Elizabeth *et al.*
Bulletin of the National Research Centre (2022) 46:81
https://doi.org/10.1186/s42269-022-00762-8

Bulletin of the National Research Centre

RESEARCH

Open Access

Impacts of trace metals on *Ascaris* sp., endoparasites of greater cane rat, *Thryonomis swinderianus* (Temmincks, 1827), in the tropical rainforests of Odo Ona Kekere, Oluyole Local Government of Ibadan, Oyo State, Nigeria

Ayodele Elizabeth[1], Akinsanya Bamidele[1*], Ogunyebi Amos Lanrewaju[3], Akeredolu Excellence[1] and Isibor Patrick Omoregie[2]

Abstract

Background: Samples of hunted greater cane rat (*Thryonomis swinderianus*) were collected from Oluwo Market, Epe and Odo Ona Kekere in Oluyole Local Government Area Ibadan, Nigeria. Trace metals such as zinc, cadmium, vanadium, barium, nickel, copper, lead, cobalt, chromium, and manganese were determined in the liver, intestine, and endoparasites of *T. swinderianus* and the associated implications on the lipid profile, and antioxidant biomarkers were investigated.

Results: The study showed that the enteric parasites of the greater cane rat accumulated barium and zinc at a higher level than the host rat. This may be an ecotoxicological concern as the concentrations may exceed the acceptable limits in the near future if the rate of accumulation continues without remediation. The histopathological standpoint evidences indicate that the tissue alterations appear to be higher with increase in trace metal concentrations in tissues analyzed. The tissue alterations also commensurate with the intensity of the parasitic infections. 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 greater cane rat. Furthermore, this investigation revealed that the accumulation of barium and zinc may be implicated in the oxidative stress tendencies observed in the parasites which is an early warning for the protection of the host. At the fairly higher concentrations, toxicity of these metals characterized by the oxidative stress in the parasite may be tremendous enough to eliminate the parasite and reduce their abundance in the host rats. The deleterious impact of the multi-stress conditions in the natural habitat was evident in this study. The significantly highest concentrations of zinc and barium in the parasites than the intestines and liver of the greater cane rat may partly be implicated in the outstandingly higher cholesterol and low-lipid lipoproteins indicate dyslipidemia, which results from cellular damage due to stress. Results showed that the levels of MDA in the investigated tissues were fairly stable, the upregulated SOD in the tissues of the parasite may mitigate the parasitic infection in the host.

Conclusion: This study has demonstrated an empirical prognosis on the deleterious accumulation of barium and zinc. The results have also indicated the possibility of controlling the parasitic infection in the greater cane rat using

*Correspondence: bamidele992@gmail.com
[1] Department of Zoology, University of Lagos, Akoka, Lagos State, Nigeria
Full list of author information is available at the end of the article

the metal burden in the tissues of the rat to its advantage. The report has provided useful information by identifying the actual metals of concern and the associated early warning signals which necessitates proactive decisions toward sustainable conservation of the greater cane rat.

Keywords: Dietary intake, Infected, Uninfected, Enteric parasite, Multi-stress conditions, Bioaccumulation

Background

Greater cane rats (*Thryonomis swinderianus*) are so-called because they are herbivorous rodents that feed predominantly on sugar cane. The diet of the rat also contains tuber crops such as yam, cassava, potatoes, cocoyam, etc. The diet also comprises which range of cereals and whole grains, such as wheat, maize, millet, sorghum, varieties of beans, and most nuts. In some parts of the world, greater cane rat is rather referred to as grass cutter due to its great appetite for a wide spectrum of grasses.

Husbandry of *T. swinderianus* is economically viable and nutritionally beneficial and generally sustainable. The meat of the rat owes its revered quality to its high protein and low fat content. These are some of the bases for the high demand for its protein, which in turn is one of the leading causes of poaching. Asides the threat to biodiversity poaching poses to wildlife, it is also a leading cause of zoonoses and animal–human viral transmission which often degenerates to epidemics of great concerns and pandemics.

It is estimated that 80 million greater can rats are hunted annually in West Africa, amounting to about 300,000 metric tons of meat. This marked rate of overexploitation may have outstripped the reproduction rate of the animal, which might have also been hampered by habitat alteration. The utter perturbation of the caused by man may be linked to the dwindling supply of the cane rat meat, which is in turn implicated in the widespread of unsustainable hunting methods such as bush burning, bait poisoning, and the use of firearms and explosives, which are all rooted in the shortage or inaccessibility of the special animal protein (Owen and Dike 2012). In most part of the sub-Sahara Africa, the habitat of greater cane rat has been identified in many ecosystems, ranging from rain forests of the South to the semi-arid regions of the extreme north (Olajesu et al. 2019). Most game animals, generally referred to as bush/wild meat, 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 intrusions and perturbations may greatly impact sedentary and docile animals like the greater cane rat (Olajesu et al. 2019). Furthermore, greater cane rats also live in marshy areas and along river and lake banks (Anon 2013). Its indiscriminate herbivory makes the rat also an agricultural pest of great socio-economic concern.

In West Africa, particularly Nigeria, the rise in demand for the cane rat meat is characterized by vigorous ecologically deleterious hunting techniques, occasioned by inflation and poverty. Opara and Fagbemi (2010) pointed out that grasscutter has risen to become a leading bushmeat in the wildlife market due to the exotic quality of its meat and the acclaimed unique medical and nutritional values among other bush meat products. However, the poor hunting techniques employed by the local hunters may compromise the palatability of the meat. If the meat products from cane rats are not properly investigated and screened on the basis of fitness for consumption, pollutants from the gunpower, explosives, and other hunting chemicals used may endanger the consumers of the bushmeat. Furthermore, poor processing methods generally adopted in the sub-Sahara Africa, such as burning off the animal hair in flame fueled by various substances such as fossil fuel, fuel wood, used engine oil, waste automobile tires, and plastics may poison the consumers of bushmeat. These fueling materials contain toxic substances such as heavy metals which can contaminate the meat and render them unfit for human consumption (Okiei et al. 2009). Consequently, Mustafa (2019) investigated the concentrations of trace metals in the lung, liver, and kidney of greater cane rat. They discovered that most part of the meat had concentrations of cadmium, cobalt, and copper above the established regulatory limits. They therefore recommended mitigation of poaching of these animals for consumption. The semi-aquatic nature of the rats may be a leading factor underlying their exposure to trace metals in the environment. A number of anthropogenic activities in the wild of Odo Ona forests such as application of agrochemicals, build-up of automobile emissions on the express ways at the fringes of the forest may contaminate the land, air, and water on which the animals depend. Durojaye et al. (2014) discovered impermissible concentrations of Fe, Cu, Cd, Pd, Mn, Cr, and Zn in the skin, liver, lung, and kidney of *T. swinderianus* sampled in Omo forest reserve of Ogun State.

Logging and clearance of vegetation canopy change 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).

Other studies have shown empirical evidences that 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 implication on the edibility of the meat product. For example, burning of animal hair using fossil fuel or used engine oil which is common in the sub-Sahara Africa may render the products unsafe for consumption.

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 bush meat is met by hunting using guns, cutlasses, chase dogs, baiting with chemicals, and bush burning (Oduro and Kankam 2002; Khalafalla et al. 2011). Although some metals are essential for the adequate metabolic function of animals, they may, however, be toxic at concentrations greater than their threshold of essentiality (Isibor et al. 2020a).

Ecosystems undergo constant changes due to geometric rise in human population. The steady increase has been accompanied by commensurate rise in anthropogenic perturbations that are deleterious to health and the environment. Human interferences underlie habitat degradation and fragmentation which threaten biodiversity, especially the antelope species from its common range (East 1999; IUCN 2009).

Olajesu et al. (2019) pointed out that wild consumed animals are exposed to different parasitic infestations which can be transmitted to humans that may come into contact with them. Conversely, parasitic infection may, however, depurate the toxic burdens in animal host. 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 research have reported ectoparasites and endoparasites in wild animals in Nigeria (Ajayi et al. 2007; Opara and Fagbemi 2008, 2010; Opara 2012). As the natural barrier between wild and man is either crossed or eroded, the likelihood of zoonotic outbreak increases. However, the metal burden accumulated from the environment may either be toxic to the endoparasites or sequestered by same. Either way, the host may benefit reduced parasitic intensity or toxicant concentration, respectively. These two outcomes are not classically exclusive, i.e., they may occur simultaneously.

The docile nature of greater cane rat underlies their vulnerability to habitat degradation due to human intrusion. As docile herbivores, greater cane rat are susceptible to contamination of grazing fields by agrochemicals and artificial fertilizers which may spread through surface runoff. Roan 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% the balanced dietary requirement 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 greater cane rat in the forests of Odo Ona Kereke, 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*.

Assessing the levels of zinc, cadmium, vanadium, barium, nickel, copper, lead, cobalt, chromium, and manganese in the greater cane rat 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, and lipid profile analysis may further shed some light on the conservation status of the animals in Odo Ona Kereke forests with regard to the prevailing environmental conditions. This study therefore aimed at assessing the levels of metal accumulation in the greater cane rat and the associated impacts on the tissues and lipid profile.

Methods

Study location

Samples of hunted greater cane rat were collected from Oluwo Market, Epe, Lagos State; and Odo Ona Kekere, Oluyole Local Government Area, Ibadan, Oyo State. Oluwo Market, Epe, Lagos State lies between the coordinates 6° 35′ 3 N and 3° 59′ 43′ E. During most months of the year, there is significant rainfall in Epe, interrupted by 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 also 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, with coordinates 7°14′ 1″ N, 3° 51′ 9″ E. The sampling regime spanned through the periods of May 2018 to December 2020.

Determination of trace metals in environmental media

Collection of samples

The liver and intestine of fresh greater cane rats were excised for onward tissue digestion and analysis of trace metals. For representation of the environmental media where the metals were accumulated from, the leaves that constitute the dietary intake of the animal were collected from the natural habitat and subjected to the process of digestion and analysis of heavy metals. Surface water samples and soil samples also obtained from the natural habitat of the animal where investigations proved they were sources of drinking water and shelter, respectively, to the animals. The water and soil samples were also digested using the standard procedures and analyzed for trace metals.

Analysis of samples for trace metals

Analysis of metals in water 25 mL of the preserved water sample was measured and poured into PTFE (Polytetrafluorethylene) beaker and transferred into a fume cupboard, and 10 mL of nitric acid was added to each sample in the beakers. These mixtures were then heated on a hot plate to the lowest volume possible (10 mL). They were allowed to cool and then filtered and made up with distilled water into 50 mL volumetric flask. The digested samples were then analyzed to determine the concentrations of the selected metals with the aid of a Flame Atomic Absorption Spectrometer.

Analysis of metals in sediment Sediment samples were air-dried and sieved through 25 µm mesh. Then 1 g was weighed with the aid of a weighing balance (Model—TX3202L-V), homogenized and was transferred into a PTFE conical flask. 25 mL of ratio 3:1 hydrochloric and nitric acid (aqua regia) were added to each of the sample in a fume cupboard for digestion. It was then heated on a hot plate until the volume reduced to about 5 mL. They were filtered and made up with distilled water to 50 mL volumetric flask for the trace metal concentration analysis of zinc, cadmium, vanadium, barium, nickel, copper, lead, cobalt, chromium, and manganese, using the Flame Atomic Absorption Spectrometer (Philips model PU 9100).

Analysis of metals in liver, intestine and parasites Frozen tissues were thawed and two (2) grams wet-weight sample of liver and intestine (from both infected and uninfected fish) were weighed, and the enteric parasites were separately pulled to obtain same weight. These samples were separately placed in a beaker and digested with 25 mL of ratio 1:1 hydrogen peroxide and nitric acid. The mixture was heated to about 5 mL and allowed to cool afterward. It was then filtered and made up with distilled water to the 50 mL. Flame Atomic Absorption Spectrometer (Philips model PU 9100) was then used in analyzing the concentrations of zinc, cadmium, vanadium, barium, nickel, copper, lead, cobalt, chromium, and manganese with detection limits of 0.5 µg g^{-1}, 0.01 µg g^{-1}, 0.01 µg g^{-1}, 0.03 µg g^{-1}, 0.1 µg g^{-1}, 0.05 µg g^{-1}, 0.1 µg g^{-1}, 0.05 µg g^{-1}, 0.01 µg g^{-1}, and 0.5 µg g^{-1}, respectively. All the analytical procedures adopted were strictly in compliance with the guidelines of Whiteside (1981).

Identification of gastrointestinal parasites

Gastrointestinal contents of *T. swinderianus* were collected from the bushmeat processing sections of the market. The gut was partitioned into stomach, caecum, and 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 L 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 (Opara and Fagbemi 2008). The parasites were identified based on standard morphological characteristics and the representative images of the parasites Identification of intestinal parasites was undertaken at the pathology laboratory of the Department of Veterinary Pathology, University of Ibadan, Nigeria.

Live parasite specimens collected from the dissected host guts were kept in a deionized water until parasite proboscis were everted. Afterward, 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 ascending concentrations of ethanol (70%, 80%, 90%, 90%, 100%), 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 micrometer. The width was measured as the maximum width, while the trunk length was measured without including the proboscis, neck, or bursa.

Specimens were fixed in 70% ethanol, then placed in critical-point drying baskets and dehydrated using ethanol series of 95% and 100% for at least 10 min per soak followed by critical-point drying Lee (1992). Samples were gold coated and observed under a scanning electron microscope XL30 ESEMFEG (FEI, Hillsboro, Oregon, USA). Digital images of the structures were obtained with the aid of Olympus BH2 compound light microscope (Olympus Optical Co., Tokyo, Japan), equipped with an AmScope camera MU900 (United Scope, Irvine, California), in conjunction with digital imaging software. Detailed studies were then carried out on the parareceptacle structure by sectioning the specimens using plastic and diamond knives under the scanning electron microscope.

Using Omar et al. (2016) 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. (2016).

A coprological survey was also conducted where a total of eighty fecal samples were put in sample bottles with saline solution added to it and twenty fecal samples were collected. Aliquots were taken and examined under the light microscope to observe eggs or parasites larvae; intensity was estimated by the number of parasites per mL.

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

Determination of biochemical biomarkers

Determination of cholesterol

Total cholesterol in the liver, intestine and parasites of the rats was determined using enzymatic end point method described by Roeschlau et al. (1974).

Determination of high-density lipoprotein-associated cholesterol (HDL)

The high-density lipoprotein-associated cholesterol was spectrophotometrically measured using a series of coupled reactions as described by Burstein et al. (1980).

Low-density lipoprotein-associated cholesterol (LDL)

All reagents used in the analysis were provided as ready to use. The method of Assman et al. (1984) was adopted in analysis of low-density lipoprotein-associated cholesterol, which is a combination of polyvinyl sulfate precipitation and enzymatic method.

Determination of protein (PRO)

The protein content of the liver and intestine of 16 uninfected and 49 infected fish was estimated using Biuret method as described by Umemoto (1966).

Triglycerides

Triglycerides were analyzed in the 65 fish samples using the enzymatic method described by Tietz (1990).

Glucose

The glucose concentrations in the liver and intestine of the 16 uninfected and 49 infected fish were determined within 30 min of collection using the method of Wedermeyer and Yasutake (1977).

Catalase (CAT)

Catalase (CAT) was assayed calorimetrically at 620 nm and expressed as moles of hydrogen peroxide (H_2O_2) consumed/min mg^{-1} protein as described by Quinlan et al. (1994). The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M pH7.0 phosphate buffer, 0.1 mL of Plasma and 0.4 mL of 2 M H2O2. The reaction was stopped by the addition of 2.0 mL 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 (1974). The method is based on the ability of SOD to inhibit the autoxidation of pyrogallol. In 970 µL of buffer (100 mM Tris—HCl, 1 mM EDTA, pH 8.2), 10 µL of homogenates and 20 µL pyrogallol 13 mM were mixed. Assay was performed in thermostated cuvettes at 25 °C and changes in absorption were recorded by a spectrophotometer (Spectronic 20D) at 480 nm. SOD activity was determined estimating the amount of enzyme that inhibited the auto-oxidation of 50% the total pyrogallol in the reaction.

Reduced glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of Ellman (1959). To the liver homogenate 10% TCA was added and centrifuged. 1.0 mL of supernatant was treated with 0.5 mL of Ellmans reagent (19.8 mg of 5,5′-dithiobis nitro benzoic acid (DTNB) in 100 mL of

0.1% sodium nitrate) and 3.0 mL of phosphate buffer (0.2 M, pH8.0). The absorbance was read at 412 nm.

Malonaldehyde (MDA)

Malondialdehyde (MDA) an index of lipid peroxidation was determined by adding 1.0 mL of the supernatant was added to 2 mL of (1:1:1) TCA-TBA HCL reagent (thioarbituric acid 0.37%, 0.24 n HCL and 15% TCA) tricarboxylic acid-thioarbituric acid-hydrochloric acid reagent boiled at 1000C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. 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 1.5×105 M cm^{-1}.

Quality assurance/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 the same preparation method as that employed for the samples with the frequency of 1 in 20 samples per matrix. The SRM results for each analyte was 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. Each batch of sample analysis was run with certified reagent from same Lot/Batch with Lot number properly documented. 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.

All glassware 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 greater cane rat were subject to analysis of variance (ANOVA) using Microsoft Excel 2020 to test for the significant differences among the infected and uninfected greater cane rat with regards to the concentration of trace metals, lipid profile, and antioxidant biomarkers. The Bonferroni post hoc test was employed in determining 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 liver (Fig. 1A) and intestine (Fig. 1B) of the infected and the uninfected greater cane rat in the forest of Odo Ona Kereke. The mean concentration of Ba in the parasites was

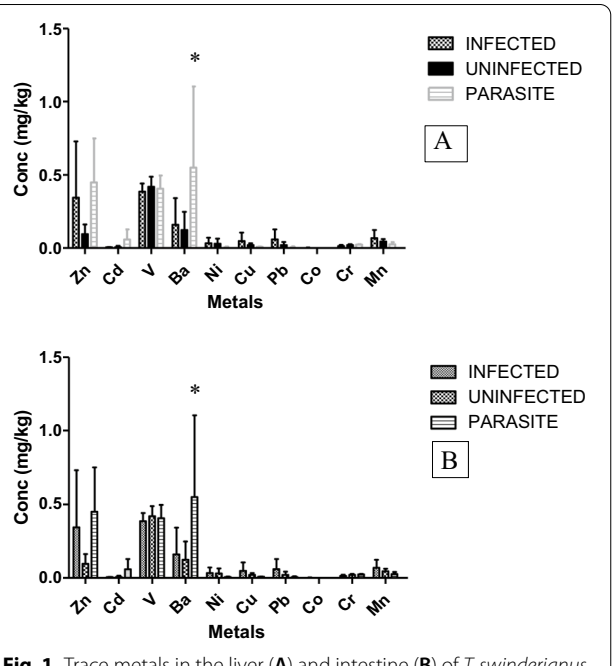

Fig. 1 Trace metals in the liver (**A**) and intestine (**B**) of *T. swinderianus*

significantly very much higher ($p > 0.001$) than the mean concentration detected in the liver (Fig. 1A) and intestine (Fig. 1B) of the uninfected rats. The concentration of Ba in the parasite was also significantly very much higher ($p < 0.001$) than the concentrations in the liver (Fig. 1A) and intestine (Fig. 1B) of the infected rats. The concentration of Zn in the parasites was significantly much higher ($p > 0.01$) than the concentrations detected in the liver (Fig. 1A) and intestine (Fig. 1B) of the uninfected rats. The concentration of other trace metals was not significantly different between the parasite and the liver and intestine of the uninfected rats. Ultimately, no significant difference occurred among the concentrations of all the trace metals between the liver and the intestines infected and the uninfected rats.

The level of low-density lipoprotein (LDL) in the parasite was significantly very much higher ($p < 0.001$) than the level in the liver (Fig. 2A) and intestine (Fig. 2B) of the uninfected *T. swinderianus* rats. The level of LDL in the parasite was significantly much higher ($p < 0.01$) than the level in the liver (Fig. 2A) and intestine (Fig. 2B). Conversely for triglyceride, the level in the parasite was also significantly much lower ($p < 0.01$) in the parasite than in the level in the liver (Fig. 2A) but very much lower ($p < 0.001$) than in the intestine (Fig. 2B) of the of the uninfected greater cane rats. Conversely, the level of triglyceride in the parasites was very much significantly lower ($p < 0.001$) than the level in the liver (Fig. 2A) and intestine (Fig. 2B) of the infected counterparts. The concentration of cholesterol in the parasite was significantly very much higher ($p < 0.001$) in the parasites than the level detected in the liver (Fig. 2A) and in the intestine (Fig. 2B) of the uninfected rats, while it was just higher ($p < 0.05$) than the concentration observed in the liver (Fig. 2A) and significantly very much higher ($p < 0.001$) than the level observed in the intestine (Fig. 2B) of the infected counterparts. No significant differences occurred in the levels of all other parameters considered for the lipid profile between the liver (Fig. 2A) and intestine (Fig. 2B) of the infected and uninfected *T. swinderianus* rats.

In the parasites, the upregulation of SOD was significantly very much higher ($p < 0.001$) in the parasites than the levels observed in the liver (Fig. 3A) and the intestines (Fig. 3B) of the uninfected rats. The level of SOD was also significantly very much ($p < 0.001$) higher in the parasite than the level observed in the liver (Fig. 3A) and the intestine (Fig. 3B) of the infected rats. Interestingly, the level of SOD in the intestine (Fig. 3B) of the infected rats was significantly higher ($P < 0.05$) than the level observed in the uninfected counterparts. No significant

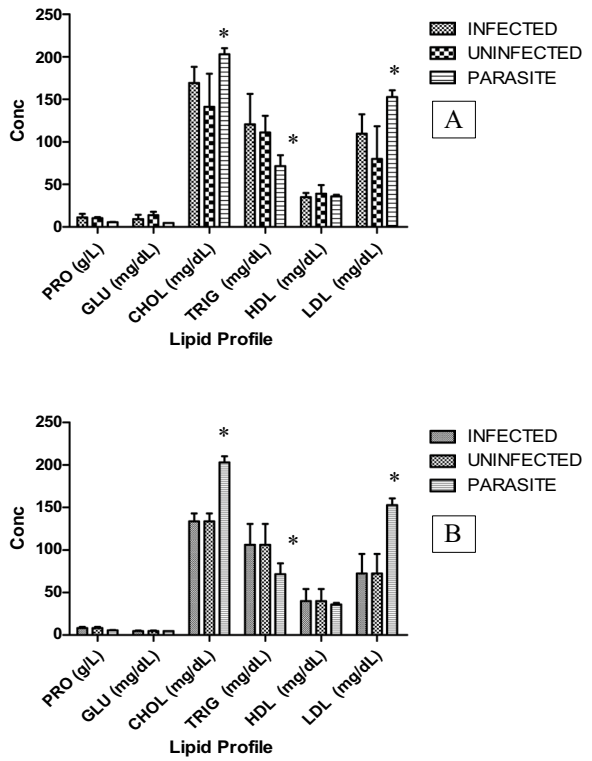

Fig. 2 Lipid profile in the liver (**A**) and intestine (**B**) of *T. swinderianus*. PRO = protein, GLU = glucose, CHOL = cholesterol, TRIG = triglycerides, HDL = high-density lipoproteins, LDL = low-density lipoproteins

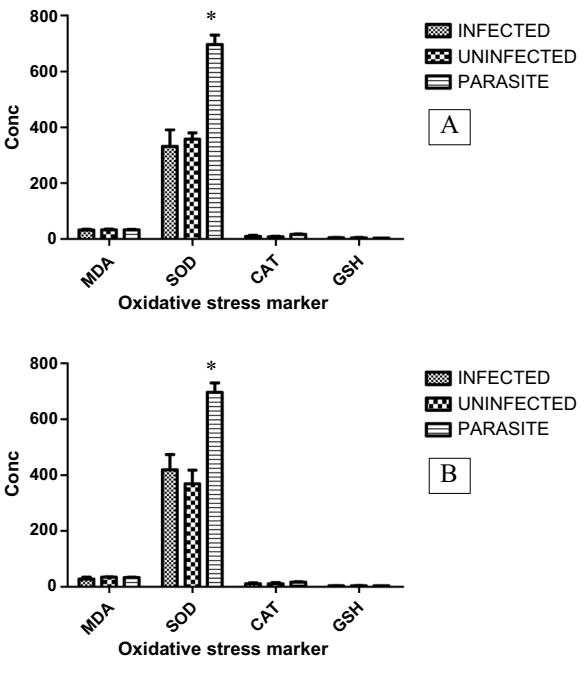

Fig. 3 Oxidative stress biomarkers in the liver (**A**) and intestine (**B**) of *T. swinderianus*

difference occurred in the other antioxidant parameters in the infected and uninfected liver (Fig. 3A) and intestine (Fig. 3B).

The order of bioaccumulation factors of the trace metals (Table 1) in the liver, intestine, and parasites of the infected and uninfected cane rats was parasites (Ba) > infected intestine (Ba) > parasite (Zn) > infected liver (Ba) > infected liver (Zn) > uninfected liver (Ba) > infected intestine (V). The highest bioaccumulation factor (8.887) was recorded in the parasites. The bioaccumulated trace metal was barium which was significantly accumulated in all the tested biological media, except the intestine of the infected cane rat, where the accumulation of the metal was close to significant. No significant bioaccumulation occurred in the remaining matches of metals with the environmental media. The intestine of the uninfected cane rats was the only biological medium where no significant bioaccumulation was recorded for any of the trace metals investigated.

Discussion

The study showed that the enteric parasites of the greater cane rat accumulated barium and zinc at a higher level than the host rat. This may be an ecotoxicological concern as the concentrations may exceed the acceptable limits in the near future if the rate of accumulation continues without remediation (Walker and Morgan 2014). 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, and excessive use or abuse of medications that interfere with absorbed barium in humans.

Table 1 Bioaccumulation factors (BAF) of trace metals from the environment in the examined biological media

Metals	Inf liv	Uninf liv	Inf int	Uninf int	Parasites
Zn	**2.401**	0.664	0.648	0.576	**3.130**
Cd	0.005	0.011	0.008	0.006	0.107
V	0.855	0.930	**1.095**	0.905	0.900
Ba	**2.574**	**1.990**	**4.687**	0.950	**8.887**
Ni	0.100	0.088	0.114	0.113	0.013
Cu	0.105	0.044	0.023	0.027	0.018
Co	0.043	0.015	0.011	0.007	0.004
Pb	0.001	0.000	0.000	0.000	0.000
Cr	0.189	0.309	0.693	0.308	0.347
Mn	*	*	*	*	*

Emboldened numbers are significant bioaccumulations, asterisks represent unavailable bioaccumulation factors

Inf liv = liver of infected rat, unif liv = liver of uninfected rat, inf int = intestine of infected rat, uninf int = intestine of uninfected rat.

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 recorded only in the liver and intestine of the greater cane rat is noteworthy. The continuous bioaccumulation of zinc, vanadium, and barium by the greater cane rat may threaten the wellbeing of the animals in the future contributing to the multi-stress conditions; hence, there is need for identification, evaluation, and prediction of the health effects of chronic low-level and moderate-level exposures to these metal in the greater cane rat and their consumers in the higher trophic levels, especially humans (Olajesu et al. 2019; Abara et al. 2021). 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.

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. 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 tissues analyzed. The tissue alterations also commensurate with the intensity of the parasitic infections. These results indicate that the greater cane rat of Odo Ona Kereke 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 greater cane rat 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 (Isibor et al. 2020b). 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. (2021) who discovered predominant *Strongyloides* eggs in the fecal samples of greater cane

rat 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 greater cane rat 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 greater cane rat.

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 greater cane rat. 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 (Akinsanya et al. 2019) 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 (Vijayavel et al. 2004; Nabi et al. 2017) 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 greater cane rat. 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 (Fosmire, 1990).

Vanadium also exhibited significant bioaccumulation factor in the intestine of the infected rats. Vanadium (V) has a wide range of diverse applications that makes it readily detectable in the environment (Gimba and Dawam 2015). 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 Bi et al. 2015). 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 to can lead to a variety of adverse health effects including cancer. Acute exposure, i.e., high levels over a short period of time, to cadmium can result in flu-like symptoms such as chills, fever, and muscle pain, which can damage the lungs. However, chronic exposure, i.e., low level over an extended period of time as observed in the parasite, can inflict toxicity on the kidney, lung, and other vital organs. The current study conforms appreciably to the observations of Mustafa (2019) who discovered high concentrations of copper, chromium, cadmium, and cobalt exceeding above the established/permissible regulatory limits. Durojaye et al. (2014) also discovered impermissible concentrations of Fe, Cu, Cd, Pd, Mn, Cr, and Zn in the skin, liver, lung, and kidney of *Thryonomys swinderianus* sampled in Omo forest reserve of Ogun State. In the current study, although the concentrations of all trace metals investigated were below the stringent regulatory limits of established and certified bodies around the world, barium, vanadium and zinc may be metals of future concerns due to their attendant bioaccumulation in the grasscutter. The observed resilience of the parasite *Strongiloides* spp. characterized by the marked accumulation of zinc and barium may be of great importance in the sequestration of the metal burden in the host. The tendency of appreciable bioaccumulation of vanadium also qualifies the parasite as a candidate for further investigation.

Greater cane rat's notable hardiness and indiscriminate herbivory further qualify them as candidate for domestication. Husbandry of greater cane rat is a viable strategy to bridge the gap in the availability of the meat products for human satisfaction. Growth and development of greater cane rat farming is a promising approach to meet the ever-growing demands for bushmeat and may also limit the transmission of diseases from wildlife to man which often occurs in the process of exploration for bushmeat, natural resources and other gaming activities.

This study revealed that the accumulation of barium and zinc may be implicated in the oxidative stress tendencies observed in the parasites which is an early warning for the protection of the host. At the fairly higher concentrations, toxicity of these metals, characterized by the oxidative stress in the parasite may be tremendous enough to eliminate the parasite and reduce their abundance in the host rats.

Conclusions

This study has demonstrated an empirical prognosis on the deleterious accumulation of barium and zinc. The results have also indicated the possibility of controlling the parasitic infection in the greater cane rat using the metal burden in the tissues of the rat to its advantage. This report is useful for proactive decisions by stakeholders to make pragmatic plans and policy toward sustainable conservation of the greater cane rat.

Abbreviations

SOD: Superoxide dismutase; MDA: Malondialdehyde; GIT: Gastrointestinal tracts; CAT: Catalase.

Acknowledgements

The authors thanked the driver, Mr Idris for his contributions. This research was done by us at the University of Lagos, Department of Zoology, Parasitology Unit.

Authors' contributions

AB conceptualized the work and edited the corrected manuscript and also went to the field. AE and AEX went to the field and participated in the parasitological analysis. OA contributed financially to the research. IP did the statistical analysis and wrote the manuscript. AB edited and corrected the manuscript. All authors have read and approved the final manuscript.

Funding

No funding received for this research.

Availability of data and materials

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

Declarations

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 since the mammals were purchased at Oluwo market from the hunters from different states after the ethical approval has been obtained.

Competing of interests

The authors declare that they have no competing interests.

Author details

[1]Department of Zoology, University of Lagos, Akoka, Lagos State, Nigeria. [2]Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria. [3]Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos State, Nigeria.

Received: 31 December 2021 Accepted: 11 March 2022
Published online: 28 March 2022

References

Abara PN, Adjeroh LA, Nwachukwu MO, Osinomumu ID (2021) Preliminary survey of the intestinal helminths of greater cane rat and antelope (bush meat) in Omagwa Rivers State. IAA J Appl Sci 7(1):49–56

Ajayi OO, Ogwurike BA, Ajayi JA, Ogo NI, Oluwadare AT (2007) Helminth parasites of rodents caught around human habitats in Jos, Plateau State, Nigeria. Int J Nat Appl Sci 4(1):8–13

Akan JC, Abdulrahman FI, Sodipo OA, Chiroma YA (2010) Distribution of Heavy Metals in the Liver, Kidney and Meat of Beef, Mutton, Caprine and Chicken from Kasuwan Shanu Market in Maiduguri Metropolis, Borno State. Res J Appl Sci Eng Technol. 2(8):743–748

Akinsanya B, Isibor PO, Kuton MP, Saliu JK, Dada EO (2019) Aspidogastrea africanus Infections, comparative assessment of BTEX and heavy metals bioaccumulation, and histopathological alterations as biomarker response in *Chrysichthyes nigrodigitatus* (Lacépède, 1803) of Lekki Lagoon, Nigeria. Sci Afr 3(2019):1–10

Anon (2013) Cane rat. Wikipedia. the free encyclopedia. Pg1.30/10/13. They live in marshy areas and along river and lake banks and are herbivores, feeding on aquatic grasses in the wild, (Anon, 2013c).

Assman G, Jabs H, Kohnert U, Nolte W, Schriewer H (1984) LDLcholesterol determination in blood serum following precipitation of LDL with polyvinyl sulphate. J Anal Chim Acta 140:77–83

Burstein M, Scholnick HR, Morfin R (1980) Rapid method for the isolation of lipoproteins from serum by precipitation with polyanions. Scand J Clin Lab Invest 40:583–595

Dural DA, Bakare OK, Ayodele IA (2007) Trade in the wild mammalian Species for Traditional medicine in Ogun State. Nigeria Global. J Med Res 12(3):6–21

Durojaye AS, Olufemi MA, Rasheed YO, Ibukun AA (2014) Bioaccumulation of heavy metals in cane rate (*Thryonomis swinderianus*) in Ogun State, Nigeria. J Toxicol Environ Health Sci 6:154–160

East R (1999) African antelope database 1998. IUNC/SSC Antelope Specialist Group Publication

Ellman GL (1959) Tissue sulphydryl groups. Arch Physiol Biochem 82:70–77

Fonweban JN, Njwe RM (1990) Feed Utilization and life weight gain by African giant rat (*Crisitomys gambianus*, Water House) at Dschana in Cameroon. Tropicult. 8:118–120

Fosmire GJ (1990) Zinc toxicity. Am J Clin Nutr 51(2):225–227. https://doi.org/10.1093/ajcn/51.2.225

Gimba UN, Dawam NN (2015) Epidemiological status of intestinal parasitic infection rates in children attending Gwagwalada Township Clinic, FCT-Abuja, Nigeria. Am J Res Commun 3(2):97–110

Harlia E, Balia RL (2010) The Food Safety of Livestock Products (Meatball, Corned Beef, Beef Burger and Sausage) Studied from Heavy Metal Residues Contamination. Anim Prod 12(1):50–54

Hunt WG, Watson RT, Oaks LJ, Parish CN, Burnham KK, Tucker RL, Belthoff JR, Hart G (2009) Lead Bullet Fragments in Venison from Rifle-Killed Deer: Potential for Human Dietary Exposure. PLoS ONE 4(4):e5330–e5330

Isibor PO, Tunde TOT, Enuneku AA, Akinduti PA, Dedeke GA, Adagunodo TA, Obafemi YD (2020a) Principal components and hieratchical analyses of trace metals and total hydrocarbons in gils, intestines, and muscles of *Clarias gariepinus* (Burchell, 1822). Sci Rep 10:5150

Isibor PO, Akinsanya B, Soyinka O, Kuton MP, Obe A, Saliu JK (2020b) *Raphidascaroides brasiliensis* (Nematoda: Anisakidae) infection and bioaccumulation of polycyclic aromatic hydrocarbons in *Gymnarchus niloticus* (Cuvier, 1829) in Lekki Lagoon, Nigeria. Egypt J Aquat Biol Fish 24(1):99–118

IUCN (2009) IUCN red list of threatened species. Version 2009.1. IUCN

Lee RE (1992) Scanning electron microscopy and X-ray microanalysis. Prentice Hall, p 458

Khalafalla FA, Ali FH, Schwagele F, Abd-El- Wahab MA (2011) Heavy metal residues in beef carcasses in Beni-Seuf abattoir, Egypt. Veter Ital 47(3):351–361

Marklund S, Marklund G (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469–474

Mustafa MO (2019) Comparative assessment of heavy metal content in organs and flesh of marketed cane rat (*Thryonomys swinderianus Temminck*, 1827) along five highways in South-Wstern Nigeria. J Res for Wildl Environ 11(3):26–34
Nabi S, Tanveer S, Ganie SA (2017) Glutathione-S-transferase, superoxide dismutase (GST, SOD) levels, protein content and lipid peroxidation in *Schizothorax plagiostomus* under the infection of Pomphorhynchus in Nallah Sukhnag of Kashmir valley. Pak J Biol Sci 20(9):442–446
Oduro W, Kankam, B. (2002) Environmental and public health hazards of traditional grasscutter (*Thryonomys Swinderianus*) hunting. In: Weidinger KA (ed) proceedings of a workshop on promoting Grasscutter production for poverty reduction in Ghana, pp 19–22.
Okiei W, Ogunlesi M, Alabi F, Osiughwu B, Sojinrin M (2009) Determination of toxic metal concentrations in flame –treated meat products, ponmo. Afr J Biochem Res 3(10):332–339
Olajesu SO, Akinyemi AF, Lateef F, Lameed GA (2019) Population density, diversity and abundance of antelope species in Kainji Lake National Park, Nigeria. Open J Ecol 2019(9):107–116
Omar MA, Evans RP, Magloire B, Heckmann R (2016) Morphological and molecular description of *Tenuisentis niloticus* (Meyer, 1932) (Acanthocephala: Tenuisentidae) from *Heterotis niloticus* (Cuvier) (Actinopterygii: Arapaimidae), in Burkina Faso, with emendation of the family diagnosis and notes on new features, cryptic genetic diversity and histopathology. Syst Parasitol 93:173–191. https://doi.org/10.1007/s11230-015-9615-7
Opara MN (2012) Zoonotic role of the greater cane rat, Zoonosis Dr. Jacob Lorenzo-Morales (Ed.). InTech. http://www.intechopen.com/books/zoonosis/zoonotic-role-of-theGreatercanerat
Opara MN, Fagbemi BO (2008) Observations on the gastrointestinal helminth parasites of the wild greater cane rat (*Thryonomysswinderianus*, Temminck) in Imo State, Nigeria Int. J Trop Agric Food Syst 2(1):105–110
Opara MN, Fagbemi BO (2010) Patho-physiological effects of experimental *Trypanosoma congolense* and *Trypanosoma vivax* infections in the Grasscutter (*Thryonomys swinderianus*, Temminck). Nat Sci 8(10):87–101
Owen OJ, Dike VA (2012) Grasscutter (Thryonomys swinderianus) Husbandry in Nigeria. A Review of the potentialities, opportunities and challenges. Department of Animal science, Rivers State University of Science and Technology, Port Harcourt, Nigeria. J Environ Issues Agric Dev Ctries 4(1).
Roeschlau P, Bernt E, Gruber JW (1974) Enzymatic procedure for cholesterol determination. J Clin Chem Clin Biochem 12:403
Tietz NW (1990) Colorimetric method of triglyceride estimation. In: Tietz NW, Finley PR (eds) Clinical guide to laboratory tests, 2nd edn. W.B. Saunders Company, pp 554–556
Umemoto S (1966) A modified method for estimation of fish muscle protein by Biuret method. Bull Jpn Soc Sci Fish 32:427–435
Vijayavel KRD, Gomathi K, Durgabhavani MP, Balasubramanian, (2004) Sublethal effects of naphthalene on lipid peroxidation and antioxidant status in the edible marine crab *Scylla serrata*. Mar Pollut Bull 48:429–433
Walker JG, Morgan ER (2014) Generalists at the interface: nematode transmission between wild and domestic ungulates. Int J Parasitol Parasites Wildl 3:242–250
Wedermeyer HA, Yasutake WT (1977) Clinical methods for the assessment of the effects of environmental stress on fish health. Tech Pap US Fish Wildl Serv 89:1–20
Wolfe ND, Daszak P, Kilpatrick A, Burke DS (2005) Bush meat hunting, deforestation, and prediction of zoonotic disease. Emerg infect Dis 11:1822–1827
Zouh Bi ZF, Touré A, Oka Komoin C, Karamoko Y, Fantodji A (2015) Prevalence of ticks infesting Greater cane rats (*Thryonomys swinderianus* Temminck, 1827) in the south of Côte d'Ivoire. J Appl Biosci 87:8085–8093

Publisher's Note