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Biological Monitoring Of Heavy Metals In Goats Exposed To Environmental Contamination In Bagega, Zamfara State, Nigeria.

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ABSTRACT

Environmental pollution from anthropogenic sources such as mining has been a recurrent cause of heavy metal contamination. Lead contamination from artisanal gold mining in Zamfara State has been associated with severe human mortalities. However, the effects of the contamination on animals are yet to receive adequate attention. The purpose of this study was to evaluate the levels of lead, cadmium, chromium, selenium and copper in blood and other tissues of goats as sentinels in the contaminated sites, and to assess epidemiological/public health implication on human consumption of the goats. Mining activities were ascertained by observation in Bagega, Blood and tissue samples from two hundred and eighty two naturally-exposed goats (NEG) from Bagega, and 60 unexposed goats as controls (UEG) from Tsafe were analysed for heavy metals The levels of the heavy metals were evaluated using atomic absorption spectrophotometer (AAS) in blood and tissues. Results revealed large scale mining in the area. The mean concentrations of heavy metals (µg/dl) in digested blood were 225.79±14.23 for lead, cadmium (35.85±10.47), chromium (55.69±23.50) and copper (130.82±41.26) in exposed goats, which were significantly higher, while selenium (723.85±67.32) was significantly lower compared with the control goats. Lead and cadmium concentrations in the tissues were higher in exposed goats than control goats. The levels of heavy metal were higher than the maximum acceptable limits s reported by JECFA, in both exposed and control goats (2010). The results suggest that heavy metal contamination in exposed goats in Bagega, Zamfara State, Nigeria was associated with mining and environmental pollution. The exposed and control goats are not fit for human consumption according to international standards.

KEYWORDS: Heavy metal poisoning, Goats, Gold mining sites, Nigeria, Zamfara State

INTRODUCTION

The Zamfara lead poisoning saga was due to indiscriminate small-scale artisan gold mining activities inwhich the ores were contaminated by lead [17]. Mining activities were reported in and around Bagega village in Anka LGA of Zamfara State in Nigeria, and within many of the mud-walled houses where humans and animals cohabit. There had been reports of mortality of more than 400 children of the impoverished rural region with thousands more been severely poisoned by the reported lead associated contamination [34, 16, 25]. It is possible that the lead contaminated gold ore contain other toxic metals. Lead in the earth crust is commonly found with deposits of other metals such as zinc, cadmium, silver and copper [2]. Studies have revealed high levels of lead cadmium, and zinc contaminants in hand dug wells in Dareta village of Anka local Government Area in Zamfara State, which was also reported to be contaminated [31]. Therefore, prompting the need for the investigation of the components of the contaminant in Bagega village.

The anthropogenic sources such as mines, industries and municipal waste have been reported as major sources of contamination of the environment, and most of these metals are not biodegradable and as such are retained in the environment for extended period of time [8, 24]. Heavy metals such as lead, cadmium, chromium, mercury and arsenic, have been reported to cause global-scale environmental contamination with subsequent poisoning of the humans and animals [32]. Therefore the need to bio monitors the environment through its habitants (sentinel) to assess the levels of exposures.

Exposure to heavy metal pollution has been a recurring problem in many parts of the world where minerals (such as gold and tin) and gem stone mining are on-going or had been carried out [23, 11, 1]. Heavy metals, especially those found in conjunction with ores and minerals of economic importance are released into the environment in the course of extraction, purification and processing [1, 17, 25]. Whereas inside the core and under top soil these metals are not often considered as sources of contaminants, but when exposed by anthropogenic activities, they could contaminate the environment [9] and humans.

Although mining generates economic wealth, it causes negative environmental impact in and around the mining environment, especially with heavy metal contamination. The heavy metals are toxic elements that can be assimilated, stored and accumulated, affecting all living organisms and have toxic impact on all levels of the food chain [28], including livestock. Whereas a number of studies have been carried out on the human exposure to heavy metals, animal studies are limited although it could be an important pathway of exposure to humans. This study is therefore focused on the biomarker of exposure in goats as an animal sentinel and the public health implication therein.

Lead poisoning occurs by absorption through the respiratory and gastrointestinal systems, then carried in the blood and transported to soft tissue and later into bone tissues where it accumulates over time. The half- life of inorganic lead in the blood is about 30 days and between 10 and 30 years in bones [10]. Lead is mainly excreted in the urine and faeces. The blood lead levels have been associated to tissue levels [29]. Food animals with tissue levels $\geq$ 0.1 mg/kg are regarded as not fit for human consumption and may intoxicate [12].

Small ruminants (sheep and goats) from northern Nigeria make up a large proportion of the total Nigerian small ruminant population and also a steady source of meat and animal protein for the population [14]. These small ruminants, especially goats live in close proximity with humans due to the semi-intensive type of husbandry system commonly practiced. These goats are therefore good sentinels as they are equally exposed to the reported lead contamination, because they reside in the same contaminated environment as the exposed humans. This close association in the contaminated environment exposes the animals to the same level of hazard as humans, which could translate to heavy metals in the food chain with possible grievous implications to human consumers, including those far away from the site of contamination who depend on the goats as source of animal protein.

The objective of this study was assess the impact of mining activities in Bagega, Zamfara State on heavy metals exposure to goats (blood and tissue samples) as sentinels in the mining environment with the view of assessing the epidemiological implication on consumers of goats from the region.

MATERIALS AND METHODS

Description of study area:

Bagega is a village in Anka Local Government Area (LGA), Zamfara State, Nigeria approximately 33 km from Anka as shown in Figure 1. It is one of the biggest villages in Anka LGA. Bagega community, one of the 13 communities in the Bagega ward occupies a land area of 46.2 hectares [34]. GPS coordinate of the present ore processing site visited is N 11.86251, E 006.00468 which lies outside the village. Tsafe is also a LGA in the same State as Anka LGA and about 149.9 km from Anka LGA and 178.8 km from Bagega on a driving route. It is one of the LGAs in the State without reports of heavy metal contamination. The State has a livestock population of nearly six million comprising of over 1 million cattle, 857,000 sheep, over two million goats, 1.7 million poultry, and about 18,000, 21,000, and 46,000 horses, camels and donkeys respectively [34]. Bagega is a predominantly Islamic community with a district head. Majority of the inhabitants are crop and livestock farmers, with small ruminants raised under semi - intensive system, where the animals are housed in the same compound as humans. Sampling was done in the month of May, 2013.

Animals:

Two hundred and eighty two, naturally-exposed goats (NEG), and 60 unexposed goats as controls (UEG), were used. The unexposed goats were sourced from a geographically related area in Tsafe, Anka LGA, and Zamfara State with no history of mining or lead contamination and also shared other similar demography with the exposed animals. The exposed goats were selected with selection and exclusion criteria, which include, whether they had a history of being in the environment since birth or have been brought into the area for not less than three months. The goats with the history of being newly acquired or without complete history and signalments were excluded in the study. The same selection and exclusion criteria were used for the control goats.

Blood:

282 paired blood samples were collected by jugular venepuncture into heparinized bottles and transported to the laboratory for heavy metal analysis. The animals were properly restrained and 5mls of blood was drawn from the jugular vein of the goats, and was immediately transferred into an EDTA bottle for onward transfer to the laboratory in a cold chain.

Tissues:

Organs from 40 randomly selected goats were collected from the only slaughter slab in Bagega where a large number of animals from the community are gathered and slaughtered only on Fridays. Organs from 15 goats were similarly collected from control animals from Tsafe village. The samples were from the intestine, kidney, liver, muscle, spleen and testis. Approximately 5gms of each organ were collected into containers, kept in frozen cold packs for onward transportation to the laboratory for analysis.

Analysis of Heavy metals level in the blood:

The blood samples were digested by the wet acid digestion method and analysed by Atomic Absorption Spectrophotometer (AAS) as described by Aguilera *et al.* [3]. 1 ml of whole blood was measured into each of clean test tubes; 1 ml of concentrated Nitric acid (HNO_3) containing 0.1% triton-100 was added and allowed to mix thoroughly. The test tubes were thereafter plugged with cotton wool and left on the bench overnight. On the second day, the mixture was heated in a water bath at 100°C for 20 min and thereafter allowed to cool. The digested blood samples were transferred to a measuring cylinder and the volume made up to 25 ml with distilled water and stored for analysis as described by Aguilera *et al.* [3].

Lead, cadmium, chromium, selenium and copper concentrations in the digested blood samples were measured using Atomic Absorption Spectrophotometer, (Alpha 4, and CHEMTECH 4200, Chem. Tech. Analytical Co. England) with graphite furnace and background correction (SR-BDG). Signals were read at 283.3 nm with background Zeeman correction. Standards and whole blood samples were both diluted (1:10) with an aqueous matrix modifier solution (containing 0.2% HNO_3, 0.5% Triton X-100, and 0.2% $NH_3H_2PO_4$). All blanks, standards, and samples were analysed in duplicates. The calibration curve gave a linear response across this range with a correlation coefficient of 0.999. The averages of the duplicate values were taken for statistical analysis.

Analysis of heavy metal levels in tissues:

1 g of each frozen tissue / organ was grinded to fine powder and 5 ml HNO_3 was added in a clean test tube. The mixture in the test tubes was plugged with cotton wool and left on the bench overnight to completely digest the tissue. Analysis of heavy metals in each tissue digest was carried out as described above for blood digests and additionally, 1 ml hydrogen peroxide was added to prevent foaming on day 2 and the digest completed on same day.

Quality assurance procedures:

All reagents were of analytical grade (Merck, Germany) and distilled de-ionized water was used to prepare solutions, glass wares were washed and soaked in detergent solution for two days and later soaked in 5:2 mixtures of HCl and H2O2, and the rinsed in deionized water. There was precision and accuracy of the analytical procedure, duplicate sample, analytical duplicates, and blanks were used.

Statistical analysis:

Data collected were analysed using descriptive statistics, 2-way analysis of variance (ANOVA), Correlation coefficient test and Duncan’s multiple range tests using SPSS version 22 [19]. Significant differences were measured at $p< 0.05$.

RESULTS

It was observed that active and large scale mining was on-going in Bagega, with the presence of huge mining wastes deposited around the mining and processing sites which was barely 30 km from the residential area of the village. The observed ore-processing activities included breaking of the rocks, grinding, washing, drying, using liquid mercury to extract gold flakes (separating) and using heat to vaporize mercury from the gold mixture after amalgamating. The dust generated from the grinding of rocks to get a fine powder during processing in the mining site was observed to spread a long distance that covers the village and beyond including the residential areas of the village. Goats were found roaming around the processing site and drinking from old mine processing pits (Figure1). The only earth dam that serves the entire Bagega community for domestic purposes and animal needs was also previously used for the process of mining with remnants of mining equipment observed along the borders.

Fig. 1: A goat feeding on tree shrub from an old used washing pit in the mining site.

The blood of the 282 (100%) of the NEG and 37 (62%) of the UEG had varying levels of lead and other heavy metals as shown (Table 1). The levels of lead, cadmium and copper were significantly higher ($p<0.05$) in the NEG compared to those in the UEG group while the levels in both groups were higher than the acceptable limits. However, selenium levels in NEG were significantly lower ($p<0.05$) than the UEG.

The correlation between lead and other heavy metals revealed a significant increase ($p< 0.05$) of cadmium and copper with increasing levels of lead in the NEG (Table 1). There was an inverse relationship between lead and selenium ($r = - 0.076$), as selenium significantly decreased ($p<.05$) with increasing levels of lead in the NEG. However, there was no significant relationship between chromium and lead though chromium insignificantly ($p>0.05$) decreased with increasing levels of lead. The levels of copper were significantly related to the increasing levels of cadmium in the NEG. Selenium levels were not significantly related with the increasing levels of copper as shown in Table 2.

The means of lead and cadmium (µg/g) in the tissues were significantly ($p<0.05$) higher in the NEG compared to the UEG as shown in table 3. The levels of lead and cadmium in the NEG kidneys 72.81±4.92 (µg/g) and liver (58.19±3.36 µg/g) were significantly higher ($p<0.05$) than those in the other organs (Table 3). The lowest levels of lead and cadmium (22.31±18.00) were observed in the testis as shown in Table 3. The levels of lead and cadmium in these organs are higher than the acceptable limits for human consumption [12].

The lead and cadmium tissue levels in all the samples including the confirmed exposed (NEG) and the unexposed group (UEG) exceeded the acceptable level of 0.1 mg/kg stipulated by JECFA [12].

Table 1: Mean values and ± SE of blood levels of heavy metals (µg/dl) in naturally exposed and unexposed goats

	Lead (µg/dl)	Cd (µg/dl)	Chromium (µg/dl)	Selenium (µg/dl)	Copper (µg/dl)
NEG (n=282)	22.57±1.42 [a]	3.58±1.04 [a]	5.56±2.35	72.38±6.73	13.08±4.12 [a]
UEG (n=60)	8.34±1.14	1.96±0.43	5.03±1.57	100.34±10.38 [a]	11.05±3.76

NEG – Naturally exposed goats, UEG – Unexposed goats
a = $p<0.05$

Table 2: Correlation between blood heavy metal levels (µg/dl) in the naturally exposed goats (NEG).

	Lead	Cadmium	Chromium	Selenium	Copper
Lead	1	.500*	-.058	-.124*	.154*
Cadmium		1	-.049	-.101	.167*
Chromium			1	.125*	.129*
Selenium				1	-.076
Copper					1

Table 3: Mean tissue and ± SE of lead and cadmium (µg/g) in wet weight of tissues of exposed and unexposed goats

	Lead (µg/dl)		Cadmium (µg/dl)	
	NEG	UEG	NEG	UEG
Intestine (n =16)	53.25±2.15	8.60±0.92	13.63±0.79	3.84±1.22
Kidney (n =18)	72.81±4.92*	14.07±1.10	22.82±2.20	4.86±0.61
Liver (n =21)	58.19±3.36*	9.86±1.82	21.11±1.62	4.25±0.54
Muscle (n =12)	52.56±2.42	6.48±1.26	12.74±6.59	3.61±0.83
Spleen (n =13)	42.62±1.53	4.53±1.28	15.84±1.07	0.85±0.14
Testis (n =8)	22.31±1.80	8.84±1.50	8.89±9.70	1.24±0.58

n=number of sample

Discussion:

This study has established that mining activities was, and is still on-going in the investigated area. This study has also revealed elevated levels of heavy metals (lead, cadmium, chromium and copper) in the blood, with lead and cadmium in organ samples of goats from the village where the mining occur compared to controls goats. Lead and cadmium levels detected in tissues of exposed goats exceeded the recommended levels in food animals [12].

But it is worthy of note that the data in both exposed and control areas showed levels above the acceptable limits, indicating that both environments are contaminated but at different levels and by the same means of exposure or other means that are yet to be identified, since the area has not been reported to be exposed to mining.

In contrast to the reported increase in lead and cadmium levels, the levels of selenium in the blood of the reported exposed goats reduced relatively, showing a negative correlation of lead and cadmium to selenium levels.

Reports of mining activities in the same site has been recorded in previous studies [31,25], and at different sites in other studies [20,22,34]. The environment in and around the ore processing sites were also observed to be contaminated due the mine processing activities, the dust generated from the grinding of the rock to fine powder could be a contributing factor [25]. Osher *et al.* [22] had reported that mining and smelting of ores have increased the prevalence and occurrence of toxic elements through dust emissions, mine tailing and waste water. As observed in this study, Plumlee *et al*. [25] had reported that the toxicity in Zamfara may have been so acute due to the artisanal mine processing method used, such as grinding of lead contaminated rocks using crude mills inside and around the village as observed and reported in this study. Considering that it takes about 18 tons of ore to create a single wedding ring [2], the associated amount of mine waste is considered enormous and becomes a means of massive environmental contamination especially when the ore is contaminated [25,2]. Lead and other associated metals contamination in this study were associated with the contaminated ore. The elevated level of lead in the blood in the present study is in agreement with the results obtained from the human studies by Talha [31] which reported high levels of lead in the human population that inhabit the same mining areas. Williams and Edobor [36] had also reported levels as high as 152.42 mg/kg of lead in fish from the Lagos lagoon, a polluted water body in Nigeria.

Blood lead level is considered to be the best indicator of the concentration of lead in soft tissues, reflecting recent and, to some extent, past exposure to lead toxicity and gives information linked with the effects of environmental exposure on health [15]. Therefore there is concern on the effect of heavy metals on the ecosystem, with continuous release of these metals into aquatic and terrestrial ecosystems from anthropogenic sources [13]. These metals pollute the environment and find their way into the body systems leading to serious threat of toxicity, bioaccumulation and bio magnifications with impact on the food chain [4].

Studies have shown that the levels of heavy metals in the environment and in the living system are increasing due to anthropogenic activities that pollute the environment and subsequently, the inhabitants [7, 32, 28, 9].

A high level of other metals (cadmium, chromium and copper) in the blood of goats living around the mines compared to controls goats is an evident that the environmental contaminant included lead and other metals. The detection of an array of heavy metals in the exposed animals could be due to co-existence of metals in nature and subsequent exposure of such metals when contamination occurs, as have been reported by studies of Udiba *et al*. [33].

The negative correlation with selenium seen in this study is an important pointer to the effect and interaction of lead and or other reported heavy metals toxicity on selenium. The negative correlation could be due to the use up of selenium as an anti-oxidant in the oxidative state caused by the increasing levels of heavy metals [6]. Selenium has also been reported to exert a protective effect against cadmium or lead toxicity [18, 27]. Other experiments have also shown that goats exposed to lead showed significant reductions in selenium levels in the blood [23]. The beneficial role of Selenium in heavy metal-induced oxidative stress has been reported in human studies [36, 6].

Lead and cadmium levels detected in tissues of NEG exceeded the recommended levels in food animals [12] an indication of the public health significance in the food chain as reported in other works [36, 30]. The very high levels in the tissue of goats can be considered a risk to public health due to the bio- accumulation of these metals in goat that the human population consume as a ready and cheap source of animal protein. The European Commission Regulation 466/2001 accepts 0.1 ppm as the maximum tolerable concentration of lead in beef.

In this study, the liver and kidney had the highest levels of lead and cadmium. It has been reported that when animals are exposed to lead, this is deposited most especially in liver, renal cortex and medulla [26], and the endocrine system [30]. It has been reported that once absorbed, lead is distributed particularly to the liver and kidneys, and then stored in the bones over time. It has also been reported that liver is the largest repository of lead (33%) followed by the kidney cortex and medulla and then to the bones after several months [5].

Conclusion And Recommendations:

The results of this study have confirmed active artisanal mining in Bagega, Zamfara State, Nigeria with associated contamination and subsequent poisoning of goats in the exposed area at levels far above the JECFA recommended levels and posed a potential public health hazard.

It is recommended that mines and industries producing lead and cadmium and other heavy metals, must practice safe processing procedures and be equipped with appropriate methods of waste disposal to decrease risk of human and animal exposure to heavy metals. Attention should be paid to ante-mortem and post-mortem screening of animals for human consumption, thereby advocating the inclusion of heavy metal levels screening in meat inspection by the use of specific test kits. There is also a need to put in place programmes to establish and regulate the levels of lead and other heavy metals in our environment and implement continuous surveillance of the environment.

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