

Level of lead contamination in the blood of Bali cattle associated with their age and geographical location

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Manuscript received: 15 September 2020. Revision accepted: 8 December 2020.

Abstract. Berata IK, Susari NNW, Sudira IW, Agustina KK. 2021. Level of lead contamination in the blood of Bali cattle associated with their age and geographical location. *Biodiversitas* 22: 23-29. Lead contamination is increasingly common and endangers human as well as animal health. Cattle, which is a source of protein for humans, are very sensitive to lead exposure in a polluted environment. Therefore, this study aims to determine the level of lead contamination in the blood of Bali cattle related to their geographical location and age. A total of 300 cattle was drawn as the research sample, consisting of 150 each from the low and the highland. Each comprises of 50 young (<2 years old), 50 at puberty (2-3 years old), and 50 old cattle (>3 years old). The blood sample was taken from their jugular vein and collected in tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulants. The lead content was measured using the atomic absorption spectrophotometer (AAS) method. The results showed average data for the lowlands, which include 0.430 ± 0.411 ppm, 0.792 ± 0.356 ppm, and 1.234 ± 0.533 ppm for young, puberty, and old, respectively. The highlands include 0.047 ± 0.074 ppm, 0.057 ± 0.061 ppm, and 0.089 ± 0.169 ppm for young, puberty, and old, respectively. Furthermore, the ANOVA showed a significantly higher ($P < 0.05$) lead level in the blood of lowlands cattle than the highlands. We also found that in the lowlands, the lead level increased significantly ($P < 0.05$) with the cattle's age, while the highlands show no difference. Based on the results, it is concluded that cattle in the lowlands are more exposed to lead than cattle in the highlands. Also, the increase in the blood lead level associated with age occurs only in cattle of the lowlands.

Keywords: Age, Bali cattle, geographic, heavy metal, lead

INTRODUCTION

Lead contamination is very dangerous to human health, animals, and the environment, and its consumption at a certain level results in various health issues (Wani et al. 2015). Lead metal accumulates because its metabolism is difficult. Its contamination in humans and animals disrupts the body's enzymes and physiological work; therefore, it remains dangerous even when consumed at small levels (Oymak et al. 2017). Leads in humans and animals result in damaged erythrocytes, anemia (Jang et al. 2011), oxidative stress cell, which interferes with DNA, and various organ systems (Jaishankar et al. 2014; Sharma et al. 2014). The disorders of various organs include cardiovascular disorders (Draszawka-Bolzan 2014), spermatozoa production (Lamondo et al. 2014), system of hepatic, urinary, immune, respiration (Martin and Griswold 2009; Blagojević et al. 2012), hemopoietic (Ferreira et al. 2015; Turkay et al. 2015), the motor nervous (Sharma et al. 2014), and central nerve damage, which reduces the power of cognition, especially in children as well as death (Brochin et al. 2008; Martin and Griswold 2009; Barnham et al. 2014). Furthermore, heavy metals are also carcinogenic (Jadoon and Malik 2017); therefore, various efforts to prevent contamination in food, including beef, need to be ensured. Consequently, every country has rules

about the maximum limit of heavy metal contamination in food for safe consumption (Choi 2011).

Previous research of different livestock showed data containing lead contaminants, and cattle are one of the animals that are sensitive to exposure to heavy metals, including lead (Abdulkhalik et al. 2012). Also, cattle raised in urban landfills have a higher risk of being contaminated than those in rural areas. Furthermore, besides blood contamination, it is also detected in the tissues of internal organs (Berata et al. 2017). The major cause is feed factor as evidenced by the presence of cattle exposed to lead in conventional farming (Makridis et al. 2012). Research on geographic clusters reported that lead contamination in humans is higher in urban than in the surrounding area (King et al. 2015). However, its poisoning in humans that results in death due to motor neuron disease (MND) is closely related to geographical factors (Sánchez-Díaz et al. 2018).

Furthermore, cattle sensitivity to lead contamination is related to their age. However, there are no definitive reports. Also, the relationship of age factors to the bioconcentration level of lead in animals was studied, which include oysters (Petroody et al. 2017), and fish (Farkas et al. 2003). The level of lead contamination as a function of age in humans has shown that the young are more susceptible to contamination than adults (Blagojević

et al. 2012). The relationship between the age of cattle and the level of impurity from heavy metals is important in selecting cattle for seedling and slaughter as well as to ensure healthy and lead-free contamination.

MATERIAL AND METHOD

Ethical statement

This research was accepted in the Animal Ethics Committees of The Faculty of Veterinary Medicine Udayana University, Bali, with Ref. No. 2709A/UN14.2.9/PD/2019.

Research sample

A total of 300 Bali cattle was used, consisting of 150 each in the lowland and the highland, respectively. From both locations, 50 cattle each of young age (<2 years old), puberty (2-3 years), and adult (>3 years) were selected. Furthermore, the age of the cattle, the number of teeth, and the number of rings on the horn were determined by direct interviews with the breeder (Duittoz et al. 2016; Torell et al. 2003). Lowland cattle were selected from the Denpasar and Badung areas, with 50-200 meters altitude, and the highland from Kintamani Bangli, which is 1500m above the sea level.

Blood collection

Blood was drawn from the jugular vein by venoject and collected in a tube filled with ethylenediaminetetraacetic acid (EDTA) as anticoagulants (Besung et al. 2019). All samples stored at 20 °C for maximum of 7 days before testing. The measurement of lead metal content was conducted using the atomic absorption spectrophotometry (AAS) method at the Analytical Laboratory of Udayana University, Denpasar, Indonesia.

Measurement of lead level

The blood samples were processed for the measurement level of lead by using the atomic absorption spectrophotometry (AAS) method. They were divided into two parts, 0.5 mL each for positive control and a sample to be evaluated. Also, 0.25 mL of 1 mg/L standard control was added as positive control and was evaporated on a hot plate at a temperature of 100°C until it dried. Then, the spike and samples were placed in a furnace, and half of their surface was covered. In the process, the temperature was raised gradually by 100°C every 30 minutes up to 450°C and maintained for 18 hours. After that, the spike was removed and cooled at room temperature. Subsequently, 1 mL HNO₃ 65% was added and were shaken carefully till the ash dissolved in acid and was then evaporated on a hot plate at a temperature of 100°C until they were dried. Afterward, the samples and spike were placed in the ash furnace, and its temperature was raised gradually by 100°C every 30 minutes up to 450°C and maintained for 3 hours and then cooled at room temperature. Furthermore, 5 mL of HCl 6M solution was added and then shaken carefully to ensure that all the ashes

were dissolved by acid and were then evaporated on a hot plate at a temperature of 100°C until dried. Subsequently, 10 mL of 0.1M HNO₃ was added and cooled at room temperature for 1 hour, and the solution was transferred into a 50 mL flask polypropylene before mixing with a matrix modifier solution and then with 0.1 M HNO₃ until it reached the mark limit. A standard lead working solution was prepared at a minimum of five points concentration and was read on graphite furnace atomic absorption spectrophotometry at a wavelength of 288.3 nm. Furthermore, the Pb concentration in µg/g was calculated using the formula (SNI 2354.5: 2011):

$$\text{Concentration} = \frac{(D - E) \times Fp \times V}{W}$$

Where:

D: Sample concentration µg/L from the AAS reading results

E: Concentration of sample blank µg/L from AAS reading

Fp: Dilution factor

V: Final volume of the prepared sample solution (mL), change to liter units

W: Sample weight (g)

Data analysis

The research data were tabulated and analyzed with ANOVA to determine whether there are significant differences ($P < 0.05$) followed by Duncan's multiple range test. Furthermore, all data analyses were conducted using the Statistical Package for Social Sciences (SPSS).

RESULTS AND DISCUSSION

Results

The results from the lead metal content measurements based on geographic location and age of cattle are presented in Table 1. It is shown that the contamination level is higher in cattle of the lowlands (0.819 ± 0.547 ppm) than in highlands (0.064 ± 0.113 ppm). Also, the data analysis shows that the older the cattle, the higher the lead level in their blood, both in lowland and highland areas. Each geographic location of cattle shows a similar pattern; that is, the contamination level is higher according to the cattle age.

Figure 1 shows a comparison of the lead level between the geographical location and the age of Bali cattle. SD: standard deviation.

In general, the level of lead was significantly different ($P < 0.01$) between the age category, with the lowest at a young age, followed by puberty and the highest at adults (Table 2).

The results of the analysis of variance based on geographic and the level of lead in lowland cattle showed a significant difference ($P < 0.01$) between young, puberty and adult (Tables 3 and 4), which is not true in highland ($P > 0.05$) (Table 5).

Table 1. Results data of lead level in cattle blood based on location and age of cattle

No.	The level of lead in the cattle blood (ppm)					
	Lowland location			Highland location		
	Young	Puberty	Adult	Young	Puberty	Adult
1	0.005	0.292	1.016	0	0.04	0.004
2	0.001	1.741	1.623	0.044	0.056	0.901
3	0.064	1.016	1.295	0.061	0.019	0.057
4	0.006	1.002	1.616	0	0.026	0.011
5	0.006	0.675	1.012	0.08	0.098	0.042
6	0.151	1.197	1.165	0.34	0.046	0.005
7	0.01	1.72	1.642	0	0.089	0.016
8	0.062	1.216	1.597	0.161	0.19	0.304
9	0.002	0.423	1.666	0.032	0.096	0.479
10	0.002	1.344	1.012	0.01	0.122	0.043
11	0.004	0.89	1.688	0.027	0.064	0.006
12	0.034	0.788	1.433	0.042	0.001	0.21
13	0.001	1.169	1.54	0.015	0.008	0.601
14	0	0.921	1.518	0	0.062	0.021
15	0.081	0.684	1.016	0.084	0.008	0.044
16	0.056	0.875	1.654	0.024	0.003	0.123
17	0.714	0.431	1.689	0.301	0.09	0.064
18	0.42	0	1.504	0.06	0.176	0.152
19	0.628	0.817	0	0.009	0.063	0.043
20	0.365	1.048	1.128	0	0.029	0.19
21	1.002	0.281	1.003	0.022	0.016	0
22	0.002	0.744	0.002	0.006	0.067	0.039
23	1.014	1.016	0.001	0.067	0	0.076
24	0.316	1.002	1.39	0.019	0.078	0.018
25	1.001	0.675	1.822	0.078	0.241	0.008
26	1.023	0.148	1.764	0.241	0.01	0.105
27	0.802	0.722	1.025	0	0.062	0.008
28	0.515	1.016	1.001	0.071	0.145	0.06
29	1.164	0.423	1.197	0.145	0.088	0.02
30	0.001	0.344	1.841	0.089	0.014	0.002
31	1.05	0.89	1.68	0.014	0.016	0.074
32	0.808	0.788	0.09	0.019	0.018	0.312
33	1.119	1.146	1.708	0	0.019	0.068
34	1.111	0.921	1.199	0.021	0.078	0.011
35	0.801	0.684	1.867	0.004	0.241	0.083
36	1.029	0.875	1.786	0.004	0.01	0.021
37	0.927	0.431	2.188	0.066	0.062	0.006
38	0.619	0.711	1.229	0.004	0.145	0.168
39	0.266	0.817	1.29	0.01	0.088	0.015
40	0.419	1.081	1.115	0.012	0	0.003
41	0.114	0.722	1.018	0.004	0.013	0.002
42	1.001	0.641	1.176	0.004	0.014	0
43	0.801	0.474	1.545	0.063	0.002	0.008
44	0.029	0.139	1.281	0.002	0.032	0.015
45	0.227	0.704	0.068	0.01	0.022	0.003
46	0.515	0.618	1.109	0.011	0	0.002
47	0.243	1.008	1.191	0.021	0.018	0
48	0.206	0.704	0.068	0.01	0.022	0
49	0.515	0.618	1.109	0	0.01	0.001
50	0.248	1.002	1.111	0.053	0.014	0
Mean± SD	0.430 ± 0.411	0.792 ± 0.356	1.234 ± 0.533	0.047 ± 0.074	0.057 ± 0.061	0.089 ± 0.169
Mean± SD		0.819±0.547 ppm			0.064±0.113 ppm	

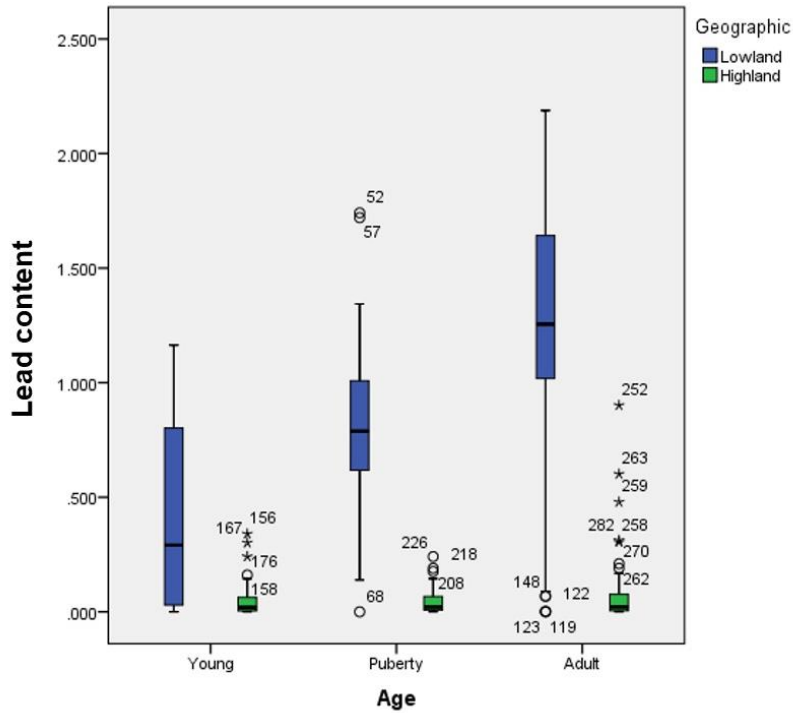


Figure 1. Comparison of lead (ppm) levels in blood between young, puberty and adult cattle each at lowland and highland location

Table 2. Analysis of variance of the level of lead between lowland and highland location

Parameters		Sum of squares	df	Mean square	F	Sig.
Lead level * Location	Between Groups	42.674	1	42.674	237.607	0.00
	Within Groups	46.479	298	0.156		
	Total	89.153	299			

Table 3. Analysis of variance of the lead level in lowland location

Parameters		Sum of squares	df	Mean square	F	Sig.
Lead level * Age of cattle	Between Groups	15.719	2	7.860	40.70	0.00
	Within Groups	28.194	146	0.193		
	Total	43.913	148			

Table 4. Duncan's test of the age category in lowland location

Age of cattle	N	Subset for alpha = 0.05		
		1	2	3
Young	50	0.43876		
Puberty	50		0.79188	
Adult	50			1.23376

Note: Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 49.662, b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 5. Analysis of variance of lead level in lowland location

Parameters		Sum of Squares	df	Mean Square	F	Sig.
Lead level * Age of cattle	Between Groups	0.048	2	0.024	1.903	0.153
	Within Groups	1.845	147	0.013		
	Total	1.893	149			

Discussion

The level of lead was higher in lowlands cattle than the highland, which indicates more pollutant sources in the field, both from the air and animal feed. Furthermore, lowland locations where cattle are sampled are urban areas (Denpasar and Badung). Therefore, pollutant levels are higher than those in the highlands (Bangli). Analogous with this study is the level of lead in the blood of pregnant women at Duke and Durham Regional Hospital Obstetrics, showing higher levels of lead in mothers in urban (lowlands) compared to the surrounding rural areas (King et al. 2015).

The level of lead contamination of an area's heavy metals is predictable from its flora, commonly called a bioindicator (Berlekamp et al. 1998; Chen et al. 2010). Furthermore, several plants are useful for remediation of lead contamination in the soil. However, the consumption of plants, which are bio accumulators by livestock, exposes them to lead (Sharma and Dubey 2005). This situation is reported to cause heavy metal contamination in intensively maintained cow's milk (Malhat et al. 2012). Also, when plants absorb heavy metals from the soil, they enter the bodies of farm animals according to an ecosystem's cycle. In line with this, humans get exposed to heavy metals when they consume contaminated beef (Pinho and Ladeiro 2012). Also, feed factor, which is a source of heavy metal contamination, is evidenced by the discovery of lead in the blood of wild cattle (Roggeman et al. 2013).

Furthermore, research on the motor neuron disease (MND) in humans caused by heavy metals was reported to be the highest (20.9%) due to contamination of lead with urban geographies in Spain (Alonso et al. 2011; Santurtún et al. 2016). Therefore, it is very important to map heavy metal sources on an ongoing basis as was done in Germany (Berlekamp et al. 1998; Etabe et al. 2010). Heavy metal contamination can occur at any time and will take place continuously.

Furthermore, there is a similar pattern between cattle in the low and highlands. The youngest has low exposure to lead and the highest on adults. Also, a comparison between young cattle in the low and highlands showed a significant difference ($P < 0.01$) between the young, puberty, and adulthood, respectively (Tables 6), and this is due to the variation in levels of lead pollution in the environment where cattle are grazing. Furthermore, there is a possibility due to the acquisition of cattle to her calf during the embryonal period through transplacental. However, it is

reported that transplacental lead contamination tends to occur in humans (Brochin et al. 2008).

The results showed that the duration of exposure significantly affected the level of lead contamination, as old cattle meant that they were in the environment for a long time. Studies on fish have reported that the older the age and the bigger the body size, the higher the contamination (Farkas et al. 2003; Govind and Madhuri 2014). Furthermore, many factors influence the level of contamination, including geographic and fish species (El-Moselhy et al. 2014), physiological fish (Govind and Madhuri 2014), tissue types (Garcia et al. 2011; Muselin et al. 2010; Okareh et al. 2015), and certain infections (Kruchynenko et al. 2018). In contrast to the results of experiments using rats, where it was reported that the young were more exposed to lead than the older rats, but the younger animals have higher metabolic rate causing the heavy metals to be rapidly accumulated (Blagojević et al. 2012), and this caused persistent immunotoxicity (Miller et al. 1988). This also applies to ducks due to exposure to lead heavy metals in Argentina (Ferreyra et al. 2015). Similarly, studies on rock oysters (*Saccostrea cucullata*) found that young age were more exposed to lead (Petroody et al. 2017). This may be due to animal species factors, as reported that the response of exposure between fish species by heavy metals varies greatly (El-Moselhy et al. 2014). Furthermore, breeds in one animal species are also very influential on the level of contamination by lead, which occurs in buffaloes (Narozhnykh et al. 2018).

Based on the distribution of contamination data, there appears to be a similar pattern in the age of puberty, which is more homogeneous compared to young and adult (Table 1) and this may be due to physiological factors (Govind and Madhuri 2014; Duittoz et al. 2016) and feed patterns (Leontopoulos et al. 2015). The physiological factors are closely related to the hormonal system, where during puberty, gonadotropin-releasing hormone (GnRH) affects the resistance to heavy metal contamination (Pereira et al. 2017; Pribadi et al. 2014). Among the types of animals, there are also variations in sensitivity to heavy metal contamination, and can be used as bioindicator or biomonitoring to the level of environmental pollution in the vicinity. Several types of animals are used as bioindicators including buffalo (Narozhnykh et al. 2018), cattle, sheep, camels (Abdelbasset et al. 2014; Skiba et al. 2017), and antler deer (Gizejewska et al. 2017).

Table 6. Analysis of variance of lead level among ages in both locations

Age of cattle	Parameters	Sum of squares	df	Mean square	F	Sig.
Young	Between Groups	3.663	1	3.663	41.976	0.00
	Within Groups	8.553	98	0.087		
	Total	12.216	99			
Puberty	Between Groups	13.515	1	13.515	207.396	0.00
	Within Groups	6.386	98	0.065		
	Total	19.901	99			
Adult	Between Groups	29.135	1	29.135	181.717	0.00
	Within Groups	15.713	98	0.160		
	Total	44.848	99			

In conclusion, Bali cattle raised in the lowlands are more contaminated with lead than the highlands. Also, they experience more contamination of heavy metals in young compared to puberty and adult. While there is no significant difference between young, puberty and adult in the highlands

ACKNOWLEDGEMENTS

The authors are grateful to the Directorate General of Research and Public Service, Ministry of Research, Technology and High Education in Indonesia for funding this study.

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