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A case of diquat poisoning in pigs

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Abstract: Diquat is a bipyridyl compound which belongs to the group of herbicides. Its activity is based on the liberation of the superoxide anion radical and, subsequently, hydrogen peroxide, leading to tissue destruction by oxidative stress. Acute poisoning is associated with high mortality within several hours to a few days. The reported case of poisoning occurred on a commercial farm. The fattening pigs of the Landrace and Large White breeds were affected. The pigs were kept on a deep litter. Reglone (active ingredient diquat dibromide, 200 g/l) was used on the farm fields to desiccate the clover crop. The dry clover straw was harvested and stored for approximately a month and then used as a litter. In total, 50 pigs were affected. The onset of the poisoning was very fast. Within eight hours after the litter administration, 20 animals died. The only clinical sign seen was severe haemorrhagic dermatitis. The pathological examination revealed acute superficial haemorrhagic dermatitis on the belly, the snout and the ears of the affected pigs. Hyperaemia of the tonsils, pharynx and oesophagus was diagnosed, as well as a pronounced hyperaemia of the stomach fundus. In the distal part of the trachea, there was a dense, white foam. The lungs were congested, with focal emphysema. The liver was slightly hyperaemic. The histological examination revealed a massive haemostasis in liver and diffuse acute polymorphonuclear hepatitis. The other organs were without changes. The examination revealed acute poisoning caused by the large body surface areas contacting with a toxic substance. The pigs that survived were immediately removed to a non-contaminated area. The changes on their skin were not so extensive compared to the dead ones. Within 5–7 days after the exposure to diquat, the skin lesions healed.

Keywords: herbicide; bipyridyl derivates; desiccant; haemorrhagic dermatitis; oxidative stress; Reglone

The aim of this manuscript is to describe a case of severe poisoning by diquat which appeared in the pigs after dermal exposure. To our knowledge, only one case of fatal poisoning by diquat administered via this route has been described in literature for cattle (Whiting et al. 2001). All the other cases describe, more or less, the severe toxic effects of diquat in humans or animals after ingestion. The authors think that the additional information about the peracute diquat poisoning, which happened after the absorption through the skin, a route of exposure usually considered of little importance, can contribute to the broader knowledge on the diquat actions and dangers.

This case of poisoning occurred on a commercial farm located in the Czech Republic, which grows fattening pigs of the Landrace and Large White breeds. The pigs were kept on a deep litter.

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Description of the case

History. This case of poisoning occurred on a commercial farm with both animal and crop production. Reglone (active ingredient diquat dibromide, 200 g/l) was used to desiccate the clover crop at a dose of 3.5 litres per hectare (700 g of diquat dibromide per hectare). After drying the crop and the clover seed harvest, the dry clover straw is, under usual conditions, ploughed under the soil as a fertiliser. As the year was poor in the grain harvest, the farm suffered from a lack of grain straw. Thus, the clover straw was harvested as a substitute and stored in a warehouse for approximately 3–4 weeks. When they ran out of the common litter, they used the dry desiccated clover as the bedding for one morning.

Clinical symptoms. The onset of the poisoning was fast. Within two hours, severe changes on the skin (haemorrhagic dermatitis) developed. No other signs of damage were visible. In total 50 pigs (all those who had been given this litter, a weight of 30–120 kg) were affected. The pigs were immediately removed to a non-contaminated area and at that moment they were all moving and behaving normally. Unfortunately, twenty of them died within the afternoon of that day. The only symptom seen was that the pigs later appeared apathetic with a loss of energy, they were lying on the new bedding and then died. In the pigs which survived, the changes on their skin were not so extensive compared to the dead ones. Within 1–2 days, the exudative skin changes dried and only a partial redness remained, which disappeared within 5–7 days post exposure. No treatment was administered to the surviving pigs.

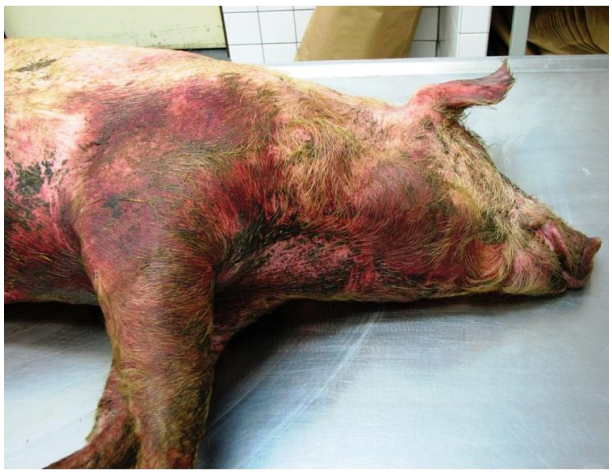

Figure 1. Haemorrhagic dermatitis on the neck, ears and front limbs of the affected pig

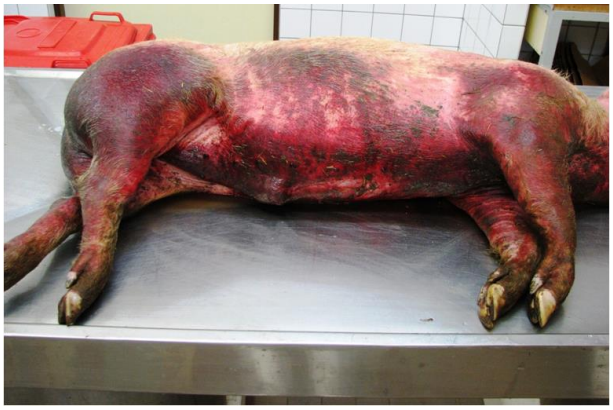

Figure 2. Haemorrhagic dermatitis on the belly and limbs of the affected pig

The pathological-anatomical examination and further analyses. One of the dead pigs was sent to the State veterinary institute for an autopsy. The nutritional condition of this individual was very good, the skeleton was without traumatic changes. Acute superficial haemorrhagic dermatitis was marked on the ventral side of the body, limbs, snout and ear bolts (Figures 1 and 2). The affected skin was glossy, with no bristles and was of a dry, tough, burnt – like consistency with diffuse dark cherry red colouring. In the interdigital areas, there was a mild haemorrhagic dermatitis which only affected the skin. The subcutis was without any inflammatory changes.

Hyperaemia of the tonsils, pharynx and oesophagus was found. There was a dense, white foam in the distal part of the trachea. The lungs contained air, were congested, with focal emphysema. The bronchial passage was open. The heart was contracted and filled with crusts of jelly-like appearance, the myocardium and the endocardium were without changes.

The liver was slightly hyperaemic, but not enlarged. The spleen and kidneys were without any pathological changes. The stomach was filled with digested feed mixture. There was a pronounced local hyperaemia of the stomach fundus (Figure 3). The intestines were without any changes.

The histological examination revealed that, in the abdominal area, the skin was severely hyperaemic with bleeding occurring in the epidermis and upper layers of the dermis, had acute diffusion loss of the corneal layers and with acute lysis of the exposed keratinocytes, but without any in-

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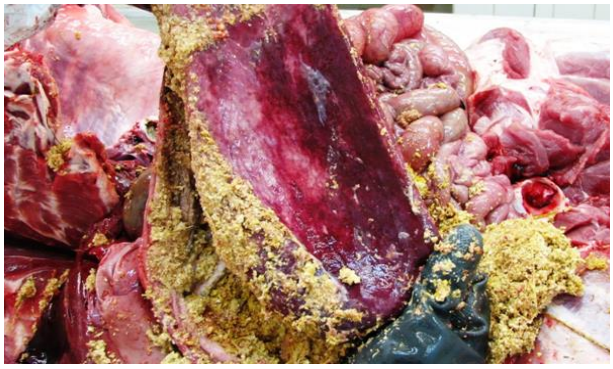

Figure 3. The pronounced local hyperaemia of the stomach fundus of the affected pig

flammatory cell infiltration in the tissue. In the skin samples of the interdigital areas, an acute diffuse loss and deep lysis of the epidermis extending to the base of the dermal papillae was seen. The dermis was strongly hyperaemic without any inflammatory cellular infiltration. Massive haemostasis and diffuse acute polymorphonuclear hepatitis were found in the liver tissue. No other changes were found in the organs of the autopsied pig.

The biochemical analysis of the blood was not performed due to the quick progress of the poisoning. Further laboratory analyses for the presence of diquat in the body samples were not performed as other sources of poisoning were excluded by the local investigation.

Diquat

Diquat is a non-selective herbicide from the bipyridinium/bipyridyl class, which is used also as a preharvest desiccant. It is a substance derived from quaternary ammonium substances. The herbicidal activity of diquat is more pronounced in daylight, aerobic conditions, high humidity and temperature (Magalhaes et al. 2018).

The herbicide is usually supplied as an aqueous solution of dibromide salt monohydrate, but the active part of the herbicide is the diquat^{2+} cation (Pateiro-Moure et al. 2013). Concentrated solutions are corrosive (Magalhaes et al. 2018).

Diquat is able to rapidly penetrate through the leaf surface (Pateiro-Moure et al. 2013). Because of its strong adsorption onto the soil particles, diquat is not taken up by roots. There is no reported metabolism of diquat in the cells of plants. The rapid desiccation of the green parts after application disables such processes (Magalhaes et al. 2018). During the photodegradation of the desiccated plants, the metabolite 1,2,3,4-tetrahydro-1-oxopyrido[1,2-a]-5-pyrazinium ion (TOPPS) is quickly formed from the diquat in the dead plant tissues (EFSA 2015).

In animals, diquat can be absorbed by all routes, but only to a limited extent. Absorption from the gastrointestinal tract is less than 10% and most of the dose is excreted unchanged in the faeces. Also, the absorption may significantly decrease with the presence of food. Absorption from the lungs after inhalation and by the skin is usually very low due to its hydrophilic nature, meaning that systemic toxicity is less common in such cases. However, due to the fact that concentrated solutions are corrosive, the absorption increases (up to 3.8% via the skin route) during the prolonged contact as the skin/mucosal irritation and the upper layer damage facilitate the penetration of the herbicide through the skin and mucous membranes (Magalhaes et al. 2018). After absorption, diquat is rapidly distributed throughout the body. Diquat is minimally metabolised in animals to less toxic mono- and dipyridine derivatives being the main metabolic pathway. Both the unchanged diquat and its metabolites are then eliminated via the urine within 48 hours (Magalhaes et al. 2018).

Oral LD_{50} ranges from 20–30 mg/kg BW (body weight) in cattle and cats to 200 mg/kg BW in rats and chickens (Clark and Hurst 1970; Lorgue et al. 1996). Dermal lethal dose (LD) is not assessed for farm animals, but based on the real case described by Whiting et al. (2001), it is approximately 50 mg/kg to 100 mg/kg BW in cattle.

The maximum residue limit (MRL) for diquat presence in the crops varies between 0.05 mg/kg in most of the commodities to 10 mg/kg for linseed and barley (EFSA 2015). The maximum residue limit ensures a safe dose of diquat in the food/feedstuff for humans or animals, respectively. In cereals at harvest, the residues of diquat range between 48–136 mg/kg in straw and substantially decrease within 6 days after application if the plants stay on the field (photodegradation). The tentative MRL for grain straw is assessed at 40 mg/kg (EFSA 2015). In lentil straw, the diquat residues range between 12.9 mg/kg to 17.3 mg/kg one day after application and decrease to 1.1 mg/kg to 6.0 mg/kg within 2 weeks on the field (Cessna 1991). It is not known whether the decrease in the diquat concen-

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tration also happens during the storage of straw inside the building without sunlight. Diquat is the predominant compound of the total residues in the straw, followed by the metabolite TOPPS, while diquat monopyridone and diquat dipyridone are only found at minor levels (EFSA 2015). Nothing is known about the toxicity of the metabolites/degradation products of diquat, but they are assumed to have the same toxicological profile as the parent compound.

The mechanism of the effect. The mechanism of toxic action of diquat is based on the cyclic reduction and oxidation reactions, which produce reactive oxygen species (ROS) and cause the depletion of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The reduction of the diquat^{2+} ion is caused by the acceptation of a single electron from NADPH and the NADP^{+} and a highly unstable diquat$^{+\bullet}$ are formed. The diquat radical then transfers an electron to the molecular oxygen (O_2) and $O_2^{\bullet-}$ (a superoxide radical) is created while the diquat is converted back to diquat^{2+}. This process is repeated multiple times and generates large quantities of $O_2^{\bullet-}$, which is changed to hydrogen peroxide (H_2O_2) and molecular oxygen by the superoxide dismutase (Saeed et al. 2001; Magalhaes et al. 2018). Moreover, both diquat$^{+\bullet}$ and $O_2^{\bullet-}$ release iron from ferritin, converting Fe^{3+} into Fe^{2+}, which is then involved in the Fenton reaction responsible for the formation of a potent hydroxyl radical, HO$^{\bullet}$ (Abe et al. 2006). Besides ROS, diquat is also able to produce reactive nitrogen species (RNS) (Fu et al. 2001). When the antioxidant capacity of the body is exceeded, the created ROS and RNS induce the oxidative damage of the tissues. Because of its potent prooxidant activity, diquat is used in many toxicological studies to induce oxidative stress in experimental animals and their tissues (Zhang et al. 2006; Yin et al. 2015).

Clinical symptoms. As the kidney is the primary excretory pathway for the absorbed diquat, it usually causes kidney damage. The clinical symptoms of diquat poisoning in humans mainly involve severe gastroenteritis and those connected with acute renal failure: anorexia, nausea, vomiting, diarrhoea, oliguria to anuria, and later uremic syndrome including haemorrhagic diathesis, mental changes, coma. In severe cases rapid respiratory failure, fluid loss and hypovolaemia, cardiovascular collapse, dysrhythmias, seizures and coma with a brain haemorrhage and infarction may develop (Vanholder et al. 1981; Jones and Vale 2000; Saeed et al. 2001; Tsamadou et al. 2009). When ingested, the corrosive damage to the oral mucosa as mouth haemorrhagic ulceration and mucosal oedemas of the tongue and oropharynx may develop.

Lack of appetite, diarrhoea, hypo- and agalactia, loss of weight, decline of production, lung oedemas with dyspnoea, and enteritis were described after the sub-acute poisoning by diquat in cattle and sheep in Hungary (Balla 1986).

In the case of the cattle exposed to diquat via the skin (Whiting et al. 2001), anorexia and a severe drop in the milk production appeared within 12 h post exposure. By 24 h, the animals were staggering, looked dehydrated, were completely anorectic, and had dilated pupils. Later, recumbency, muscle fasciculation and signs of colic with kicking at the abdomen developed and the animals died.

According to Crabtree et al. (1977), early death in rats exposed to high doses of diquat can be correlated with the rapid fluid accumulation into the gastrointestinal tract. If the poisoning follows a prolonged course, multiorgan failure is the probable source of the death.

Pathomorphological findings. Whiting et al. (2001) described that, in cattle, after the dermal exposure to diquat, marked dehydration was seen. The autopsy revealed excessive cerebrospinal fluids around the brain, the intestinal tract diffusely distended with fluids, the dark colour of the apical part of the left lung. No other gross lesions were identified. Histologically, the kidney was affected by a subacute diffuse tubular nephrosis. Large multifocal areas of acute non-inflammatory myocardial floccular degeneration with severe contraction band necrosis were seen in numerous sections of the heart. Sections of the left apical lung lobe revealed a focal area of subacute bronchopneumonia characterised by extensive oedemas and fibrin in the inter- and intralobular spaces. The lung tissue was haemorrhagic with toxic inflammatory cells filling the alveoli. The histological examination did not confirm any primary organ toxicity or identify a specific target organ system.

Laboratory diagnosis. There are no specific markers for diquat poisoning. In the case described by Whiting et al. (2001) in dairy cattle, the biochemical profile revealed severe hyponatremia, hypokalemia, and hypochloremia, marked hypocalcemia, a marked increase in the creatinine and urea, and a moderate increase in the creatine kinase

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(CK). The examined individual was in severe metabolic crisis, but it survived for 5 d after exposure and so may not accurately represent the pathological condition of the animals that die acutely.

Gas chromatography and mass spectroscopy may be used to detect diquat itself or the breakdown products of the diquat, but the performance of this analysis is rare in the clinical veterinary practice.

Therapy. Treatment of this poisoning in veterinary medicine usually includes the administration of low-molecular antioxidants and the first aid is aimed at decreasing the diquat absorption (mainly using activated charcoal or bentonite) and/or enhancing its excretion.

Because of the massive fluid losses into the gastrointestinal tract and its potential circulatory and renal consequences, special attention must be given to the adequate hydration of the patient, ideally with monitoring of the central venous pressure. Unfortunately, haemodialysis was found to be ineffective in removing diquat from the circulation, but it is used to remove the toxic metabolites in the case of diquat induced renal failure (Saeed et al. 2001).

However, these procedures are of low efficacy and the mortality rate in this poisoning is high. Unfortunately, the development of a specific antidote for diquat, and the even more toxic, but similar compound paraquat, seems to be unachievable at the moment.

DISCUSSION AND CONCLUSIONS

Diquat was, in this case, sprayed on the clover field at the dose of 700 g/hectare. The average yield of the clover straw is approximately 5000 kg/hectare. The probable concentration of the diquat (and/or its metabolites) in the clover straw was, thus approximately 140 mg/kg. This is a value similar to the grain straw diquat residues reported by EFSA (European Food and Safety Authority) which range between 48–136 mg/kg, but exceeds the tentative MRL assessed for the grain straw, which is set at 40 mg/kg (EFSA 2015). In the Czech Republic, diquat is not used for the desiccation of cereal crops. Thus, such grain straw can be used as a safe litter for farm animals which is not true for clover straw in this case as it was desiccated chemically.

In the described case, the lack of grain straw on the farm led to the use of the clover straw as a substitute. Photodegradation (3 days of desiccation on the field before harvest) and other types of diquat decomposition were expected by the farmer as the straw was stored approximately one month after the harvest before its use as a litter. Diquat is, unfortunately, not metabolised by the plant tissues as they die quickly. Nothing is known about the degradation processes which happen in the straw not exposed to sunlight, and the photodegradation products created within a few days between the desiccant application and the harvest are – due to the lack of information – considered of the same toxicity as the parental substance (EFSA 2015), so the affected pigs were exposed to the high concentration of the diquat present in the litter.

Diquat at higher concentrations can act as a corrosive substance. This was proven in the described case where haemorrhagic dermatitis with acute diffusion loss of corneal layers and acute lysis of keratinocytes, in the interdigital areas even deep lysis of the epidermis extending to the base of the dermal papillae, were seen. Dermal absorption of diquat is very low, but can increase after the corrosive damage during the prolonged contact because the upper layer damage facilitates the penetration of the herbicide through the skin and mucous membranes (Magalhaes et al. 2018). In this case, the lethal dose cannot be assessed as we do not know how much diquat or its metabolites were present in the straw (no laboratory analysis was performed) and how much was really absorbed and present in the animal tissues (no laboratory analysis was performed as well). But considering the animals were exposed to the diquat containing straw for a prolonged time of approximately 2 h (before the first signs of damage were seen and the animals were removed), and as the affected area of their body surface was more than 50% in the animals which died, the dose absorbed dermally was probably high. Mucosal absorption may have contributed to the situation, as the hyperaemia of the tonsils, pharynx, oesophagus and stomach fundus were seen suggesting a possible oral exposure as well. Up to 20% of the daily feed intake in pigs may consist of their bedding, and the clover straw may appear attractive to the pigs, so they probably ingested part of this bedding too, which is supported by the pathological findings in the digestive tract. On the other hand, probably only small portion of the bedding was ingested, as no gross plant material was found in the stomach of the autopsied pig (Figure 3). Inhalation of the dust from the bedding containing diquat is

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also possible as congestion of the lungs and focal emphysema were described in the dead pig, and the breathing difficulties could have contributed to the apathy and death of the affected animals. However, the main route of the diquat exposure remains the skin absorption.

No kidney damage or clinical signs of gastroenteritis or renal failure were seen in this case, despite the fact that it is typical in other diquat poisoning cases (Vanholder et al. 1981; Jones and Vale 2000; Saeed et al. 2001; Tsamadou et al. 2009). The clinical signs were non-specific and except for the skin damage which probably caused discomfort and pain of the affected area, nothing was described by the keepers until the afternoon when animals refused to walk and eat shortly before their death. Deterioration of the health status and death came very quickly compared to most of the described cases. For example, when compared with the only case reported in the scientific literature on the topical exposure to diquat in animals, where anorexia and a severe drop in the milk production appeared within 12 h post exposure and the death occurred 24 h or more later after the dermal exposure (Whiting et al. 2001), in our case the fatal outcome was seen after approximately 8 h after the exposure.

The skin damage seen in the described case may be attributed to the ROS production and the cytotoxicity of the diquat. The death of the skin cells caused by the local action of the diquat and the loss of a protective barrier probably lead to the increased absorption of the active substance further into the body where it caused liver damage (polymorphonuclear hepatitis). Moreover, the skin damage itself could have led to the death, as there was damage to a large area of the natural barrier (more than 50%), which can be compared to thermal burns, where total the body surface area involvement of > 30–40% may be fatal without treatment (CHEMM 2019). The result of such vast damage may be thermoregulation deficiency, loss of fluid, hypovolaemia, shock, respiratory or circulatory failure or even multiorgan failure (Williams et al. 2009).

Based on the clinical symptoms and pathological findings, the probable cause of death of the pigs was the circulatory failure due to the massive loss of fluids during the intradermal bleeding, hypovolaemia and shock. As other sources of health damage were excluded based on the local investigation and speed of the progress, the peracute poisoning by the diquat present in the litter is responsible for the fatal outcome. Thus, dermal exposure to diquat for a prolonged period of time must also be considered as an important route of intoxication.

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