# Some Clinicopathological and Microbiological Studies on Lead Toxicity in Bull

 $^1$ Nevin E. Sharaf,  $^2$ Mona S. Zaki,  $^1$ Hend R. Gomaa,  $^3$ Nabila El. Batrawy and  $^4$ Olfat M. Fawzi

<sup>1</sup>Department of Environmental and Occupational Medicine, National Research Center, Dokki, Giza, Egypt

<sup>2</sup>Department of Hydrobiology, National Research Center, Dokki, Giza, Egypt

<sup>3</sup>Department of Microbiology, Animal Reproduction Research Inst.

<sup>4</sup>Department of Biochemistry, National Research Center, Dokki, Giza, Egypt

Abstract: The problem of lead toxicity originated in a private farm in *El-Katta* "Giza governorate", due to ingestion of plant polluted with lead. About 8 out of 50 bull animals showed lead toxicity. The animal's age was 6 months. The animals suffered from depression, pressing head against objects, dilatation of eye pupils, total blindness (in 2 cases) with normal light reflex in both eyes, edema in briskets, enteritis with bloody diarrhea and pupil dilation. Also there were lacrimation, pale dirty mucous membrane and sunken eyes. Serum analysis from these animals revealed high lead concentration. In addition too, significant decrease in the levels of testosterone, LH, FSH PCV, haemoglobin, R.B.C.s and total proteins were also decreased. Highly degeneration of kidney and liver accompanied with elevation of AST, ALT, Urea, creatinine, cortisol, sodium and potassium. Moreover, *Sterptococcus sp.*, *S. epidermidis* and *S. Aeruginosa* were isolated. We conclude that the cause of animals morbidity and mortality in this farm was not due to bacterial infections but due to lead toxicity and we can say that polluted environment, especially with lead, can cause severe harm to animal health, in addition to serious danger on human health, by eating food polluted with lead.

Key words: Lead toxicity in bull · environmental pollution · biochemical and microbial changes

### INTRODUCTION

Lead is a major environmental pollutant and its toxicity continues to be a major public health problems and there is a growing consensus that lead cause toxic injury to human at a level of exposure that was considered to be safe only a decade ago. As the chronic exposure to lead, even at low levels, can result in slow progressive, in most of time, irreversible damage to haematopoeitic, nervous and renal systems [1]. Recent researches predicted that high levels of toxic metals in scalp hair, due to environmental pollution, in addition to deficiency in trace metals, play a role in the development of heart diseases [2].

In recent years, research efforts are directed towards quantification of the impact of lead exposure on human health, particularly from environment. The diagnosis based on blood lead levels doesn't always give an accurate estimate of total body burden of lead, so it's important to detect sub cellular damage using reliable sensitive biomarkers [3]. Animals are very good indicator of the environmental pollution, as they inhabit

the same space as humans and are exposed to the action of the same pollutants, for that reason, it's appropriate and advantageous to evaluate the negative impact of the polluted environment by heavy metals and their influences load on human health by parallel evaluation of their load on animals [4, 5], so measuring of lead in blood animals [6], in cows and especially in lactating one, proved to be a good indicator of environmental contamination and also for food contamination from polluted animal [7].

## MATERIALS AND METHODS

Animals: 8 young bull of 6 months old were used in the present study. They were obtained from a private farm in *El-Katta* "Giza governorate". The animals suffered from depression, pressing head against objects, dilatation of eye pupils, total blindness (in 2 cases) with normal papillary light reflex in both eyes, edema in briskets, enteritis with bloody diarrhea and papillary dilation. Also there were lacrimation, pale dirty mucous membrane and sunken eyes.

**Haematological studies:** Blood samples were collected from the jugular vein on EDTA as anticoagulant for determination of Hb, PVC, ESR, RBC's count and WBC's count, according to Jain [8].

**Biochemical and Hormonal studies:** The activities of aspartic aminotransferase (AST) and alanine aminotransferase (ALT) as well as cholesterol, urea and creatinine levels were determined according to the method of Varley *et al.* [9] by using commercial kits (Bio Merieus, France).

Total serum protein was estimated according to Drupt [10]. Serum cortisol was analyzed by a Gamma counter using 125 I cortisol radioimmunassay kit (Baxter Health Care Corporation USA) according to the method described by Pickering and Pottinger [11]. Potassium, Sodium and lead concentrations were determined by atomic absorption spectrophotometry.

**Bacteriological studies:** Swabs from internal organs (liver, kidney, intestine and lungs) were collected under aseptic condition. The inoculated plates were incubated at 37°C for 24-48 h. The suspected colonies were picked and purified by further subculturing after which they were stained with Gram stain, for further biochemical identifications, the subjected isolates were classified according to Buchman *et al.* [12] and Wilson and Miles [13].

**Soil Forage:** The lead content of the soil and forage was measured by atomic absorption according to the method of Rodrigues and Castellon [14].

**Statistical analysis:** The obtained data were subjected to the student t-test according to Gad and Well [15].

#### RESULTS

In the present study, haematological examination showed significant decrease in haemoglobin, PVC and RBC's count and significant increase was found in ESR and W.B.C's count in all animals (Table 1).

Biochemical results detected significant increase in AST, ALT, urea, creatinine, cortisol, sodium, potassium and lead (Table 2). While, there was significant decrease in total protein level (Table 2). Serum LH, F.S.H and testosterone hormones were significantly decreased (Table 3).

Table 1: Effect of lead toxicity on some hematological parameters (Mean values±SE) in bull

Parameters	Control (8)	Bull (8)
P.C.V	31.00±1.20	31.00±1.40
E.S.R	$1.10\pm0.01$	1.80±1.30*
R.B.C's count 106 /ml	6.33±0.28	4.40±1.24*
W.B.C's count 103 /ml	8.35±0.23	11.03±0.49*
HB g/dl	$8.10\pm0.01$	7.13±0.14*

<sup>\*</sup>P < 0.01

Table 2: Effect of lead toxicity on some biochemical parameters (Mean values±SE) in bull

Parameters	Control (8)	Bull (8)
AST U/I	131.00±1.23	174.00±2.45*
ALT U/l	31.00±1.46	62.00±1.62*
Total Protein g/ dl	10.40±1.68	8.00±0.27*
Urea mg/dl	2.90±0.62	3.09±0.82*
Creatinine mg/dl	$0.98\pm0.03$	1.70±0.02*
Cortisol mg/dl	$0.60\pm0.01$	1.67±0.23*
Na <sup>+</sup> M.E.Q	97.00±2.20	114.00±3.30*
K+ M.E.Q	4.70±1.30	6.20±1.90*
Lead ppm	$0.87 \pm 0.12$	1.68±0.10*

<sup>\*</sup>P < 0.01

Table 3: Effect of lead toxicity on L.H, F.S.H and testosterone hormones (Mean values±SE)

Parameters	L.H (mu/ml)	F.S.H (mu/ml)	Testosterone (ug/dl)
Control	3.14±0.32	5.40±0.32	5.10±0.042
Affected animal	2.10±0.14*	4.10±0.42*	3.80±0.23*

<sup>\*</sup>P < 0.01

Table 4: Mean level of lead in soil and vegetables in the studied area

	Polluted area concentration		
Samples	of lead in p pm	Control Area	
Soil	293.72±32.24*	74.23±9.12	
Forage	164.30±6.32 *	64.01±6.23	

<sup>\*</sup>P < 0.01

Table 5: Bacterial determined in internal organs liver and kidney in bull

Bacterial Isolated	Degree of presence
Streptococcus sp.	30%
S. epidermidis	40%
S. aeurogenosa	25%

The lead content was found to be 293.72 ppm in the polluted soil and 164.3 ppm in forage (Table 4).

Bacteriological results revealed that the most predominant isolated micro-organisms was *Streptococcus*, *S. epidermis and S. Aeuroginosa* (Table 5).

#### DISCUSSION

From our results, we noticed a decrease in the level of hemoglobin, PVC and R.B.C's count, owing to the fact that lead intoxication causes a documented defect in haem synthesis. The results obtained agreed with several authors [8, 16] because lead pollution has an inhibitory effect on globin synthesis, inhibits iron to form haem and inhibits delta amino levulinic acid dehydratase in red cells.

Moreover, we can conclude that lead toxicity has dangerous effect on animals in the studied areas where the lead content of soil measured was about 293.73 ppm and in contaminated pasture was 164.3 ppm, the lead poisoning in the studied bull may be due to grazing this contaminated pasture which may be contamination by industrial wastes [17]. Hob and Kirn [18] reported that, lead concentration in plants was 80-160 ppm and in soil was 100-300 ppm which was considered as toxic level. Referring to the FAO-WHO[19] recommendations, the acceptable daily lead intake is 0.05 ppm. and our finding agreed with that of Bryant and Rose [20]; Fayed and Abdallah [21] and Zaki *et al.* [22].

Bacterial microorganism e.g. Streptococcus sp, S. Epidermis and S. Aeuroginasa were isolated from internal organs, similar finding were reported by Fingold and Martin [23] and Ducan and Prasse [24] who stated that bacterial microorganism are present in animals and birds suffering from high pollution with lead due to immunological suppression.

Both clinical signs and ocular changes, which were observed in the present work, might be attributed to the toxic effect of lead on the C.N.S as mentioned by Krameller-Froetcher, [25] and Schlerka [26], this toxic effect was characterized by severe cerebral disturbances leading to blindness in some cases which may be due to cerebrocoritcal oedema, or may be also due to associated optic neuritis and optic atrophy. We can concluded, that all the clinical symptoms reported in the studied bull might be due to acute lead intoxication, as mentioned by Ozmen and Mor [27].

The biochemical results detected in Table 2, showed significant increase in AST, ALT, while there was significant decrease in total protein level (Table 2). These findings agreed with those found by Swarap *et al.* [28], as the as the elevation in transaminases activities and the decrease in total protein level may be attributed to the liver injury, so the exposure to lead in polluted environments alters serum biochemical parameters indicative of liver functions.

The biochemical results, detected in Table 2, showed significant increase in urea and creatinine, which are indicative of abnormal kidney functions, agreed with those of Goswami and Gachhui [29].

The disturbed liver and kidney functions have been seen on the last stage of lead toxicity, this agree with Zaki *et al.* [22].

The hormonal results, detected in Table 3, showed significant decrease in L.H., F.S.H. and testosterone, which are affected in the early stages of lead poisoning [30, 31].

In conclusion lead toxicity cause atrophy of liver, kidney, gonads blindness, in addition to the locomotor disturbances. And the cause of animal morbidity and mortality in this farm was not due to bacterial infections but due to lead toxicity. We can also say that polluted environment, especially with lead, can cause severe harm to animal health, in addition to serious danger on human health, by eating food polluted with lead.

This work supported from internal project 10/8/5 (P.I Dr. Mona S. Zaki) belonging to National Research Center, Dokki, Giza, Egypt.

#### REFERENCES

- 1. Silbergeld, E.K., 1997. Preventing lead poisoinning in children. Ann Rev. Public Health, 18: 801-810.
- Afridi, H.I., T.G. Kazi, G.H. Kazi, M.K Jamali and G.O. Shar, 2006. Essential trace and toxic element distribution in the scalp hair of Pakistani myocardial infarction patients. Biol. Trace Elem. Res., 113(1): 11-34.
- Endo, G., S. Honiglechi and I. Kiyota, 1990. Urinary NaG in lead exposed workers. J. Appl. Toxicol, 10: 235-38.
- Korak, M., E. Kralova, P. Sviatko, J. Bilek and A. Bugarsky, 2002. Study of the content of heavy metals related to environmental load in urban areas in Slovakia. Bratsil Lek. Listy, 103: 231-237.
- Rashed, M.N. and M.E. Soltan, 2005. Animal hair as indicator for heavy metal pollution in urban and rural areas. Env. Monit. Assess, 110: 41-53.
- Zadnik, T., 2004. Lead in top soil, hay, silageand blood of cows from farm near a former lead mine and current smelting plant before and after installation of filters. Vet. Hum. Toxicol., 46: 287-90.
- Swarp, D., R.C. Patral, R. Naresh, P. Kumar and P. Shekhar, 2005. Blood lead levels in lactating cows reared around polluted localities; transfer of lead into milk. Sci. Total Environ., 347: 106-110.

- Jain, S.D., 1986. Evaluation of Haemogram in healthy and diseased sheep. Res. Vet. Sci., 33,21.
- Varley, H., A.H. Gwenbek and M. Bell, 1980. Practical Clinical Chemistry Vol. I Genera] I top-'scomnoner test 5<sup>st</sup> ed. London, William Medical Books Ltd.
- 10. Drupt, F., 1974. Estimation of total protein. Pharm. Biol., 9: 77.
- 11. Pickering, A.D. and J. Pottinger, 1983. Analysis of Hormone, Gen Comp Ender., 49: 232.
- Buchman, R.E., W.E. Gibbuns and R.Y. Stajner, 1975.
   Bergeyes Manual of Determinative Bacteriology.
   Ed. Millions and Wilkins Co. Baltimore.
- Wilson, G.S. and A.A. Miles, 1975. Topley and Wilsonies Principles of Bacteriology and Immunity. 6<sup>th</sup> Ed, Edwards and Annald, Publishers Ltd., London.
- Rodrigues, M.F. and Castellon, 1982. Lead and cadmium levels in soil and plants near highways and their correlation with traffic density. Environ. Pollute., (B) 4: 281-290.
- 15. Gad, W. and G. Well, 1976. Statistical Methods. 6<sup>th</sup> Ed., Iowa State Univ., Press Iowa, USA.
- Soliman, M.N., 1983. Toxicity resulting from lead compounds in veterinary practice. Ph. D. Thesis, Cairo University, Egypt.
- Lemos, R.A., D. Driemeier, E.B. Guimacaes, I.S. Dutra, A.E. Mori and C.S. Barros, 2004. Lead Poisoning in cattle grazing pasture contaminated by industrial Waste, Vet. Hum. Toxicol., 46: 326-328.
- Hob and Kirn, 1988. Elevated levels of a lead and other metals in roadside soil and grass and their use to monitor heavy metal depositions in Hong Kong. Environ. Pollut., 49: 34-51.
- FAO/WHO 1983.6<sup>th</sup> joint expert committee on food additives, evaluation of mercury, lead, cadmium and the food additives diethyl pyrocarbonate and acetyl gallate, WHO food additivitie Ser. No. 4.
- Bryant, S.L. and R.W. Rose, 1985. Effect of cadmium on the reproductive organs of the male Ram. Aust. J. of Biol. Sci., 28: 305-311-315.
- Fayed, A.H. and E.B. Abdallah, 1997. "Technique for studying the morphology of mammalian spermatozoa which are eosinophilic in a different live/dead stain. J. Report. Fertile., 29: 443.

- Zaki, M.S., A.M. Haman and F.S. Bayaumi and M.N. Shalaby, 2001. Some clinicopathological and microbial studies in lead toxicity on caws. Egypt Comp Path and Clinic Pathology, 14: 29-35.
- 23. Fingold, S.M., 1982. Diagnostic Microbiology. 6<sup>th</sup> ed., Mosby Co. St. Lowis, Toronto, London.
- Ducan, J. and K.W. Prasse, 1986. Vet laboratory Medicine Clinical Pathology. 2<sup>nd</sup> ed. Iowa State Univ., Press. Ames., Iowa, USA.
- Krametter-Frooestscher, R., F. Tataruch, S. Hauser, M. Leschnick, A. Url and W. Baumgartner, 2007. Toxic effects in herd of beef cattle, following exposure to ash residue contaminated by lead and mercury. Vet. J., 174: 99-105.
- Scherleka, G., F. Tataruch, R. Krametter-Frooestscher, A. Url, D. Kossler, S. Hogler and P. Schmidt, 2004. Acute lead poisoning in cows due to feeding of lead contaminated ash residue, Berl Mumch Tierarztl Wochenschr, 117: 52-6.
- Ozmen, O. and F. Mor, 2004. Acute lead intoxication in an old Battery Factory. Vet. Hum. Toxicol., 46: 255-256.
- Swarp, D., R. Naresh, V.P. Varshney, M. Balgangatharathilagar, P. Kumar, D. Nandi and R.C. Patral, 2007. Changes in plasma hormone profile and liver functions in cows naturally exposed to lead and cadmium around different industrial areas. Res. Vet. Sci., 82: 16-21.
- Goswami, K., R. Gachhui and A. Bandopadhyay, 2005. Hepatorenal dysfunction in lead pollution. J. Environ. Sci. Eng., 47: 75-80.
- 30. Gorbel, F., M. Boujelbene, F. Makni Ayadi, F. Gucimazi, F. Croute, J.P. Soleilhevoup and A.l. El-Feki, 2002. Cytotoxic effects of lead on the endocrine and exocrine sexual function of pubescent male and female rats. Demonstration of apoptotic activity, C R Biol., 325: 927-40.
- Srivastava, V., R.K. Dearth, K.J. Hiney, L.M. Ramisez,
   G. Bratton and W.L. Dees, 2004. The effects of low level Pb. on steroidogenic acute regulatory protein in the pubertal ratovary. Toxicol. Sci., 77: 35-40.