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Lead Poisoning in Cattle: Reassessment of the Minimum Toxic Oral Dose*

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The lethal dosage of lead (Pb) in any of its several forms ingested as a single bolus is about 220-400 mg Pb/kg body weight (b. w.) for calves (CLARKE & CLARKE 1967). However, the minimum daily level of intake that will result in intoxication has been less well defined. ALLCROFT & BLAXTER (1950) succeeded in producing a chronic Pb intoxication in calves with 5-6 mg Pb/kg b.w. but only after almost three years of daily intake. HAMMOND & ARONSON (1964) evaluated cattle poisoned on farms in the vicinity of a Pb smelter and estimated that 6-7 mg Pb/kg b.w./day can cause intoxication and death in approximately two months. Dosages of 3.0-3.5 mg Pb/kg b.w./day had no observed effect on cattle on these same farms for the period of an entire winter. The investigators suggested that 6-7 mg Pb/kg b.w./day is the minimum intake which will eventually cause poisoning in cattle. These data have become widely accepted by diagnostic laboratories (HATCH & FUNNELL 1969), researchers (DINIUS et al. 1973, LYNCH et al. 1976, WRIGHT et al. 1976), and currently available veterinary toxicology textbooks (BARTIK & PISKAC 1981, BUCK et al. 1978, RADELEFF 1970). In recent studies evaluating thiamin-Pb interactions, a daily Pb intake below 6 mg Pb/kg b.w. caused intoxication and death in calves in less than twenty days (BRATTON et al. 1981). These surprising results prompted additional interest in low level Pb intake in calves. The purpose of this report is to present additional quantitative data relevant to this subject.

MATERIALS AND METHODS

Twenty-two Holstein male calves (9-12 weeks old) weighing approximately 55 kg were directly utilized in this study. Blood samples only were evaluated from an additional seventeen calves. All calves were housed in individual wooden or wire stalls and fed twice daily with a commercial milk replacer diet (Milk Max C-50, ConAgra, Inc., Omaha, Neb.) balanced in all known dietary needs (NATIONAL RESEARCH COUNCIL 1978). Lead in the milk replacer was < 0.1 mg/kg. Calves were fed such that the average daily gain prior to the beginning of Pb dosing was 0.36 ± 0.19 kg/day.

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Calves were divided into three groups: Group I - non Pb dosed controls, and Groups II and III treated with lead acetate at dosages of 2.7 and 5.0 mg Pb/kg b.w./day, respectively. A single calf was dosed with 20 mg Pb/kg b.w./day, a dose previously shown to cause death in 8-22 days (WELLS et al. 1976). Based on weekly body weight, Pb acetate was measured for each individual calf and dissolved in 100 mls of deionized water. This solution was given to the calves by nurse bottle at noon each day. This dosing time was one-half way between morning and afternoon feeding; thus, during a time of fasting.

Blood samples were collected via jugular venipuncture from the calves in Group III and from an additional seventeen calves maintained and dosed in the same manner as the calves in Group III. Samples were collected just prior to the initial Pb dose and then 1, 3, 6, 12 and 24 hours after the first dose of Pb. Further blood samples were taken 24 hours following a second, fourth, and seventh dose of Pb. Heparin was used as the anticoagulant in samples collected for Pb analysis. EDTA tubes were used for determination of erythrocyte and leukocyte counts, hemoglobin concentration, and packed cell volume. Clot tubes were used for total protein evaluation.

When the majority of the calves in Pb treated groups (Groups II and III) showed signs of intoxication, all calves in the respective group were anesthetized via intravenous pentobarbital and exsanguinated by severing the carotid arteries. The following tissues were taken for Pb analysis: bone (4th rib and femoral head), kidney, liver, pancreas, spleen, sciatic nerve, spinal cord, brain (cerebrum, cerebellum, brain stem), biceps femoris muscle, heart (ventricle only), and blood. Large organs were homogenized in toto and aliquots taken for analysis, while smaller organs were analyzed intact. One calf from each group was perfused for histopathological assessment and tissues were not collected from these calves for Pb analysis. Consideration of morphological data is deferred.

Lead analysis was performed by atomic absorption spectroscopy following dry ashing and organic extraction as described by YEAGER et al. (1971) and modified and validated by ŻMUDZKI (1977). Recovery of Pb from the various tissues averaged 93.3% and analysis of test samples obtained from the Center for Disease Control was consistently within 15% of reported values.

RESULTS AND DISCUSSION

The calves in Group II were dosed for 20 days before the majority displayed signs of intoxication, while 9 of 11 calves in Group III developed signs in 7 days and the one calf dosed with 20 mg Pb/kg b.w. died on the 5th day. The clinical assessment of calves is shown in Table 1.

Similar signs of toxicosis were observed in all calves that displayed abnormalities, regardless of dosage. The calves first became depressed and developed hypoglossal paresis which resulted in problems with suckling. Within the next 12-24 hours the calves became unsteady, developed a trunkal ataxia, and displayed muscular tremors, especially around the head and forelimbs. The calves

next developed generalized seizures, opistotonus, and died in respiratory failure during staticus epilepticus. The period of time between development of hypoglossal paresis and death varied inversely with the dosage of Pb. Seizures and death occurred within 48-72 hours following hypoglossal paresis in calves given 2.7 mg Pb/kg b.w., within 24-36 hours in calves given 5.0 mg Pb/kg b.w., and the calf given 20 mg Pb/kg b.w. died within 8 hours after the onset of nursing difficulty. Signs such as hyperirritability, head pressing, bellowing, frenzy, and diarrhea commonly reported with experimental and natural bovine Pb poisoning (WHITE & COTCHIN 1948, LEARY et al. 1970), failed to occur in this study. Blindness and salivation which are often reported as the most common signs of Pb poisoning (BUCK et al. 1978, LEARY et al. 1970) were observed in only two calves in the present study.

Table 1. Clinical Evaluation of Pb Poisoned Calves

Pb Dose mg/kg	Total Calves in Group	Days Dosed with Pb		Signs Observed		Fate	
		Calves	days	Calves	(+)or(-)[a]	Calves	days
0.0	5	5	0	5	(-)	2 Killed	8
						3 Killed	21
2.7	5	1	7	4	(+)	1 Died	7
		1	8	1	(-)	1 Died	9
		3	20			3 Killed	21
5.0	11	11	7	9	(+)	4 Died	7
				2	(-)	7 Killed	8
20.0	1	1	5	1	(+)	1 Died	5

a (+) = presented 2 or more signs compatible with Pb poisoning
(-) = no signs

Erythrocyte and leukocyte counts, packed cell volumes, hemoglobin concentrations, and total protein levels fluctuated during the study but no significant differences ($P > 0.05$) were found between pretreatment figures and treatment means of individual animals. Moreover, the changes observed in all calves were compatible with changes previously observed in cattle of this age (TENNANT et al. 1974, WINGFIELD & TUMBLESON 1973). Reticulocytes, nucleated erythrocytes, and basophilic stippling of erythrocytes were not observed in stained blood films.

Laboratory confirmation of excessive Pb in tissues of treated calves is shown in Table 2. There was considerable variation in Pb concentration in some tissues but not in others. In most tissues the mean Pb concentration increased directly as the daily intake of Pb increased; however, individual variation was such that only blood, muscle, and sciatic nerve were significantly different ($P < 0.05$) between calves in Group II and Group III.

Table 2. Lead Concentration in Calf Tissues (mean standard deviation and range)[a]

Tissue	Control n=4	2.7 mg Pb/kg n=4	5 mg Pb/kg n=10	20 mg Pb/kg n=1
BONE	0.22±0.07 0.18-0.32	49.02±14.15 30.00-75.34	54.92 ± 20.15 32.63-105.81	108.52
KIDNEY	0.11±0.02 0.09-0.13	49.49±32.54 20.72-90.36	88.00 ± 19.50 51.55-114.24	82.92
LIVER	0.13±0.04 0.09-0.18	19.00±11.76 5.42-29.96	30.51 ± 11.67 15.13- 54.98	37.11
PAN-CREAS	0.07±0.02 0.05-0.09	3.14 ± 1.13 2.00- 4.26	6.11 ± 3.42 2.38- 12.91	5.66
SPLEEN	0.08±0.02 0.05-0.10	0.73 ± 0.17 0.53- 0.87	1.67 ± 0.69 1.07- 3.51	2.52
SCIATIC NERVE	0.06±0.01 0.04-0.07	0.53 ± 0.15 0.33- 0.65	0.96 ± 0.19 0.70- 1.41	1.58
SPINAL CORD	0.06±0.01 0.04-0.07	0.32 ± 0.21 0.18- 0.56	0.34 ± 0.10 0.22- 0.50	0.82
CERE-BRUM	0.07±0.02 0.06-0.10	0.66 ± 0.16 0.54- 0.89	0.81 ± 0.27 0.50- 1.18	1.43
CERE-BELLUM	0.07±0.02 0.05-0.09	0.51 ± 0.10 0.38- 0.60	0.72 ± 0.27 0.41- 1.09	1.41
HEART	0.07±0.02 0.05-0.10	0.33 ± 0.18 0.17- 0.53	0.59 ± 0.25 0.31- 1.04	1.64
MUSCLE	0.07±0.02 0.05-0.09	0.17 ± 0.05 0.11- 0.22	0.34 ± 0.09 0.17- 0.49	0.93
BLOOD	0.03±0.01 0.03-0.04	0.47 ± 0.29 0.30- 0.90	1.57 ± 0.62 1.08- 3.21	2.41

a - All results are in milligrams of Pb/kg of wet tissue (ppm)

The Pb residues in bone are greater than residues in liver and kidney in continuous oral exposures to Pb. This situation is reversed in acute non-cumulative exposures (ALLCROFT 1950, BUCK et al. 1978). In this study bone Pb was consistently higher than liver Pb. Kidney Pb was the same as bone Pb in calves dosed with 2.7 mg

Pb/kg, higher than bone Pb in calves dosed with 5.0 mg Pb/kg, and lower than bone Pb in the calf dosed with 20 mg Pb/kg. Because only one calf was given 20 mg Pb/kg, this bone-kidney relation was disregarded and the deaths in this study were considered acute and compatible with previous studies. The greatest residues of Pb in the treated calves were in the kidney and liver. The pancreas, an organ not generally measured in experimental or field cases of bovine Pb poisoning, contained the third highest Pb concentration. The pancreas was followed by the spleen, the organ generally considered next after liver. In cases of acute Pb poisoning, tissue levels of 10 and 15 ppm are frequently encountered in the liver and kidney, respectively (BUCK et al. 1978). The soft tissue residues in this study were in excess of these levels confirming excessive exposure to Pb.

The Pb concentrations in Table 2 represent all calves whether signs were present or not and whether the calves were killed or had died. In every case the lowest calf in the range was the calf or calves which failed to display clinical signs. The range indicates a large individual variation as to Pb absorption, excretion, or metabolism. Interestingly, the tissue levels observed in this study, even in calves receiving 2.7 mg Pb/kg b.w., were 5 to 10 times greater than similar tissues from other studies that dosed calves with 6 mg Pb/kg (ALLCROFT 1950, DINIUS et al. 1973, WRIGHT et al. 1976). On the other hand, they are compatible with tissue levels observed when extremely high levels of Pb were ingested (HAMMOND & ARONSON 1964, KELLIHER et al. 1973, WHITE & COTCHIN 1948).

A rapid rise in blood Pb concentration during the first several hours after a single dose of 5.0 mg Pb/kg is shown in Table 3. The rapid rise indicates rapid absorption from the gastrointestinal tract in most calves, but the standard deviation indicates a high degree of inherent variability.

Table 3. Blood Pb Levels in Calves treated with 5 mg Pb/kg b.w. (µg/100 ml, n = 27)

	Pre	1 dose					2	4	7
	Pb	1 hr	3 hr	6 hr	12 hr	24 hr	doses	doses	doses
Mean	4	4	21	74	46	48	72	69	108
SD	1	2	16	84	35	26	35	31	50
Range	3-7	3-6	4-64	7-264	7-124	9-110	23-156	25-145	35-3.21

Only a small proportion of the amount of a Pb compound ingested is absorbed (1-2%) from the gastrointestinal tract of calves

(ALLCROFT 1950, BUCK et al. 1978). In this study the rapid rise in blood Pb after a single dose of 5.0 mg Pb/kg b.w. and the high tissue levels obtained in 5 to 7 days would appear to contradict these findings. LYNCH et al. (1976) dosed calves 3 times weekly with Pb and fed the calves a milk replacer diet. Their blood Pb values are very similar to those obtained in this study. These results support the concept developed in other species that milk diets play an important role in absorption and deposition of Pb (KELLO & KOSTIAL 1973, BUSHNELL & DELUCA 1980). However, the Pb in this study was not given by gelatin capsule as in other studies, but by nurse bottle. This dosing method should have allowed direct passage of Pb to the abomasum, a factor which should also be considered in evaluating why 5.0 and 2.7 mg Pb/kg caused such severe signs in so short a time. Likewise, the dosing time must be considered since giving Pb in a time of fasting increases absorption (SINGAHAL & THOMAS 1980). While each of these factors probably played a significant role in increasing the low level effects, confirmation must await further study.

These results show (1) that under some conditions daily Pb intakes below 6 mg Pb/Kg can kill calves in short periods of time. (2) Daily Pb intakes of 2.7 mg Pb/Kg can kill calves on milk diets in 20 days or less while 5.0 mg Pb/Kg/day consistently causes signs of intoxication and death in 7 days. (3) Absorption rate of Pb is rapid and tissue depositions are high in calves on milk replacer diet. (4) The data suggest that diet, dosing method, and dosing time must be carefully considered in evaluations of minimum toxic dose. (5) The consistent production of seizures at these low daily Pb intakes suggests that this calf model may be valuable in the study of Pb encephalopathy. Whether or not daily intakes of Pb below 2.7 mg Pb/kg can cause poisoning is currently under investigation.

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