Bull. Environ. Contam. Toxicol. (1987) 39:622–629

Bulletin of Environmental Contamination and Toxicology

Chronic Lead Poisoning in Steers Eating Silage Contaminated with Lead Shot—Diagnostic Criteria

D. A. Rice,[1] M. F. McLoughlin,[1] W. J. Blanchflower,[1] and T. R. Thompson[2]

[1]Veterinary Research Laboratories, Stormont BT4 3SD, Northern Ireland and
[2]116 Movilla Road, Newtownards BT23 3RJ, Northern Ireland

Lead ingestion is one of the most common causes of poisoning in cattle (Clarke et al. 1981). Toxicity results most commonly from the consumption of a single high dose of lead although cumulative toxicity resulting from the ingestion of small doses over a prolonged time also occurs.

The sources of lead most commonly involved in disease outbreaks are paint, batteries, felt, linoleum and oil (Osweiller and Van Gelder 1978b). It has traditionally been held that ingested metallic lead does not present a major toxicity risk to cattle because of its low solubility in the rumen and reticulum. This belief was based on the work of Allcroft (1951) who was unable to demonstrate clinical or biochemical evidence of poisoning in calves fed metallic lead. More recent evidence suggests that lead shot, if present in silage, can induce toxicity when such silage is eaten by cattle. Howard and Braum (1980) reported death and disease in dairy cows fed contaminated corn silage in the USA and Frape and Pringle (1984) describe a similar outbreak in the UK, where grass silage was fed. In both instances difficulty was experienced in making a diagnosis because of inconclusive results of clinico-pathological tests.

This communication describes a similar outbreak in steers eating lead shot contaminated grass silage. It presents and discusses the limitations of the criteria used for arriving at a diagnosis, including the use of whole blood amino levulinic acid dehydratase (ALAD) concentrations in fresh whole blood and after reactivation with dithiothreitol.

There are differences of opinion, in the literature, regarding the response of erythrocyte ALAD to ingested lead in the bovine. Consequently the results of a small lead feeding trial are also reported here. These results demonstrate a large ALAD response to lead ingestion and justify the use of this test in the confirmation of field cases of lead poisoning in cattle such as the one reported here.

MATERIALS AND METHODS

Kidneys from two dead steers, approximately 12 months old, were submitted for laboratory confirmation of lead poisoning. One steer had been found dead with no previous history of disease. The other had died within 12 hours of developing classical clinical signs of acute lead poisoning including head pressing, blindness, convulsions and staggering. This animal died in spite of immediate treatment with intravenous sodium calcium edetate and a favourable prognosis made. These dead steers were 2 of a batch of 14 which had been introduced to a winter diet of grass silage, as their sole source of food, 6 weeks previously. This precision chopped silage had been made from a field which had been used for clay pigeon shooting for a number of years. In previous years the owner had produced hay from the field and had experienced no disease problems in the cattle to which it had been fed.

The kidneys were submitted along with 4, 2 kg samples of the suspect silage and with heparinized blood samples. Twelve of these bloods were from the remaining clinically normal cattle in the batch and one was a pre-treatment sample collected from the animal in which clinical signs were observed prior to death.

Kidney and silage lead estimations were carried out on acid digests using a Varian Model AA10 Atomic Absorption Spectrophotometer. Blood lead levels were measured on 5 x dilutions of blood in 0.1% Triton X solution using a Perkin Elmer Model HGA500 Graphite Furnace and Model 272 Atomic Absorption Spectrophotometer.

Whole blood was also analysed for thiamine deficiency by measuring the percentage increase in transketolase activity as a response to added thiamine, the TPP effect (Hoffman et al. 1971).

Whole blood ALAD activity was determined on fresh blood samples by modification of the method of Wigfield and Farant (1981) which determines not only the ALAD concentration in the fresh sample but also the increase in concentration when the ALAD is reactivated using dithiothreitol. The modifications involved increasing the volumes of blood used by 20 fold and of the other reagents by 10 fold. The incubation period was also increased from 60 min to 150 min to improve sensitivity. Units are expressed as micromoles of porphobilinogen generated/hour/litre of erythrocytes at 37°C and as the percentage increase in ALAD activity as a response to reactivation with dithiothreitol.

The lead feeding trial set up at this laboratory to measure the response of whole blood ALAD to ingested lead was carried out on six, 5 month old Jersey steers. These were eating a hay/

concentrate ration. Two of these received no additional lead and acted as controls, 2 received 3 mg lead/kg bodyweight/day and 2 received 6 mg/kg bodyweight/day. Lead was fed as lead acetate, mixed in with the concentrate feed for 3 consecutive days. Heparinized blood samples were collected on a sequential basis from all calves.

Heparinized blood samples were also collected from 12 normal steers at this laboratory. These were of an age similar to those in the field outbreak and were additionally similar in that they had been eating a grass silage diet for the previous 2 months.

Blood samples from all control and experimental animals were analysed for lead, ALAD activity and the percentage increase in ALAD activity on reactivation.

RESULTS AND DISCUSSION

The effect of lead acetate feeding on blood lead and fresh and reactivated ALAD activity are shown in Fig 1. All 3 parameters rapidly responded to oral lead acetate. The parameters returned towards pre-treatment levels only slowly after lead withdrawl from the feed. The magnitude and duration of the effect was related to the dose of lead administered. The peak blood lead values were 1.38 and 1.14 µmol/l for the calves receiving 6 and 3 mg of oral lead/kg bodyweight/day respectively. The corresponding lowest levels of ALAD activity resulting from lead supplementation were 15 and 16 µmol/l and the greatest response to reactivation with dithiothreitol 1600% and 1350% respectively. The ALAD concentration of the blood of control calves did not remain constant throughout the 50 day sampling period but increased throughout. This may have been due to an age factor since ALAD activity in erythrocytes is known to vary with age (Braton and Zmudski 1984). These results are in agreement with those of Pinault and Milhauld (1983) and Bratton and Zmudski (1984) who have observed decreases in blood ALAD in lead poisoned calves. Our results are different in degree to theirs in that their studies measured decreases in activity of 60-80% of the values observed in control animals, whereas 3-4 fold decreases were observed in this study. It would appear that Osweilder and Ruth (1978a) were wrong in their ascertion that blood ALAD does not respond to lead poisoning in cattle. This experiment therefore confirmed the potential usefulness of blood ALAD for the diagnosis of lead poisoning in the bovine and justified its current use in the field investigation of lead shot associated poisoning described here.

Lead shot was found in all 4 sub-samples of silage. Kidney cortex lead concentrations in each of the dead steers was 13 mg/kg for that found dead and 18 mg/kg for that in which clinical signs were observed. These kidney lead values are

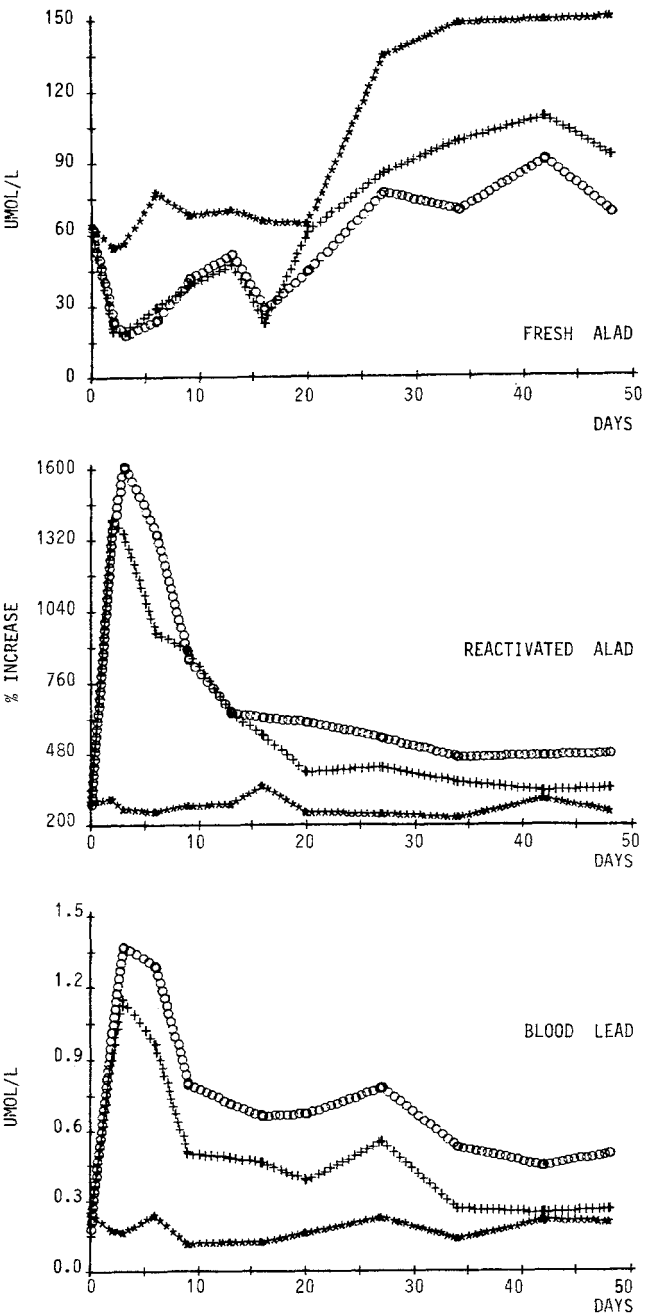

Figure 1 Response of blood lead and ALAD activity in 3 groups of calves fed lead acetate. Calves received no lead (*) or 3 mg (+) or 6 mg (o) lead/kg bodyweight/day for 3 days.

well below the mean value of 50.3 ppm found in 105 field cases of lead poisoning by Osweiller and Van Gelder (1978b). They are also below the 30 mg/kg level used in this laboratory for confirmation of lead poisoning and are on the threshold of the very lower limit of 15 ppm established by Buck et al. (1976).

The blood lead level of the animal which died from lead poisoning was 1.9 µmol/l. This was similar to the values found in its 12 clinically normal cohorts (Table 1), which ranged from 1.4 to 3 (mean 2.3) µmol/l. Again, all of these were lower than the mean blood lead concentration of 3.9 µmol/l found in lead poisoned cattle by Osweiller and Van Gelder (1978b) and are on the lower limit of the positive range used at this laboratory (1.75 to 18 µmol/l). Using these blood and tissue lead levels, there was evidence to suspect lead exposure of the cattle in this outbreak but the low concentrations made confirmation of the diagnosis difficult. This was particularly so because of the acute nature of the disease and because of the lack of response to treatment.

Because of the clinical signs observed in one animal, the only likely alternative diagnosis to lead poisoning was cerebrocortical necrosis resulting from thiamine deficiency. The TPP effect on erythrocyte transketolase activity showed no evidence of thiamine deficiency.

Table 1 Amino levulinic acid dehydratase (ALAD) activity (µmol/l) in erythrocytes and whole blood lead (µmol/l) concentrations of 12 steer exposed to lead shot compared with 12 age and diet matched controls

Steers		Fresh ALAD	Reactivated ALAD	% Increase	Blood lead
Exposed to oral lead shot	Mean	45	185	436	2.3
	SD	33	112	165	0.5
	Range	22-65	87-319	197-741	1.4-3.0
Age and diet matched controls	Mean	287	343	75	0.4
	SD	301	150	33	0.2
	Range	102-469	201-495	49-146	0.2-0.9
Significance		$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$

The mean fresh blood ALAD concentrations and reactivated blood ALAD concentrations of the 12 lead exposed cohorts of the dead steers are also shown in Table 1. The corresponding values for the 12 age and diet matched control animals are shown in the same table. Fresh whole blood ALAD activities were lower in the lead exposed steers, 45 µmol/l, as opposed to 287 µmol/l in the controls. Similarly, when reactivated with dithiothreitol the increase in ALAD activity was 436% in affected cattle and

75% in controls. It would be logical to conclude therefore that since the lead intake of these cattle was sufficient to inhibit the activity of ALAD in red cells, it was also possible that it had reached sufficiently high concentrations in tissue to inhibit the sulphydryl groups in other important enzyme systems and therefore produce disease. These depressed blood ALAD concentrations together with the clinical and other clinio-pathological findings, therefore helped to confirm lead poisoning in this case.

Although blood ALAD concentrations were useful in arriving at a diagnosis in this field outbreak of disease, the results show certain inconsistencies. These are in agreement with those mentioned by Bratton and Zmudski (1984). The dithiothreitol stimulated increase in ALAD concentration in blood of cattle receiving lead was marked in both field (436% increase) and experimental (900-1600% increase) toxicity. However, an increase also occurred in control animals being 75% increased in the silage fed yearling control steers and 200-300% in the hay/concentrates fed 5 month old cattle. Whilst these increases are lower than those found in toxicity cases it adds further weight to the advice of Bratton and Zmudski (1984) who suggested that ALAD results from disease outbreaks should be compared with values obtained from age and diet matched controls. If such controls are available then the use of this assay will prove to be a useful additional test to add to the diagnostic profile currently used for diagnosing cumulative lead poisoning.

This study confirms the opinions of Frape and Pringle (1984) and Howard and Braum (1980) that lead shot in silage presents a threat to livestock consuming it. When the lead shot was removed, the four samples of silage *per se* from this farm contained 0.41, 0.17, 0.25 and 0.09% lead (mean 0.23%). This study has not determined whether it was this soluble lead alone which caused toxicity or whether the lead pellets present in the rumen/ reticulum were dissolved and also contributed to the lead being absorbed. Certainly the 0.23% soluble lead in the silage would have contributed approximately 18 g of oral lead per day to a steer eating 8 kg (dry matter) of silage. It is possible that this lead alone would have been sufficient to cause toxicity, especially in light of the work by Zmudski et al. (1983) who have shown that the minimal lethal dose of lead for calves is at most 2.7 mg/kg bodyweight/day (approximately equal to 950 mg/day for a 300 kg steer). However, they administered lead acetate which is a very soluble and rapidly absorbed source and may behave very differently from the lead which was present in silage. Until further work is carried out to establish the relative availabilities of the soluble lead in silage and the metalic lead in the rumen of silage fed cattle, then the matter will not be resolved. However, in the interim the assertion made by Allcroft (1951) that metallic lead in the

rumen does not present a toxicity risk to cattle should not be considered definitive.

Bjorn et al. 1982 have demonstrated that lead shot does not present a toxicity threat to cattle when they graze land on which shooting has taken place. Previous experience on the farm affected by this present outbreak suggests that it was not a problem when the farmer made hay from the field. Presumably this was because the hay bailer did not pick up lead pellets from the ground. However, this work confirms that when silage is made from grass from lead shot contaminated fields, there is a definite danger. This presumably is due to the suction created by forage harvesters picking up the lead shot from the ground together with the cut grass. Our results, together with previous reports, present sufficient evidence to justify the prohibition of silage making from such contaminated pastures in future.

REFERENCES

Allcroft R (1951) Lead poisoning in cattle and sheep. Vet Rec 63: 583-590.

Bjorn H, Gyrd-Hansen N, Kraul I (1982) Birdshooting, lead pellets, and grazing cattle. Bull Eniviron Contam Toxicol 29: 174-176.

Bratton G R, Zmudzki J (1984) Laboratory diagnosis of Pb poisoning in cattle. A reassessment and review. Vet Hum Toxicol 26 (5): 387-392.

Buck W B, Osweiler G D, Van Gelder G A (1976) Clinical and diagnostic veterinary toxicology 2nd Edition. Van Gelder G A (Ed), Kendale/Hunt, Dubque, Iowa.

Clarke M L, Harvey D G, Humphrey D J (1981) Veterinary Toxicology 2nd Edition. Bailliere Tindall, London.

Frape D L, Pringle J D (1984) Toxic manifestations in a dairy herd consuming haylage contaminated by lead. Vet Rec 114: 615-616.

Hoffman I, Knapp A, Rietz K, Milner C (1971) Determination of transketolase activity in blood. Clin Chem Acta 33: 415-421.

Howard D R, Braum R A (1980) Lead poisoning in a dairy herd. Proc Annu Meet Am Assoc Vet Lab Diag 23: 53-58.

Osweiler G D, Ruhr L P (1978a) Lead poisoning in feeder calves. J Am Vet Med Assoc 172: 498-500.

Osweiler G D, Van Gelder G A (1978b) Epidemiology of lead poisoning in Animals. In: Oehme F W (ed) Toxicity of heavy metals in the environment, part 1. Marcel Dekker Inc, New York and Basel, p143.

Pinault L, Milhaud G (1983) Evaluation of subclinical lead poisoning in dairy cattle. In: Ruckebusch Y, Toutain Y L, Koritz G D (Eds) Veterinary Pharmacology and Toxicology. Service de Pharmacologie - Toxicologie, Ecole National Verinarie de Nantes, BP527, 44206 Nantes Cedex, France, p 715

Wigfield D C, Farant J P (1981) Assay of D-aminolaevulinate dehydratase in 10 µl of blood. Clin Chem 27: 100-103.
Zmudski J, Bratton G R, Womac C, Rowe L (1983) Lead poisoning in cattle. Reassessment of the minimum toxic oral dose. Bull Environ Contam Toxicol 30: 435-441.
Received May 29, 1987; Accepted July 14, 1987.