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RECENT TRENDS IN DIAGNOSING POISONING IN DOMESTIC ANIMALS

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ABSTRACT

In Veterinary Medicine, suspected poisoning often cannot be detected and certified by a Toxicology Laboratory due to many procedural errors and unclear requests for analysis. Therefore, the current study provides guidelines to obtain a definitive diagnosis on poisoning in domestic animals illustrating the multi-step approach to achieve such goal. The Authors describe the components and procedures needed for acquiring a good clinical anamnesis. Moreover, the utility and modality to perform a necropsy are described along with the method to collect and dispatch biological materials to Veterinary Toxicology Laboratory. At the end, the analytical techniques currently employed in detection of major toxic substances, responsible for frequent poisoning in domestic animals, are briefly described.

Key words: Toxicological diagnosis; poisoning; necropsy; domestic animals.

INTRODUCTION

Many substances can cause intoxication in domestic animals. Data on current trends in animal poisoning revealed that pesticides, especially cholinesterase inhibitors and anticoagulant rodenticides, were the main cause of poisoning for domestic animals, followed by human and animal medications (barbiturates, paracetamol, NSAIDs, antiparasitic compounds), household products (solvents, detergents, caustic agents), cosmetics, plants, industrial substances, such as fuels, heavy metals, and ethylene glycol (Berny et al., 2010; Caloni et al., 2012; McLean and Hansen, 2012; Sanchez-Barbudo et al., 2012).

As there are several cases of suspected poisoning in Veterinary Medicine, veterinarians often use wrong procedures to contact the Veterinary Toxicology Laboratory. Biological samples which are inadequate for type and quantity, are often sent to the analytical laboratory without a detailed clinical history profile which is needed to direct the detection of the xenobiotics suspected to have caused the intoxication. Therefore, the current study which is aimed especially to veterinarians specialised in animals practice, focused on the description of a correct multi-step approach to achieve an accurate definitive poisoning diagnosis on domestic animals.

Poisoning often cannot be diagnosed timeliness and is only alleged in many cases. When a veterinary clinician suspects for intoxication, symptomatic treatment should be carried out as soon as possible. The veterinarian should contact poison information services which are also a source of information in case of suspected poisoning. They may be able to help to narrow down the list of possible toxic substances. However, the toxicological diagnosis is a path that must be taken especially when it allows to detect and eliminate the

intoxication source in order to save other animals or humans from being exposed to the same toxic substance (Stegelmeier, 2011).

Even if there is no single protocol to be applied to the poisoning treatment which may vary according to the several toxic substances, it seems essential, in any case, the close cooperation among the involved parties: owner, veterinarian and toxicologist. The veterinarian must provide the analytical laboratory with information about complete history, environment, clinical signs and suspected exposure route to toxicant (Galey, 1995). Veterinarian should perform physical examination of the animal recording vital signs, evidence of pain, clinical signs, evidence of skin or oral irritation and so on, and take samples of body fluids for additional laboratory examination and toxicological analysis. Anyway, if the animal dies, the veterinarian must provide samples taken during the necropsy, very important for poisoning diagnosis. In fact, the detailed description of the pathologic lesions in the animal organs, possibly also supported by histological examinations, allows the pathologist to make a diagnosis of suspicion which allows the veterinary clinician to narrow the field of analytical research to only those specific substances which may have caused the animal's death. Finally, the toxicologist focuses on the substances to detect and interpret the results based on the information and samples (Galey, 2000).

Patient identification and medical history: The veterinarian must look for detailed information for a case history (Puschner and Galey, 2001). The animal identification is essential and includes: animal's and owner's name, species, breed, sex, age, weight, microchip number or tattoo, if available (table 1). It is well known that the sensitivity of a specific species exists

to many substances or, in some cases, different responses in relation to breed. For instance, it is well known that cats are susceptible to paracetamol for the deficiency of this animal species in glucuronyl S-trasferase enzyme which is involved in the glucuronide conjugation and metabolism of drugs. Toxic metabolites cause methemoglobinemia and hepatotoxicity (McConkey et al., 2009); Bedlington Terriers are known for their tendency to accumulate copper in the hepatocytes (Coronado et al., 2008) while several dog breeds such as Collies, Australian Shepherd Dog or Shetland Sheepdog are sensitive to ivermectine and other avermectins that cross into the central nervous system provoking neurotoxicity (Volmer and Meerdink, 2002). Regarding the age, young subjects are more susceptible to various toxic hazards due to incomplete development of liver enzymes which affect biotransforming reactions with consequent less inactivation of xenobiotics. Older animals may be more susceptible because the tissue content with biotransforming enzymes and elimination pathways are less efficient than adult animals. Preexisting illness and pathologies may predispose the animal to the effects of certain toxic substances. It is also important to be informed of any drugs used for the treatment of the animal because some medications along with active compounds of natural origin contained in herbal drugs may interfere with the metabolism and excretion of chemicals or interact with them resulting in additive or synergistic effects (Russo et al., 2009).

Other important information that the veterinarian should obtain to reconstruct a complete medical history, is the total number of animals of the herd and those sharing the same environment, the number of animals potentially exposed to toxic substances and those probably intoxicated. This kind of information helps to identify the source of the toxic substance, such as in the case of food contamination. It is also important to know roughly when the exposure occurred and the chronological appearance of clinical signs and the potential dose that an animal could have taken, along with the formulation of the toxic compound, if possible. Other medical information useful for diagnostic purposes is connected to the animal's behaviour, the relationship the owner has with other people, as neighbours (intentional poisoning), the possibility for the animal to attend open places unguarded, the vaccination status (the symptoms may be caused by infectious diseases), the presence of children in the house (they could administer drugs or other chemicals to animals (Severino, 2010).

Moreover, it is necessary to indicate housing conditons (indoor, outdoor, urban or rural areas, industrial setting) and if the animal go regularly on pastures, gardens, parks. In such places, the animal could be exposed to or ingested the toxic substance. In the end, complete and detailed medical history allows the toxicologist to narrow the search for the chemicals which

are likely to have caused the clinical symptoms. Establishing a list of possible toxicants and give them priority in connection to the probability of causing the observed symptoms is essential for the selection of samples for toxicological analysis; such approach helps to keep analytical costs low at the same time (Guillart *et al.*, 1999).

Ante-mortem sampling: The samples to be taken for toxicological analysis may vary depending on the substance that is suspected to have caused the intoxication. If the toxicokinetics of suspected toxicant is known, it is possible to make a targeted withdrawal; otherwise, the clinician has to send out whole blood, serum, urine, stomach content, hair, along with environmental samples as food, water and suspicious poisoned baits for laboratory analysis (Galey, 2000; Meiser, 2005).

Biochemistry, hematology and other test such as the coagulation test may be useful in increasing suspicion of intoxication (Collicchio-Zuanaze *et al.*, 2010). When taking blood, it is very important to note its colour and consistency. Chocolate brown blood may indicate methemoglobinemia; watery not coagulable blood and bleeding from the venepuncture site suggest anticoagulant rodenticides (Patterino *et al.*, 2004).

Blood should be refrigerated until the time for the analysis; do not expose blood samples to sunlight. Whole blood is the most suitable biological matrix for the evaluation of anticholinesterase activity to diagnose, for example, poisoning by organophosphorus pesticides or carbamates (Berman *et al.*, 2011) and for the determination of heavy metals (Bischoff *et al.*, 2010).

EDTA is an anticoagulant commonly used for most clinical and analytical determinations. Heparin is considered the natural anticoagulant as present at low levels in the blood and tissues: it acts by inhibiting thrombin and other coagulation factors. The sample obtained using this anticoagulant can be used for any type of biochemical analysis, including the blood gas analysis. Sodium citrate, instead, is used in coagulation tests and the determination of each coagulation factors (Mohri and Rezapoor, 2009).

Hepatic injury is the most common manifestation of xenobiotics (drugs, environmental contaminants, etc.) toxicity. As a consequence, serum biochemical parameters, when employed accurately, can provide important and useful information for poisoning diagnosis. They allow the clinician to assess not only the extent and severity of liver damage, but also the type of liver damage, for example membrane injury versus cholestasis and hepatic function (Ramaiah, 2007). The count of blood cells and the blood chemistry determinations are important elements in order to identify the affected organ and exclude diseases that may mimic the effects of a toxicant. Thus, for example, an increase

of white blood cells could be due to an infectious disease, but not to a poisoning. High level of urea in the blood might suggest that the animal ingested nephrotoxic chemical, such as ethylene glycol (Goicoa *et al.*, 2003), whereas high levels of calcium and phosphorus could let the veterinarian suspect colecalciferol poisoning (Talcott *et al.*, 1991).

The urine sample should always be taken; urine analysis makes it possible to detect the presence of drugs administered for therapeutic purposes (or unlawfully) or other chemicals to which animals are exposed accidentally or intentionally (Smith and Lang, 2000). Thus, for example, the presence of paranitrofenol in the urine can indicate exposure to parathion (Abu-Qare and Abou-Donia, 2000; Barr et al., 2002), the quantitative analysis of -aminolevulinic acid in urine allows early detection of exposure to lead (Knight and Kumar, 2003; Kang et al., 2009). Brauer and others (2009) used the urinalysis by gas chromatography-mass spectrometry (GC/MS) for the diagnosis of barbiturate intoxication in dogs. Moreover, the stomach contents obtained following induction of emesis or gastric lavage could be useful for a diagnostic purpose. It must be refrigerated or frozen until the analysis. The macroscopic and microscopic evaluation of gastric contents may be useful for the detection and identification of residues of toxic plants (Severino, 2009). Feces are suitable for analysis too. Hair is usually overlooked but it can be useful for assessing exposure to topical agents such as organophosphate pesticides or products containing permethrin. The blood serum, urine, gastric content and hair must be kept refrigerated or frozen in separate containers, hermetically sealed until the analysis. Sending blood or urine samples in syringes is not recommended and is not suitable to be transported. The blood samples must be collected in special tubes (vacutainer) with or without anticoagulants; whole blood can be kept and shipped in the same vacutainer tubes, whereas, serum, obtained after centrifugation of the blood, is decanted and kept in plastic tubes suitable for freezing. For a good analysis, spin all serum or plasma samples and remove them from the clot as soon as possible. In the end, these samples should be labeled accurately and frozen until the time of analysis. Even environmental samples such as food, water, litter or other suspect materials must be kept refrigerated or frozen until the analysis. Especially for water samples, it is recommend to use glass containers because this is an inert material, whereas, plastic and metal containers should be avoided. For extremely sensitive tests, such as the search for heavy metals, glass containers should be rinsed with acetone.

Necropsy: The necropsy of an animal should be carried out when it is found dead or died suddenly, so a suspicion of poisoning may be considered as a real hypothesis. Necropsy should be made as soon as possible in order to

avoid that the onset of the cadaveric phenomena of autolytic nature may damage organs and tissues to withdraw for laboratory investigations and compromise an objective assessment of histopathologic lesions.

Changes observed in the various tissues allow the pathologist to confirm or deny the suspicion of poisoning by the clinician or endorsed by the animal's owner. This is the first important milestone of necropsy. Many times, especially in case of "sudden" death of a companion animal, owners tend to identify a possible poisoning as the cause of the death also identifying hypothetical responsible among neighbours and other people. The finding of certain lesions during the necropsy such as red-colored mucosa, petechial haemorrhages on the serosa, acute congestive and degenerative condition in kidneys and liver, hyperaemic and haemorrhagic phenomena on gastric and enteric mucosa on the meninges as well, edema of the lung, allow the pathologist to formulate a diagnosis of acute toxic condition that may be associated with the hypothesis of a suspected poisoning. For example, during the necropsy, the finding of bilateral yellowish discolouration of the inner part of the renal medulla with deposit of green yellowish uroliths in the medulla and renal pelvis, diffuse thickening of the urinary bladder, and ulceration of the gastro-enteric mucosa could show a melamine poisoning (Cocchi et al., 2010).

Only the necropsy, however, is not enough for a poisoning diagnosis which remains at the stage of "suspicion". In addition to the detection of these aspecific lesions, highlighting other lesions connected to detrimental action of certain groups of toxicants can lead the pathologist to advance the suspect of poisoning, and to assume the group of substances responsible for the intoxication. For example, the finding of petechial hemorrhages in the pancreas associated with the presence of an intense state of muscle rigidity is strongly indicative of strychnine poisoning; the presence of bleeding or hemothorax and/or hemoperitoneum should suggest a poisoning by anticoagulant rodenticides, whereas, the presence of black necrotic areas on the gastrointestinal mucosa leads to the suspicion of arsenic poisoning. Lesions localized on oral mucosa could be due to plants rich in oxalates, such as Dieffenbachia picta, Philodendron scadens and Codiaeum variegatum pictum. Calcium oxalates are irritating for mucosas and cause intense pain in the mouth, stomatitis, salivation, paralysis of the tongue and tough oedema (Severino, 2009).

The necropsy and the subsequently sampling of tissues for histological and toxicological investigations, should be followed by drafting of a necropsy report which should be sent to the toxicology laboratory. In this report, the pathologist lists in detail all the lesions found in the organs and makes an early diagnosis indicating the type of toxic substances which are believed to be responsible for the animal's death. This considerably

narrows down the search made by the toxicologist who otherwise would be subject to a huge and impossible work, considering the enormous variety of toxic substances present in nature and accessible to both humans and animals (Johnson, 2001).

Post-mortem sampling: In case of sudden death of an animal, leading to suspect a poisoning, the necropsy should be carried out by a pathologist. However, the veterinarian often performs the necropsy personally for economic or logistic reasons. In any case, it is not superfluous to point out that the amount of samples to be taken during the necropsy is of great importance; the diagnosis is often hampered by insufficient samples along with an incorrect choice of the necroscopic samples. Adequate amounts of tissues not only permit to determine more substances which have similar activity and tropism, but also to store part of the samples in order to repeat the analytical determinations and confirm the results obtained by different methods. Furthermore, it is much more useful to draw an excess amount of samples and, eventually eliminate them later, rather than take small quantities and not be able to provide any for further analysis, if necessary. The type of tissue, the sample size and the storage conditions affect the quality of analytical results (Poppenga, 2008).

The tissues which concentrate the toxic substances, in connection to the particular toxicokinetic properties of the suspected chemical, are most useful for diagnostic purposes. In general, samples of liver, kidney, adipose tissue, brain, eye and stomach content should be always taken for analytical toxicology (table 2). The urine should be collected with a syringe directly from the bladder. Blood should be taken right from the heart. In case of suspected poisoning by strychnine, it could be useful to add a sample of muscles and pancreas. In the end, a full set of tissues should be taken for histologic examination in case the animal dies without a certain reason.

Each sample should be kept in separate containers to avoid cross-contamination and identified in an accurate and comprehensive manner reporting on the label all the information needed for its recognition and its origin, such as the animal's name, its owner, the date of levy and the type of tissue (Volmer and Meerdink, 2002). Samples taken during a necropsy must be refrigerated or frozen until analysis, and at the end the samples for histopathological examination shall be about 1-2 cm thick and must be fixed in a 10% formalin solution. A common mistake is to include a too large tissue in insufficient quantities of formalin causing tissue autolysis with subsequent and inevitable impairment of the histopathological examination; for the histological evaluation, in fact, should be respected the following proportion: one piece of tissue and nine pieces of formalin.

Packing and shipping the samples to the Veterinary Toxicology Laboratory: Biological samples taken during the clinical examination or necropsy should be placed in plastic containers. Plastic bags or disposable gloves should not be used as they might tear and cannot be sealed to protect the sample from the environmental contamination. Different samples should always be placed in different containers; moreover, it is not recommended adding antiseptics, preservatives or fixative, such as formalin, except where it is necessary (histological examination): formalin, in fact, makes the sample unsuitable to the conventional techniques used in analytical toxicology. In order to preserve the sample, it is also important not to bring absorbent material (cotton wool, gauze, paper) in direct contact with it as it could dry the sample.

The different samples should be placed in another external container, usually made of polystyrene (good thermal insulation), and sent out to the toxicological laboratory as quickly as possible using freezer packs or dry ice, if available. As mentioned previously, the tissues should be sent to the laboratory in a frozen state, whereas the samples of whole blood and serum must be refrigerated and transported in insulated containers, able to ensure the low temperatures. It is not recommended shipping samples during the weekends or during holiday periods as they may lie under thermal inadequate condition for several days. In the end, it is important to bear in mind that it is necessary to send samples in accordance with the current national regulations on the transport of biological materials (Lorgue et al., 1996).

Analytical toxicology: Thousands of chemicals and natural products could cause poisoning in domestic animals and the search of all compounds to identify the one responsible for the poisoning is impossible.

It is advisable, however, a preliminary comparison (at least by telephone) between the veterinarian and toxicologist on the diagnostic approach and the most appropriate analysis to be carried out.

After receiving the biological samples, the analytical toxicologist can direct the search of the toxicants based on anamnesis, clinical signs, and necropsy findings, if the animal died. Then, the toxicologist is able to assign different priorities and ensure logical progression searching for those substances which are suspected to have caused the intoxication. He may retain samples of tissue for subsequent evaluations, if necessary.

Moreover, the toxicologist selects the most appropriate analytical method based on the required sensitivity and the matrix to be processed; the quality of the analytical method is the strength to make a definitive diagnosis.

As previously mentioned, several factors external to the laboratory, particularly the quality and amount of the samples, are directly affecting the analytical results (Poppenga and Braselton, 1990).

Diagnostic methods used in Veterinary Toxicology: With the progress of scientific research, analytical instrumentations have become increasingly sensitive and accurate. In recent years, various analytical methods of screening, fast, and simple diagnostic tests have been developed which make the research of a large number of chemicals quick and relatively cheap.

Below some techniques currently used for toxicological diagnosis for the detection of xenobiotics in biological samples and different methods of screening, some of which are still under study, are listed (Satoh *et al.*, 2008; Varriale *et al.*, 2009).

Chromatographic techniques – Chromatography (gas chromatography - GC, high performance liquid chromatography - HPLC, thin layer chromatography - TLC) is used to formulate the definitive diagnosis by enabling the isolation and quantification of various substances (pesticides, drugs and/or their metabolites, plant compounds) in the biological samples, such as tissues and body fluids (Maurer, 2004; Meiser, 2005; Brauer et al., 2009; Perez et al., 2010).

However, the most widely used method for the detection of toxic substances in biological samples is HPLC often coupled with DAD, FLD or Mass Spectrometry (Fourel et al., 2010; Armentano et al., 2012; Grobosch et al., 2012). HPLC represents the most reliable method to detect residues of anticoagulant in animal tissues (i.e. liver, blood, gastric content, bloody intestinal content) and bait, feed and water samples as well. This method implies high recoveries of the analytes, which can simultaneously be detected. It shows good reproducibility and is most suitable for forensic analyses (Meiser, 2005). Moreover, Fourel et al. (2010) validated a liquid chromatography-tandem mass spectrometry method based on ion-trap technology with electrospray ionization (ESI) and multiple reaction monitoring (MRM) technique for the identification and quantification of anticoagulant compounds, including rodenticides, in dog plasma. The method gave good results in terms of specificity, linearity, and percent recovery and was useful as diagnostic tool in several animal species. Nevertheless, these techniques are generally time-consuming because the extraction and clean-up procedures are needed before the analysis is carried out providing the results in few days. Liquid-liquid partitioning, solid-phase extraction (SPE), supercritical fluid extraction (Liau et al. 2007) and immunoaffinity cleanup (IAC) are used for the purification of extracts. An advantage of liquid-liquid partitioning is that it removes high background interferences, even if it is very time consuming. IAC is nowadays the most common clean up method used in analytic toxicology thanks to its specificity and selectivity. Furthermore IAC can be reused up to eight times to reduce costs in clean up and analysis. Finally, SPE and IAC have been often used together with the HPLC system leading to solvent reduction, time saving and minimization of possible losses.

Spectroscopic techniques – The atomic absorption spectroscopy (AAS) is the method of choice for the detection of metals, such as Pb, Cd and Hg in biological samples and body fluids, such as blood, urine, tissues and hair (Dunlap et al., 2007; Gow et al., 2010; Ivanenko et al., 2012). AAS allows rapid and accurate determination of metals and trace elements obtaining the results in few hours thanks to the modern mineralization procedures (for example microwaves digestion system) that have reduced the analytical time (Lopez-Alonso et al., 2007). Recently, new analytical procedures for a rapid detection of some metals in biological samples have been proposed, including immunological methods, such as specific monoclonal antibody-based indirect competitive ELISA (Wang et al., 2012), sensors (Ly et al., 2011; Kim et al., 2012), and microarrays (Liu et al., 2012). An alternative approach for the analysis of metal concentration in biological samples could be based on the evaluation of metallothioneins (MT). MTs are lowmolecular mass proteins which are involved in metal detoxifications and homeostasis and prevention of oxidative stress damage (Vasak, 2005). Different analytical methods, such as optical detectors, mass spectrometric detection coupled to the various ionization techniques, including inductively coupled plasma (ICP) and electrospray ionization (ESI) have been proposed for MT characterization and sensitive determination (Pedrero et al. 2012; Ryvolova et al., 2012). Although such methods could represent very useful and feasible tools to diagnose metal poisoning in animals, they still need to be validated.

Screening methods for quantitative determination of toxic substances in biological samples – In recent years, progresses in scientific research in the field of diagnostic toxicology have led to develop many simple, fast and cheap methods of screening, permitting the quick analysis of a large number of samples. They are characterized by fast sample preparation and short time of analysis, sometimes only few minutes. Among the methods studied so far, there are colorimetric assays which detect the presence of a toxic substance in biological samples through a chromogenic reaction. A colorimetric assay was recently developed to detect serum paracetamol concentration in acute intoxicated humans (Senarathna et al., 2012). Such assay resulted easy, very fast (results in less than 30 minutes) and cheap. Moreover, there was a good correlation between this colorimetric assay and conventional method (HPLC). Considering cats' susceptibility, further studies should be carried out to

apply such diagnostic tool in veterinary practice. Spectrophotometry in the ultraviolet (UV) or visible is useful to highlight the presence of a substance in an appropriate solution based on the capacity of multiple links in it to absorb light at a specific wavelength (Hallbach and Guder, 1991; Lee et al., 2004; Fukui et al., 2005); different immunochemical techniques, such as enzyme linked immuno-sorbent assay, ELISA (Cocchi et al., 2010) and biosensors that represent a qualiquantitative method able to detect a wide variety of toxic substances including pesticides, drugs and contaminants in different biological matrices by issuing a signal (electrical or optical) which develops after the recognition of the analyte (Horswell et al., 2003; Marchesini et al., 2007; Redshaw et al., 2007; Peng et al., 2009).

Acetaminophen is still an important cause of acute liver failure both in humans and animals (McConkey et al., 2009; Hinson et al., 2010). After the exposure, approximately 5% of acetaminophen is metabolized in liver by the cytochrome P450 2E1 pathway into N-acetyl-para-benzoquinone-imine, or Nacetyl-p-benzoquinoneimine (NAPQI), responsible of hepatotoxicity (James et al., 2003). Ward et al. (2012) have recently proposed early diagnostic indicators of hepatic injury as micro-RNA (miRNA) fragmatns aimed to reduce the diagnostic time. miRNAs are short, chemically stable, noncoding molecules, acting as regulators which bind to untranslated mRNA to produce gene silencing. Such indicators could be dosed in serum from intoxicated patients by real time PCR and commercial available kit and screening libraries.

Ethylene glycol poisoning can occur in domestic animals (Caloni *et al.*, 2012). However, the diagnosis of ethylene glycol poisoning is not always immediate. Thus, measurement of ethylene glycol in serum is definitive. Nevertheless, the detection of this simple compound is not simple and carried out mainly by chromatographic methods. To make the diagnosis easier, new screening methods have been developed, such as enzymatic and colorimetric ones which can be performed directly on body fluids like blood, serum and saliva providing the results in a short time (Porter, 2012).

Table 1. Information to be attached to the samples before sending to the laboratory of veterinary toxicology

Species, breed, sex, age, weight, medical history, current drug treatments, herbal products administered, number of animals exposed, number of animals showing symptoms, type, duration and severity of symptoms, duration of exposure, dose and formulation (if known) of the compound, any other relevant information.

Table 2. Sampling

Ante-mortem samples: whole blood, blood serum, urine, stomach contents, hair. It should also take a sample of food, water and suspicious baits.

Post-mortem samples: liver, kidney, adipose tissue, brain, eye, stomach contents, urine, intracardiac blood and blood serum, bile. Other tissues can be taken if specifically requested by the laboratory.

Conclusions: In conclusion, poisoning due to several toxicants is common in domestic animals. Although veterinary toxicology is constantly evolving, published data about the current trends in domestic animal poisoning and methods used for the detection of the most commonly toxicants are still missing. Such information is very important to better manage the intoxicated patients in order to reduce their mortality. The creation of databases including calls on suspected poisoning in domestic animals are of great concern and the analytical techniques for the detection of toxic substances make the diagnosis easy and quick. Nevertheless, poisoning sometimes cannot be diagnosed on time and is only suspected in many cases. A tight co-operation among the involved parts (owner, veterinarian and toxicologist) is needed to determine a toxicological diagnosis. In particular, detailed individual and environmental anamnesis, meticulous clinical examination or necropsy and accurate collection of samples to be sent to the analytical laboratory are necessary actions.

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