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PATHOLOGY

Outbreak of monensin poisoning in equines: clinical signs, histopathologic findings and chromatographic diagnosis

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ABSTRACT: Ionophores are polyether antibiotics used in animal production for ruminants and birds to promote growth and exert coccidiostat action. Despite the benefits of their use, poisonings have also been reported in several species. Equines are very sensitive, and most cases involve mistaken intake of feed intended for ruminants. This research described an outbreak of ionophore poisoning in seven horses and a mule that ingested livestock concentrate, confirmed by a chromatographic diagnostic method. A six-year-old horse was presented with clinical signs of sialorrhea, sweating, ataxia, prostration, anorexia, muscle tremors, lateral recumbency, posterior lateral decubitus, which began the day before. Laboratory tests showed increased AST (aspartate aminotransferase) and CK (creatine kinase) activities. Electrocardiogram revealed ventricular tachycardia. Due to the severity of the condition, the patient was euthanized on the day of admission, and a necropsy was performed, in which skeletal and cardiac muscle tissues were collected. Histology revealed multifocal necrosis in cardiomyocytes, necrosis, edema and hemorrhage in skeletal muscle. A visit was made to the property to evaluate other animals and collect samples for laboratory tests. All remaining animals underwent physical examination, two of which showed serious clinical signs. Three animals showed normocytic normochromic anemia, four increased creatinine, two had increased AST activity and all had increased CK activity. Among the poisoned animals, two died on the property. Liver sample from the first euthanized horse was examined by liquid chromatography coupled with mass spectrometry, identifying 51.59 µg/kg of monensin. It is concluded that, despite the recovery of the animals, the toxic action of the ionophore can cause injuries that affect equine health and welfare. The feasibility of chromatographic analysis is essential for a definitive diagnosis and must be subsequently developed together with reference values for tissue residues and maximum tolerated doses.
Key words: horses, ionophores, muscle necrosis, toxicity.

Surto de intoxicação por monensina em equídeos: sinais clínicos, achados histopatológicos e diagnóstico cromatográfico

RESUMO: Ionóforos são antibióticos poliéteres utilizados na produção animal para ruminantes e aves para promover o crescimento e exercer ação coccidiostática. Apesar dos benefícios de seu uso, intoxicações também foram relatadas em diversas espécies. Os equídeos são muito sensíveis e a maioria dos casos envolve ingestão inadequada de alimentos destinados a ruminantes. Este trabalho descreve um surto de intoxicação por ionóforo em sete cavalos e um burro que ingeriram concentrado de gado, confirmado por método de diagnóstico inovador. Um cavalo de seis anos de idade apresentou sinais clínicos de sialorreia, sudorese, ataxia, prostração, anorexia, tremores musculares, decúbito lateral, decúbito lateral, iniciados no dia anterior. Foram realizados exames laboratoriais e eletrocardiograma, que evidenciaram aumento da atividade de AST e CK, além de taquicardia ventricular. Devido à gravidade do quadro, o paciente foi eutanasiado no dia da internação, sendo realizada necropsia, na qual foram examinados tecidos musculares esqueléticos e cardíacos. A histologia revelou necrose multifocal em cardiomiócitos, necrose, edema e hemorragia no músculo esquelético. Foi realizada visita à propriedade para avaliação de outros animais e coleta de amostras para exames laboratoriais. Todos os cinco animais restantes foram submetidos a exame físico, dois dos quais apresentaram sinais clínicos graves. Três animais apresentaram anemia normocítica normocrômica, quatro apresentaram aumento da creatinina, dois apresentaram aumento de atividade de AST e todos apresentaram aumento de atividade de CK. Entre os demais animais intoxicados, dois morreram na propriedade. A amostra de fígado do primeiro cavalo eutanasiado foi examinada por cromatografia líquida acoplada à espectrometria de massa, identificando 51,59 µg/kg de monensina. Conclui-se que, apesar da recuperação dos animais, a ação tóxica do ionóforo pode causar lesões que afetam a saúde e o bem-estar do animal. A viabilidade da análise cromatográfica é essencial para um diagnóstico definitivo e deve ser posteriormente desenvolvida juntamente com valores de referência para resíduos teciduais e limite máximo tolerável.
Palavras-chave: cavalos, ionóforos, necrose muscular, toxicidade.

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INTRODUCTION

Ionophores are antibiotics widely used in animal production both as coccidiostats and growth promoters (RODER, 2011; BURNETT, 2012). Amongst the main ionophores used as food additives in animal production are monensin, lasalocid, salinomycin, and narasin. They are products of fungi fermentation, specifically *Streptomyces* spp. (RODER, 2011; NOGUEIRA et al., 2009; BURNETT, 2012). Their capacity of forming liposoluble complexes with polar cations, such as potassium, sodium, calcium, and magnesium, aids these ions' transport through cellular membranes, thus affecting ionic gradient and leading to effects on organelles (EKINCI et al., 2023), especially mitochondria inhibiting oxidative phosphorylation (GAO et al., 2018).

Ionophores are safe when administered at recommended doses. However, there is a considerable variation in sensitivity to these compounds, depending on the species involved and the type of ionophore used (SILVA et al., 2022). Equine species are particularly sensitive to monensin toxicity, which occurs following consumption of only 2-3 mg/kg (SILVA et al., 2022). Even a regular commercial ruminant dose is enough to poison a horse (EKINCI et al., 2023). There are no maximum limits of detection for this substance in animal samples in Brazilian law. However, other species have already been reported to suffer from ionophore poisoning, such as mules, cattle, sheep, dogs, camelids, and poultry. These poisonings occur because of a larger consumption of ionophores, primarily stemming from errors in food mixing or unintentional offering to susceptible animals (EKINCI et al., 2023; ENSLEY, 2020).

Clinical signs commonly observed in horses are anorexia, depression, sweating, and ataxia. Death may occur within 12 to 48 hours after the onset of clinical signs (NOGUEIRA et al., 2009; FRITZ & HALL, 2024). Diagnosis is often based on history and clinical signs, commonly associated with skeletal and/or cardiac muscle necrosis. No clinical signs or postmortem findings are pathognomonic of the poisoning. However, chromatographic analysis can establish a definitive diagnosis. Previous methodologies included high-performance liquid chromatography (HPLC) of bovine liver samples (PASTORE et al., 2022). Currently, there are no recommended doses of monensin for equine and no maximum limit residue level described in Brazilian law. This study reported an outbreak of monensin in a property in Goiás State, Brazil, followed by detection and confirmation using Liquid chromatography-tandem mass spectrometry (LC-MS/MS) from liver sample.

MATERIALS AND METHODS

One horse (EQ1), a six-year-old Mangalarga Marchador, approximately 400 kg of body mass, was admitted to the Hospital Veterinário of the Universidade Federal de Goiás (VH-UFG), under an ionophore poisoning suspicion. According to the owner, seven horses and a mule from his property in Goiânia (Goiás, Brazil) were provided with a significant amount of expired bovine feed the day before the admittance. A few hours after exposure, one of the horses developed sialorrhea, sweating, ataxia, and lateral recumbency. The next day, the owner referred the horse to the veterinary hospital, still in lateral recumbency, and presenting lethargy, anorexia, and shivering.

RESULTS

During clinical examination, the horse (EQ1) was unable to stand up, depressed, with hyperemic ocular mucosa and normal oral mucosa, a capillary refill time of 3 seconds, heart rate of 86 bpm, respiratory rate of 60 mpm, rectal temperature of 39,1 ºC, hypomotility of digestive tract and a moderate tympanism. Due to the history stated by the owner, associated with clinical signs, a presumptive diagnosis of ionophore poisoning was outlined. Thus, blood was sampled for a complete blood cell count (CBC) (Table 1) and serum biochemistry (creatine kinase, aspartate aminotransferase, creatinine). Electrocardiography (ECG) was performed according to the Dubois configuration adapted to the left lateral recumbency position (AYALA et al., 2000).

Blood hematology and fibrinogen showed results within normal ranges for the species (GRONDIN & DEWITT, 2010). Serum biochemistry (Table 2) showed values of creatine kinase (CK) and aspartate aminotransferase (AST) activities above range values. CK values were 350 times greater than those of reference values (8203 UI/L, 2.4 - 23.4 UI/L, KANEKO et al., 2008). ECG showed significant ventricular tachycardia.

Considering the patient's clinical status and the worsening of clinical signs, euthanasia and necropsy were performed on the same day. The following day, a team of veterinarians from HV-UFG visited the property to advise the owner about the nutritional management of horses and to evaluate the other animals. At that time, the property had six

Table 1 - Total Blood Cell Count of equines (CBC) and mule under ionophore poisoning suspicion.

CBC								
Erythrogram	EQ1	EQ2	EQ3	EQ4	EQ5	EQ6	AS	Reference values #
Red Blood Cell	8.39	6.17	6.50	6.30	6.00	5.87	6.30	6.80-12.90 (x10^6/µL)
Hemoglobin	14.9	11.1	11.7	9.9	10.2	9.7	12.9	11.0 - 19.0 (g/dl)
Hematocrit	46	34	36	30	30	30	39	32 - 53.0 (%)
MCV	54.8	55.1	55.4	47.6	50.0	51.1	61.9	37 - 58.0 (fl)
MCHC	32.4	32.6	32.5	33.0	34.0	32.3	33.1	31 - 36 (%)
RDW	16.8	15.6	16.9	15.6	15.7	16.6	15.8	15 - 18 (%)
Platelets	108	135	192	161	114	120	300	80 - 400 (x10^3/µL)
Total Plasma Protein	7.0	7.0	7.4	6.0	6.0	6.4	7.0	5.8 - 8.7 (g/dl)
Fibrinogen	200	100	100	100	100	100	200	100 - 400 (mg/dL)
Observations		*	-	**	-	***	-	
Leukogram								Reference values #
Total leukocytes	9,100	10,100	9,200	7,900	8,000	8,600	9,900	5,500 - 12,000 (x10^3/µL)
Myelocytes	0	0	0	0	0	0	0	0
Metamyelocytes	0	0	0	0	0	0	0	0
Band neutrophil	0	0	0	158	0	0	0	0 - 240 (x10^3/µL)
Segmented neutrophil	7,189	5,454	6,164	3,950	4,800	5,590	4,752	1,925 - 9,000 (x10^3/µL)
Eosinophil	0	404	552	869	640	516	792	110 - 1,440 (x10^3/µL)
Basophil	0	0	0	0	0	0	0	0 - 360 (x10^3/µL)
Lymphocytes	1,547	3,939	2,300	2,844	2,320	2,236	4,158	825 - 6,000 (x10^3/µL)
Monocytes	364	303	184	79	240	258	198	110 - 1,200 (x10^3/µL)
Observations		*	-	**	-	***	-	

#GRONDIN & DEWITT, 2010.
*Acantocytes (+); hypersegmented neutrophils; reactive lymphocytes (+); activated monocytes (+++);
**Acantocytes (+); reactive lymphocytes (++); platelet clumps (+);
***Acantocytes (+); hypersegmented neutrophils; activated monocytes (+++); reactive lymphocytes (++). MCV: Mean Corpuscular Value; MCHC: Mean corpuscular hemoglobin concentration; RDW: red cell distribution width.

horses and a mule. Before the team arrived, a horse had died under the same suspicion, with significant nasal foam and suspected pulmonary edema (Figure 1). No necropsy was performed due to the advanced state of autolysis.

During clinical examination, five animals had a normal state of consciousness, and one was depressed. Horse 2 (EQ2) presented with tachycardia, tachypnea, weakness, ataxia, lethargy, sternal recumbency (Figure 1), and died on the property after two days. Horse 3 (EQ3) presented tachycardia, tachypnea and was restless, with hypomotility and epiphora. Horse 4 (EQ4) presented an alternating state between alert and lethargy, tachycardia, tachypnea, pale mucous membranes, and hypermotility. Horse 5 (EQ5) presented with lethargy, tachypnea, hyperemic ocular mucosa, and hypermotility. Horse 6 (EQ6) also presented a state of consciousness alternating between alert and lethargy, tachypnea, hyperemic mucous membranes, and epiphora. The mule (AS) presented with tachypnea, a mild degree of dehydration (< 6%), and hyperemic ocular mucosa. During this visit, blood samples were

Table 2 - Serum biochemistry of equines with suspected ionophore poisoning.

Serum biochemistry analysts	----------Results----------							Reference values (KANEKO et al., 2008)
	EQ1	EQ2	EQ3	EQ4	EQ5	EQ6	AS	
Creatinine	1.26	4.70	1.79	3.39	1.63	3.05	2.57	1.20 - 1.90 (mg/dL)
AST	594	353	330	204	349	251	219	226 - 366 (UI/L)
CK	8203	159	157	95	88	65	98	2.4 - 23.4 (UI/L)

AST: aspartate aminotransferase; CK: creatine kinase.

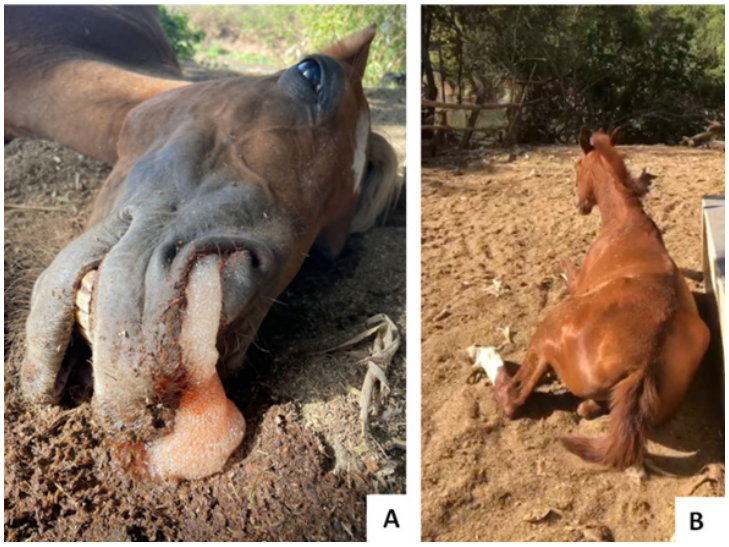

Figure 1 - Horses under suspected ionophore poisoning. A. Dead horse presenting significant nasal foam, suspected of pulmonary edema, 3 days after ionophore consumption. B. Horse in sternal recumbency.

collected for CBC and serum biochemistry (Table 1 and 2). Regarding biochemical analysis of the remaining six animals, four animals showed an increase in creatinine; two showed a decrease in AST activity; and all showed an increase in CK activity (Table 2).

Some recommendations were suggested to the horse's owner, such as feed disposal, cleaning the feeding trough, and observing all animals that had consumed the cattle feed. Additionally, we suggested that animals have access to clean and abundant water to prevent dehydration. It was also recommended supplementation with vitamin E and selenium. The remaining animals were not referred to the veterinary hospital due to the owner's financial limitations. Among five surviving animals, one horse still presented with weight loss and certain ambulation difficulty, three months after the outbreak.

Necropsy was performed on the first animal reported in this case. Upon macroscopic examination, the heart showed multifocal ecchymosis and suffusions in the left ventricle, predominantly at its' base. At the apex, there was a discolored, irregularly marginalized, and focally extensive area. Skeletal muscles of the pectoral region had a diffuse and bright dark red color. In the center of the superficial cut, there was a marked dark and bright red area, with fragmented muscle fibers, suggestive of hemorrhagic muscular necrosis (Figure 2A). Effusion was also observed in the pleural and peritoneal cavities (Figure 2B).

Upon histopathological evaluation, cardiac striated muscle showed multifocal and moderate cardiomyocyte coagulation necrosis, with slight hemorrhage (Figure 3A and B). Striated skeletal muscles of the temporal region showed marked hyaline, segmental, and flocculated necrosis, with diffuse and marked edema and hemorrhage between collagen fibers (Figure 3C and D).

Liver sample was collected, refrigerated, and sent to the Federal Laboratory of Agricultural Defense of Minas Gerais, Brazil, where it underwent chromatographic analysis to detect abamectin, doramectin, eprinomectin, ivermectin, moxidectin, fipronil, fipronil sulfone, sisapronil and monensin. A QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach with acetonitrile, NaCl, $MgSO_4$, sodium citrate tribasic dihydrate and sodium citrate dibasic sesquihydrate was used for analytes extraction, and the purification was made with a mixture of PSA and C18 as adsorbents. The identification and quantification were carried out by liquid chromatography coupled mass spectrometry (LC-MS/MS), as described and validated by PASTORE et al. (2022). Analyses were performed using a Waters UHPLC system coupled to a Quattro Premier XE Triple Quadrupole mass spectrometer, with an electrospray ionization (ESI) source and identified 51.59 µg/kg of monensin and 10.75 µg/kg of sulfone fipronil (Table 3).

DISCUSSION

In the present study, we reported an outbreak of monensin poisoning of seven horses

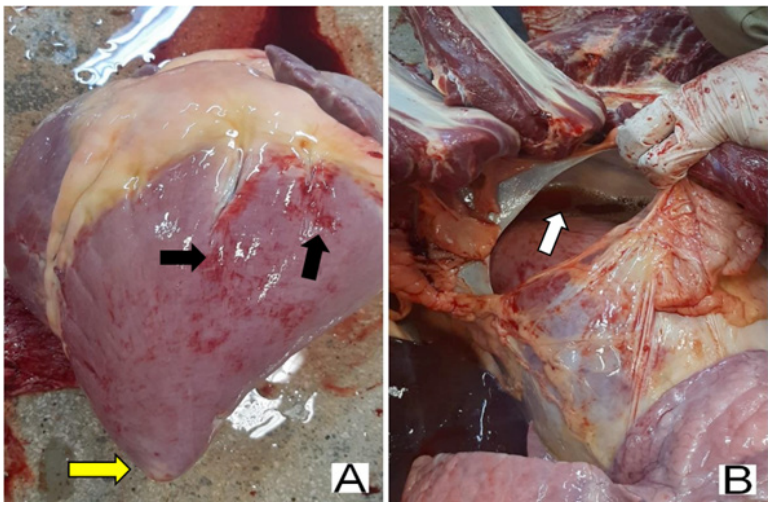

Figure 2 - Macroscopic images of the euthanized horse with monensin poisoning. A. Heart showed multifocal ecchymosis and suffusions in the left ventricle, predominantly at its base (black arrows). At the apex, there was a discolored, irregularly marginalized, and focally extensive area (yellow arrow). B. Effusion was also observed in the pleural and peritoneal cavities (white arrow).

and one mule after consumption of livestock feed. Although, the condition does not have any specific clinical signs, cases of monensin poisoning can present anorexia, diarrhea, and neurological changes including incoordination, muscle tremors, depression, and decubitus as the main clinical signs (RODER, 2011; BRITO et al., 2020). These signs are not pathognomonic of the poisoning;

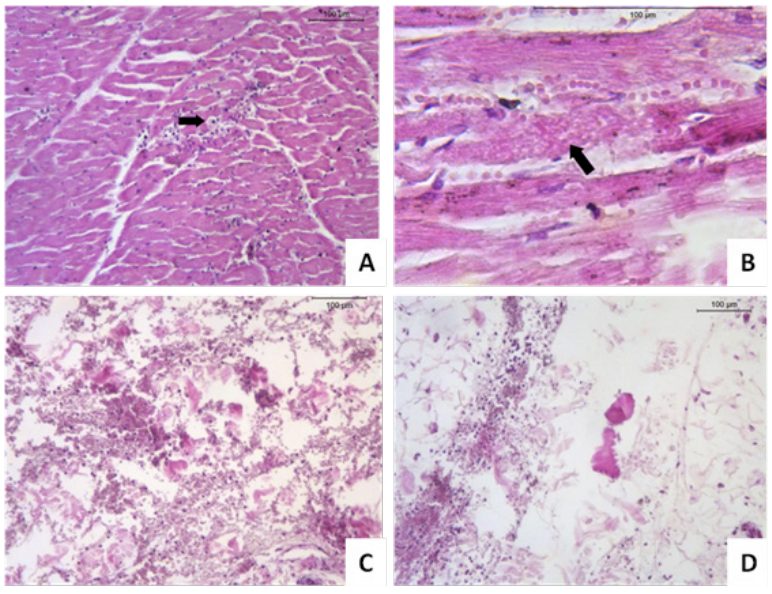

Figure 3 - Histology samples of the left ventricle and skeletal muscle from a horse with monensin poisoning (HE). A. Moderate multifocal necrotic fragmentation of cardiomyocytes associated with discrete hemorrhage, and discrete mononuclear inflammatory infiltrate (black arrow). B: Necrotic cardiomyocyte (black arrow). C and D. Skeletal muscle. Focal floccular segmental hyaline necrosis and extensive hemorrhage, neutrophilic inflammatory infiltrate, and edema between hyaline fibers, which are presented corrugated, and loose, with diffuse distribution. HE, obj.40x.

Table 3 - Results from LC-MS/MS analysis of the horse's liver suspected of ionophore poisoning.

Toxic agent	----------Result----------	----------LQ----------	----------MRL----------	----------Unit----------
Abamectin	NQ	5	-	µg/kg
Doramectin	NQ	5	100	µg/kg
Eprinomectin	NQ	5	-	µg/kg
Ivermectin	NQ	10	100	µg/kg
Moxidectin	NQ	5	100	µg/kg
Fipronil	NQ	5	-	µg/kg
Fipronil sulfone	10.75	5	-	µg/kg
Monensin	51.59	5	-	µg/kg
Sisapronil	NQ	10	-	µg/kg

LQ: Limit of quantification; NQ: Not Quantifiable; MLR: Maximum Residue Limit.
Reference: Normative Instruction No. 162, of July 1, 2022 of the National Health Surveillance Agency (Published in the Federal Official Gazette of Brazil, No. 126, of July 6, 2022).

therefore, toxicological diagnosis is relevant. In our report, definitive diagnosis was provided through liver sample analysis of LC-MS/MS, revealing a significant concentration of this ionophore. Our study contributes to the diagnosis of ionophore poisoning and future advances in regulatory documents.

The first horse exhibited severe clinical signs a few hours after being fed with livestock concentrate. The animal was euthanized due to a poor prognosis. In addition, six horses and a mule were given the same feed and showed clinical signs. Supplying concentrated feed intended for cattle to other species, especially horses, is unsafe. Apart from not being the target species, these animals are sensitive to the consumption of ionophores, which are commonly part of the composition of these products (RODER, 2011; NOGUEIRA et al., 2009).

The patient admitted to VH/UFG displayed clinical signs such as lethargy, sweating, ataxia, reluctance to move, muscle tremors, weakness, lateral recumbency, arrhythmia, tachycardia, and tachypnea. Horses 2 and 3 also exhibited some of these clinical signs, aligning with the pattern seen in ionophore poisoning. These clinical manifestations stem from the mechanism through which ionophores impact the organism. They form lipid-soluble complexes that are dynamically reversible with mono or divalent cations, causing shifts in ionic gradients across the cell membrane (ENSLEY, 2020). Typically, these effects target specific tissues, notably skeletal muscles and the heart (NOGUEIRA et al., 2009). Additionally, monensin has been shown to increase the permeability of Na^+, K^+, and H^+ ions to cytosol of muscle cells with excitatory effects due to depolarization of presynaptic nerve endings and Ca^{++} influxes, leading to cardiac and skeletal degeneration and necrosis (BRITO et al., 2020). In this account, one patient exhibited clinical signs within 24 hours, while the other animals took 24 to 48 hours to manifest clinical signs.

The ionophore activity may change normal ion concentration gradient, and this causes cellular ion imbalance, pH change, and disruption of the plasma membrane (EKINCI et al., 2023).

Ionophore toxicity is based on its impact on mitochondria and plasma membranes. This imbalance in ion concentration results in inhibition of mitochondria-mediated oxidative phosphorylation. Myocardium and skeletal muscle cells are the most affected, possibly due to their high metabolic activity (EKINCI et al., 2023). Electrocardiogram performed on EQ1 showed sustained ventricular tachycardia. These results thus indicated that the impact on target tissues is frequently linked to ionophore poisoning, encompassing cardiac and striated skeletal muscles. Consequently, heightened activity of muscle-derived enzymes like CK, AST, and LDH may be noted, alongside increased levels of cardiac troponin I, hypocalcemia, and hypokalemia. Furthermore, anomalies in the electrocardiogram, among other changes, such as ventricular tachycardia, atrioventricular block, increased S wave amplitude, absence of the P wave, intermittent premature ventricular contractions, and atrial fibrillation could be evident (NOGUEIRA et al., 2009; FRITZ & HALL, 2024).

Clinical-pathological changes include elevated levels of muscle enzymes with possible increases in serum urea. Additionally, serum calcium concentrations may decrease to life-threatening levels in ponies or horses poisoned with monensin. The ion

imbalance, especially intracellular sodium excess, activates the sodium calcium exchanger, triggering the release of calcium from intracellular stores, leading to serum Ca^{++} reduction (RODER, 2011). CK activity was elevated in all animals, creatinine was high in four, and AST activity was elevated in three animals.

To enhance the diagnostic support and prognosis, additional tests were performed, including a complete blood count (CBC), serum biochemistry and electrocardiogram. No hematological alterations were observed in the complete blood count (CBC) of EQ1, unlike the laboratory tests of other animals, where normocytic and normochromic anemia were observed. Anemia is possibly associated with microhemorrhages in muscle tissue, as observed in the necropsy findings.

An amount of 51.59 µg/kg of monensin was identified in the liver using LC-MS/MS technique. Oral monensinLD50 in horses is 2-3 mg/kg (body weight) (MATSUOKA et al., 1996), yet previous research does not provide a direct relation to liver concentrations. This difference between lethal dose and the concentration of monensin identified by chromatography can be justified by the ingested dose, toxicokinetics, sample harvesting methods, and post-mortem degradation (BAUTISTA et al., 2014). LC-MS/MS technique enables quantitative analysis of a wide variety of compounds and is one of the most used analyses for assessing coccidiostats in both animals and feed (BURNETT et al., 2012; MORETTI et al., 2013). Additionally, it is a cost-effective technique with fast processing and high sensitivity (DAI & HERRMAN, 2010). Besides liver samples used in the reported case, it is possible to use myocardial and stomach content samples (BAUTISTA et al., 2014). Samples can be collected from blood, serum, urine, gastrointestinal content and also from the suspect feed (BAUTISTA et al., 2014; DAI & HERRMAN, 2010).

Ionophore poisoning lacks a specific antidote. However, administration of vitamin E and selenium were described as effective in cases of monensin poisoning (RODER, 2011; NOGUEIRA et al., 2009). This is controversial, as currently there is no consensus in the matter, as some authors recommend administration before the onset of lesions, and others highlighted that the treatment was not very effective once clinical signs have begun (DOONAN et al., 1989; FRITZ & HALL, 2024). Vitamin E and selenium deficiency are responsible for causing cardiac necrosis; therefore, supplementation with these nutrients can be recommended in cases of differential diagnosis (DOONAN et al., 1989). Additionally, supportive treatment is important, particularly ensuring proper hydration through fluid therapy and treating other clinical signs presented (FRITZ & HALL, 2024).

It is important to highlight that animals exposed to sublethal doses of monensin that survive critical clinical phases may present variable sequelae, including exercise intolerance and persistence cardiac abnormalities (GY et al., 2020).

CONCLUSION

We described an outbreak of ionophore poisoning in equine that resulted in three deaths and a novel approach of liver analysis by LC-MS/MS to detect and confirm monensin presence. The viability of chromatographic analysis is paramount for a definite diagnosis and should be further publicized along with reference values for tissue residues and maximum tolerated doses.

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DECLARATION OF CONFLICT OF INTEREST

Authors declare no conflicts of interests.

AUTHORS' CONTRIBUTIONS

Conceptualization: AAP, WG and OGF. Data acquisition: WG, LBC and BJRF. Design of methodology and data analysis: WG, CMGO and OGF. WG and OGF prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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