ACTA Scientific Veterinary Sciences
Volume 1 Issue 2 September 2019
Research Article

Distribution of Lead Heavy Metal in Blood and Tissues of Bali Cattle Reared in Landfill in Denpasar, Indonesia

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Received: August 12, 2019; **Published:** August 27, 2019

Abstract

Garbage dump in landfills is growing to be problem worldwide. It also becomes popular as a place to rear animals as happen in Denpasar – Bali and various cities in Indonesia. Such animals are at risk to be contaminated by lead heavy metal. This might arrive in human consumption, which leads to intoxication. We examined lead concentration in blood plasma and tissues of bali cattle reared in landfill, Denpasar. A total of 25 samples of blood plasma were taken from the jugular vein of the animals. Blood sample was collected in a tube filled with EDTA 0.5%. Five out of the 20 cattle were necropsied to collect the muscle, liver, kidney, lungs and spleen tissues. The tissues and blood plasma were then checked for lead concentration using atomic absorption spectrophotometry (AAS). We found that lead concentration varied in blood plasma and tissues. The average contamination level of lead in blood plasma was 3.767 ppm, while in muscle, liver, kidney, lung and spleen were 0.6944 ± 0.13 ppm, 0.7576 ± 0.25 ppm, 0.9173 ± 0.21 ppm, 1.1064 ± 0.08 and 1.7423 ± 0.19 ppm, respectively. the conclusion is that the levels of heavy metal lead in the blood exceed the safe concentration for consumption, while the tissue is still safe for consumption.

Keywords: Landfill; Lead; Atomic Absorption Spectrophotometry AAS; Bali – Indonesia

Introduction

Lead heavy metal can be very harmful to the health of animals and human. A source of lead heavy metal contamination in humans is from food, especially meat food that contains high heavy metal. Lead heavy metal poisoning in animals and human may cause hepatotoxicity, nephrotoxicity, genotoxicity and neurotoxicity [1]. Lead heavy metal poisoning in children has been reported caused growth retardation, hyperactivity and declined cognitive ability [2].

Garbage dump in landfills is growing to be problem worldwide. It also becomes popular as a place to rear animals as happen in Denpasar – Bali and various cities in Indonesia. Such animals are at rik to be contaminated by lead heavy metal. This might arrive in human consumption, which leads to intoxication. Cattle that are reared in landfills are high likely contaminated with Pb heavy metal. In addition, landfills located close to the main city, the trash does not only consist of organic trash, but also inorganic substances, including Pb heavy metal. Pb heavy metal that is existing in landfill can be derived from waste industry, polluted water (2) and plants [3,4].

Carcass and internal body organ of cattle are generally for meat and offal that are used for human consumption. The meat is mainly derived from the skeleton muscles of the cattle, while the innards can consist of liver, intestines, lungs, kidneys and spleen. In some areas, blood cattle can also be consumed. In Bali, blood cattle are used for a traditional food called "lawar". Therefore, the meat and offal require more attention to be free from lead heavy metal contamination.

Indonesian national standard [5] has determined the maximum level of lead heavy metal in meat is 2.0 ppm. A preliminary study shows a positive correlation between high contamination levels of lead heavy metals with high Serum Glutamate Oxaloacetat Transminase enzymes (AST) detection, which indicates that the lead heavy metal has distributed in to the cattle's body (unpublished data). This study purposes were to determine the levels of lead heavy metals in blood plasma, meat and offal of cattle that were reared in a landfill, Denpasar.

Materials and Methods

Research samples

An observational study was done using a total of 25 bali cattle which were kept in a landfill, Denpasar since they were born. The blood plasma of the samples were collected. Five out of 25 cattle samples were sacrificed and necropsied in an abattoir. The tissue organ of muscle, liver, kidney, lungs and spleen were collected for the evaluation of lead heavy metal contamination

Blood plasma collection

Blood plasma of the cattle samples was collected from jugular vein using 10 ml vacuumed venoject tubes. Before the tubes were used, they were added with ethylene diamine tetraacetic Acid (EDTA) 0.5%. Then, the collected plasma was delivered to Analitical Laboratory, Udayana University for lead heavy metal measurement.

Tissue collection

Once the the cattle were sacrificed and necropsied, the tissue organs consist of muscle, liver, kidney, lungs and spleen from five cattles were collected and kept in a cool box before being further sent to the Analitical Laboratory, Udayana University for lead heavy metal measurement.

Measurement of lead heavy metal level

The tissue samples, such as: muscle, liver, kidney, lungs and spleen, were processed for the measurement level of lead heavy metals by using atomic absorption spectrophotometer (AAS) method [6]. The samples were divided into two parts, 0.5 ml for positive control and 0.5 ml as sample to be evaluated. Standard solution 0.25 ml of 1 mg/l was added as positive control. The control was evaporated on a hot plate at a temperature of 100ºC until it dried. Then, the spike and the samples were inserted into a furnace and covered half of their surface. In the process, the temperature furnace was raised gradually 100ºC every 30 minutes up to 450ºC and maintained for 18 hours. After that the spike was removed from the furnace and chilled at room temperature. Next, 1 ml HNO3 65% was added, before they were shaken carefully so that all the ash dissolved in acid and then they were evaporated on a hot plate at a temperature of 100ºC until they were dried. The samples and spike put them back into the ash furnace. Its temperature was raised gradually 100ºC every 30 minutes up to 450ºC and maintained for 3 hours. After they were formed white ash, the spike and samples were cooled at room temperature. A 5 ml of HCl 6 M solution was added to each sample and spike then shaken carefully so that all the ashes were dissolved by acid. Then they were evaporated on a hot plate at a temperature of 100ºC until dried. A 10 ml of 0.1 M HNO3 was added and cooled at room temperature for 1 hour, the solution was transferred into a 50 ml flask poly propylene before they were added with matrix modifier solution, then added with 0.1 M HNO3 until it reached to the mark limit. Lead heavy metal working standard solution was prepared at least five points concentration. Working standard solution, samples, and spike were read on graphite fumace atomic absorption spectrophotometry at a wavelength of 288.3 nm for lead heavy metal measurement.

Data analysis

Data measurement result of lead heavy metal in blood plasma and tissues were analyzed by quantitative analysis of one way ANOVA and if there is a significant difference ($p < 0.05$) followed by Duncan test.

Results and Discussion

Data measurement of lead heavy metals level in blood plasma samples are presented in Table 1. Measurement result of lead heavy metals level in blood plasma samples obtained varies from the highest was at 10.2912 ppm, and the lowest was 0.0010 ppm. Mean of lead heavy metal level in blood plasma was 3.7672 ± 4.1408 ppm. One way ANOVA test results showed no significant difference ($p > 0,05$) among cattle age.

The mean of lead heavy metal level in blood plasma of cattle on varies age groups is presented in Figure 1. Although the statistical analysis was not significantly different, but numerically it appears mean of lead heavy metal level in the blood plasma was lowest at 2 year age group and the highest in 4 year groups. The 1, 3, and 5 year age groups were seem equal to each other.

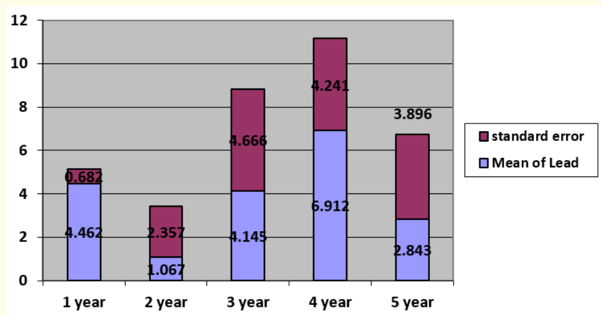

Figure 1: The mean lead concentration in the blood plasma of Bali cattle reared in Denpasar land fill at various age groups. The concentration is at ppm. The standard deviation of each group is shown.

Cattle code	Age (year)	Level of lead heavy metal (ppm)
1	1.0	5.005
2	1.0	5.001
3	1.0	4.064
4	1.0	3.456
5	1.0	4.786
6	2.0	0.002
7	2.0	0.002
8	2.0	0.014
9	2.0	5.316
10	2.0	0.001
11	3.0	10.292
12	3.0	7.741
13	3.0	0.016
14	3.0	0.002
15	3.0	2.675
16	4.0	10.016
17	4.0	9.623
18	4.0	9.295
19	4.0	5.616
20	4.0	0.012
21	5.0	0.003
22	5.0	0.002
23	5.0	0.001
24	5.0	7.390
25	5.0	6.822
Mean		3.886 ± 3.748

Table 1: Data of measurement result of lead heavy metal in the cattle's blood plasma.

Data of the measurement result of lead heavy metals level in blood plasma and the tissues are presented in Table 2 and Figure 2. Measurement result of lead heavy metals level in tissues from 5 cattle samples were obtained an average results that was in the blood plasma = 6.7365 ± 2.29; spleen = 1.7423 ± 0.19 ppm, Lung = 1.1064 ± 0.08; kidney = 0.9173 ± 0.21: liver = 0.7576 ± 0.25; muscle = 0.6944 ± 0.13 ppm. Duncan's test results showed that the levels of lead heavy metal in blood plasma significant difference ($p<0,05$) between blood and other tissues. There was no significant difference between the levels of lead heavy metal in the spleen, lungs, kidneys, liver and muscles.

Tissue Organ	The lead heavy metal level (ppm)					Mean ± SE
	Cattle a	Cattle b	Cattle c	Cattle d	Cattle e	
Spleen	1.765	1.502	2.027	1.764	1.653	1.742 ± 0.19
Lung	1.134	1.070	1.196	1.140	0.991	1.106 ± 0.08
Kidney	0.583	1.050	1.131	0.9213	0.901	0.917 ± 0.21
Liver	0.692	0.450	1.140	0.7570	0.760	0.758 ± 0.25
Muscle	0.682	0.702	0.677	0.7120	0.700	0.694 ± 0.13
Blood	5.005	7.740	10.291	5.616	5.001	6.737 ± 2.29

Table 2: Distribution of the lead heavy metal level in the cattle's tissues.

The measurement results of the lead heavy metal levels in the blood plasma (Table 1) are varied with the highest at 10.2912 ppm and the lowest at 0.0010 ppm, even though the cattle samples were taken from the same location. This difference can be caused by variations in the age of the cattle and the period the cattle samples that have been kept in the landfill, selectivity of the cattle in choosing the feed and the heterogenic distribution of lead heavy metals contained in the waste. The results on the correlation analysis between age and levels of heavy metals show no association between them. However, this seems to be related to the distribution of the lead heavy metal in the waste in the landfill, which is very heterogeneous. The period of cattle kept in the landfill area is high likely only related to their age, because the samples that were selected and used have been limited only for the cattle that were kept since they were born in the landfill. The most likely affect the levels of lead heavy metals varied is the selectivity of the cattle in choosing for their food and the distribution of lead heavy metal in the waste in the landfill. Characters level of selectivity in choosing food cattle reported by [7], that the breed affects the variation exposure to lead heavy metals and other heavy metals. Similar results also reported in fish, variation levels on lead heavy metal contamination on each fish, even though they remained collected from the same location [8].

The mean of lead heavy metal levels in the blood plasma is 6.7365 ± 2:29 ppm, is much higher than in the tissues (Table 2). The mean of lead heavy metal level in the tissues such as spleen, lungs, kidneys, liver and muscle observed below a threshold level of ISO-7387-2009 ppm (maximum 2.00). High level of lead heavy metal in blood plasma is associated with properties that lead heavy metal may substitute iron (Fe) in hemoglobin [9]. Level variations of lead heavy metal in the tissues reportedly related to the tissue in binding with lead heavy metal [7]. A similar trend was reported [10] that the period of lead heavy metal accumulation in the fish tissues was varied greatly. Lead heavy metal accumulation rates from the highest to the lowest found in the tissues were the liver, kidney, gills and muscle. The interesting finding is the contamination of lead heavy metal in the muscle tissue in cattle and fish is similar; but only at the lowest level. Muscle tissues in cattle and fish were a common part of human's food. Overall, levels of lead heavy metal level in tissues are still below the threshold limit of the maximum ISO-787-2009 that is 2.00 ppm [5] Thus, the beef is still safe to consume.

The results showed there is a different result with lead heavy metal examination done in the cattle tissues in abattoirs, Nigeria [11]. This study reports that mean content of lead heavy metal was 4.36 ± 0.79. Lead heavy metal contamination in the cattle in Nigeria demonstrated its harmful effect on the health of consumers. Meanwhile [12] reported that a 4.404 ppm of the lead heavy metal contamination was examined in dairy cattle in Egypt, which can rise some questions: where is the source of lead heavy metals contamination ?, because they were intensively reared dairy cows. In this regard [3] reported that lead heavy metal could be derived from plants that were eaten by animals. The presence of heavy metal in plants was also reported by [4]. From these studies, it can illustrate that lead heavy metal contamination source is also varied.

Other than the general part of muscle that being consumed, there are organs liver, spleen, kidneys, lungs are also commonly consumed. The average lead heavy metal contamination in the cattle tissues, the highest contamination found in spleen tissue, then followed by lungs, kidneys, liver and muscle. The spleen is an organ of the body's defenses as well as a haemopoietic organ. Not only the spleen, the liver is also a haemopoietic and detoxification organ. In regard to the mechanism of lead heavy metal associated with in Fe substitution in hemoglobin, so that hemopoietic tissues are the main tissue as the lead heavy metal place. This is consistent with a study reported by [9] that heavy metal may accumulate in the tissues as haemopoietic organs.

Not only anemia, the high level of lead heavy metal that exceed the threshold may cause central nervous system disorders [13] and liver damage [14] in humans. Therefore, regarding the lead heavy metal contamination in cattle tissues organs, beef seems to be safer for consumption compared to the cattle offal or blood.

Lead contamination in the blood plasma seems to be age dependent. Young calf under one year old has high plasma lead contamination that might be coming from milk [3]. As the animal is weaned, the lead contamination is the lowest at 2 years old than increased to 4 year old. The lead contamination then decreased at 5 years old.

The low lead contamination in 2 year old cattle can be caused by beef behavior factor, which at that age is the time of puberty of Bali cattle [15]. The calves at puberty usually break away from their host environment, thus avoiding exposure to leads from the mother's milk and contaminated feeding. When a 3 year old cow is the first time a Balinese cattle breeds, so the movement becomes decreased and allows the cow to be contaminated leads and accumulate drastically until the age of 4 years. While low lead contamination in 5 year old cattle showed decreased lead substitution power to Fe in hemoglobin. This is explained by [16] that leads stimulate the occurrence of erythrophagocytosis that causes anemia. Anemia is a low state of hemoglobin, which causes only few leads to substitute Fe.

The result of this study brings new insight in cattle raising throughout the world, especially in rural environment. The production of garbage does provide source of food for production animals. However, our data indicate that the meat and internal organ of animals might contain dangerous substance for human health. More risk exists in the use of animal offal for human food, a common praxis in Indonesia, as well as in other developing country. In developed countries, offal is discarded or used as animal food and not for human consumption [17]. The lead concentration in offal is supposed to be accumulative that might lead to toxicity after long periode of consumption [18]. We provide evidence that the lead level is the lowest in muscle compared to offal such as liver, spleen, kidney and lung as previously published [19].

Anova of lead content based on cattle age					
Lead content					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	92959386,240	4	23239846,560	1,903	,149
Within Groups	244193350,400	20	12209667,520		
Total	337152736,640	24			

Table 3

Anova of lead content among some tissues					
Lead content					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	102879137,467	5	20575827,493	3,365	,019
Within Groups	146737724,000	24	6114071,833		
Total	249616861,467	29			

Table 4

Duncan test as a further test of the ANOVA test between lead levels in the tissue				
	Tissues	N	Subset for alpha = 0.05	
			1	2
Duncan[a]	Lung	5	1.106,20	
	spleen	5	1.742,20	
	Muscle	5	1.976,20	
	Liver	5	2.122,40	
	Kidney	5	2.575,60	
	Blood	5		6.730,60
	Sig.		,410	1,000
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 5,000.				

Table 5

Conclusion

The mean lead heavy metal level in the blood plasma of cattle reared in a landfill, Denpasar is 3.7672 ± 4.1408 ppm indicating above the maximum threshold of SNI-7387-2009 (2.00ppm). The highest contamination of lead heavy metal in tissue organs of the cattle reared in a landfill, Denpasar is in the spleen = 1.7423 ± 0:19 ppm and the lowest is in the muscles = 0.6944 ± 0:13 ppm, which is still below the threshold provisions of SNI-7387-2009.

Suggestion

Need to do further routine research on lead heavy metal contamination in animals and their products, starting from animals from the pen, abattoirs, and beef markets. Therefore, consumers can get safe beef and offal for consumed.

Aknowledgement

Authors thank to the Directorate General of Research and Public Service, Ministry of Research, Technology and High Education in Indonesia for the funding support on this research.

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