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Research Article
Clinical and Pathological Changes in Sheep During a Monensin Toxicity Outbreak in Brasilia, Brazil

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Abstract
A monensin poisoning outbreak resulting from a failure in the feed formulation was investigated in a sheep herd in Brasilia, Brazil. Forty sheep died, with a morbidity rate of 33.33% and mortality rate of 100%. Poisoned animals showed attitude, postural, mucosal and cardio-respiratory changes, as well as neutrophilia, lymphopenia and high serum aspartate aminotransferase (AST) and creatine phosphokinase (CPK) levels. At necropsy, the main lesions observed were pale and whitish areas on one or more skeletal muscle groups, in addition to myocardium. Degenerative-necrotic, circulatory and inflammatory changes, fibroplasia and regeneration of muscle fibers were the main histopathological alterations in sheep spontaneously poisoned by monensin. The frequency of skeletal muscle group and myocardium lesions was determined. Striated skeletal muscle groups of monensin-poisoned animals showed degenerative necrotic lesions that were more frequent and intense compared to the myocardium. The detailed characterization of the changes and lesions in sheep intoxicated by monensin may contribute to the diagnosis of toxicosis and as well as to the understanding of the clinical and pathological events involved.

Key words: Sheep, monensin, ionophores, poisoning, toxicity, muscles

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INTRODUCTION

Monensin is one of the most used ionophore antibiotics in veterinary medicine, although the use of this agent can potentially expose animals to a higher risk of poisoning (Chapman *et al*., 2010). When properly used, ionophores are effective as a growth promoter in ruminants, however, its margin of safety is small and it can cause great economic losses due to toxicosis (De Miranda Neto *et al*., 2011; Roder, 2011; Mohammadi *et al*., 2012). Ionophore use is generally considered safe at recommended doses. Nevertheless, there are reports of poisoning in ruminants (Nation *et al*., 1982; Bourque *et al*., 1986; Jones, 2001; Gonzalez *et al*., 2005; Franca *et al*., 2009; Deljou *et al*., 2014), poultry (Pavarini *et al*., 2011; Zavala *et al*., 2011) and horses (Bautista *et al*., 2014). Monensin poisoning can be the result of dosage miscalculation, incorrect mixing of feed, improper recipient identification and use in non-recommended species (Novilla, 1992; Roder, 2011; Zavala *et al*., 2011; Bautista *et al*., 2014).

Despite monensin poisoning occurring in farm animals, there are few studies that describe how toxicosis affects sheep. Thus, to understand alterations promoted by ionophore toxicity, it is important to evaluate epidemiology during outbreaks, as well as detailed characterization of lesions, distribution and intensity. For this purpose, this study described clinical-pathological changes during a natural monensin poisoning outbreak in a sheep herd from Brasília, Brazil.

MATERIALS AND METHODS

An outbreak of monensin poisoning was investigated in a sheep mixed breed flock in Brasília, Distrito Federal and Midwest Brazil. The herd was kept in *Panicum* spp., pastures and received supplementation with concentrate at 1.5% of BW. Misuse of monensin occurred during concentrate formulation, due error in dose calculation, resulting in the addition of 750 ppm of the product in the final mixture. It was estimated that the sheep's (20 kg average b.wt.) average ionophore consumption was 11.25 mg kg^{-1} b.wt. Clinical signs appeared four to six hours after intake and the death of the first animals occurred at 24 h, when use of the concentrate was suspended. Lambs continued to die until seven days after ionophore exposure.

During the outbreak investigation, a clinical examination of eight poisoned sheep was conducted and blood samples were collected to test the CBC and serum activity of aspartate aminotransferase (AST) and creatine phosphokinase (CPK) measurements. In addition, morbidity and lethality rates were calculated.

Twenty-seven sheep were necropsied at the Veterinary Pathology Laboratory of the University of Brasília (UnB), out of a total of forty animals that died during the outbreak. Fragments of skeletal muscle groups, heart and other organs (central nervous system, lungs, liver, kidneys, spleen, lymph nodes, stomach, pre-stomachs, intestines and adrenal glands) were collected for histopathological evaluation.

Samples of cardiac and skeletal muscle and other organs were analyzed under optical light microscopy to determine the pattern and distribution of lesions. A semiquantitative analysis of necrosis, muscle fiber regeneration by satellite cell proliferation indications, fibroplasia, mineralization, circulatory changes and inflammatory cellular components was performed in cardiac muscle tissues. Fisher's exact test was used to compare the frequency of injuries among the skeletal striated muscle samples and between cardiac and skeletal muscle group histopathological changes (GraphPad Prism 6.0).

RESULTS

In our study, 48.3% of the poisoned sheep evaluated during the outbreak were female and 51.8% were male. The sheep were between four and five months of age, with a morbidity rate of 33.33% and a mortality rate of 100%, with 40 total lamb deaths. Poisoned animals showed attitude, postural, mucosal and cardio-respiratory changes, neutrophilia, lymphopenia and high serum AST and CPK levels (Table 1). Acute monensin poisoning diagnosis was established based on high doses of ionophore exposure, in addition to clinical signs and pathological changes.

Table 1: Clinical signs and laboratory findings in sheep accidentally poisoned by monensin (Brasília-DF, Brazil)

Animal	Sex	Decubitus	Apathy	OMH	Tachycardia	Tachypnea	Neutrophilia	Lymphopenia	AST	CPK
A	M	-	+	+	+	+	+	+	-	+
B	F	+	-	+	+	-	+	+	+	+
C	F	-	+	+	+	-	+	+	+	+
D	F	-	+	+	+	+	-	-	-	+
E	F	+	+	+	+	-	+	+	+	+
F	M	+	+	+	-	-	+	+	-	+
G	M	+	-	+	+	+	-	-	-	+
H	F	+	-	+	+	+	+	+	+	+

M: Male, F: Female, OMH: Ocular mucosa hyperemia, AST: Aspartate aminotransferase, CPK: Creatine phosphokinase, +: Present or increased, -: Absent or unchanged

Multifocal to diffuse pale (Fig. 1a) or whitish areas in one or more skeletal muscle groups (59.2%) were the major muscle changes observed at necropsy. Similar alterations were present in 48.1% of the cardiac muscle samples (Fig. 1b). Petechiae were observed in the myocardium, primarily at the heart base in 11.1% of the intoxicated animals, pulmonary congestion and edema were found in 59.2% and an evident liver lobular pattern was found in 29.6% of these animals.

Degenerative-necrotic (myofibers segmental necrosis-Fig. 2a), circulatory and inflammatory changes, fibroplasia and

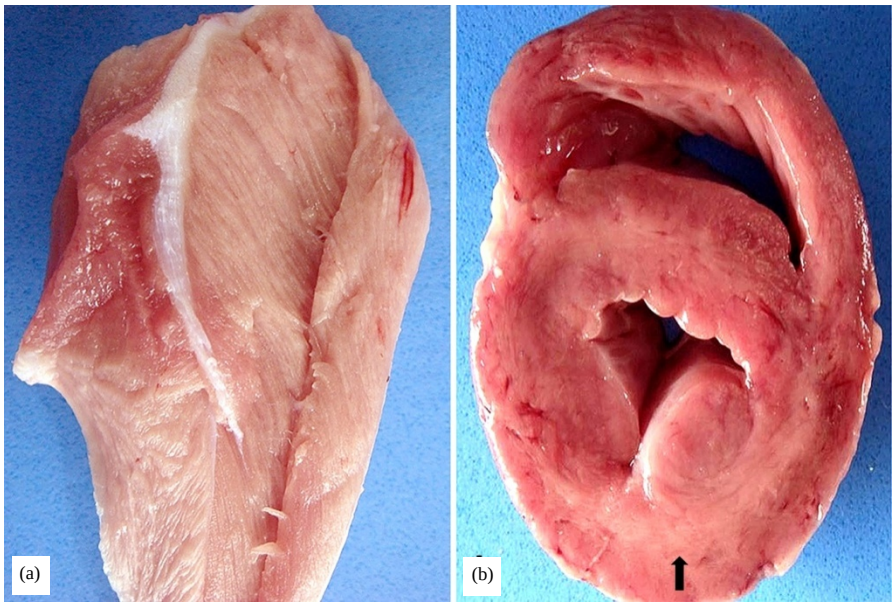

Fig. 1(a-b): Diffuse pallor, (a) Femoral quadriceps muscle in sheep with monensin poisoning and (b) Diffuse myocardium pallor, more intense in the left ventricle (arrow), sagittal section, heart in sheep with monensin poisoning

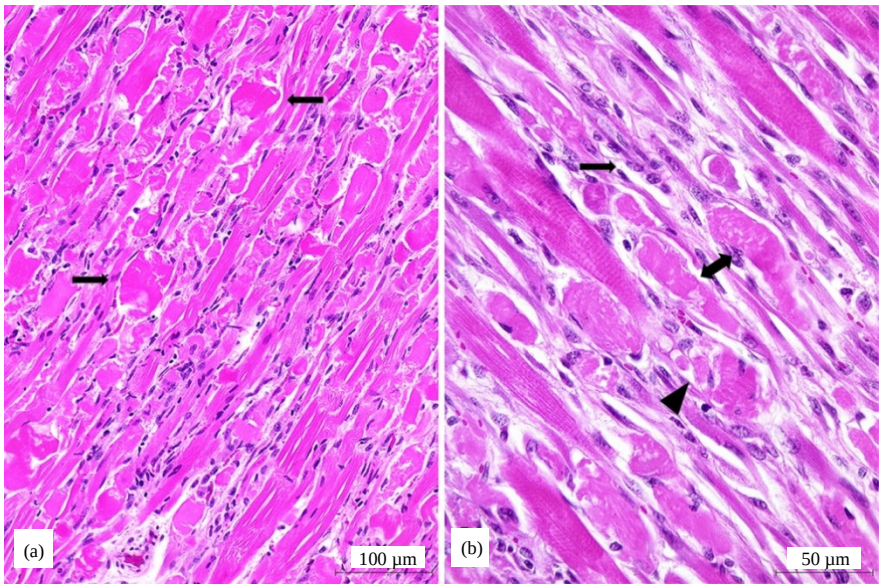

Fig. 2(a-b): (a) Myofiber segmental necrosis (arrows), femoral quadriceps muscle in sheep with monensin poisoning (H and E) and (b) Mononuclear cells' inflammatory infiltrate (small arrow), satellite cells proliferation (head arrow) and myofibers necrosis (double arrow), gluteus muscle in sheep with monensin poisoning (H and E)

Table 2: Frequency of histopathological changes (%) in striated muscle samples of sheep poisoned by monensin (Brasília-DF, Brazil)

	Necrosis							Infiltrate Inflammatory		
Muscle tissue	HDCS	FRAG	Regeneration	Fibroplasia	Mineralization	Congestion	Hemorrhage	HIST	LINP	PLASM
Heart (n = 27)	55.5[a]	55.5[a]	NA	48.2[a]	7.4[a]	74.1[a]	37.0[a]	81.5[a]	63.0[a]	66.7[a]
Skeletal muscles (n = 73)	84.9[b]	83.5[b]	83.5	86.3[b]	15.0[a]	67.1[a]	27.4[a]	91.8[b]	45.2[a]	54.8[a]
Muscle group										
Gluteus (n = 13)	84.6	76.9	84.61	100.0	15.4	92.3	38.4	84.6	38.4	76.9
Femoral quadriceps (n = 13)	92.3	100.0	92.3	76.9	0.0	69.2	7.7	92.3	30.8	30.8
Subscapularis (n = 13)	92.3	69.2	84.6	100.0	30.8	84.6	46.1	92.3	30.8	30.8
Infrascapular (n = 5)	60.0	60.0	60.0	60.0	20.0	80	20.0	80.0	40.0	60.0
Brachial triceps (n = 9)	88.9	88.9	100.0	88.9	11.11	55.5	33.3	100.0	66.6	77.8
Tongue (n = 20)	75.0	75.0	75.0	80.0	15.0	40	20.0	95.0	55.0	60.0

HDCS: Hyalinization of muscle fibers and disappearance of cross striations, FRAG: Fragmentation of muscle fibers, HIST: Histiocytes, LINP: Lymphocytes, PLASM: Plasmocytes, n: Number of samples, NA: Non-assessed. Different frequencies are represented by different letters in the same column ($p \leq 0.05$)

Table 3: Histopathological lesions (%) in the myocardium (n = 27) of sheep accidentally poisoned by monensin (Brasília-DF, Brazil)

	With lesion				
Lesion	+	++	+++	Total	Without lesion
HDCS	29.6	22.2	3.7	55.5	44.5
FRAG	22.2	22.2	11.1	55.5	44.5
Fibroplasia	44.4	3.7	0	48.2	51.8
Mineralization	3.7	3.7	0	7.4	92.6
Congestion	51.8	22.2	0	74.1	25.9
Hemorrhage	22.2	11.1	3.7	37.0	63.0
Histiocytic infiltrate	1.8	22.2	7.4	81.5	18.5
Lymphocytic infiltrate	48.1	14.8	0	63.0	37.0
Plasmacytic infiltrate	44.4	22.2	0	66.7	33.3

HDCS: Hyalinization of muscle fibers and disappearance of cross striations, FRAG: Fragmentation of muscle fibers, n: Number of samples. +: Mild, ++: Moderate, +++: Severe

regeneration of muscle fibers were the main histopathological alterations in sheep spontaneously poisoned by monensin, varying in intensity from mild to severe (Table 2).

There were no differences in the frequencies of the skeletal muscle group changes ($p \geq 0.05$). The myocardium showed lesions similar to those in skeletal striated muscles (Table 3). Hyalinization of muscle fibers with disappearance of cross striations, fragmentation of muscle fibers, fibroplasia and histiocytic infiltrate were observed less frequently in the myocardium when compared with these changes in skeletal muscle samples ($p \leq 0.05$).

Histological examination of the lungs showed hemorrhage (74.1%), in addition to congestion and alveolar edema in 77.8% of the animals. Vacuolization of hepatocytes (77.8%) with diffuse distribution (76.2%) or a periportal pattern (23.8%) and centrilobular focal to multifocal necrosis of hepatocytes (33.3%) were observed in the poisoned sheep. Congestion (74.1%) and hemorrhage (25.9%) were also present in the livers of intoxicated sheep. The other organs analyzed showed no significant alterations.

DISCUSSION

The investigation of a sheep monensin poisoning outbreak demonstrates the risks of ionophore antibiotic misuse. The estimated levels of monensin supplied to the animals were close to the single dose of 12 mg kg^{-1} used for experimental lamb poisoning (Confer *et al*., 1983) and the LD_{50} dose (11.9±1.2 mg kg^{-1}) for sheep (Confer *et al*., 1983; Roder, 2011).

Morbidity during the outbreak was elevated and all the animals that became ill died while intoxicated. In accidental monensin sheep poisoning, morbidity can vary from 20-30% (Bourque *et al*., 1986; Jones, 2001). The high morbidity and mortality rates during the outbreak occurred possibly due high ionophore levels in the ration. Clinical signs during the monensin toxicosis outbreak were similar to those reported in sheep and other ruminants intoxicated by ionophores (Nation *et al*., 1982; Confer *et al*., 1983; Rozza *et al*., 2007; Franca *et al*., 2009; Roder, 2011; Deljou *et al*., 2014). Difficulty moving, lack of coordination and increased apathy occurred possibly due to incapacitation caused by extensive muscle damage (Franca *et al*., 2009).

Tachypnea and tachycardia in poisoned animals in Brasília were previously described in sheep affected by accidental monensin toxicosis (Jones, 2001). The dyspnea may be present in sheep poisoned by other ionophores associated with pulmonary edema, or due to functional impairment of muscles involved in breathing (Franca *et al*., 2009; Roder, 2011). Monensin poisoning causes the release of catecholamines and

changes in the myocardium contractile phase (Dorne *et al*., 2013), which could explain the tachycardia observed in poisoned sheep.

Stress response due to extensive muscle necrosis may release endogenous cortisol, promoting neutrophilia and lymphopenia (Souza *et al*., 2008), as observed in the poisoned sheep. High serum levels of creatine phosphokinase (CPK) and aspartate aminotransferase (AST) are frequently observed in sheep intoxicated by monensin due to extensive myopathy (Confer *et al*., 1983; Bourque *et al*., 1986).

Degenerative-necrotic lesions in the skeletal muscles and myocardium were the main pathological changes of monensin-poisoned sheep and are similar to those described in domestic animals with ionophore toxicosis (Confer *et al*., 1983; Bourque *et al*., 1986; Jones, 2001; Rozza *et al*., 2006, 2007; Franca *et al*., 2009; Varga *et al*., 2009; Pavarini *et al*., 2011). Liver and lung congestion, as well as cytoplasmic vacuolization of hepatocytes with liver coagulation necrosis foci (Franca *et al*., 2009), were possibly secondary to toxicosis changes in heart function (Varga *et al*., 2009).

Tissue damage observed in ionophore poisoning occur mainly due to changes in ion transport (Na^+, K^+ and Ca^{++}), inhibiting energy metabolism and promoting increase in sodium and water cellular influx, intracellular calcium deposit release and cell death (Roder, 2011; Dorne *et al*., 2013). Monensin promotes mitochondrial damage due to changes in osmotic metabolism control, increasing cell membrane and sarcoplasmic reticulum permeability (Confer *et al*., 1983).

Striated skeletal muscle groups of monensin-poisoned animals had degenerative-necrotic lesions that were more frequent and intense compared to the myocardium. Detailed frequency of injuries in various muscle groups in ionophore toxicity of sheep is unknown, however, the dorsolumbar muscle groups, buttocks and shoulders seem to be most affected (Bourque *et al*., 1986). Sheep intoxicated by monensin have lesions predominantly in skeletal striated muscles and these lesions are more intense in the hind limbs, in contrast to cattle where the main changes are in the myocardium (Nation *et al*., 1982).

Regeneration of skeletal muscle fibers, myocardium fibroplasia and mineralization were observed in the muscle groups of intoxicated sheep and are seen in ruminants with ionophore poisoning (Nation *et al*., 1982; Confer *et al*., 1983; Franca *et al*., 2009). In addition, fibroblast proliferation predominated in intoxicated animals that took longer to die. On the other hand, mineralization of necrotic muscle fibers occurred with low frequency when compared to the other muscle changes in poisoned sheep, but may be highly variable in monensin poisoning (Nation *et al*., 1982; Confer *et al*., 1983).

Despite the widespread use of monensin in food animals, it is important to be careful with its use, ensuring the correct dose calculation and product homogenization in the ration. The erroneous administration of ionophores reaching toxic doses can be extremely lethal, causing significant economic losses to sheep flocks. The complete characterization of sheep monensin-intoxicated clinical changes and lesions may contribute to toxicosis diagnoses and help with understanding the clinical and pathological events involved.

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