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SUSPECTED GLYPHOSATE POISONING IN A DOG

SUSPEITA DE INTOXICAÇÃO POR GLIFOSATO EM CÃO

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Abstract

Glyphosate is a widely used pesticide that has been associated with poisoning in both humans and animals. Its toxicity remains a topic of controversy, particularly regarding its carcinogenic potential. This study aimed to investigate a suspected case of glyphosate poisoning in a dog, providing a detailed account of the case history, clinical evolution, laboratory findings, and genotoxic assessment. A seven-month-old dog was referred to the Veterinary Hospital, presenting with mental confusion and ataxia. The pet guardian reported altered consciousness and incoordination twelve days after Roundup® was applied to the farm's grass. A complete blood count revealed normocytic normochromic anemia, a packed cell volume of 20%, thrombocytopenia (50,000/µL), and severe leukocytosis (447,200/µL), with 95% of the white blood cells consisting of blasts of unidentified origin and 5% segmented neutrophils. Myelogram analysis showed a moderately cellular bone marrow sample with a predominance of primitive cells of undetermined origin (>95%), suggesting a diagnosis of leukemia. To further investigate the potential link between leukemia and glyphosate exposure, whole blood was collected in a heparinized tube for a comet assay. The test indicated moderate DNA damage. Chemotherapy was recommended; however, treatment was not pursued for personal reasons, and the patient died five days after the consultation. A necropsy was not authorized. This report contributes to the understanding of glyphosate poisoning and its potential health consequences, particularly its association with hematologic malignancies in canines.

Keywords: comet assay, leukemia, myelogram, Roundup, glyphosate poisoning.

Resumo

O glifosato é um pesticida amplamente utilizado, associado a intoxicações em humanos e animais. Sua toxicidade é controversa, especialmente em relação ao potencial carcinogênico. O presente estudo teve como objetivo investigar um caso suspeito de intoxicação por glifosato em um cão, incluindo a descrição detalhada de um caso a partir da história, evolução clínica, exames anatomopatológicos e diagnóstico genotóxico. Um cão de sete meses foi encaminhado ao Hospital Veterinário apresentando confusão mental e desequilíbrio. O tutor relatou mudanças de consciência e incoordenação, doze dias após a aplicação do Roundup® na grama da fazenda. O hemograma revelou anemia normocítica normocrômica, hematócrito de 20%, plaquetopenia (50.000 / µl) e leucocitose intensa (447.200 / µl), composta por 95% de blastos de origem não identificada e 5% de neutrófilos segmentados. O mielograma mostrou amostra moderadamente celular, com alta predominância (mais de 95%) de células primitivas de origem indeterminada. Os resultados sugeriram o diagnóstico de leucemia. Na tentativa de elucidar a origem da leucemia, corroborando a exposição do paciente ao glifosato, o sangue total foi coletado em tubo de heparina para o ensaio cometa. O teste indicou dano moderado ao DNA do paciente. O tratamento quimioterápico foi indicado, entretanto não foi realizado por motivos pessoais e o paciente faleceu cinco dias após a consulta. O presente relato visa contribuir para o entendimento da intoxicação por glifosato e principalmente com suas suas consequências, especialmente em relação a doenças hematológicas em caninos.

Palavras-Chave: ensaio cometa, leucemia, mielograma, Roundup, intoxicação por glifosato.

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Introduction

Glyphosate is the most widely used herbicide in the world, applied in both agricultural and domestic settings. Accidental poisonings have been reported in both animals and humans (DAWSON et al., 2010; KARTHIKRAJ et al., 2019; KUNAPAREDDY; KALISETTY, 2021). Previous studies indicate that dogs and cats exposed to glyphosate-based products exhibit gastrointestinal and respiratory effects, primarily due to the surfactants used in these formulations (BATES; EDWARDS, 2013). Additionally, clinical signs such as vomiting, diarrhea, and lethargy have been observed. In humans, evidence suggests that glyphosate has potential carcinogenic and immunogenic effects (PEILLEX; PELLETIER, 2020; RANA et al., 2023); however, these findings have yet to be confirmed through large-scale cohort studies (ANDREOTTI et al., 2018).

In other mammalian species, such as pigs, glyphosate exposure has been shown to impact the number of galaninergic neurons in the intestine and alter the expression of galanin receptors in the small intestinal wall (PALUS et al., 2024). In rodents, exposure has been linked to impairments in spatial orientation, navigation, learning, and memory (CHÁVEZ-REYES et al., 2024; 2025).

The debate surrounding glyphosate toxicity has intensified over the years. In 2015, the International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic to humans." However, a joint evaluation by the WHO and FAO in 2016 concluded that glyphosate was not carcinogenic in rats but could not rule out carcinogenic potential in mice at very high doses. In the same year, a systematic review found insufficient evidence to support an association between Roundup® exposure and lymphohematopoietic cancers (CHANG; DELZELL, 2016).

It is crucial to consider several factors when assessing glyphosate poisoning. Some studies on the compound have been met with controversy due to methodological limitations, lack of scientific rigor, and conflicts of interest, particularly regarding funding sources. Nonetheless, animal exposure to glyphosate poses a potential health risk. Thus, this study aims to report a clinical case of suspected glyphosate poisoning in a dog in Brazil. To investigate this case, we conducted a comprehensive evaluation, including physical, neurological, and genotoxic examinations.

Material and Methods

Case study

A seven-month-old, 10.2 kg male, mixed breed canine, was referred to the Veterinary Hospital of the Federal University of Goiás, presenting mental confusion and imbalance. During the anamnesis, the guardian reported changes in consciousness plus uncoordinated head and hind limb movements. It was also reported hyporexia and normodipsia, with no history of diarrhea, fainting, epileptic seizures, trauma, or previous illnesses.

The patient lived in a rural environment, and when asked about access to toxic products, it was reported that twelve days before the consultation, they had used the product Roundup® in the grass of the farm to kill weeds. The animal had access to the grass area and the street, and was fed only homemade food.

Clinical exams

A depressed state of consciousness and reactive superficial mandibular and cervical lymph nodes were observed on physical examination. Mucosal staining, capillary filling time, skin turgor, temperature, and cardiac and pulmonary auscultation were normal. The neurological examination revealed a reduced response to threat in both eyes, preserved facial sensitivity, normal to reduced swallowing and jaw tone, and preserved withdrawal reflex and superficial pain in the four limbs. When assessing the patient in motion, proprioceptive ataxia, broad-based station, and head tremors when moving around were observed.

Based on history, anamnesis, and clinical signs, some infectious diseases, such as distemper, ehrlichiosis, toxoplasmosis, leishmaniasis, and herbicide poisoning, were suspected. To better understand this case, a rapid test for distemper (Alere®), complete blood count (CBC), search for Lentz corpuscles, creatinine, and alanine aminotransferase (ALT) were requested.

Comet Assay

The comet assay was performed on peripheral blood as a biomarker of genotoxicity. The alkaline assay was based on Singh et al. (1988), with some modifications (RAMOS et al., 2021). A total of 15 µL of blood was homogenized in 120 µL of low-melting-point agarose. This sample was placed on slides, and after solidification, the slides were immersed in a cold lysis buffer in a light-protected chamber, where they remained for 15 hours. After this period, the slides were removed from the lysis solution and placed in a horizontal electrophoresis tank, where they were incubated in an alkaline buffer at 4°C for 30 minutes. Electrophoresis was then performed for 30 minutes at 25 volts and 250 mA, maintaining a temperature of 4°C. The slides were neutralized using a Tris solution, repeated three times at 5-minute intervals. After neutralization, the slides were washed with distilled water and then fixed by immersion in absolute ethanol for 10 minutes. Once dried, the DNA was stained with 100 µL of SYBR® Green I solution. A total of 100 cells per slide (two slides in total) were analyzed using an Axio Imager D2® fluorescence microscope (Carl Zeiss, Berlin, Germany) by a single observer, using a 20x objective. Three genomic damage parameters were assessed: tail length, percentage of DNA in the tail, and Olive tail moment.

Data Analysis

The data from this case are presented in absolute and relative frequencies, as well as compared to findings from other studies analyzing the same parameters/biomarkers. Furthermore, for the Comet Assay, the data are presented as mean ± standard deviation (SD).

Results and Discussion

This study highlights a fatal case of suspected glyphosate poisoning in a dog. The rapid test result was negative, and the CBC showed normocytic normochromic anemia, packed cell volume (PCV) of 20%, with the slight presence of codocytes and thrombocytopenia (50.000/µl). Anemia is characterized by a reduction in red blood cells or in the blood's ability to transport oxygen, due to decreased packed cell volume, red blood cell count, or hemoglobin concentration below reference values (PADALINO et al., 2016). In this context, some studies indicate an association between anemia and exposure to pesticides (YUSHANANTA et al., 2020; ASIF SYED et al., 2021).

It was also possible to observe intense leukocytosis (447.200/µl), composed of 95% of blasts of unidentified origin and 5% of segmented neutrophils. The observed blasts showed mild to moderate anisocytosis and pleomorphism, mild to moderately basophilic cytoplasm, and mild to moderate vacuolization in all cells. Its nucleus/cytoplasm ratio varied from mild to moderate; the nuclei had moderate anisocariosis, moderate pleomorphism (round, oval, and monocytoid), possible figures of aberrant mitosis, and rare binucleations. The nucleoli, possibly prominent, rounded, or angular nucleoli, were observed, sometimes multiple, with discrete anisonucleolysis. Rare lymphoglandular corpuscles were present, mild to moderate cell fragility and recurrent cell apoptosis (Figure 1).

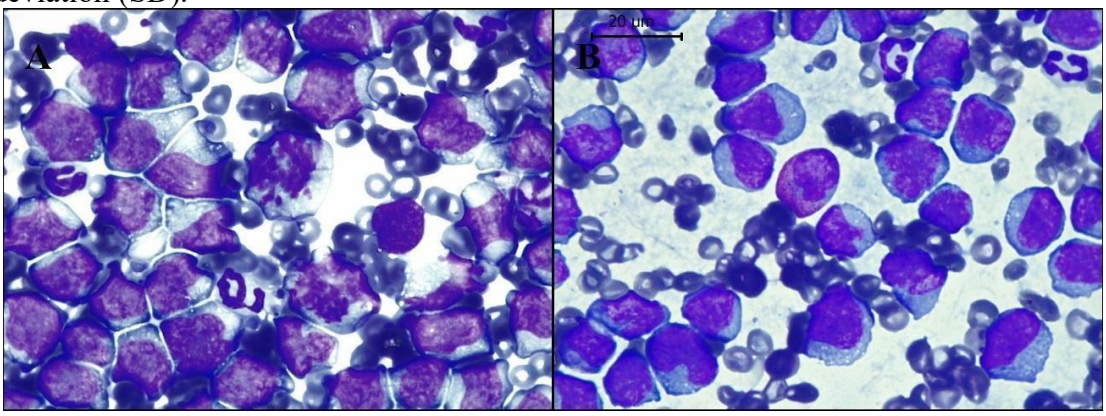

Figure 1. Microscopic evaluation of cells from a dog suspected of glyphosate poisoning. A) Photomicrograph of blood smear of intense cellularity composed of 95% of blasts of unidentified origin and 5% of segmented neutrophils. 100x. B) Cytological sample of moderate cellularity, with predominance of primitive cells of undetermined origin.

Based on the patient's history and complementary exams, leukemia was investigated. Leukemia is a clonal proliferation of hematopoietic stem cells in the bone marrow (DAVIS et al., 2014). Thus, for further clarification, the patient was directed to perform the myelogram. Optical microscopy showed a moderately cellular sample, with a high predominance (more than 95%) of primitive cells of undetermined origin isolated in cell islands. Cellular characteristics included mild to moderate anisocytosis, high to medium nucleus/cytoplasm ratio, and intense cytoplasmic and nuclear basophilia. There was intense cellular fragility and rare lymphoglandular corpuscles; some cells had a morphology similar to "hand mirror." Rare myeloid precursors and possible mature neutrophils, very few erythroid precursors, and no megakaryocytic precursors were observed (Figure 1). Such findings indicated an exacerbated proliferation of the blast population with a break in the expected proportionality of the medullary compartments, confirming the suspicion of leukemia.

Leukemia is a complex neoplasia that is often associated with previous exposure to toxic components such as benzene (SNYDER, 2012). Acute myeloid leukemia is a rare and progressive cancer in dogs. Recent studies revealed that diagnosis can be based on more than 20% blasts in bone marrow, as described in the present report (DAVIS et al., 2018). The most common clinical signs are inappetence, lethargy, and lymphadenopathy, and clinical pathology findings suggest an increase in blast circulation, anemia, and thrombocytopenia, also corroborating our findings. The mean survival time was 19 days, ranging from 1 to 121 days.

In an attempt to elucidate the origin of the leukemia, corroborating the patient's exposure to glyphosate, whole blood was collected in a heparin tube for the comet assay (HALDER et al., 2002). For the comet assay (Figure 2), the results indicate moderate damage ($\bar{x}\pm SD$), as observed across all parameters, such as Tail Length (10.06±7.14), Olive Tail Moment (2.25±2.01), and %DNA in the tail (21.51±15.36). In this regard, in at least one of the parameters, the mean was consistent with other studies that demonstrated significant DNA damage due to pesticide exposure (HONG et al., 2017; KWIATKOWSKA et al., 2017; LOPES et al., 2021). Furthermore, DNA fragmentation may also indicate cytotoxic potential, as previously described (ESCOBAR et al., 2007). Other studies also highlight the genotoxicity attributed to herbicides in dogs. Both glyphosate and 2,4-D were genotoxic to canine cells at concentrations of 0.10 μM and above (TINDLE et al., 2025).

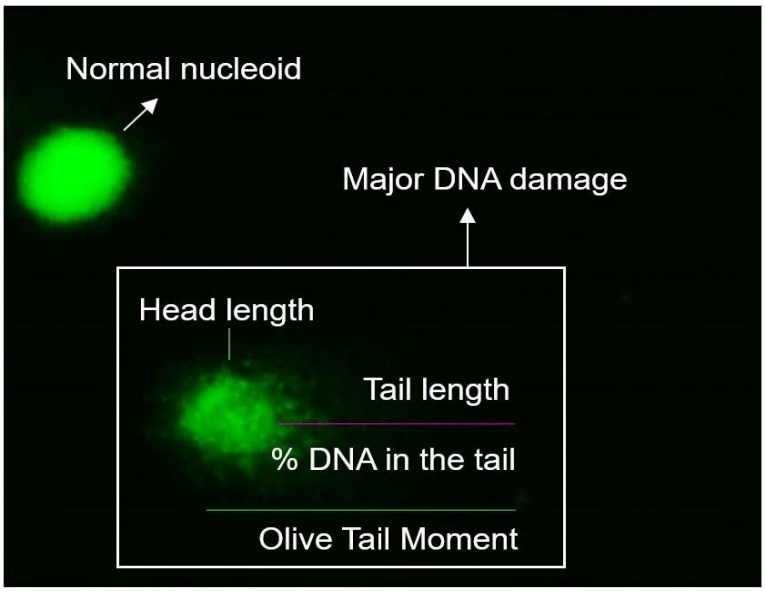

Figure 2. Photomicrograph of the Comet Assay showing one normal nucleoid and another fragmented in a dog suspected of glyphosate poisoning.

Glyphosate exposure, as previously stated, may be cancerous, particularly regarding the surfactants used in the current formulation of the product (GASNIER et al., 2009). Its full toxic potential, especially regarding cancer presentations (CHANG; DELZELL, 2016; DAVOREN; SCHIESTL, 2018), is yet to be established in both humans and animals. The dog

reported in the present document was young, with no previous conditions and a recent exposure to a supposedly large amount of glyphosate in its environment. Both hematology and myelogram support the diagnosis of leukemia, and the comet assay further suggests a toxic agent as a possible factor in the cancer development.

Among herbicides, glyphosate has been reported as the main culprit in poisoning cases involving domestic animals such as dogs, cats, horses, goats, and sheep (CALONI et al., 2016). However, acknowledging some limitations of this study, it is important to highlight the financial constraints that prevented additional investigations using flow cytometry and immunocytochemistry to determine the cellular origin. Furthermore, chemotherapy was recommended; however, the treatment was not carried out for personal reasons, and the patient died five days after the consultation. Necropsy was not authorized. Further toxicological analysis from serum samples was attempted but was not successful due to the difficulty in glyphosate detection and the lack of specialized services in Brazil.

Considering the susceptibility of domestic animals to contact with pesticides and other xenobiotics, this study highlights the risks of intoxication. Exposure to household xenobiotics may occur through oral ingestion or inhalation, although some pesticides are not volatile due to their molecular weight. However, inhalation of glyphosate surfactant herbicide by a dog has been documented, resulting in hypersalivation, tremors, and hyperthermia, followed by death (CORTINOVIS et al., 2015). In the present study, it was not possible to determine the route and duration of exposure. Finally, environmental contamination by household chemicals represents a global challenge, and bioindicators such as pets can be essential tools for effective monitoring and risk assessment (BERTERO et al., 2020).

Conclusion

In this study, we report a case of suspected glyphosate poisoning in a dog. Although the data indicate a severe acute response of leukemia and DNA damage, further studies are still needed in the future. The findings contribute to the understanding of glyphosate poisoning and its consequences in canines. This case reinforces the importance of implementing biosecurity measures in rural areas and training veterinarians to recognize early signs of pesticide poisoning in domestic animals.

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