopic)	**Lungs** (microscopic)
Canidae	Severe vascular ectasia; cerebral and meningeal congestion, pituitary haemorrhage and in the Turkish saddle.	Hyperaemia, pronounced perivascular and perineuronal oedema, nonspecific vacuolations at the level of the neuropil	Vascular ectasia of the coronary arteries, necrotic miliary foci following red or white infarcts	Hyperaemia, hyalinization and fragmentation of cardiomyocytes accompanied by necrobiosis phenomena, oedema and interfibrillar microhaemorrhages	Obvious vascular pattern on a congestive-haemorrhagic background, easy decapsulation	The presence of numerous oxalate crystals, birefringent, single or multiform, with a clustered or rosette-like arrangement, in the lumen of the proximal convoluted tubules	Severe panlobar pulmonary congestion, positive docimation	Hyperaemia, haemorrhagic infiltrations with obliteration of alveolar structures
Feline	Vascular ectasia, cerebral congestion.	Hyperaemia, pronounced perivascular and perineuronal oedema	Coronary artery ectasia, red-black uncoagulated blood in the atria	Hyalinization and fragmentation of cardiomyocytes accompanied by necrobiosis phenomena, oedema and interfibrillar microhaemorrhage's	Obvious vascular pattern on a necrotic-degenerative background	Oedema in the glomerular filtration space, the presence of numerous oxalate crystals, birefringent, single or multiform, with a clustered or rosette-like arrangement in the lumen of the proximal convoluted tubules and in the renoepithelia	Pulmonary congestion in the diaphragmatic lobes and compensatory emphysema in the lobes	Haemorrhagic infiltrates and obliteration of the alveolar pattern, bronchiectasis.

xic compound were identified, the result would be ethylene glycol poisoning.

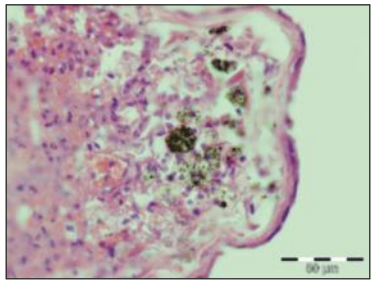

Fig. 9. Oxalate crystals in the pulmonary tissue in a dog intoxicated with ethylene glycol (lung, HE, x20)

Also, the differential aspects between the macroscopic and microscopic picture in ethylene glycol poisoning in dogs, compared to cats (Table 1).

CONCLUSIONS

Following the correlation of the anatomopathological examination (necropsy) with the histopathological examination and with the complementary laboratory examinations (toxicological examination) in the animals examined during the two years studied, we were able to draw conclusions regarding the specific aspects of ethylene glycol poisoning in pets. The macroscopic aspects observed at the necropsy present a general picture specific to this type of poisoning, namely that the main lesions are circulatory and degenerative, mainly centred on the heart and parenchymal organs: the lung and the kidney. The major differences compared to the other types of poisoning with toxic principles, such as anticoagulant raticides, are as follows: the lack of effusions from the abdominal and thoracic cavities, as well as the hemopericardium; the main lesions were those of parenchymal degeneration at the renal level with the presence of specific, birefringent crystals, grouped in the form of a rosette, present at the level of the proximal convoluted tubules and in the renoepithelia of the renal tubules, a specific aspect of this type of intoxication; and, as a novelty element, we observed the appearance of crystals of the same type in the lung and brain, which proves the crossing of the blood-brain barrier of the toxic product.

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