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Impacts of trace metals on Roan Antelope, *Hippotragus equines* and its Endoparasite *Strongyloides spp*., sampled in the tropical rainforests of Odo Ona Kekere, Ibadan, Oyo State, Nigeria

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ABSTRACT

Background: Roan antelopes, together with other mammals, serve as man's proteins. Parasitic infections affect their growth and development and therefore reduce their yield. Samples of hunted roan antelopes (*Hippotragus equines*) were collected from Oluwo bushmeat Market, Epe, and Odo Ona Kekere in Oluyole Local Government Area of Ibadan, Nigeria. The intestine and liver of the roan antelopes were assessed based on endoparasites, accumulated trace metals, lipid profile, antioxidant biomarkers, and histopathology. The study was conducted to determine the impacts of trace metals *on* the enteric parasite *Strongyloides spp.* as an early warning to metal toxicity impacts on the antelopes and the consumers.
Results: The study showed that the enteric parasites of the Roan antelope accumulated barium at a higher level than the host. The parasites showed great potentials for storage of cadmium and nickel, with the second-highest bioaccumulation factors in the study (>2), after zinc with bioaccumulation factor > 3. Vanadium's significant bioaccumulation factors are recorded only in the liver and intestine of the roan antelopes. The negative impact of the multi-stress conditions was evident in this study. For example, the significantly highest concentrations of zinc and barium in the parasites than the intestines and liver of the roan antelopes may partly be implicated in the outstandingly higher cholesterol, and low-lipid lipoproteins indicate dyslipidemia, which results from cellular damage due to stress. In stress conditions, some physiological reactions occur, including changes in hormones and components in the blood. These events might lead to higher cholesterol levels which may result in dyslipidemia. As seen in this study, although the levels of MDA in the investigated tissues were reasonably fairly stable, the upregulated SOD in the investigated tissues of the parasite served as an early warning signal of stress in the roan antelopes.
Conclusion: The study revealed that *Strongloides spp* might be a reliable bioindicator of the metal burden in the roan antelopes. The enteric parasite may also serve as a good biosequestration tool to alleviate the toxic load of cadmium and nickel from the roan antelope. The synergistic impacts of cadmium and nickel on the parasites might reduce the infection intensity in the host. This study has demonstrated an empirical early warning against the deleterious accumulation of vanadium, barium, and zinc, which might rise beyond acceptable levels in the future, thus providing prognostic data for proactive decisions

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by stakeholders to make pragmatic plans and policy towards a sustainable conservation of the roan antelopes.

List of abbreviations

SOD	Superoxide dismutase
MDA	Malondialdehyde
CAT	Catalase
GIT	Gastrointestinal tracts

Background

Game animals, generally referred to as bush/ wildmeat, greatly support meat production and serve as a rich source of animal protein to the poor in the rural communities of Africa (Fonweban and Njwe, 1990). As habitat degradation and fragmentation encroach the conservation areas, the wellbeing of wildlife is progressively impacted. Poaching, among other anthropogenic perturbations and intrusions may greatly impact sedentary and docile animals like the roan antelopes [16]. Logging and clearance of vegetation canopy changes the ecosystem structure and function, thereby rendering the wildlife vulnerable to poaching. Furthermore, the process of hunting by the poachers may render the bushmeat unfit for consumption as some methods involve the use of chemical baits and gunpowder to incapacitate the animals. These hunting practices could expose consumers of the bushmeat products to trace metals such as Zn, Cd, V, Ba, Ni, Cu, Co, Pb, Cr, Mn, etc. (Hunt et al., 2009). Trace metals are persistent toxic micropollutants that bioaccumulate in exposed organisms and biomagnify up the pyramid of biomasses (Dural et al., 2007).

Bushmeat may be contaminated by animal drugs, pesticides, feed and other agricultural or industrial chemical substances (Khalafalla *et al.,* 2011). Events of heavy metal contamination of bushmeat products during processing have been widely reported (Akan *et al.,* 2010; Harlia and Balia, 2010). Meat production processes may also have an implication on the edibility of the meat product. For example, the burning of animal hair using fossil fuel such as kerosine ~~or diesel~~ which is common in sub-Saharan Africa may render the products unsafe for consumption. Methods such as burning off the hairs of the animals in flame fueled by various substances such as wood mixed with spent engine oil (Spent engine oil is poured on wood for starting up fire), plastics mixed with refuse or tyres. These materials contain toxic substances such as heavy metals, which can contaminate the meat and render them unfit for human consumption (Okiei *et al.,* 2009). Despite these associated issues with bushmeat, the commercial benefits and nutritional value play direct role in the livelihoods of about 150 million people in the world (Hunt et al., 2009). Significant percentage of the high demand for bushmeat is met by hunting using guns, cutlasses, chase dogs, baiting with chemicals, and bush burning (Oduro and Kankam, 2000). In other cases, contaminated animal feed and livestock rearing in proximity to polluted environments were reportedly responsible for heavy metal contamination in meat (Khalafalla *et al.,* 2011). Copper, nickel, chromium and iron, are essential in very low concentrations for the survival of all forms of life. Essential trace metals may, however be toxic at concentrations more significant than the threshold of essentiality [10].

Ecosystems undergo constant changes due to the geometric rise in human population. The steady increase has been accompanied by a commensurate rise in anthropogenic perturbations that are deleterious to health and the environment. Human interferences underlie habitat degradation and fragmentation, threatening biodiversity, especially the antelope species from its standard common range [6,12].

Olajesu *et al.* [16] pointed out that wild consumed animals are exposed to different parasitic infestations which can be transmitted to their offspring and other mammals including humans that may come into contact with them if domesticated. In the event of high habitat alterations due to multi-stress conditions, the inherent species may suffer immunosuppression which may enhance their susceptibility to parasitic infections. Various researches have reported ectoparasites and endoparasites in wild animals in Nigeria [2,17–19]. As the natural barrier between wild animals and man is either crossed or eroded, the likelihood of zoonotic outbreaks increases. However, the metal burden accumulated from the environment may either be toxic to the endoparasites or sequestered by the same. Either way, the host may benefit from reduced parasitic intensity or toxicant concentration, respectively. These two outcomes are not classically exclusive, i.e. they may occur simultaneously.

The docile nature of antelopes underlies their vulnerability to habitat degradation due to human intrusion. As docile herbivores, antelopes are susceptible to contamination of grazing fields by agrochemicals and artificial fertilizers which may spread through surface runoff. Roan *antelopes* are predominantly intermediate height grazers and feeding is hampered at heights lower than 8-10 cm above ground level. The peak of grazing occurs during the cooler hours of the day in the mid-dry seasons which constitutes 5-10% to the total dietary intake. Broad leave forbs with a broad spectrum of palatable grass species constitute 5%of the balanced dietary requirements of the animal. The width of the leaf provides a wide surface area

for adsorption of air pollutants. Other plants that are resilient to desertification are not in the natural diet of the animal. The most dominant dietary grasses for the roan antelopes in the forests of Odo Ona Kekere, Oluyole Local Government of Ibadan, Oyo State, Nigeria include the red grass-*Themeda triandra*, spear grass-*Heteropogon contortus*, tassel three-awn-*Aristida congesta*, wool finger grass-*Digitaria pentzii*, blue buffalo grass-*Cenchrus ciliaris* and white buffalo grass-*Panicum coloratum*. and short grasses such as couch grass-*Cynodon dactylon*.

Naturally, *H. equines* require 9-10 litres of clean water daily for sustenance, hence are susceptible to unclean water. Anthropogenic activities such as the use of agrochemicals around the catchment areas, unsustainable hunting processes, etc., however threaten these ecological requirements of the animals and may further pose threats to the consumers. Although animals, show great preferences for cleaner water and food but in polluted environments, they unavoidably depend on the contaminated resources in the absence of preferred alternatives. Unhealthy conditions in the forests are directly attributable to the advancement of anthropogenic perturbations towards biodiversity hotspots. The ecophysiological consequences may include evident immunosuppression among the animals dwelling within the ecosystem. However, there is replete detailed research done on the conservation of roan antelopes. In Nigeria, bio-conservation of this species may be initiated through identification and mitigation of the prevailing perturbations in the natural habitat of the animal. Immunosuppression in the roan antelopes may initiate susceptibility to parasitic infections, which may further deteriorate the health of the individuals and negatively impact the conservation status of the population large.

Assessing the levels of trace metals in the roan antelopes and understanding the dynamics of the accumulation in their tissues which serve as animal protein to humans is key to making informed decisions on protecting the wild animals and the dependent human health. Other useful tools such as oxidative stress biomarkers, lipid profile, and histopathological analysis may further shed some light on the conservation status of the animals in Odo Ona Kekere forests regarding the prevailing environmental conditions. This study therefore aimed at assessing the impacts of metal accumulation on parasitized roan antelopes. The study explored the possibility of aiming the toxicity of Zn, Cd, V, Ba, Ni, Cu, Co, Pb, Cr, and Mn at the endoparasites (*Strongyloides spp*) to reduce the parasite intensity in the roan antelopes (*Hippotragus equines*).

Methods

Study location

Samples of hunted roan antelopes were collected from Oluwo Market, Epe and Odo Ona Kekere in Oluyole Local Government Area Ibadan. Epe is a town and Local Government Area (LGA) in Lagos State, Nigeria located on the north side of the Lekki Lagoon and about 90 km from Ibadan; it has a road connection to Ijebu ode and Ikorodu. It lies between latitude 6°35′ 3N and 3°59' 43′E. During most months of the year, there is significant rainfall in Epe. There is only a short dry season. The average temperature is 26.3°C and precipitation is 1990 mm per year. The climate comprises the rainy season between March to October and dry season from November to February.

Roan antelope samples were procured on a monthly basis from hunters within the catchment area of Odo Ona Kekere in Oluyole Local Government Area Ibadan in Ibadan North, Oyo, Nigeria (7°14′1" N, 3°51′9" E)from May 2018 to December 2020.

Identification of gastrointestinal parasites

Gastrointestinal contents of *Hippotragus equines* (roan antelope) were collected from the bushmeat processing sections of the market. The gut was partitioned into stomach, caecum, small and large intestines; the small and large intestines were unfolded by detaching them from the mesentery. The gastrointestinal tract (GIT) was dissected, and contents emptied in sterile dishes. The linings of each region of the GIT were scraped, washed in saline solution (9 g salt dissolved in 1 litre of water), and examined for any helminth attaching to it. A hand lens was used to examine the intestinal content of the mammals for adult parasites. Helminth parasites were recovered with a pair of forceps and fixed in 70% alcohol for parasite identification. The parasites were identified based on standard morphological characteristics Live parasite specimens collected from the dissected host guts were kept in a deionized water until parasite proboscis were everted. Afterwards, they were fixed in 70% ethanol. The specimens were stained in Mayer's acid carmine, distained in 4% hydrochloric acid in 70% ethanol, then dehydrated in serial concentrations of 70%, 80%, 90%, 90%, 100%ethanol, and cleared in 100% xylene, then in 50% Canada balsam and 50% xylene. Each step was at the interval of 24 h. Whole worms were then mounted on slide and analyzed. All measurements were recorded in micrometre. The width was measured as the maximum width, while the trunk length was measured without including the proboscis, neck, or bursa.

Using Omar *et al.* (2006) as the parasite identification manual, some of the taxonomic identification keys used include possession of proboscis which is spineless at the anterior and apical ends. The posterior end of the proboscis and conical neck exhibited depressions like sensory structures. Furthermore, the apical end of the proboscis possesses an apical epidermis cone, while the posterior of the proboscis had thin latero-dorsal and massive ventral hooks as described by Omar *et al.* (2006).

The predominant parasites identified in the host was *Strongyloides spp.* which was recruited into the study.

Determination of biochemical biomarkers

Total cholesterol was determined using enzymatic end point method described by Roeschlau *et al.* [22]. The high-density lipoprotein-associated cholesterol(LDL-C) was spectrophotometrically measured using a series of coupled reactions as described by Burstein *et al.* [5]. All reagents used in the analysis were provided as ready to use. The method of Assman *et al.* [4] was adopted in analysis of low-density lipoprotein-associated cholesterol, which is a combination of polyvinyl sulphate precipitation and enzymatic method.

Determination of protein (PRO)

The protein content of the liver and intestine of ~~16~~ uninfected and ~~49~~ infected ~~fish~~ Roan Antelopes were estimated using Biuret method as described by Umemoto [23].

Triglycerides

Triglycerides were analyzed in the mammal tissue samples using the enzymatic method described by Tietz [21].

Glucose

The glucose concentrations in the liver and intestine of the ~~16~~ uninfected and ~~49~~ infected ~~fish~~ Roan Antelopes were determined within 30 min of collection using the method of Wedermeyer and Yasutake [26].

Catalase (CAT)

Catalase (CAT) was assayed calorimetrically at 620nm and expressed as moles of hydrogen peroxide (H2O2) consumed /min/ mg protein as described by Quinlan *et al.*(1994). The reaction mixture (1.5ml) contained 1.0ml of 0.01M pH7.0 phosphate buffer, 0.1ml of Plasma and 0.4ml of 2M H2O2. The reaction was stopped by the addition of 2.0ml of dichromate-acetic acid reagent (5%potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). The specific activity of catalase was expressed as moles of reduced per minute per mg protein.

Superoxide dismutase (SOD)

Superoxide Dismutase activity in liver homogenates was determined using the procedure described by Marklund and Marklund [13]. The method is based on the ability of SOD to inhibit the autoxidation of pyrogallol. In 970µL of buffer (100 mMTris - HCl, 1mM EDTA, pH 8.2), 10µL of homogenates and 20µL pyrogallol 13mM were mixed. Assay was performed in thermostated cuvettes at 25°C and changes in absorption were recorded by a spectrophotometer (Spectronic 20D, Philips model PU 9100, manufactured in England, 2018) at 480nm. One unit of SOD activity was defined as the amount of enzyme that can inhibit the auto-oxidation of 50% the total pyrogallol in the reaction.

Glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of Ellman [7]. To the liver homogenate 10% TCA was added and centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellmans reagent in 100ml of 0.1%sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH8.0). The absorbance was read at 412nm.

Glutathione peroxidase (GPx)

Glutathione peroxidase catalyses the reduction of hydrogen peroxide and lipid peroxide into water and lipid alcohol through the oxidation of reduced glutathione (GSH) into glutathione disulphide (GSSG) (Arthur, 2000). Samples were incubated using hydrogen peroxide in the presence of glutathione for a particular time period. The amount of utilized hydrogen peroxide is then determined by directly 5, 5′- estimating GSH content using Ellman's reagent.

Malonaldehyde (MDA)

Malondialdehyde (MDA) an index of lipid peroxidation was determined by adding 1.0ml of the supernatant was added to 2ml of (1:1:1) TCA-TBA HCL reagent (thioarbituric acid 0.37%, 0.24n HCL and 15% TCA) tricarboxylic acid-thioarbituric acid-hydrochloric acid reagent boiled at 1000C for 15mins, and allowed to cool. Flocculent materials were removed by centrifuging at 3000rpm for 10mins. The supernatant was removed and the absorbance read at 532 against a blank. MDA was calculated using the molar extinction coefficient for MDATBA-complex of 1.5 $\times$ 105M/cm.

Histopathological analysis

The liver and intestine tissues were preserved in Bouin's fluid for 6 h and then decanted. Afterwards, 10% of phosphate buffered formalin was added to preserve the tissue. Random selection was made from the preserved tissues for analysis. The selected tissue was routinely dehydrated in an ascending series of alcohol of 1% at 30 min interval. The liver and intestine tissues were then embedded in molten paraffin wax and allowed to solidify. The blocked tissues were sectioned at 4-5 microns, processed and stained with haematoxylin and eosin (H&E) stains. The stained tissues were washed off in tap water. The tissues were then mounted using DPX mountant dried an examined under the binocular dissecting microscope (American Optical Corporation, Model 570) at the pathology laboratory of the department of veterinary pathology, University of Ibadan, Nigeria where the samples were taken for analysis and recording.

Table 1
Comparative concentrations of trace metals in the liver of infected and uninfected roan antelopes and parasites.

	Infected		Uninfected		Parasite		
Metals	Mean	SD	Mean	SD	Mean	SD P-value	FEPA [8]
Zn	0.460	0.367	0.434	0.336	0.045	0.056>0.05	100
Cd	0.028	0.032	0.015	0.014	0.025	0.021>0.05	<1
V	0.596	0.163	0.522	0.138	0.218	0.051>0.05	-
Ba	0.159^{B}	0.140	0.123^{B}	0.051	0.585^{A}	0.118**<0.001**	-
Ni	0.042	0.066	0.112	0.098	0.086	0.066>0.05	10
Cu	0.234	0.132	0.225	0.249	0.005	0.002>0.05	30
Co	0	0	0	0	0.004	0.008>0.05	-
Pb	0.064	0.057	0.066	0.065	0.001	0.002>0.05	2
Cr	0.020	0.007	0.025	0.003	0.011	0.006>0.05	50
Mn	0.054	0.025	0.066	0.019	0.016	0.007>0.05	1

Legends: Values with different superscripts are significantly different.

Quality control

All standards, replicates, and blanks were prepared at the same time and used immediately to prevent contamination or compromise of quality. The standards were calibrated, and the calibration curves were verified with ICV standard. The ICV standard was prepared from an independent (second source) material at or near the mid-range of the calibration curve. The acceptance criteria for the ICV standard were ±20% of its true value. The analysis data for the ICV was kept on file with the sample analysis data. The calibration curve was verified at the end of each analysis batch and after every 20 samples using continuing calibration verification (CCV) standard and a continuing calibration blank.

A certified Standard Reference Material (SRM) was prepared with each analytical batch of samples using thesame preparation method as that employed for the samples with the frequency of 1 in 20 samples per matrix. The SRM results for each analyte were validated to be within the specifications supplied by the vendor or within 75 - 125% of the true value. Samples that exceeded the linear calibration were diluted and reanalyzed to sensitive line for which quality control data was already established

All reagents used were analar grade which were permissible and standard reagent for laboratory analysis as obtained from the vendor with their certificate of analysis. Gases purchased from the gas vendor were of high purity as shown in the certificate of analysis. Standard regents, of high purity with certificate of analysis were obtained from certified manufacturers. Storage and handling of all reagents and standards are strictly in compliance with the safety precautions necessary as indicated in the MSDS of each respective reagent/standard.

All glasswares were treated with chromic acid before washed with detergent. They were then cleaned all glassware by detergent washing with hot water, and rinse with tap water, distilled water and acetone and oven-dried at 150 to 200°C for 30 min. The volumetric flask was rinsed with dichloromethane only. After drying and cooling, they were sealed and stored in a clean environment to prevent post-cleaning contamination.

Ethical permission

Ethical approval was obtained from the University of Lagos College of Medicine health research ethics committee with reference number CMUL/HREC/05/20/724.

Statistical analysis

The descriptive statistics of the trace metals, lipid profile and antioxidants of the live and intestine of the roan antelopes were subject to analysis of variance (ANOVA) using Graph pad Prism 2020 to test for the significant differences among the infected and uninfected roan antelopes with regards to the concentration of trace metals, lipid profile, and antioxidant biomarkers. The Bonferroni posthoc test was also conducted using the same software to determine the actual locations of the significant difference at the probability level of 0.05.

Results

Bioaccumulation of trace metals

There was no significant difference ($p>0.05$) between the concentration of trace metals in the infected and the uninfected roan antelopes in the forest of Odo Ona Kekere. The concentrations of Ba in the liver of the infected and uninfected roan antelopes were however higher ($p<0.001$) than the concentration in the parasites (Table 1).

The concentration of Ba in the enteric parasites of the roan antelope was outstandingly higher than the concentrations in the liver and the intestine. The concentrations of all the metals were however lower than the established limits of FEPA [8] for bush meat products.

Table 2
Comparative concentrations of trace metals in the intestine of infected and uninfected roan antelopes and parasites.

	Infected		Uninfected		Parasite		
Metals	Mean	SD	Mean	SD	Mean	SD P-value	FEPA [8]
Zn	0.094^{B}	0.061	0.079^{B}	0.033	0.045^{A}	0.056<**0.01**	100
Cd	0.012	0.019	0.022	0.006	0.025	0.021>0.05	<1
V	0.507	0.271	0.381	0.136	0.218	0.051>0.05	-
Ba	0.086^{B}	0.093	0.173^{B}	0.090	0.104^{A}	0.118<**0.01**	-
Ni	0.030	0.058	0.174	0.004	0.086	0.066>0.05	10
Cu	0.012	0.005	0.022	0.007	0.005	0.002>0.05	30
Co	0	0	0	0	0.004	0.008>0.05	-
Pb	0.020	0.053	0	0	0.001	0.002>0.05	2
Cr	0.022	0.006	0.017	0.005	0.011	0.006>0.05	50
Mn	0.089	0.059	0.066	0.043	0.016	0.007>0.05	1

Legends: Values with different superscripts are significantly different.

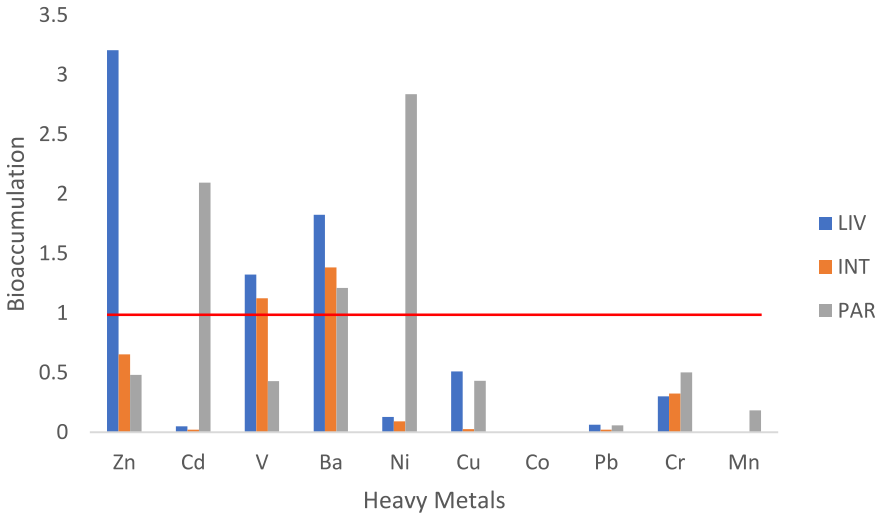

Fig. 1. Comparative bioaccumulation of trace metals in the liver, intestine and parasites of roan antelopes
Legend: Bars above 1 (horizontal line) has significant bioaccumulation.

The concentrations of Zn and Ba in the parasite were significantly higher (<0.01) than the concentrations in the intestines of both the infected and uninfected roan antelopes, both at (Table 2). There was no significant difference (p>0.05) between the concentration of trace metals in the intestine of the infected and the uninfected roan antelopes in the forest. The concentrations of trace metals in the infected, uninfected, and parasites of the roan antelopes were within the regulatory limits of FEPA [8]. The concentration of Ba in the parasite was outstandingly higher than the remaining trace metal concentrations in both the infected and uninfected roan antelopes, followed by the concentration of Zn in the parasite.

Despite the relatively low concentrations of Zn, Cd, V, Ba, and Ni, compared to the FEPA [8] regulatory limits these trace metals exhibited significant bioaccumulation rates in some of the tissues analyzed (Figure 1). The levels of bioaccumulation of Zn in the liver of the roan antelope were significant. Significant bioaccumulation of V also occurred in the liver and intestine. Bioaccumulation of Ba was significant in all the tissues analyzed, while Ni was significant in the parasite only. Significant bioaccumulations of Cd and Ni in the parasite were accompanied by extremely low bioaccumulations in the liver and intestine of the roan antelope. These instances were the only occasions with outstandingly high concentrations in the parasite coincided with extremely low concentrations in the liver and intestine of the roan antelope.

Assessment of lipid profile

As illustrated in Table 3, the level of cholesterol in the parasite was significantly higher than in the liver of the infected roan antelopes (<0.05), it was also very much higher than the level in the uninfected roan antelopes (<0.001). However, the level of triglyceride significantly much higher (p< 0.01) in the liver of the uninfected roan antelopes than the parasites, while the triglyceride observed in the liver of the infected roan antelopes was significantly very much higher (p<0.001) than the level in the parasites. There was no significant difference in the level of triglyceride between the liver of the infected and uninfected roan antelopes. The level of low-density lipoprotein detected in the parasite was significantly very much higher than the levels in the infected and uninfected roan antelopes.

Table 3
Comparative lipid profile in the liver of infected and uninfected roan antelopes and parasites.

Lipid profile	Infected		Uninfected		Parasite	
	Mean	SD	Mean	SD	Mean	SD
PRO (mg/dL)	11.1	1.755	10.3	2.307	5.467	1.345
GLU (mg/dL)	9.238	3.451	13.79	0.583	4.66	1.157
CHOL (mg/dL)	169.2^{b}	32.438	141.2^{b}	44.358	203^{a}	2.597
TRIG (mg/dL)	120.9^{a*}	23.961	111.2^{b}	20.251	71.5^{c}	4.875
HDL (mg/dL)	35.12	16.856	38.99	7.310	35.98	1.232
LDL (mg/dL)	109.9^{b}	37.652	80.02^{c}	41.442	152.7^{a}	1.921

Legends: PRO= protein, GLU= glucose, CHOL= cholesterol, TRIG= triglyceride, HDL= high-density lipoprotein, LDL= low-density lipoprotein. Values with different superscripts are significantly different ($p<0.05$).

Table 4
Comparative lipid profile in the intestine of infected and uninfected roan antelopes and the enteric parasites.

Lipid profile	Infected		Uninfected		Parasite	
	Mean	SD	Mean	SD	Mean	SD
PRO (mg/dL)	8.957	1.390	8.200	0.755	6.500	1.345
GLU (mg/dL)	4.685	1.068	4.550	0.561	5.114	1.157
CHOL (mg/dL)	171.814^{b}	27.097	158.650^{b}	12.092	204.133^{a}	2.597
TRIG (mg/dL)	54.323^{b}	31.646	76.387^{a}	29.795	73.127^{a}	4.875
HDL (mg/dL)	48.331	11.431	59.340	4.579	21.483	1.232
LDL (mg/dL)	112.617	24.311	77.780	22.618	168.023^{a}	1.921

Legends: PRO= protein, GLU= glucose, CHOL= cholesterol, TRIG= triglyceride, HDL= high-density lipoprotein, LDL= low-density lipoprotein. Values with different superscripts are significantly different ($p<0.05$).

Table 5
Comparative analysis of oxidative stress in the liver of infected and uninfected roan antelopes and the enteric parasites.

Antioxidant	Infected		Uninfected		Parasite	
	Mean	SD	Mean	SD	Mean	SD
MDA (nmol/ml)	30.521	4.838	24.903	9.105	31.677	1.792
SOD (min/mgprotein)	294.943^{b}	72.353^{b}	243.633^{b}	17.724	496.267^{a}	55.765
CAT (min/mg protein)	5.814	1.638	5.734	0.893	13.313	2.433
GSH (µmol/ml)	4.609	1.248	6.166	1.529	3.297	0.296

Legends:MDA= malondialdehyde, SOD= superoxide dismutase, CAT= catalase, GSH= reduced glutathione. Values withdifferent superscripts are significantly different ($p<0.05$).

The parasites exhibited higher levels of cholesterol and low-density lipoproteins than what was detected in the livers of the infected and uninfected roan antelopes, except in the case of triglyceride where the highest level was detected in the liver of the infected roan antelopes.

The levels of cholesterol and low-density lipoprotein in the enteric parasites of the roan antelopes was significantly very much higher ($p<0.001$) than the levels in the intestine of the infected and uninfected roan antelopes (Table 4). The levels of triglyceride in the parasite and uninfected roan antelopes were significantly much higher ($p<0.01$) than the level in the infected roan antelopes. There were no significant differences in the other lipid profile parameters analyzed.

A graphical representation of the lipid profile in the intestine and enteric parasite of the roan antelopes shows outstandingly higher levels of cholesterol and low-density lipoproteins in the parasites than the intestines of the infected and uninfected roan antelopes. The intestines of the infected and uninfected roan antelopes however exhibited higher levels of triglycerides than detected in the parasites.

Oxidative stress biomarkers

The level of SOD in the parasite was significantly very much higher ($p<0.001$) than the levels in the liver of the infected and uninfected roan antelopes (Table 5). There was no significant difference among the other oxidative stress biomarkers across the group of tissues. The relative dynamics of the markers. Among the oxidative stress markers, SOD was outstandingly upregulated and the highest level was detected in the parasites.

As illustrated in Table 6, the level of SOD in the parasite was significantly very much higher than the levels in the infected and uninfected roan antelopes ($p<0.001$). No significant differences occurred in the levels of MDA, CAT, and GSH among the uninfected roan antelopes, infected roan antelopes and their enteric parasites. The outstanding oxidative stress biomarker was SOD, with marked level observed in the parasite > infected roan antelope> uninfected roan antelope (Figure 2).

Table 6
Comparative analysis of oxidative stress in the intestine of infected and uninfected roan antelopes and the enteric parasites.

Antioxidant	Infected		Uninfected		Parasite	
	Mean	SD	Mean	SD	Mean	SD
MDA (nmol/ml)	29.614	2.593	31.093	4.232	31.677	1.792
SOD (min/mg protein)	348.371	34.590	366.900	24.076	496.267***	55.765
CAT (min/mg protein)	9.804	1.340	9.226	1.227	13.313	2.433
GSH (µmol/ml)	3.933	1.528	3.629	0.050	3.297	0.296

Legends:MDA= malondialdehyde, SOD= superoxide dismutase, CAT= catalase, GSH= reduced glutathione. Values with * are significantly higher than others ($p<0.05$), values with ** are significantly much higher than others ($p<0.01$), values with *** are significantly very much higher than others ($p<0.001$).

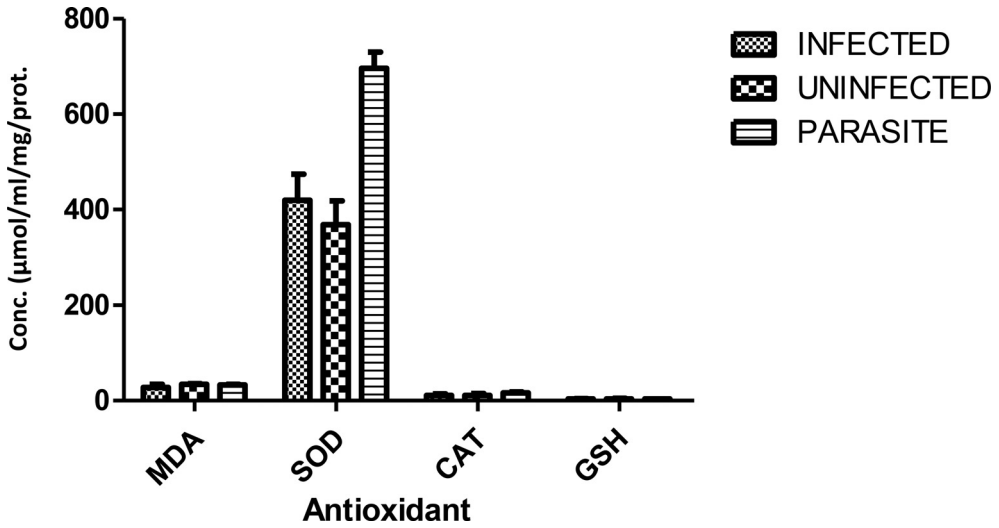

Fig. 2. The levels of oxidative stress biomarkers in the intestine of the infected and uninfected roan antelopes and the enteric parasites.

The intestinal histopathological photomicrograph of the uninfected roan antelopes with relatively low concentrations of trace metals shows no tissue alterations (Figure 3A). Photomicrograph of the infected intestines with low trace metal concentrations shows some evidence of reduction in the mucosal layer (Figure 3B), while the photomicrograph of the infected intestines with high trace metal concentrations also shows reduction in the mucosa layer but with addition tissue alteration signals such as loss of gastric glands and hemorrhagic lesion (Figure 3C). The histopathological analysis of densely infected intestines with high concentrations of trace metals shows vascular congestion and the presence of detritus in the lumen (Figure 3D). The photomicrograph of the uninfected intestines with high concentrations of trace metals shows apical blunting of focal areas and necrosis of the villous structure (Figure 3E), while the photomicrograph of uninfected intestines with low trace metal concentrations also shows apical blunting of focal areas and necrosis of the villous structure (Figure 3 F).

Figure 3A= histopathological photomicrograph of uninfected intestine and very low or no trace metals concentrations. 3**B**= photomicrograph of **infected intestines with low metal concentrations** show a reduction in the mucosa layer **3C**= photomicrograph of **infected intestine with high metal concentrations** show reduction in the mucosa layer (double arrow) with loss of gastric glands and hemorrhagic lesion (black arrow) within the mucosa **3D**= photomicrograph of **highly infected intestine with high metal concentrations** show vascular congestion (blue arrow) and presence of detritus within the lumen (slender arrow) 3**E**= photomicrograph of **uninfected intestine with high metal concentrations** show focal areas of apical blunting and necrosis (black arrow) of the villous structure 3**F**= photomicrograph of **uninfected intestine with low metal concentrations** show focal areas of apical blunting and necrosis of the villous structure (black arrow).

Discussion

The study showed that the enteric parasites of the roan antelope accumulated barium at a higher level than the host. Even though the concentrations of the trace metals in the roan antelopes or their enteric parasites were below the established regulatory limits of FEPA [8]. Barium was the only trace metal that recorded significant bioaccumulation in all environmental media analyzed. This may be an ecotoxicological concern as the concentration may exceed the acceptable limit in the near future if the rate of accumulation continues without remediation [25]. The reported health implications of barium include cardiovascular complications, kidney diseases, metabolic disruptions, neurological disorder, and cognitive impairment. These complications are however influenced by intrinsic factors such as age, race, lifestyle, dietary intake, excessive use or abuse of medications that interfere with absorbed barium in humans.

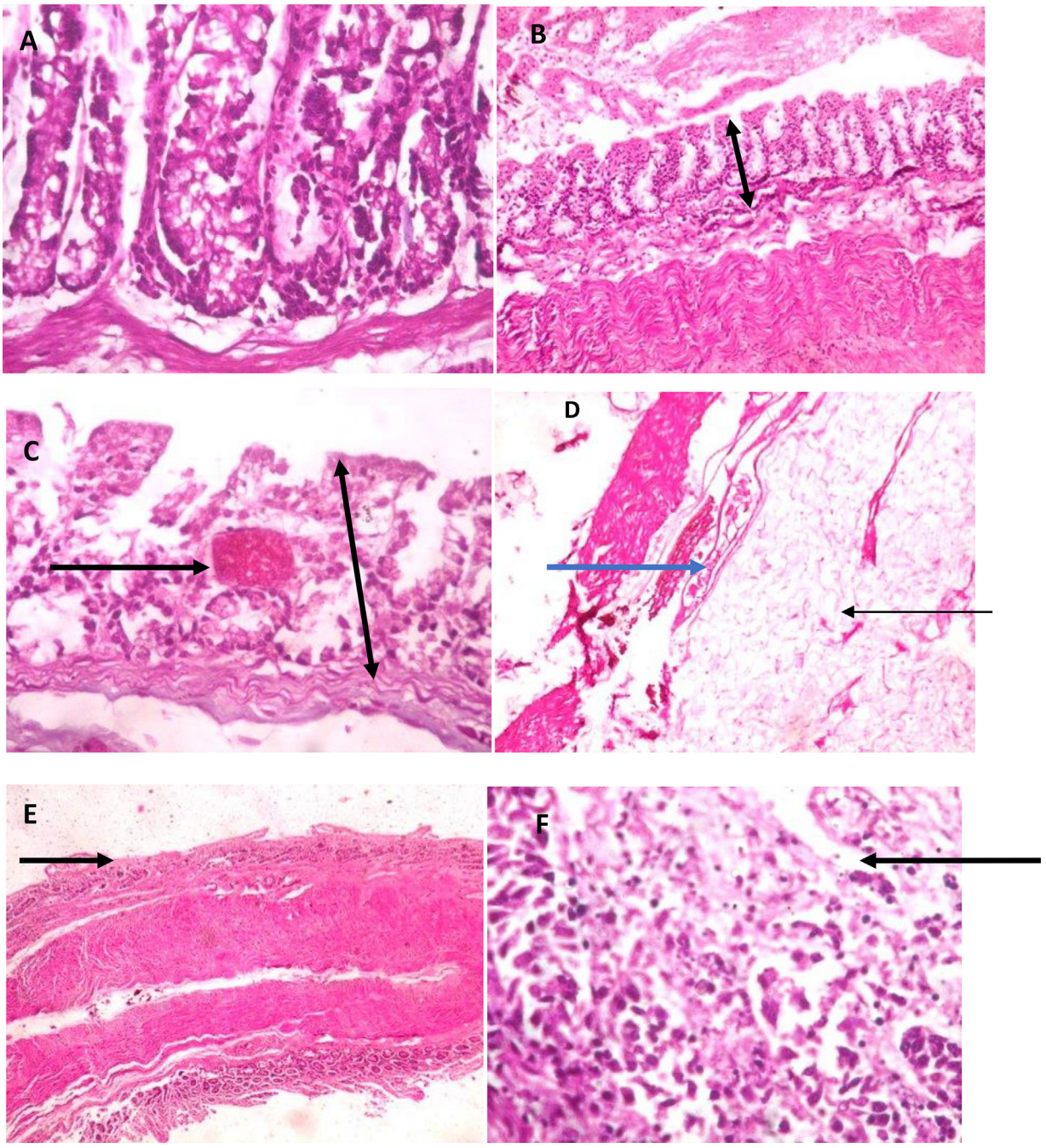

Fig. 3. Photomicrograph of the roan antelope intestine's histopathology.

The parasites showed great potentials for storage of cadmium and nickel, with the second highest bioaccumulation factors in the study (>2), after zinc with bioaccumulation factor> 3. Vanadium had significant bioaccumulation factors in the liver and intestine of the roan antelopes. The continuous bioaccumulation of zinc, vanadium and barium by the roan antelopes may threaten the wellbeing of the animals in the future by contributing to the multi-stress conditions. There is thus need for identification, evaluation, and prediction of the health effects of chronic low-level and moderate-level exposures to these metals in the roan antelopes and their consumers in the higher trophic levels, especially humans [1,16]. Hence, further research is needed to understand the bioaccumulation patterns of vanadium, barium and zinc in order to mitigate their potential health impacts in the exposed populations.

Significant bioaccumulations of Cd and Ni in the parasite were accompanied by extremely low bioaccumulations in the liver and intestine of the roan antelope. The instances where cadmium and nickel were of marked bioaccumulations the in

the parasite (*Strongyloides spp*) were accompanied by extremely low bioaccumulations in the liver and intestine of the roan antelope (*Hippotragus equines*). This implies that the enteric parasite *Strongyloides spp* might have significantly sequestered the trace metals in order to mitigate deleterious bioaccumulation of the metals by the vital organs of the roan antelope.

As technologies advance and industrialization progresses, the use of vanadium has increased, and its application has been favored by diverse industries. Due to the wide applicability of vanadium, the potential for occupational exposure to vanadium remains a concern. Similarly, there is an increased risk for environmental contamination by vanadium agents or the by-products released into the environment. The use of vanadium in sulfuric acid production results in the release of soot rich in vanadium pentoxide. Petroleum refinery, smelting, welding, and cutting of vanadium-rich steel alloy, the cleaning and repair of oil-fired boilers, and catalysis of chemical productions are other sources of increased airborne vanadium-bearing particles at far and near destinations, including forest ecosystems. Studies have demonstrated associations between exposure to airborne vanadium-bearing particles and increased risks of hypertension, dysrhythmia, systemic inflammation, hyper-coagulation, cancers, and bronchial hyper-reactivity.

From the histopathological standpoint, results imply that the tissue alterations appear to be higher with increase in trace metal concentrations in the tissues analyzed. The tissue alterations also commensurate with the intensity of the parasitic infections. These results indicate that the roan antelopes of Odo Ona Kekere may be suffering from multi-stress conditions in the forest, which may threaten the population of the animal and worse still may impact the health of the consumers negatively if the rate of bioaccumulation of trace metals are unregulated. Exposure of the roan antelopes to trace metals can be regulated through mitigation of the predominant anthropogenic activities such the application of agrochemicals and artificial fertilizers. Mitigation of poaching and illegal hunting methods; especially those that involve the use of chemical poisons.

Multi-stress conditions strongly enhance immunosuppression, which increases susceptibility to parasitic infections [11]. These infections may further be introduced to man as a new zoonotic disease. The current study conforms to the findings of Wolfe *et al*. (2005) and Abara *et al*. [1] who discovered predominant *Strongyloides* eggs in the fecal samples of roan antelopes in multi-stress conditions. The conformity of the current study with the old and recent findings point to the fact that *Strongyloides spp*. may be the foremost opportunistic parasite in the roan antelope. *Strongyloides* may therefore serve as a reliable bioindicator for proactive determination of prognostic deleterious anthropogenic perturbations. The negative impact of the multi-stress conditions was evident in this study. For example, the significantly highest concentrations of zinc and barium in the parasites than the intestines and liver of the roan antelopes may partly be implicated in the outstandingly higher cholesterol and low-lipid lipoproteins indicate dyslipidemia, which results from cellular damage due to stress. In stress conditions, some physiological reactions occur, including changes in levels of hormones and components in the blood. These events might lead to higher cholesterol levels which may result in dyslipidemia. As seen in this study, although the levels of MDA in the investigated tissues were fairly stable, the upregulated SOD in the tissues of the parasite served as an early warning signal of devastating stress level in the roan antelopes.

Suspected cellular damage in the parasites, evidenced by the high levels of cholesterol and low-lipid lipoproteins was characterized by the outstanding upregulation of SOD in the parasites above the levels detected in the liver and intestine of the roan antelopes. It is possible that the parasites may be playing protective roles on the host from the prevailing environmental stress. Oxidative damage may have occurred due to the fact that the reactive oxygen species (ROS) generated from the multi-stress overwhelmed the antioxidant defense system of the parasites. The upregulation of SOD is strongly linked to oxidative stress [3] as it is one of the foremost responsive antioxidants in the event of exposure of organisms to stressors. The antioxidant defense system releases SOD to mop-up the oxyradicals [14,24] by fostering superoxides' dismutation to H_2O_2, which are destructive to biological membranes.

Zinc had a significantly high bioaccumulation factor in the liver and may elicit metabolic complications in the roan antelopes. As an essential metal, the consequences of zinc deficiency have been recognized for many years. However, of recent the attention has been directed to the potential consequences of excessive zinc intake. Zinc is considered to be relatively nontoxic, particularly if taken orally. However, manifestations of deleterious toxicity symptoms include nausea, vomiting, epigastric pain, lethargy, and fatigue at extremely high zinc intakes [10].

Vanadium (V) has a variety of applications that make it suitable for use in ceramic production and decoration, production of pigments for a variety of products, an accelerator for drying paint, production of aniline black dye, and as a mordant in coloring textiles. Taking advantage of its hardness, resilience, ability to form alloys and its resistance to corrosion, V is also used in the production of tools, steel, machinery, and surgical implants [9]. Vanadium is employed in producing photographic developers, batteries, and semi-conductors, and in catalyst-based recycling processes. As technologies have evolved, the use of V has increased in jet aircraft and space technology, as well as in manufacture of ultraviolet filter glass to prevent radiation injury. The toxicity of vanadium depends on its physico-chemical state; particularly on its valence state and solubility. Based on acute toxicity, pentavalent NH_4VO_3 has been reported to be more than twice as toxic as trivalent VCl_3 and more than 6 times as toxic as divalent VI_2. Pentavalent V_2O_5 has been reported to be more than 5 times as toxic as trivalent V_2O_3 (Zouh*et al.,* 2105). In animals, acutely toxic oral doses cause vasoconstriction, diffuse desquamative enteritis, congestion and fatty degeneration of the liver, congestion and focal hemorrhages in the lungs and adrenal cortex.

If cadmium exposure exceeds regulatory limit in the consumers of the bushmeat due to the observed significant bioaccumulation, the unregulated exposure can lead to a variety of adverse health effects including cancer. Acute exposure to cadmium can result in flu-like symptoms such as chills, fever, and muscle pain, which can damage the lungs. However, chronic exposure as observed in the parasite can inflict toxicity on the kidney, lung, and other vital organs. Nickel is a metal

of widespread distribution in the environment. Contact with soluble and insoluble nickel compounds can cause a variety of side effects on human health. Human exposure to Ni may occur through food, water or air. Workers in Ni producing and processing industries are exposed by inhalation, and to a lesser extent, dermal contact. The nervous system is one of the main target organs for Ni toxicity; in fact, it can be accumulated in the brain. Allergy to nickel and metals is caused by the materials used in our daily life; therefore, the chances of triggering the onset of allergic reactions are high. This metal can cause an allergy that manifests as contact dermatitis, headaches, gastrointestinal and respiratory implications [15]. The synergistic deleterious impacts of cadmium and nickel on the parasites might reduce the infection intensity in the host.

Strongyloides spp may be a reliable bioindicator of the metal burden in the roan antelopes. This is a reliable early warning for proactive remediations. The enteric parasite *Strongyloides spp* may also serve as a good biosequestration tool to alleviate the toxic load of cadmium and nickel from the roan antelope. This conforms to the study of Rafia *et al.* [20] who demonstrated the metal sequestration potentials of two enteric nematodes, namely *Echinocephalus sp.* and *Ascaris sp.* in *Liza vaigiensis* fish. More recently, Akinsanya *et al.* [3] also demonstrated the biosequestration potential of enteric nematodes, *Amplicaecum africanum*on heavy metals in the toad host *Amietophrynus regularis.* Strongyloides being a member of the phylum Nematoda indicates that members of the phylum may possess unique attributes that aid absorption of heavy metals.

Conclusion

Strongyloides spp may be a reliable bioindicator of the metal burden in the roan antelopes. This is a reliable early warning for proactive remediations. The enteric parasite *Strongyloides spp* may also serve as a good biosequestration tool to alleviate the toxic load of cadmium and nickel from the roan antelope. This unique attribute is however worthy of further investigation. This study has demonstrated an empirical early warning against deleterious accumulation of vanadium, barium and zinc which might rise beyond acceptable levels in the future, thus providing prognostic data for proactive decisions by stakeholders to make pragmatic plans and policy towards sustainable conservation of the roan antelopes.

Authors contributions

AB conceptualized the work and edited the corrected manuscript and also went to the field.ET and AEX went to the field and participated in the parasitological analysis.FO also went to the field and participated in the research. OA contributed financially to the research. IPO did the statistical analysis and wrote the manuscript. AB edited and corrected the manuscript

Declaration of Competing Interest

The authors declare that there is no competing interests in this research**.**

Ethics approval and consent to participate

Ethical approval was obtained from the University of Lagos College of Medicine health research ethics committee with reference number CMUL/HREC/05/20/724.

Consent for Publication

This is not applicable to this research.

Availability of data and materials

The authors declared that all the data obtained for this research are available.

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