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Research Article

Preliminary Study on the Helminth Parasites of *Rattus rattus* sp (Rodentia) – M9 (nomen nodum) and *Arion hortensis* (Pulmonata) and Heavy Metals in their Muscle Tissues in a Terrestrial Ecosystem

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ABSTRACT
The *Rattus rattus* sp – M9 (*nomen nodum*) and *Arion hortensis* have been primary sources of protein to humans. The fact that their close association to humans may pose collateral risks, there is need to evaluate their bio-indicator potentials and their associating helminth parasites. In order to assess the heavy metals contents in the muscles these two animals were selected based on being a vertebrate and invertebrate, land and aerial dwelling, as well as hairy protective skin and soft, membranous skin respectively in order to appreciably conduct good assessment of the environment to breach the gap in knowledge. They were collected by digging of burrows and hand picking with average body lengths (cm), *R. rattus* sp 12 (16.13±2.49) and *A. hortensis* 8 (3.99±1.53) respectively. Due to difficulty in their collection not quite many samples were collected, although enough for preliminary investigation. Parasitological analysis using sedimentation method was used to examine their intestines, body fluids, and internal organs. Out of this number, 4(0.333) *R. rattus* sp. were infected with the eggs of *Trichuris muris 2* (0.167), *Ancylostoma duodenale* 2(0.167) and *Strongyloidus* sp 1(0.083) and *Ascaris suum* 1(0.083) with mixed infection of *T. muris*, *Strongyloidus* sp and *Ancylostoma duodenale* whereas 2 (0.25) samples of *A. hortensis* were infected with both adult *Phasmarhabditis hermaphrodita* and their eggs. Also, muscle tissues analysis for the presence of heavy metals (AAS) mg/kg, indicates no Hg while other heavy metals indicate safe range limits.

Keywords: *Arion hortensis;* Heavy metals; Helminthes parasites; *Rattus rattus* sp – M9*;* Terrestrial ecosystem

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INTRODUCTION

Under *Rattus* sub species, many have not been morphologically and molecularly studied. Nevertheless, DNA Barcoding of some West African mammals could not separate morphologically distinct for instance all white fur *Rattus rattus norvegicus* (JF457097) and burrowing ventrally restricted white fur and dorsal ash coloured fur *Rattus rattus* sp – M9, with high boot straps and clade separation from *Rattus leucopus* EF186566, *Rattus sordidus* EF 186593 and *Rattus nitidus* HM 217492 (Echi *et al*., 2013).

Rattus rattus sp – M9, inhabits narrow holes in mainly soils where agricultural activities are high due to abundant food materials they forage on such as vegetations, tubers, inter alia where they play important roles in sustenance of the ecosystem through food chain (Echi *et al*., 2013).

A. hortensis break down dead organic matter, releasing nutrients back into the soil (Bagyaraj *et al*., 2016). In this manner, they aid in maintaining soil health and promoting ecosystem productivity. They are herbivores and primarily feed on plant material, including leaves, stems, and flowers.

While some species can be considered pests in agriculture, they also serve as a vital food source for many other organisms, including birds, amphibians, and insects (South, 2012). Their feeding habits influence plant distribution and abundance in ecosystems (Glen *et al.,* 2000). *Arion hortensis* have been recognized as potential bioindicators for environmental quality. Their sensitivity to environmental conditions and contaminants, such as heavy metals, makes them useful tools for monitoring ecological health (Bhaduri *et al*., 2022). Changes in *Arion hortensis* populations and behaviours can signal shifts in environmental conditions. Certain *Arion hortensis* species have been used as model organisms in ecological and evolutionary research. Their relatively simple biology and responsiveness to environmental changes make them valuable for studying ecological dynamics, predator-prey interactions, and evolutionary processes (Wisz *et al.*, 2013).

Arion hortensis, despite their inconspicuous appearance, are ecologically significant organisms that contribute to nutrient cycling, plant interactions, and serve as potential bioindicators in ecosystems. Their role in terrestrial environments underscores the importance of studying their taxonomy, diversity, and ecological functions.

Anthropogenic sources of heavy metal contamination include agricultural activities, such as pesticide and herbicide application, contaminated irrigation water; municipal waste used for fertilization of crops, mineral fertilizer containing traces of heavy metals and direct waste disposal on farmland (Merian *et al*., 2004).

MATERIALS AND METHODS

Study Area

These were collected from various parts of Ikwoka, Southeast Nigeria latitude 6° 49' 2" North and longitude 7° 20' 48" East from January to October 2023. Ikwoka is in the humid tropical climatic region and is characterized by two distinct wet and dry seasons (figure 1). The soil is sandy with the potential to severe erosion. Erosion menace remains serious environmental concerns of Southeastern part of Nigeria. Therefore, burrowing animals generally excavate soft soil particles easier, faster than harder, much compact soil particles.

Sample Collection: *Rattus rattus* sp – M9 were collected by digging of the burrows and hand picking of *Arion hortensis.* They were immersed in alcohol and transported to ZEB, MOUAU laboratory where they were measured to the nearest centimeters and other further analysis.

Sample Analysis: In every case, freshly caught samples were examined for parasites and the procedure followed partly modified details in Arthur and Albert (1994). Thereafter, the contents of the intestines were transferred into a beaker and 8ml of 10% formalin was added. The beaker was shaken vigorously and the suspension was sieved into another beaker and later transferred into a centrifuge tube. The filtrate was then added 3ml of ethyl acetate and was immediately shaken vigorously for 1min. The test tube which was capped was centrifuged using 3000rpm for 1 minute. Thereafter the debris formed was loosened using applicator stick and the tube was inverted to discard supernatant fluid. The tube was now returned to its original upright positions and the fluid from the slide was allowed to drain to the bottom. The bottom of the tube was tapped to re-suspend and mix the sediment. The sediment was then transferred to a slide covered with a cover glass and examined microscopically using 10X objective lens with condenser iris covered sufficiently. The 40X objective lens was also used to examine parasites seen (Cheesbrough,1999). After parasitic examinations, the samples were returned to the vials containing ethanol prior to heavy metals analysis of their muscle tissues. The samples were burnt to ashes in a muffle furnace, after which 1g of it was placed in a beaker 10ml Aqua Regia: (Measure 75ml of Conc. HCl and 25ml conc. HNO_3 into 100ml Volumetric Flask, 3:1 was added. Then, it was stirred to dissolve completely using a glass stirring rod. The solution was then cooled and filtered into a 100ml flask and further diluted to mark with distilled water (Radojevic and Bashkin, 2004), while Atomic Absorption Spectrophotometer (AAS) mg/kg was used to read off the metal level at a particular wavelength (Lenntech, 2012).

Figure 1: Google Map showing the study area and the neighbouring communities

RESULT

The morphometric measurements indicate the *R. rattus* samples had measurements as follows: a body length of 7.94±2.75 cm, a body width of 4.57±2.05 cm, a total length (body and tail) of 15.13±5.87 cm, a head length of 2.96±0.82 cm, a body weight of 16.72±15.23 g, a tail length of 7.19±3.31 cm, a forelimb length of 1.96±0.51 cm, a hind limb length of 3.16±1.16 cm, and a head width of 3.29±0.79 cm. The morphometric measurements of the *Arion hortensis* showed body length (cm) of 3.99±1.53, a body weight (cm) of 1.5±0.54, Length of groove (cm) of 2.79±0.92, Width of groove (cm) of 0.45±0.12, and Weight (g) of 1.32±0.12.

Parasitological analysis indicates: 4(0.333) *R. rattus* sp. were infected with the eggs of *Trichuris muris 2* (0.167), *Ancylostoma duodenale* 2(0.167) and *Strongyloidus* sp 1(0.083) and *Ascaris suum* 1(0.083) with mixed infection of *T. muris*, *Strongyloidus* sp and *Ancylostoma duodenale* (Plates 1 - 3). In *A. hortensis, Phasmarhabditis hermaphrodita* was observed (Plates 4 and 5).

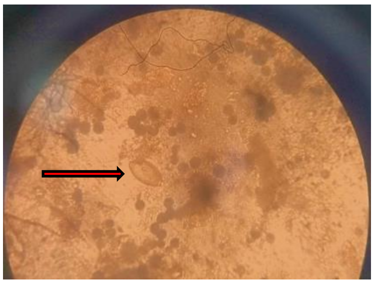
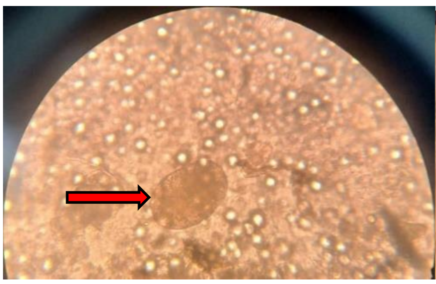


Plate 1: Egg of *Trichuris muris* **Plate 2: Egg of *Ancylostoma duodenale*** **Plate 3: Egg of *Ascaris suum***

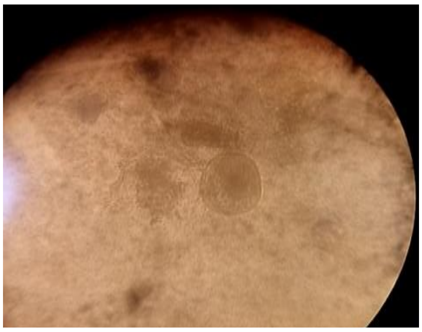


Figure 4 and 5: Showing the egg and adult *Phasmarhabditis hermaphrodita*

Three elements of heavy metals examined: Chromium (Cr), Lead (Pb) and Mercury (Hg) indicate significant variations in heavy metal content among the specimens. Chromium had the highest concentration in the samples. Rat 8 exhibited the highest chromium content, reaching 3.41 mg/kg, which significantly exceeded the WHO standard level of 1 mg/kg.

Lead concentrations were found in all samples, with Rat 8 showing the highest lead content at 0.94 mg/kg. This level exceeded the WHO standard level of 0.3 mg/kg. Elevated lead levels can have adverse health effects on both wildlife and, potentially, humans through bio-accumulation in the ecosystem.

Mercury was not detected (ND) in any of the analyzed samples, indicating specimens did not exhibit mercury contamination at the time of analysis. The heavy metals concentration in some *Arion hortensis* constitute: Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Chromium (Cr), Cadmium (Cd), Mercury (Hg), Lead (Pb), and Manganese (Mg).

These results highlighted magnesium (Mg) as the predominant heavy metal, with values of 0.82 mg/kg, 0.77 mg/kg, and 0.32 mg/kg for 1, 2, and 3 samples respectively. Additionally, iron (Fe) emerged as another noteworthy heavy metal, exhibiting values of 0.32 mg/kg, 0.28 mg/kg, and 0.14 mg/kg in 1, 2, and 3 respectively (Figure 2).

Furthermore, the increase in heavy metal concentrations in the *A. hortensis* followed a distinctive pattern. In Slug 1, the hierarchy of heavy metals in ascending order was Pb > Cd > Cr > Cu > Mn > Zn > Fe > Mg, characterized by values of 0.01 > 0.01 > 0.02 > 0.03 > 0.08 > 0.11 > 0.32 > 0.82 mg/kg, respectively. Notably, this order was consistently observed in Slug 2: Pb > Cd > Cr > Cu > Mn > Zn > Fe > Mg, featuring values of 0.01 > 0.01 > 0.02 > 0.03 > 0.06 > 0.08 > 0.28 >0.77 mg/kg, respectively. Slug 3's heavy metal concentration also adhered to the same ascending order: Pb > Cd > Cr > Cu > Mn > Zn > Fe > Mg, with values of 0.01 > 0.01 > 0.02 > 0.04 > 0.06 > 0.14 > 0.32 mg/kg, respectively.

It is important to note that the values obtained for all the tested heavy metals in Slugs samples did not exceed the World Health Organization (WHO) standard levels, indicating that the heavy metal concentrations in these Slug are within safe limits.

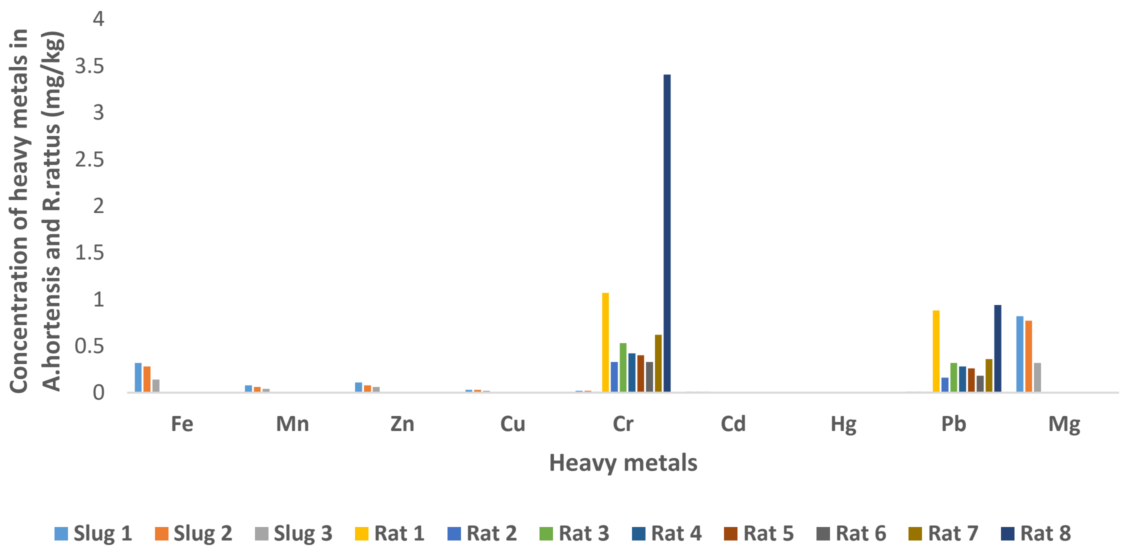

Fig 2: A bar chart showing the Heavy metal content of both *Arion hortensis* and *Rattus rattus*

DISCUSSION

The presence of *Phasmarhabditis hermaphrodita* in some Slug has potential ecological implications. These nematodes are known to affect Slug behavior, reducing their feeding activity and potentially causing death. Such behavioral changes can influence the distribution of plant species, impacting the entire ecosystem (Pieterse *et al*., 2017). The absence of parasites in certain Slug may indicate variations in susceptibility, possibly due to genetic differences or environmental factors. This highlights the complexity of parasitic interactions and their impact on individual host organisms (Antzée-Hyllseth *et al*., 2020).

The observed pattern of increasing heavy metal concentrations in the Slug samples is noteworthy. This suggests that certain heavy metals may accumulate in Slug more than others, possibly due to variations in their exposure, uptake, and metabolic processes. The consistent ascending order of heavy metals (Pb > Cd > Cr > Cu > Mn > Zn > Fe > Mg) in all three Slug, with specific concentration values, implies some degree of uniformity in the heavy metal uptake and storage mechanisms across different Slug populations.

The presence of heavy metals in Slug has broader environmental significance. Slug, being bioindicators, can reflect heavy metal contamination in their habitats. This makes them important for assessing ecosystem health and environmental management practices (Bhaduri *et al*., 2022; Jasim, 2015).

The identification of *Trichuris muris* (Tm) in the examined *Rattus rattus* samples is significant, reaffirming the importance of these parasites in the context of rodent ecology. *Trichuris muris* is well-established as a common endoparasite of rodents, and its presence in *Rattus rattus* carries implications for both the health of the host and broader ecological dynamics. *Trichuris muris* is known to influence the health, behaviour, and fitness of its rodent hosts. These parasites establish in the caecum and colon, causing infections that may lead to various physiological effects, including inflammation and alterations in nutrient absorption (Ranjbar*et al*., 2017). The recognition of *T. muris* in *Rattus rattus* samples underscores the potential impact on the overall health of these rodents. Parasites, including *T. muris,* can influence the behaviour of their hosts, potentially altering feeding habits, activity patterns, and even reproductive strategies (Grandón-Ojeda*et al*., 2022). These behavioural changes can have cascading effects on the fitness of individual rodents and, consequently, on population dynamics. Research by Ranjbar *et al*. (2017) has highlighted the broader ecological implications of endoparasite infections in rodent populations.

The identification of *Ancylostoma duodenale* in *Rattus rattus* is of significance, given the potential implications for both rodent and human health. *Ancylostoma duodenale,* commonly known as the human hookworm, is a gastrointestinal nematode that typically infects humans but has been observed to infect a variety of hosts, including rodents. The zoonotic implications of *Ancylostoma duodenale* in *Rattus rattus* in the present study shows potential for zoonosis. Populations, such as the human population, which is in close proximity to perhaps potential rodents infected by these nematodes, would be affected (Stracke *et al*., 2020). The adaptability of *Ancylostoma duodenale* to a diverse range of hosts, as evidenced by its presence in rodents, raises concerns about the potential for zoonotic transmission. This adaptability is highlighted in studies emphasizing the ability of hookworms to exploit various hosts, underlining their potential role in the transmission of parasitic infections within ecosystems (Abuzeid *et al*., 2020). The coexistence of *Rattus rattus* with human populations in certain regions creates a scenario where the presence of *Ancylostoma duodenale* in rodents could contribute to the transmission dynamics of zoonotic diseases. Understanding the ecology of these hookworms in wildlife, including rodents, is crucial for assessing the risk of cross-species transmission and potential outbreaks in human populations. Present finding emphasizes the interconnectedness of wildlife and human health, highlighting the need for integrated approaches in public health and wildlife management. Surveillance and control measure targeting zoonotic parasites in both rodent and human populations become essential in regions where these interactions are prevalent, contributing to the overall prevention of parasitic infections and safeguarding public health.

The identification of *Ascaris suum* in *Rattus rattus* is noteworthy, highlighting the adaptability of certain parasites to a range of hosts. *Ascaris suum*, a roundworm commonly found in pigs, is typically associated with domestic animals, but its presence in *Rattus rattus* emphasizes the potential for these parasites to exploit diverse hosts (Siddique *et al* ., 2015).

The presence of *Ascaris suum* in *Rattus rattus* suggests the possibility of these rodents participating in the transmission cycle of the parasite between wildlife, domestic animals, and potentially humans. This raises intriguing questions about the ecological dynamics of parasite transmission in environments where different host species coexist. The adaptability of *Ascaris suum* to infect both pigs and rodents challenges traditional host-parasite associations and prompts a reevaluation of the role of rodents in the epidemiology of this parasite (Siddique *et al .,* 2015). The ecological dynamics of *Ascaris suum* in *Rattus rattus* become particularly relevant in shared environments where wildlife, domestic animals, and humans interact. Understanding these dynamics contributes valuable insights into the complex interactions between different host species and the potential risks posed to human health. The ecological study of *Ascaris suum* in *Rattus rattus* becomes crucial for comprehending the intricate web of transmission routes and potential disease pathways in these shared ecosystems (South, 2012).

In line with previous studies, the presence of parasites in *Rattus rattus* can have profound implications for both the health of the host and the ecosystems they inhabit. These parasites can compromise the overall well-being of the rats, leading to reduced reproductive success and survival, as observed with ectoparasites like fleas (Rae *et.al*., 2005). Additionally, endoparasites can lead to a range of health issues, including gastrointestinal problems and nutrient absorption difficulties, potentially affecting the growth and longevity of the host (Rae *et.al*., 2023).

These rodents can serve as reservoirs for zoonotic diseases, with the potential for disease transmission pathways to humans (Sturm *et al*., 2006). This dual role as a reservoir and vector can have far-reaching consequences for public health and the health of wildlife populations within the ecosystem.

The parasitological examination results align with the existing studies, highlighting the rich diversity of parasites that *Rattus rattus* can host. These findings emphasize the intricate web of interactions between parasites, the host species, and the ecosystems they inhabit, underscoring the need for further research to fully comprehend the far-reaching ecological and public health implications of these relationships.

Heavy metal contamination in ecosystems is a global environmental concern. Human activities such as industrial processes, mining, and agriculture release heavy metals into the environment, where they can accumulate in soils, water, and organisms, including wildlife. The introduction of heavy metals into ecosystems has the potential to disrupt ecological processes and affect the health of both wildlife and humans (Triebskorn *et al*., 1996).

Chromium, one of the heavy metals identified in the *Rattus rattus* samples, is known for its toxic effects on organisms. In previous studies, the detrimental impact of chromium on wildlife, particularly birds and mammals, has been documented. Elevated levels of chromium can result in reduced reproductive success and impaired immune function in wildlife, which can have cascading effects on ecosystem health (Vaufleury and Pihan, 2002). The significantly elevated chromium levels observed in Rat 8 suggest potential health risks for this individual and raise concerns about the sources of chromium contamination in the ecosystem.

Lead, another heavy metal detected in the samples, has long been recognized for its harmful effects on both wildlife and humans. Birds, in particular, have been extensively studied in the context of lead poisoning due to the ingestion of contaminated food sources (Wilson, 2012). In *Rattus rattus*, elevated lead levels, as seen in Rat 8, can lead to neurological, reproductive, and immune system impairments. The presence of lead in these rats raises questions about potential exposure sources and the broader implications for ecosystem health.

Mercury, although not detected in the samples, is a heavy metal of significant concern. In ecosystems, mercury primarily enters the food web through aquatic systems, where it can bioaccumulate and biomagnify in organisms, posing risks to both aquatic and terrestrial species. Predatory species, such as *Rattus rattus*, can be exposed to mercury through the consumption of contaminated prey (WHO, 2011).

The heavy metal analysis results indicate the presence of chromium and lead in the examined *Rattus rattus* samples, with notable concentrations in certain individuals. These findings align with the broader literature on heavy metal contamination in ecosystems, emphasizing the potential risks and consequences associated with these contaminants. Further research is warranted to investigate the specific sources of heavy metal exposure in this ecosystem and to assess the broader ecological and public health implications.

CONCLUSION

The results presented here indicate presentation of *Phasmarhabditis hermaphrodita* in *A. hortensis* as the first report in West Africa and presence of the eggs of *Ancylostoma duodenale* in the fecal analysis of the *R. rattus* constitute a public health concern. These parasites represent the only known parasites of these fauna at least in Sub-Saharan Africa and due to safe ranges of metal elements in their tissues; they are safe protein sources at least to the locals. Good sense of hygiene is highly recommended for the locals to avoid zoonotic infection.

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