Massive death of wild boars caused by ethylene glycol: a case report

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ABSTRACT: During April 2012, 34 dead wild boars (Sus scrofa) were found in the Tachov region (Czech Republic) directly in the River Mze or in its close vicinity. After infectious diseases were excluded, poisoning was suspected as the cause of death. The finding of cadavers directly in or close to a river was indicative of poisoning by a substance which causes thirst. Laboratory analysis excluded dietary salt poisoning. Later, the presence of ethylene glycol was proven in the stomach contents of the wild boars using HPLC. The diagnosis was confirmed by the typical autopsy findings on the kidneys. The kidneys were firm and of a pale brown colour with scattered petechiae on the surface and haemorrhagic stripes on the cut. Histopathological examination revealed the presence of oxalate crystals in the tubules and interstitial tissue of the kidneys.

Keywords: poisoning; thirst; sodium chloride; nephrotoxicity; oxalate crystals; kidneys; Sus scrofa

List of abbreviations

AD = Aujeszky’s disease; ASF = african swine fever; CNS = central nervous system; CSF = classical swine fever; HPLC = high performance liquid chromatography; PCR = Police of the Czech Republic; RVAP = Regional Veterinary Administration of the State Veterinary Administration for region Pilsen; SVI Jihlava = State Veterinary Institute, Jihlava; SVI Prague = State Veterinary Institute, Prague; TIS = Toxicological Information Centre, Czech Republic

Cases of the mass deaths of wild animals always arouse considerable interest among the media and the general public. All parties participating in the investigation are expected to act quickly to stop further animal deaths and to clearly identify the cause of death.

These expectations of the general public were fully evident during the investigation of the mass death of wild boars in the Tachov region, which happened between 3rd and 24th April 2012 and which was exceptional because of the fact that all the dead animals were found directly in the River Mze or in its close vicinity (Figure 1). The Police of the Czech Republic (PCR); the Fire Rescue Service of the Czech Republic, Pilsen region; the Regional Veterinary Administration of the State Veterinary Administration for region Pilsen (RVAP); users of the hunting districts of Stribro, Svojsin, Benesovice, Vrbice and Vranov; the State Veterinary Institute Prague (SVI Prague); the State Veterinary Institute Jihlava (SVI Jihlava); the Veterinary Sanitation Institute Birkov; and last but not least the University of Veterinary and Pharmaceutical Sciences Brno participated in solving the case and in disposing of the cadavers. Despite the cooperation of these bodies, the investigation of the case was protracted and the identification of the cause of death took several weeks.

Ethylene glycol. Ethylene glycol, chemically 1,2-dihydroxyethane, is a substance which is used as an antifreeze agent, as a constituent of hydraulic and brake fluids and industrial de-icing chemicals, and also as a humectant and solvent in dyes and cosmetics (Dalefield 2004). It is also used in cryoprotection during embryo preservation and it is a
constituent of photographic developers and inks (Thrall and Hamar 2007).

Ethylene glycol is a liquid which is naturally colourless and odourless with a sweet taste. Its palatability for animals is often mentioned, but this is probably true only on a case-by-case basis, as a study by Doty et al. (2006) showed that higher concentrations of ethylene glycol are not preferred by either dogs or rats, even if those are subjected to a water deprivation schedule.

**Kinetics and metabolism.** Ethylene glycol is easily and quickly absorbed both via the gastrointestinal tract and the lungs. Then, it is metabolised in the liver and kidneys via a complex cascade of metabolites. The first step of the metabolism is its conversion to glycoaldehyde, which is a process catalysed by the liver enzyme alcohol dehydrogenase. Glycoaldehyde is then metabolised by aldehyde dehydrogenase to glycolic acid. Glyoxal, a minor product of this conversion, is later also metabolised to glycolic acid. In the next step, glyoxallic acid is produced from glycolic acid. This is a key step and also a very slow process, which leads to increased levels of glycolic acid in the blood. Glyoxallic acid is then converted to many products, among which oxalic acid is produced in the highest amounts and is also of the greatest toxicological importance (LaKind et al. 1999). Ethylene glycol and the products of its metabolism are excreted primarily via urine (Dalefield 2004).

**Mechanism of action and clinical signs.** Approximately 1–3 h after the absorption of ethylene glycol to the organism, the first clinical signs of poisoning start to appear. They usually follow a three-phase progression, but the schedule of individual phases for different animal species varies a great deal according to different authors (Dalefield 2004; Thrall and Hamar 2007; Porter 2012). The clinical signs of the first phase (stage) are caused by the ethylene glycol itself and by the created aldehyde (LaKind et al. 1999). Ethylene glycol is a strongly osmotically active molecule and causes increased serum osmolality, polyuria, and polydipsia. Polyuria can be evoked also by the predicted inhibition of anti-diuretic hormone release (Dalefield 2004). Polydipsia is, by contrast, usually absent in cats, or can diminish also in other animal species during a strong depression of the central nervous system (Thrall and Hamar 2007). As a consequence of the direct irritating effect of ethylene glycol on the mucosa, gastrointestinal signs may also appear.

Later, other metabolites, mainly of an acidic character, accumulate in the body and the second phase of the poisoning begins. This stage is characterised by metabolic acidosis, CNS depression, miosis, and changes in heart function. The effect on the heart may be potentiated also because oxalic acid starts to react with calcium present in the blood creating insoluble calcium oxalates, which may lead to severe hypocalcaemia.

In the third phase, the oxalates are created and deposited in several tissues, most important being their formation and accumulation in the kidneys. Also the effect of acidic metabolites on kidney tubules becomes evident, renal oedema increases, and the blood flow through the kidneys is decreased (Dalefield 2004). The internalisation of monohydrate oxalate crystals, which leads to damage or even necrosis of tubule cells (Porter 2012), together with the concurrent decreased blood perfusion of the kidneys lead to acute kidney failure.

Ataxia, nausea and vomiting, tachypnoea, tachycardia or bradycardia, hypothermia, and central nervous system depression are among other clinical signs which may be specific to individual phases of the poisoning or may overlap or coincide in different stages. In pigs, ataxia, knuckling, crouching stance, and muscle tremors are seen. Later, sternal recumbency, ascites or hydrothorax may also appear (Osweiler and Eness 1972).

Lethal doses of ethylene glycol are known for dogs (4.4–7.3 ml/kg), cats (0.9–1 ml/kg) (LaKind et al. 1999; Dalefield 2004), birds (2–7 ml/kg) (Hutchison and Dykeman 1997), calves (2 ml/kg), and adult cattle (5–10 ml/kg) (Crowell et al. 1979). Lethal doses for pigs and goats are unknown.

**Diagnostics.** During laboratory examination, increased anion gap, hypocalcaemia, hyperphosphataemia, and hyperglycaemia are typically re-
vealed. In the later stages of poisoning, increased levels of potassium, creatinine and urea are also detected in blood samples. Isothienuria (Thrall and Hamar 2007) or hypothermia, proteinuria, glycosuria, haematuria and oxalate crystals (envelope or needle-shaped) are typically present in urine samples. Oxalate crystals can be detected in the urine of most animal species approximately 6–8 h after ingestion of ethylene glycol, in cats as early as 3 h after ingestion (Dalefield 2004; Thrall and Hamar 2007). Diagnosis is usually based on the finding of oxalate crystals in urine and changes in the acid-base balance and biochemical parameters in blood and urine samples. The concentration of ethylene glycol can be measured in both blood and urine, but the results are variable. Also, due to the short half-life of ethylene glycol in the body, measurements of its concentration may result in false-negative results. It is possible and more precise to assess also the concentration of glycolic acid in blood samples. Its metabolism is slow and its concentration, compared to that of ethylene glycol, better correlates with the intensity of the clinical signs of poisoning. Unfortunately, the determination of glycolic acid using chromatographic methods is rather complicated and available in only a few laboratories (Porter 2012). The detection of ethylene glycol in the body can also be accomplished on the basis of the fact that many antifreeze liquids contain fluorescein, which is easily detectable in urine by Wood’s lamp up to 6 h after ingestion (Winter et al. 1990). Other diagnostic methods include e.g. ultrasonography of the kidneys (Dalefield 2004).

Pathological findings. Pathological examination usually reveals pale firm kidneys with pale stripes on the corticomedullary junction. Further hyperaemia of gastrointestinal mucosa and sometimes pulmonary oedema are seen. In pigs, ascitic fluid, perirenal oedema, terminal colon, urinary bladder ligaments, and mesometrium oedemas are also evident. The kidney surface can be streaked with petechiae or ecchymoses; the heart is often dilated. Pale-yellow oxalate crystals, multifocal degeneration, and atrophy or necrosis of the tubules are among the most significant histological findings (Dalefield 2004).

Therapy. The most important factor in the treatment of ethylene glycol poisoning is to administer the antidote as soon as possible. The traditional antidote is ethanol, which competes with the ethylene glycol at the active site of the enzyme alcohol dehydrogenase. Its affinity is higher than that of ethylene glycol, which leads to the excretion of ethylene glycol in an unchanged form. The second antidote, 4-methylpyrazole (fomepizole), has a similar mechanism of action – it is an inhibitor of alcohol dehydrogenase. Fomepizole is much safer than ethanol; it exhibits only minor adverse effects compared to ethanol, but it is very expensive and rarely quickly accessible in common veterinary practice. Both of these antidotes are effective within the first five hours after the ingestion of ethylene glycol and have to be administered as soon as possible. After the enzymatic change of the whole dose of ethylene glycol into its metabolites (which takes 36 h in dogs) the administration of antidotes is useless (Thrall and Hamar 2007). The prognosis can be improved if early decontamination is performed. Some authors recommend gastric lavage (repetitive in ruminants) and the administration of activated charcoal within the first 4 h after the ingestion of ethylene glycol. On the other hand, some authors state that decontamination is efficient only at a time very close (in minutes) to the time of ingestion of ethylene glycol, due to its rapid absorption (Davis et al. 1997). As haemodialysis is rarely available in common veterinary practice, the importance of an aggressive fluid therapy to maintain kidney function and correct acidosis must be emphasised (Dalefield 2004).

Case description

The first dead wild boars (Sus scrofa) were reported to the Police of the Czech Republic (PCR) on 3rd April 2012 in the afternoon by parents of children who were playing near the River Mze on the periphery of the village of Milikov. Two dead bodies of wild boars were found directly in the River Mze. On the next day, the PCR reported the finding to the Regional Veterinary Administration of the State Veterinary Administration for region Pilsen (RVAP). Primary examination of the place where the dead wild boars were found and the surroundings of the river (approximately 1 km) (Figure 2) was undertaken immediately and another three wild boar cadavers were discovered. The veterinarians present noted that the dead animals weighed approximately 50–80 kg, were in a good nutritional state, showed no signs of hunting or injury, and showed no signs typical of infectious diseases. At that moment, drowning was considered the most probable cause of death. Transportation of the ca-
davers to the Veterinary Sanitation Institute was arranged and an autopsy on all five dead bodies was carried out. During the autopsy, several specimens were taken for microbiological examination in order to exclude contagious infectious diseases. The autopsy did not reveal any pathological changes which would indicate infectious disease, and examination of the lungs excluded drowning as the cause of death.

A search in the vicinity of the river organised in the following days by users of the local hunting district led to more discoveries. On 6th April 2012, six more dead wild boars (four adults 70–100 kg, two yearlings up to 30 kg) were found further along the river but still in the cadastral area of the village of Milikov. According to their appearance, the animals died approximately one month before the discovery of their bodies. Again, transportation of the cadavers to the Veterinary Sanitation Institute was organised and an autopsy was performed on them yielding similar results as the previous one.

From the 7th to 9th April 2012, further parts of the River Mze were checked, and in the cadastral areas of the city of Stríbro and the village of Vranov seven more dead wild boars were found. The bodies were transported to the State Veterinary Institute in Jihlava for autopsy and microbiological and toxicological analysis.

From the 10th to 24th April 2012 the discoveries of 16 more dead bodies of wild boars in other cadastral areas near the river followed. The police again asked for an autopsy and analyses to be carried out and the animals were sent to the SVI Jihlava.

**Pathological findings.** Between the 4th and 24th April 2012, pathological examinations were performed successively on the 34 dead wild boars that had been found during that period. The weights of dead animals varied between 30 and 110 kg; almost half of the animals were adults with an average weight of 70–80 kg. The sex ratio was 16 males to 17 females; in one animal the sex could not be determined due to the high degree of autolysis of body organs. Most of the sows were pregnant.

A feature common to all examined animals was their very good nutritional status. Stomachs were filled with a granular-like feed of a green-grey to pale-brown colour (mainly grain meal) with a portion composed of whole grains. In all cases, stomach and intestinal mucosa were hyperaemic. Hearts were dilated and relaxed; the myocardium had strips of different colour on the cut in all animals. In some animals, mild pulmonary oedema was found. The kidneys were firm, pale (a pale-brown colour) and streaked with petechiae on the surface; haemorrhagic strips were found on the cut.

Autopsies were performed during the whole of the 20-day period in which dead wild boars were discovered. The above mentioned pathological findings were revealed mainly in those wild boars found in the first few days; in the later discoveries...
it was very hard to draw firm conclusions as the internal organs exhibited a high degree of autolysis.

Histopathological examination of kidneys from wild boars found between the 10th and 24th April 2012 revealed both autolytic processes and advanced dystrophy of the tubular epithelium as well as druses (clusters) of needle-shaped crystals in the tubules and interstitial tissue (Figures 3 and 4).

**Microbiological and toxicological examination.** Samples of blood and organs from the first dead wild boars found on 3rd April 2012 were taken to exclude the viral infectious diseases of wild boars occurring in Europe (CSF, AD). Samples were immediately transported to the SVI Jihlava. Laboratory examination excluded both CSF and AD in the animals.

To collect all possible evidence, on 6th April 2012 stomach and liver samples were also withdrawn from the dead wild boars, this time for toxicological screening. These samples were sent to the SVI Prague. The result of this toxicological examination ruled out poisoning by dietary salt (sodium chloride) or carbofuran.

Then, more samples were taken from the dead wild boars found between the 7th and 9th April 2012. These samples were sent to the SVI Jihlava for serological, virological, and toxicological analyses. Repeatedly, viral infectious diseases (CSF, AD and also ASF) and poisoning by dietary salt and carbofuran were excluded as the cause of death.

Due to the fact that between the 10th and 24th April 2012 many more dead wild boars were found, the case was referred to skilled toxicologists. Based on discussion with the experts from the SVI Jihlava, the University of Veterinary and Pharmaceutical Sciences Brno, and the Central-European Institute of Wildlife Ecology, toxicological analyses were extended to cover ethylene glycol, nitrites (NO₂⁻), and selected coccidiostatic drugs which are approved for poultry but may be toxic to pigs. The results of further toxicological examinations carried out at the SVI Jihlava identified poisoning by ethylene glycol as the cause of death. The presence of ethylene glycol was qualitatively confirmed in the gastric contents of the majority of the dead wild boars (in 10 of the 12 investigated samples). An HPLC system equipped with a refractive index detector and PhenomenexRezex RCM-Monosaccharide (300 × 7.8 mm; 8µm) column was used for ethylene glycol detection. The column temperature was 65 °C and the mobile phase (redistilled water) flow rate was 0.6 ml/min.

**DISCUSSION AND CONCLUSIONS**

Diagnosis of the poisoning was extremely complicated in this case. None of the wild boars was found alive; therefore, clinical signs of poisoning could not be observed and blood samples for biochemical and haematological examination could not be taken. Also, pathological examination was difficult due to the advanced state of decomposition of the tissues and samples. The key factor in the search for the cause of death of the wild boars was the fact that all dead animals were found close to water, indicating that the animals were thirsty. Thirst is usually caused by poisoning by dietary salt (described...
mainly in birds) (Howell and Gumbrell 1992; Wages et al. 1995). This is why laboratory testing for sodium chloride content in the liver was performed first. Although not a cause of thirst, the presence of carbofuran in samples was tested simultaneously, as carbofuran is the most common source of the intentional poisonings of wild animals in the Czech Republic. Because both of these analyses were repeatedly negative, other substances influencing the kidneys and causing thirst were considered as the source of poisoning. Such substances which are toxic in low doses and can be easily administered to wild boars were chosen for further examination.

Suspicion fell on ethylene glycol, which is sometimes used for the intentional poisoning of pet animals and small pests due to its sweet taste, low lethal doses, and availability to the general public. Ethylene glycol is a common cause of poisoning in animals, usually in cases of the accidental ingestion of antifreeze liquids. These accidental and inadvertent poisonings typically occur in late winter and early spring. In the last few years such poisonings have been documented, for example, in Italy (Caloni et al. 2012), Germany (Dircks et al. 2007), Finland (Vainionpaa et al. 2012), and South Africa (Keller and Goddard 2005). Ethylene glycol poisonings of dogs and cats are also common in the Czech Republic (Modra and Svobodova 2009) and Slovakia (Swan 2003), and queries about such poisonings are also recorded by the TIS Czech Republic, which obtained seven queries about ethylene glycol poisoning in animals between 2010 and 2013 (unpublished data). Review articles on the causes and sources of poisonings from the last decade do not mention any cases of ethylene glycol poisoning in farm or wild animals in Europe (Guitart et al. 2010a,b), but, in Canada, this form of poisoning has been described in raccoons (Mikaelian and Martineau 1997; Foley and McBurney 2002). Only cases of anticoagulant rodenticide (Berny et al. 1997) and insecticide poisonings (Guitart et al. 2010b) are described in wild boars in Europe. In domestic pigs, poisonings are typically due to mycotoxins, nitrates, ammonia, sulfane and some drugs (Guitart et al. 2010a).

Ethylene glycol is one of the most common causes of fatal poisonings in animals. Mortality is very high in ethylene glycol poisoning; death as a result of this poisoning is described in 50–70% of poisoned dogs; the mortality rate in cats is even higher (Thrall and Hamar 2007; Connally et al. 2010). Detailed information on ethylene glycol poisoning is available for humans and pet animals (dogs and cats). The poisoning of farm and wild animals by this substance is rare, although cases involving cattle (Crowell et al. 1979; Barigye et al. 2008), goats (Boermans et al. 1988), poultry (Stowe et al. 1981; Hutchison and Dykeman 1997; Radi et al. 2003; Ozcan et al. 2007) and pigs are documented.

The literature offers only one case of ethylene glycol poisoning in domestic pigs. This was described by Osweiler and Eness in 1972. Five pigs from one pen in a farm breeding 750 fattening pigs were affected. The pigs showed signs of muscle weakness, ataxia, and a crouching stance and later fell into eternal recumbency, three of later dying. Biochemical examination of blood samples was performed and increased concentrations of urea and potassium were revealed. Similar lesions were found in all the dead pigs at autopsy. Subcutaneous oedema of the abdominal wall, ascites, and perirenal oedema were described. The kidneys were pale brown with petechiae and ecchymoses on their surface. On the cut surface the kidneys were pale with stripes of haemorrhages in the cortex. The hearts were dilated and relaxed, and fluid was found in the thoracic cavity. Histological findings included signs of kidney damage: hyperaemia of glomeruli, tubular epithelium necrosis, and numerous polarising crystals in the proximal tubules. When searching for the source of the poisoning, it was revealed that some liquid had leaked into the pen of the affected pigs. This liquid was water from the hot water heating system located under the floor. As protection against freezing and pipe rupture during periods when the heating system was not used, the pipes were filled with a mixture of water and ethylene glycol. The affected pigs had ingested this liquid. The results of postmortems clearly indicated poisoning by a substance causing the deposition of oxalate crystals in the kidneys and led to the diagnosis of ethylene glycol poisoning. However, the clinical signs of the ethylene glycol poisoning described in this case differed substantially from those observed in dogs and cats (Osweiler and Eness 1972).

Pathological findings in the case of the poisoning of the Tachov wild boars supported the possibility of ethylene glycol poisoning as they revealed kidney damage – pale kidneys with petechiae on the surface and streaks of haemorrhages on the cut, and the presence of druses of needle shaped crystals, which was in accordance with the findings described by Osweiler and Eness (1972). In both cases, the hearts were dilated and flaccid. Also, pulmonary oedema was revealed in some dead wild boars. Ethylene glycol was finally confirmed...
by chromatographic analysis of the gastric contents of the dead wild boars.

As the palatability of ethylene glycol and the willingness to ingest it is a strongly individual trait and depends also on the concentration of ethylene glycol (Doty et al. 2006), it is possible that ethylene glycol was added to the feed administered to the wild boars. The digestive tracts of the poisoned wild boars contained both whole grains and grain meal, into which it is very easy to incorporate liquid ethylene glycol. The presence of the bitter additive Bitrex (denatonium benzoate), a common component of many antifreeze liquids, does not necessarily have to have any significant impact on the ingestion of the antifreeze agents themselves or baits containing ethylene glycol with this additive. According to studies on humans, the addition of denatonium to antifreeze liquids did not lead to a decreased incidence of either the accidental ingestion of antifreeze ethylene glycol liquids by children (Mullins and Horowitz 2004; White et al. 2009) or the intentional suicidal ingestion of these liquids in adults (White et al. 2008).

Diagnosis of the mass deaths of wild boars in the Tachov region was made on the basis of the identification of ethylene glycol in the gastric contents of the affected animals and on the pathological findings in the kidneys characteristic of ethylene glycol poisoning, including the histological finding of calcium oxalate druses in both the tubules and interstitial tissue of the kidneys.

According to the results of the above mentioned investigation, intentional poisoning is the probable cause of death of the wild boars, but the investigation is still open and the offender(s) has not yet been identified or convicted.

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