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Renal cortex copper concentration in acute copper poisoning in calves[1]

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ABSTRACT.- Fazzio L.E., Mattioli G.A., Costa E.F., Picco S.J., Rosa D.E., Testa J.A. & Gimeno E.J. 2012. **Renal cortex copper concentration in acute copper poisoning in calves.** *Pesquisa Veterinária Brasileira 32(1):1-4.* Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, calle 60 y 118, CC 296, B1900AVW, La Plata, Argentina. E-mail: fazzio@fcv.unlp.edu.ar

The aim of this study was to estimate the diagnostic value of renal cortex copper (Cu) concentration in clinical cases of acute copper poisoning (ACP). A total of 97 calves that died due to subcutaneous copper administration were compiled in eleven farms. At least, one necropsy was conducted on each farm and samples for complementary analysis were taken. The degree of autolysis in each necropsy was evaluated. The cases appeared on extensive grazing calf breeding and intensive feedlot farms, in calves of 60 to 200 kg body weight. Mortality varied from 0.86 to 6.96 %, on the farms studied. The first succumbed calf was found on the farms between 6 and 72 hours after the susbcutaneous Cu administration. As discrepancies regarding the reference value arose, the local value (19.9 parts per million) was used, confirming the diagnosis of acute copper poisoning in 93% of the analyzed kidney samples. These results confirm the value of analysis of the cortical kidney Cu concentration for the diagnosis of acute copper poisoning.

INDEX TERMS: Copper, calves, diagnosis, renal córtex, poisoning.

RESUMO.- [**Concentração de cobre no córtex renal na intoxicação aguda de cobre em bezerros.**] O objetivo deste trabalho foi estimar o valor diagnóstico de concentração de cobre (Cu) no córtex do rim em casos clínicos da intoxicação cobre aguda (ACP). Um total de 97 bezerros foi compilado em onze fazendas. Pelo menos, uma necropsia foi realizada em cada caso e foram colhidas amostras para análise complementar. O grau de autólise em cada necropsia foi avaliado. Os casos aparecem em criação extensiva e também em fazendas de confinamento intensivo. Os pesos dos animais variavam de 60 até 200 kg. Mortalidade variou entre 0,86 e 6,96%, em todas as fazendas estudadas, o primeiro animal morto foi observado entre 6 e 72 horas após à administração parenteral de Cu. Surgirem discrepâncias em relação ao valor de referência a ser usado. O valor local (19. 9 partes por milhão) foi usado, confirmando o diagnóstico de intoxicação aguda de cobre em 93% das amostras analisadas nos rins. Estes resultados confirmam o valor diagnóstico da concentração de Cu no rim córtex para o diagnóstico de ACP.

TERMOS DE INDEXACAO: Cobre, bezerros, diagnóstico, córtex renal, intoxicação.

INTRODUCTION

Copper (Cu) is an essential mineral nutrient, which must be supplemented in deficiency cases to maintain the animal's health and performance (Fazzio 2006). However, it can also be toxic, mainly for the liver, when accumulate in excessive quantities (Allen & Mallinson 1984, Galey et al. 1991, Sullivan et al. 1991, Minervino et al. 2008). Parenteral supplementation with Cu injectable salts is a rapid, low-cost and simple method to compensate the animal's Cu status, when the diet does not provide enough (Allen & Mallinson 1984). On the other hand, parenterally injected salts are stored in liver, avoiding intestinal interference of other compounds, such as molybdenum, iron and sulphur (Underwood & Suttle 1999, Rosa & Mattioli 2001). The right Cu dose is important since there is a narrow margin between the therapeutic dose and the toxic one (Galey et al. 1991, Sullivan et al. 1991, Mendel et al. 2007).

Many papers quote liver Cu values in bovine above 600 ppm in dry matter (DM) as indicator of natural or induced

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Cu poisoning (Gummow 1996, Hamar et al. 1997, Auza et al. 1999, Mendel et al. 2007). This value is used in cases of chronic Cu poisoning (CCP). In cases of acute Cu poisoning (ACP) this value is not useful since the cause of death is not the total amount of Cu in liver, but the high transference rate from the injection site to the organ (Radostits et al. 2002, Fazzio et al. 2004, Stöber et al. 2005). Thus, liver concentration values do not increase so much as they do in CCP (Uzal 1992 et al. 1992, Giuliodori et al. 1997, Fazzio et al. 2004). The kidney is another organ to be analyzed in cases of suspected Cu poisoning (Uzal et al. 1992, Giuliodori et al. 1997, Roubies et al. 2008, Mozaffari et al. 2009). The transference rate is lower in the kidney when compared with the liver; additionally it is more efficient in the control of Cu deposition (Giuliodori et al.1997, Roubies et al. 2008).

The aim of this study was to evaluate the diagnostic value of Cu concentration in the renal cortex in clinic cases of ACP in Buenos Aires province, Argentina.

MATERIALS AND METHODS

This study was carried out between 2008 and 2010. Eleven farms with ACP diagnosis were recorded, with a total of 97 succumbed calves. Subcutaneous copper supplementation is a routinely measure in that farms. In all cases described the copper salt used was Cu-Ca EDTA, with the exception of Case 5 in which Cu glycinate was applied.

All farms where the poisoning occurred were visited, a detailed history was recorded, including case description, anamnesis and at least one complete necropsy was conducted. Samples for complementary analysis were taken. From calves that showed signs of Cu toxicity, blood was collected with and without anticoagulant for Cu determination in plasma (Piper & Higgins 1967) and indicators of liver damage respectively (Minervino et al. 2008).

In the dead calves a subjective analysis of autolysis degree was carried out, taking into account the following scale: Degree 0 = without autolysis, Degree 1 = minimal changes, Degree 2 = moderate changes, and Degree 3 = severe autolysis (Robles & Uzal 1991). From the animals included in Degrees 0 and 1, samples of different organs were collected in buffered 10% formaldehyde for routine histopathology.

For Cu measurement, liver and cortical kidney samples were taken from dead animals. The samples were arranged in refrigerated polyethylene bags and then frozen until processing. The tissue samples were digested in a mixture of nitric and perchloric acids (2:1). Cu concentration was determined by atomic absorption spectrometry (GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia), following the manufacturer's advice. The coefficient of inter-assay variation was 3.2% when weight and digestion were taken into account. The addition of a one-tenth and twice the reference standard allowed a recovery of 101 and 98% respectively.

RESULTS

Relevant data of evaluated cases are shown in Table 1. Farms 1, 2, 3, 5, 6 and 7 were calves in extensive grazing system, while on Farms 4, 8, 9, 10 and 11 the calves had been recently introduced into intensive feedlots. In all cases there was an antecedent of Cu parenteral supplementation with different commercial products available on the market.

The observed clinic signs were tachypnea, tachycardia, depression, lethargy, apathy, lack of response to visual or auditive stimulus and sternal or lateral decubitus. In Case 1 and 4, incoordination, brusxism and opisthotonos were observed.

Table 2 shows the results of blood tests corresponding to calves showing clinic signs of ACP (Farms 1, 2, 3 and 4).

In all the farms studied, the first dead animal was observed between 6 and 72 hours after parenteral Cu administration, the deaths continuing no longer than day 7 in all cases, except

Table 1. Cases with confirmed acute copper poisoning diagnosis

Farm	Year	Breed	Breeding system	Weight (Kg)	Age (months)	Nº of calves supplemented	Nº of dead animal
1	2008	A. Angus	Extensive	100 – 130	3 - 5	238	7
2	2008	Zebu cross	Extensive	90 – 200	5 - 13	720	26
3	2008	Hereford	Extensive	140 – 170	5 - 7	438	8
4	2009	Hereford	Feedlot	110 – 155	5	614	11
5	2009	A. Angus	Extensive	160 – 170	4 - 5	1400	12
6	2009	A. Angus	Extensive	175	12	230	16
7	2009	A.Angus	Extensive	60 – 180	3 - 6	140	8
8	2009	Zebu cross	Feedlot	100 – 140	5	213	3
9	2009	Zebu cross	Feedlot	90	3	112	1
10	2010	Zebu cross	Feedlot	110	4	135	3
11	2010	A. Angus	Feedlot	135	5	121	2

Table 2. Blood tests of animals with clinical signs

Identification	Plasma	Hepatic enzyme			Bilirubin (mg/100ml)		
	Cu(µg/dl)	AST (U/L)	γGT (U/L)	AF (U/L)	DB	IB	TB
Farm 1 (n=1)	46	110	98	690	0.99	2,1	3,09
Farm 2 (n=1)	46	542	63	1,087	1,75	7,96	9,71
Farm 3 (n=2)	72	338	25	1,537	1,14	5,18	6,32
	65	489	38	1,195	0,89	1,43	2,32
Farm 4 (n=1)	105	68	78	901	2,1	2,6	4,7
Ref. Value	60 – 120	48 – 100	20 – 48	70 - 300	0,001-0,5	0,1- 0,3	0,1-0,5

AST = aspartate aminotransferase;γGT = gama glutamyl transferase; AF = alkaline phosphatase; DB = direct bilirrubin; IB = indirect bilirrubin; TB = total bilirrubin.

Table 3. Autolysis degree, liver and kidney copper concentrations (ppm DM)

	Liver (ppm)	Kidney (ppm)	Autolysis degree
Farm 1			
Necropsy 1	237	35	1
Necropsy 2	175	29.9	2
Necropsy 3	228	21	3
Farm 2			
Necropsy 1	79	28	3
Necropsy 2	100	46	2
Necropsy 3	30	24	1
Necropsy 4	119	26	1
Farm 3			
Necropsy 1	234	17	3
Necropsy 2	82	10	3
Necropsy 3	147	31	1
Farm 4			
Necropsy 1	150	36	2
Necropsy 2	207	32	1
Necropsy 3	217	23	3
Necropsy 4	84	31	0
Farm 5			
Necropsy 1	349	51	1
Necropsy 2	289	34	1
Farm 6			
Necropsy 1	ND	27	2
Necropsy 2	ND	56	2
Necropsy 3	70	42	1
Farm 7			
Necropsy 1	ND	22	3
Farm 8			
Necropsy 1	143	24	3
Necropsy 2	108	27	3
Necropsy 3	97	31	1
Farm 9			
Necropsy 1	322	66	0
Farm 10			
Necropsy 1	143	70	0
Necropsy 2	108	42	0
Necropsy 3	97	33	1
Farm 11			
Necropsy 1	143	41	0
Necropsy 2	138	34	2

ND = no data.

on Farm 2, where a calf in permanent decubitus was sacrificed for humanitarian reasons on day 9.

Mortality varied between 0.86 and 6.96 %. Livers appeared enlarged and with dark red mottling on the cut surface. In the gallbladder petechial hemorrhages were observed on the serous membranes. Other findings included petechial hemorrhages in pleura as well as in epi and endocar-dium; widespread pethechial and ecchymotic hemorrhages; congestion of the lungs, abomasum and first portion of duodenum.

Diffuse periacinar hepatic necrosis and hemorrhage was observed in every necropsied calf. In kidney, tubule cell degeneration was present.

Renal cortex and liver Cu concentration and autolysis degree for each evaluated animal are shown in Table 3.

DISCUSSION

All the cases of ACP evaluated were confirmed with specific studies on calves that showed clinical signs or were found dead. The acute course of iatrogenic copper poisoning usually leaves dead animals as the main diagnostic tool. On the dead animals liver function cannot be tested and cupraemia changes to values within normal range within few hours (Bohman et al. 1984, Underwood & Suttle 1999) as happened in the calves evaluated (Table 2). In these fatal cases of ACP, liver Cu concentration has no value, because there is no longer Cu accumulation period in the liver as occurs in CCP (Humann-Ziehank et al. 2001, Mendel et al. 2007), when the high transference rate of Cu is the lethal factor (Giuliodori et al. 1997). The liver Cu values found confirm this statement (Table 3). Therefore, the only confirmatory diagnostic tools were the macro and microscopic liver lesions. Hepatic degeneration and necrosis are typical of Cu toxicosis but are not specific.

As for the use of kidney Cu concentrations for ACP diagnosis, there are discrepancies regarding the concentration value to be used. Many authors propose values between 50 and 75ppm DM (Bulgin et al.1986, Sullivan et al.1991, Steffen et al. 1997, Auza et al. 1999, Mozaffari et al. 2009), while others (Radostits et al. 2002) proposes values higher than 25ppm DM as an indicator of Cu poisoning. The differences between authors could be attributed to the kidney basal concentration. Values of 20-25ppm DM are quoted as normal Cu concentration (Steffen et al. 1997, Korsrud et al. 1985, Bulgin et al. 1986, Mozaffari et al. 2009). However, in the geographic area where this study was conducted, in previous investi-gation Giuliodori et al. (1998) observed an average value of 12.7± 3.6ppm DM. Based on these results, they proposed for this area the value of 19.9ppm DM as a basal mean concentration of Cu. Such value is obtained by adding the average plus 2 standard deviation [12.7 + (3.6 x 2)] (Giuliodori et al. 1999). In the current study, this value would have confirmed the ACP diagnosis in 93% of the analyzed kidney samples (27 out of 29). If the reference value would have been 25ppm DM (Radostits et al.2002), the percentage of samples reflecting poisoning would have been 76% (22 out of 29), while if the threshold value would have been 50ppm DM (Auza et al. 1999, Mozaffari et al. 2009), only 14% of the samples analysed (4 out of 29) would have reflected poisoning. These results encourage the use of local reference values.

The results presented here lead to the conclusion that kidney Cu concentration in the renal cortex is a useful parameter for postmortem ACP diagnosis. Likewise, it is advisable to establish normal regional values.

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