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CASE STUDY OF DIAZINON POISONING IN WILD PIGEONS (*COLUMBA LIVIA*)

STUDIU DE CAZ PRIVIND INTOXICAȚIA CU DIAZINON LA PORUMBEI SALBATICI (*COLUMBA LIVIA*)

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ABSTRACT | REZUMAT

Diazinon is a non-systemic organophosphorus insecticide formerly used to control cockroaches, ants and other insects as well as fleas in residential, non-food premises. Diazinon was used extensively in the 1970s and early 1980s for general gardening and indoor pest control. One form of bait has been used to control scavenger wasps in the western US. Diazinon is used in flea collars for domestic animals in Australia and New Zealand. Unauthorized uses of diazinon were banned in the US in 2004 due to risks to human health, but it is still approved for agricultural uses. An emergency antidote is atropine. During the years 2022-2023, several cases of diazinon poisoning in birds were identified within the surface of Bucharest, the most important of which was the death of approximately 50 wild pigeons (Columba livia). Following the laboratory examinations represented by necropsy examination, histopathological examination and toxicological examinations, the chemical compound diazinon was identified in the gastric contents. This insecticide is still used in Romania for the insect control actions organized by the authorities, especially in the warm season to control insects (flies, mosquitoes) in parks and green spaces, being spread by vaporization and sprinkling. It is also sold in veterinary pharmacies and phyto-sanitary stores, and can be used in one's own household, but with great caution regarding the animals in the targeted area. Given its widespread use, diazinon is a frequent cause of accidental poisoning in birds in particular, but also in mammals.

Keywords: diazinon, dove, birds, congestion, sudden death

Diazinonul este un insecticid organofosforic nesistemic folosit anterior pentru a reduce numărul gândacilor, furnicilor și altor insecte, precum și pentru a controla infestațiile cu purici în clădiri rezidențiale, nealimentare. Diazinonul a fost utilizat intens în anii 1970 și începutul anilor 1980 pentru uz general în grădinărit și controlul dăunătorilor de interior. O formă de momeală a fost folosită pentru a controla viespile în vestul S.U.A. Diazinonul este folosit în zgarda pentru purici pentru animalele domestice din Australia și Noua Zeelandă. Utilizările nejustificate ale diazinonului au fost interzise în SUA în 2004 din cauza riscurilor pentru sănătatea umană, dar este încă aprobat pentru utilizări agricole. Un antidot de urgență este atropina. Pe parcursul anilor 2022-2023 au fost identificate mai multe cazuri de intoxicații cu diazinon la păsări pe raza municipiului București, dintre care cele mai importante fiind exitusul a aproximativ 50 de porumbei sălbatici (*Columba livia*). În urma examenelor de laborator reprezentate de examen necropsic, examen histopatologic și examenele toxicologice a fost identificat compusul chimic diazinon la nivelul conținutului gastric. Acest insecticid se folosește încă în România pentru acțiunile de dezinsecție organizate de către autorități în deosebi în anotimpul călduros pentru controlul insectelor (muște, țânțari) din parcuri și spații verzi, fiind răspândit prin vaporizare și aspersiune. De asemenea, acesta se comercializează în farmacii veterinare și magazine fito-sanitare, putând fi utilizat în gospodăriile proprii, însă cu foarte multă precauție privitor la animalele din zona vizată. Având în vedere utilizarea răspândită a acestuia, diazinonul reprezintă o cauză frecventă a intoxicațiilor accidentale la păsări îndeosebi, dar și la mamifere.

Cuvinte cheie: diazinon, porumbel, păsări, congestie, moarte subită

Currently, worldwide, it is estimated that approximately 3 million tons of pesticides are used, of which 48% are herbicides, 29% insecticides, 17% fungicides and the remaining 6% being represented by other categories of pesticides (2). Pesticide use statistics show a 33% increase (about 4 million tons of pesticides) in the coming years (6). It is known that organophosphorus pesticides (chlorpyrifos, chlorfenvinphos, diazinon, malathion, fenitrothion, etc.) are extremely neurotoxic, they irreversibly inhibit acetylcholinesterase (an enzyme involved in the hydrolysis of acetylcholine), with accumulation of acetylcholine in motor neurons (3). Many compounds of the dithiocarbamate type (mancozeb, zineb, urbazid) induce intraneuronal oxidative stress, leading to neuronal damage, and by releasing metal ions from the composition (Cu2+, Zn2+, Mn2+) during biotransformation, they can improve the level of balance reactive oxygen species

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(ROS), can stimulate ROS-induced oxidation of lipids and proteins or inactivate certain enzymes leading to neurotoxic effects (5).

The present article aims to present a case study carried out during the years 2022-2023 regarding a number of approximately 50 birds represented by wild pigeons (*Columba livia*) that were found dead in the territory of the city of Bucharest during this period. The pigeons were identified following calls made by citizens to the 112-emergency service, an action that led to the removal of the bodies by Police officers from the Animal Protection Service and the opening of criminal files aimed at establishing the cause of death of the respective animals. The approximately 50 birds died in distinct batches and at relatively close time intervals (for example 15 pigeons one time, 20 pigeons another time) as well as found dead in different areas of Bucharest.

Representative samples were chosen and presented for laboratory forensic medico-legal expertise which had the purpose of establishing the cause(s) of death of the investigated pigeons. Following the macro psychic examinations and complementary laboratory investigations (histopathological examination, toxicological examination, molecular biology examination for differential diagnosis) the cause of death in all cases was determined to be poisoning with the chemical compound diazinon (1).

Diazinon, an organophosphorus with anticholinesterase action, inhibits acetylcholinesterase in the central and peripheral nervous system. Inhibition of acetylcholinesterase leads to the accumulation of acetylcholine at muscarinic and nicotinic receptors, leading to effects on the peripheral and central nervous system. These effects usually occur within minutes to 24 hours after exposure, depending on the degree of exposure. Most of the localized incident reports of human exposure to diazinon have involved occupational exposure via the inhalation route, although significant exposure may also have occurred via the dermal route (7). Also, this substance is often used for disinfestation in poultry farms for consumption and production, but diazinon should not be used in poultry houses, because chickens will consume the diazinon crystals, leading to tearing, diarrhoea, dyspnoea and death. Lesions at necropsy include pulmonary oedema, fatty liver, and severe enteritis. Diazinon crystals can be seen in the contents of muscle and glandular stomachs (4). The diagnosis can be confirmed by laboratory toxicological examination of the contents of the proventriculus and ventricle. After examining the cases studied, we came to the conclusion that the poisoning of wild pigeons (*Columba livia*) in Bucharest with the diazinon compound was unintentional, as they were victims of diazinon disinfestation actions in the parks and green spaces of the city.

MATERIALS AND METHODS

The present case study had a period of one and a half years, respectively 2022-June 2023, during which 6 medico-legal veterinary laboratory examinations were carried out, all summarizing a number of approximately 50 carcasses of wild pigeons in which the cause of death was determined to be poisoning with the diazinon compound.

Carcasses of wild pigeons were not individualized; they were examined as a collective. They were received at the Animal Diagnostic and Health Institute (IDSA) as an integral part of the medico-legal veterinary laboratory expertise, expertise carried out as a result of receiving official documents from the Ministry of Internal Affairs - the General Police Directorate of Bucharest, respectively to the Animal Protection Service in which the cause of their death was requested to be identified.

Veterinary laboratory medico-legal expertise includes all the laboratory analyses that are undertaken in order to establish the cause of death, whether suspicious or not, as the case may be. As integral parts of the final analysis report, in the case of wild pigeons poisoned with diazinon, starting from the suspicion of poisoning, the following laboratory examinations were carried out: individual anatomopathological and histological examination for each individual bird, toxicological examination, parasitological examination and examination for the isolation of Avian Influenza and Avian Plague virus for the purpose of differential diagnosis. In order to carry out the additional laboratory examinations, representative samples were taken from each concerned organ during the necropsy examination. These samples were processed separately and simultaneously for the performance of several complementary tests that complement each other, namely the toxicological examination through a diazinon screening test through liquid-chromatography coupled with mass spectrometry from pathological material and the histological examination through specific methods of staining to highlight the histopathological changes that can be attributed to this toxin.

Methods

In order to identify the toxic compound present in the ingluvial contents and the contents of the muscular and glandular stomachs in the pigeons taken into the study, the anatomopathological examination was performed, in a preliminary phase, preceded by the examination of the exterior. The anatomopathological examination followed a standard necropsy procedure in birds, as follows: general examination of the carcasses, examination of the skin and feathers, examination of the apparent mucous membranes, plucking and examination of the subcutaneous connective tissue, opening and examination of the thoraco-abdominal cavity (coelomic cavity), evisceration and examination of the organs in the cavity. Fragments of organs and tissues are taken for histological examination, as well as the ingluvial and glandular and muscular stomach contents for toxicological examination, in this case for the detection of the toxic compound diazinon.

In order to identify the diazinon compound in the sample of ingluvial content from birds (in our case, wild pigeons brought for the forensic veterinary laboratory expertise), a physico-chemical method called gas-chromatography coupled with mass spectrometry was used. The technique called gas chromatography coupled to mass spectrometry (GC/MS) separates chemical mixtures (the

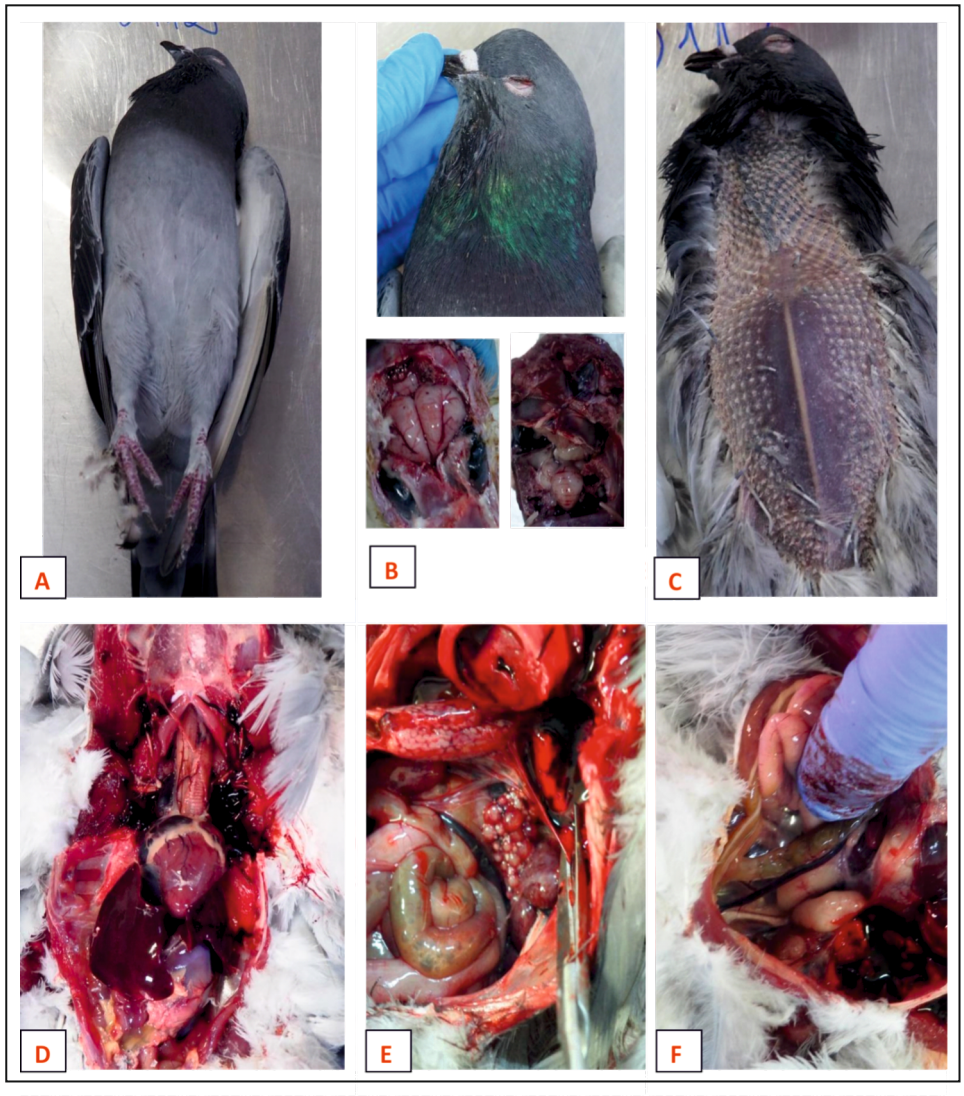

Fig. 1. Different macroscopic aspects identified following necropsy in the studied pigeons: A - Examination of the exterior, lack of sero-sanguineous discharges; B - Macroscopic appearance at the head and brain level; C - cutis skin after plucking; D - Opening of the thoraco-abdominal cavity, haemorrhagic effusions; massive pulmonary congestion; red-black noncoagulated blood; accentuated ectasia of the vessels of the cavity; E - Haemorrhage at the level of ovarian follicles, congested and haemorrhagic kidneys; haemorrhage in the serosa of the intestine; F - Severe vascular ectasia and pulmonary congestion.

GC component) and identifies the components at the molecular level (the MS component). It is one of the most accurate tools for analysing environmental samples and more. Gas chromatography works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances exit the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A "library" of known mass spectra, covering several thousand compounds, is stored on a computer (8). Mass spectrometry is considered the only definitive analytical detector, which represents the scientific evidence in the case of veterinary forensic laboratory expertise. Following toxicological examinations, the compound diazinon was expressed as being present in the sample to be analysed, therefore, this technique is qualitative and not quantitative, which demonstrates that, once identified, it is toxic to animals and humans and can produce neurological disorders followed by exitus.

Apart from the anatomopathological and toxicological examination, the histological examination of the organs most affected by diazinon poisoning is a representative laboratory test.

RESULTS AND DISCUSSIONS

Following the anato-pathological examinations carried out on the corpses received during 2022 and the first part of 2023, several common aspects of diazinon poisoning were observed in the examined pigeons, aspects that raised the issue of a differential diagnosis compared

to poisoning with coumarin derivatives, but it is known that birds are very resistant to these compounds with anticoagulant properties intended for mammals. On the other hand, the anatomopathological picture also differs significantly due to the amount of poison ingested and the physiological state of the birds at the time of ingestion. Diazinon is an organo-phosphorus compound that is part of toxicity group I, used as an insecticide. It is toxic for animals and birds, if the method of use provided for in the leaflet of the commercial product (Diazinol) is not respected, once ingested it is quickly absorbed through the digestive mucosa, the metabolism taking place in the liver. It can also act through inhalation, being absorbed at the level of the respiratory mucous membranes, in which case it can no longer be identified at the level of the proventriculus and ingluvium.

The anato-pathological changes observed following the necropsy examination in the pigeons taken into the study had as their main seat the parenchymal organs, but also other tissues such as: accentuated vascular ectasia at the level of subcutaneous and intradermal vessels, hyperaemia at the level of bilateral eyeballs; apparently anaemic mucous membranes; at the level of the cranial cavity, haemorrhagic miliary foci were observed on the dorsal face of the skull, haemorrhagic infiltrations at the level of the meninges of the brain and the spinal cord, haemorrhages in small foci at the level of the central nervous tissue, sero-haemorrhagic secretions at the level of the nasal turbinates and sinuses, serous or sero-haemorrhagic effusions at the level of the tracheal lumen.

When opening the thoraco-abdominal cavity, it was possible to observe the opacification of the air sacs and the parietal and visceral serosas at the level of the cavity, haemopericardium, at the level of the heart, coronary ectasia and subepicardial effusions, vascular ectasia of the great vessels at the base of the heart, oedema and accentuated congestion at the level of the pulmonary tissue, slight haemorrhagic effusions in the coelomic cavity, liver congestion, enlarged liver, sero-mucous catarrh in the intestine and hyperaemia in the mucosa of the small intestine, splenic congestion, haemorrhagic kidneys. In the case of male pigeons, congestion of the testicles and their increase in volume was observed, and in the case of female pigeons, pronounced haemorrhage at the level of the ovarian sacs. For each individual case, examinations complementary to the anatomopathological examination and the toxicological examination were carried out, with the aim of excluding some infectious, bacterial or viral diseases, which could have major implications from an epidemiological point of view, as follows: from organ samples (heart, liver, kidney, lung, brain, trachea, spleen) through molecular biology techniques, the possibility of

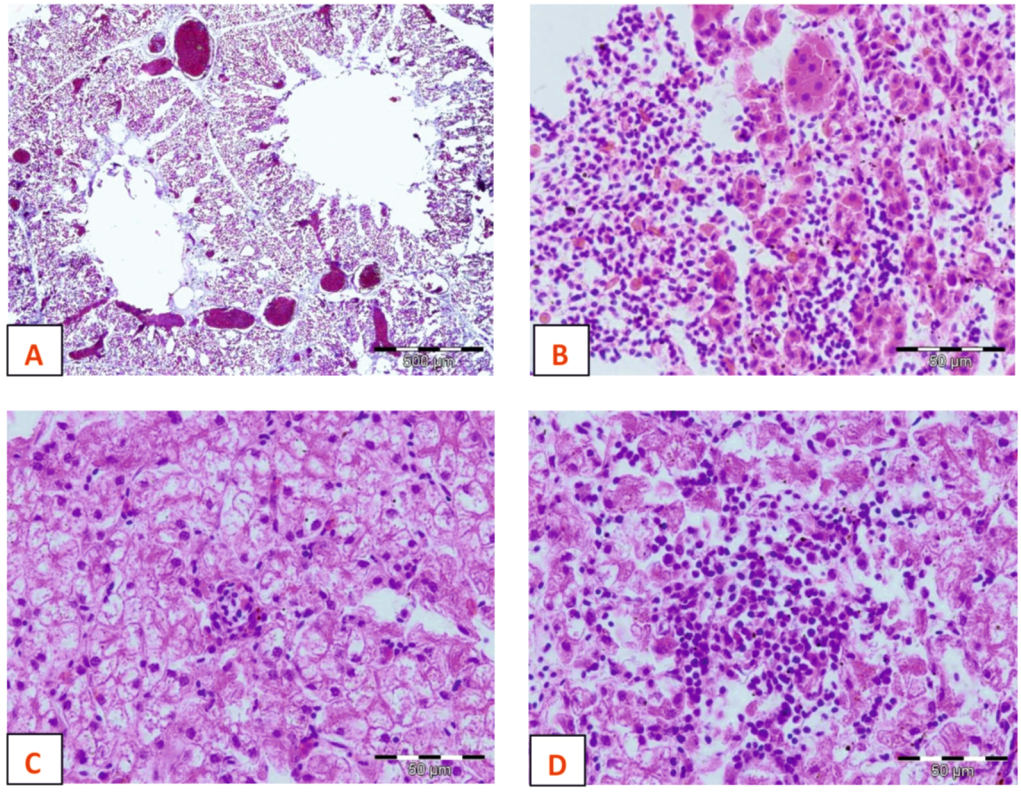

Fig. 2. Microscopic aspects observed following the analysis of tissue fragments taken from the pigeons under study: A - obliteration of the alveolar space by an accentuated haemorrhagic infiltrate, zonal microcenters with hyperaemia and intraalveolar oedema (lung, HE, x40); B - lymphoid infiltrate and discrete interstitial haemorrhagic infiltrate (kidney, HE, x400); C -vacuolar-ballooning degeneration (liver, HE, x400); D - severe granulovacuolar degeneration with disappearance of cell borders (liver, HE, x400)

death due to Avian Influenza or Newcastle Disease was ruled out, and laboratory parasitological and mycological examinations were also carried out. In all cases, the molecular biology examinations resulted in the absence of the antigen specific to each viral disease. In some of the examined pigeons, in addition to the detection of the toxic compound diazinon in the content of the glandular and muscular stomach, Candida spp. could also be isolated from lung, liver and kidney samples, without implications in the animal's outcome, but with the probability that this comorbidity may have influenced the physiological state of the animal from the moment of ingestion of the toxic. However, no difference was observed as the moment of exitus or expression of intoxication (supracute, acute or chronic) determined by the existence of mycosis at the level of the internal organs or of different types of parasites existing at the intestinal level and identified by the complementary laboratory examinations (Fig. 1).

The histopathological examination of the organs revealed aspects frequently found in diazinon poisoning in birds. Among the general histopathological changes encountered in the case of diazinon poisoning in the main parenchymal organs, we mention: in the heart, interstitial oedema, interfibrillar haemorrhages and microcenters with cardiomyocyte hyalinization were observed; at the level of the lung, the obliteration of the alveolar space by an accentuated haemorrhagic infiltrate, zonal microcenters with hyperaemia and intraalveolar oedema is observed; at the level of the kidney, haemorrhages in the structure of the renal lobules, ectasia of the interlobular veins of the renal portal system, as well as haemorrhages in the cortico-medullary; at the level of the liver, haemorrhagic infiltrations, severe granulo-vacuolar degeneration with the disappearance of cell boundaries are observed. All the histological aspects reinforce the macroscopic picture, that of haemorrhages and haemorrhagic infiltrate at the level of the central organs of the investigated birds, a fact that is concluded by specifying the cause of death as surely that of diazinon poisoning (Fig. 2).

Following the toxicological laboratory examination, the presence of diazinon was identified, through the specific techniques, in the ingluvial content and that of the glandular stomach, and the results were interpreted by the presence of this compound in the sample to be analysed, a fact that represents a qualitative and not a quantitative result. So, if even traces of this toxic compound were identified, the result is diazinon poisoning (the existence of scientific probatory).

CONCLUSIONS

Following the research carried out, we were able to observe data on the frequency of poisoning with the diazinon compound (respectively Diazinol - the commercial product most frequently used by the population), data that prove that this compound is extremely toxic for birds in particular. It is also possible to specify the rapidity of mortality in the pigeons taken in the study, a fact that can be explained by the induction of an acute or even peracute intoxication, demonstrated by the following specific morpho-pathological aspects: severe congestion and haemorrhages at the level of parenchymal organs; cerebral congestion; haemorrhage in the large cavities: coelomic cavity (thoraco-abdominal) and pericardial cavity. From a histological point of view, haemorrhagic infiltrations with the deletion of the alveolar pattern in the lungs are found; hepatocytic granulo-vacuolar degeneration accompanied by microhaemorrhages and venocentrolobular hyperaemia in the liver; massive congestion with influx of blood into the kidneys; opacification and congestion of the air sacs; haemorrhage in the ovarian follicles in the case of females or in the testicular parenchyma in the case of males. As a general conclusion regarding the cause of death in the birds taken in the study and by correlating the anatomopathological investigations with the laboratory examinations, it is found that the death of the pigeons was caused by neurogenic and haemorrhagic shock as a result of diazinon poisoning. The substance identified (diazinon) in the body of the investigated birds is used as an insecticide and is toxic to animals and birds, if the method of use provided in the leaflet of the commercial product (Diazinol) is not respected.

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