

Cestodes fauna of silvery mole rats (*Heliophobius Argentiocinereus Emini*) in Morogoro Region, Tanzania

Debora Elias Shija^{1*}, Jahashi Saidi Nzalawahe², Mungo Kisinza Ngalameno², Eliakunda M Mafie³

Abstract

Background: Silvery mole rat (*Heliophobius Argentiocinereus*) is a subterranean rodent widely distributed throughout Sub-Saharan Africa. They have been reported to host several gastrointestinal parasites, however, the status of parasites from these rats in Tanzania has not been widely studied. This study aimed to investigate the prevalence of cestodes parasites in silvery mole rats from the Morogoro region.

Methods: A cross-sectional study was conducted in the Mlali and Mji mkuu wards of the Mvomero and Morogoro urban districts in Tanzania, from March to June 2023. Silvery mole rats were collected through manual excavation of their burrows in agri-ecological areas. The captured rats were euthanized using Diethyl Ether and dissected to remove the gastrointestinal tract (GIT). GIT contents were gathered. Adult worms were collected and preserved in 70.0% ethanol. A flotation and aceto-carmin staining techniques were employed to process gastrointestinal contents and adult tapeworms respectively. Both cestode eggs and adult tapeworms were identified based on general morphological features using taxonomic keys. The prevalence of cestodes was computed, and a chi-square test was applied to assess association, a p-value ≤ 0.05 was considered significant.

Results: One hundred thirty-seven silvery mole rats were collected, adult females 65% (89/137) were dominant among collected rats. One species of gastrointestinal cestode was detected with an overall prevalence of 20.44% (28/137) and identified as *Hymenolepis diminuta*. A total of 392 adult worms and 1450 eggs per gram (EPG) for *H. diminuta* were collected from the gastrointestinal tract. A significant association was shown between cestodes infection with both host age and reproductive category ($p < 0.05$).

Conclusion: This study has established that *Hymenolepidae* (*H. diminuta*) is prevalent in silvery mole rats of the Morogoro region, suggesting that this animal could be among the sources of hymenolepiasis disease in the community. Therefore, further studies are needed to investigate cestodes fauna in community members of the respective study area.

Keywords: *Hymenolepis diminuta*, Prevalence, Morogoro, Silvery Mole Rats, Endoparasites, Tanzania

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Background

Small mammals are important carriers of different parasites of which some have medical and veterinary importance. The silvery mole rat (*Heliophobius Argentiocinereus*) is one of the six genera in the subterranean African mole rat (Bathyergidae family). They are widely distributed throughout Africa, and endemic to Sub-Saharan Africa [1-3]. Similar to other subterranean rodents, silvery mole rats are herbivores, feeding on underground organs of a variety of plants including roots, bulbs, and corms [1]. Rarely, do silvery mole rats spend time above the ground and include ground vegetation in their diet [4]. Their burrow pattern is influenced by the seasonality of the year, becoming more reticulated at the peak of the dry season when the soil is dry and hard [5]. They breed seasonally giving birth after the onset of long rainfall with one to six litters per female [2,5,6]. In some local communities, they are served as an additional source of animal protein [7,8]. Cestodes are hermaphroditic and endoparasitic worms with an elongated flat body without an alimentary canal. Their bodies are comprised of three parts, including the scolex, neck, and strobila which contain the mature, immature, and gravid proglottids respectively [9]. They are named tapeworms because the sum of proglottids which is called strobila resembles a strip of tape. The larval stage develops in the definitive host and then matures to the adult stage in the

intermediate host [10]. Surveys show that rodents play a role as an obligatory intermediate host or paratenic hosts of tapeworms. Some rodent cestodes like *Taenia* spp, *Hymenolepis* spp, *Echinococcus* spp, and *Diphyllobothrium* spp are zoonotic capable of parasitizing humans and animals, and some raise serious concerns regarding human and veterinary health [11–13]. The significant sources and transmitters of zoonotic cestodes are reported to be rodents since they have adapted to human habitats and environmental changes [14]. The primary source of human and animal infestation with these tapeworms is the consumption of food contaminated with cestode eggs, larvae, or metacercaria [14]. Gastrointestinal cestodes have been studied in different groups of subterranean rodents in Africa and elsewhere [15,16]. Infections with these parasites are mostly subclinical and are not easily manifested [17]. High infestation with cestode parasites can lead to decreased activity, weight loss, diarrhea, abdominal pain, bloating, fatigue, and occasionally intestinal lesions [18]. From the first helminth study in African mole-rats, cestode species of *Taenia spalacis* was reported in *Georycus capensis* collected from Port Natal South Africa [19]. Fain [20] reported *Taenia brauna setti* from *Tachyoryetes splendens* collected in Rwanda and Burundi. *Inermicapsifer madagascariensis* was recovered in common mole rats collected from South Africa [8]. Viljoen et al. [21] discovered one cestode species of *Mathevotaenia* spp in *Cryptomys hottentotus* of Tshwane region, South Africa. Lutermann and Bennett [22] discovered two tapeworms of *Rodentolepis* spp and *Taenia* spp in *Bathyergus suillus* from Cape Town in South Africa. Lutermann and Bennett [22] reported on the presence of *Raillietina* spp in *Cryptomys hottentotus* from South Africa. Moreover, gastrointestinal cestodes have been reported in silvery mole rats, in a study conducted in Malawi discovered a cestode species of *Inermicapsifer arvicanthidis* [23]. Recently, Lutermann et al. [24] reported on *Inermicapsifer* spp recovered from *F. anelli* collected in Zambia. About 34.0% of the reported endoparasites in African subterranean rodents are cestodes, however, studies have been conducted on a few known species of less than 40% [15]. *Taenia spalacis* was the first cestode species to be reported in African subterranean rodents in a study conducted in South Africa [19]. Although there have been several reports of cestodes parasitizing subterranean rodents including the silvery mole rats from different parts of the Sub-Saharan region [23, 25-27], studies from Tanzania are limited. Most surveys were conducted exploring the biology, reproductive pattern, and craniometric analysis of silvery mole rats [1,2,28]. To the best of our knowledge, few studies surveying cestode fauna on silvery mole rats have been reported to date since 1864, this is attributed to underground lifestyle limiting studies on these animals [4,15]. However, more studies on this animal are important due to the notable interaction between, humans, animals, and subterranean rodents [7,29]. Therefore, this study carried out a preliminary survey on the prevalence of cestode parasites in silvery mole rats in the Morogoro region, Tanzania.

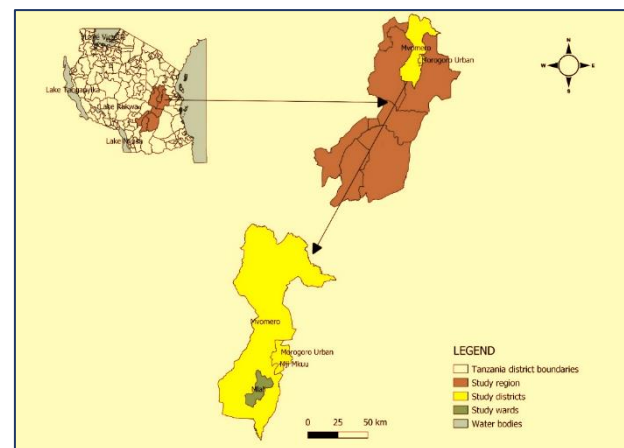
Methods

Study area

The research took place in two districts within the Morogoro region of Tanzania (Mvomero and Morogoro) from March to June 2023. Mvomero is situated at latitude 060 57'16.45- 48" S, longitude 0370 32'05.40-47" E, and at an elevation of 1266-1330

meters above sea level. Morogoro is located at latitude 06049'20" S, longitude 037039'55" E, and at an elevation of 509 meters above sea level. The terrain in these regions is mainly flat, with a bi-modal rainfall pattern averaging 600mm annually. The shorter rainy season occurs from November to January with an average of 130mm, while the longer rainy season is from March to May with an average of 470mm. The climate is characterized by hot temperatures, with an average annual temperature of 25°C ranging from 18°C to 30°C. The area is an agri-ecological zone with river valleys and basins that support the cultivation of food and cash crops, as well as grazing activities. Mvomero District boasts the largest water area in the Morogoro region, covering 1,882 sq. km [30].

Figure 1. Map of Morogoro region highlighting the study districts and wards. The



map was created using QGIS software version 3.2.6.1 and shape files from DIVA-GIS (retrieved on 5 July 2023).

Study design, and sampling strategies

A cross-sectional study was carried out in Mvomero and Morogoro districts at five different sites (Figure 1) from March to June 2023. The study animals were collected from the Mji Mkuu division of Morogoro District and in four villages of Mvomero District namely Mlali, Kipera, Mkuyuni, and Mongwe. Farm fields for sampling were purposively selected based on the presence of geophytes that form the staple diet for silvery mole rats and evident fresh mole hills as an indicator of burrowing activity by subterranean animals [1]. Burrows for capturing animals were selected randomly by opening a distinct burrow within the farm fields. All these were performed following the positive response of landowners.

Capturing, handling, and transportation of silvery mole rats

Silvery mole rats were collected manually using hand hoes by digging out the burrow, and animals found in the burrow were picked using hands with the aid of a half-cut plastic bottle. Capturing of silvery mole rats was conducted in the early morning following the help of new fresh mole hills on the surface made every last night [5] and was conducted for 3 weeks in each study site. Captured animals were kept in 20-litre plastic buckets half filled with fresh soil, and tightened with lids containing aeration pores [6], then were transported to the parasitology laboratory at SUA where gastrointestinal contents were to be collected under animals' maintenance guidelines [31].

Collection of GIT Contents

Animals were euthanized using Diethyl ether for about 3- 4 minutes before the collection of GIT contents. Animal body parameters were recorded (sex, age, and reproductive status) by examining body morphological features, and the body weight (gm) was measured using a digital weighing balance. During postmortem examination, animals were dissected by opening their body cavity to remove the gastrointestinal tract according to standard procedures described by Rusli (1988). The stomach, small intestines, and large intestines were separated, and each section was opened and flushed with physiological saline(40ml-50ml) to remove its contents, the contents were mixed well using a wood applicator and were sieved with a fine tea strainer, the obtained contents were preserved for examination. Adults' worms present in the gastrointestinal tract were collected with the aid of a hand lens, washed with physiological saline, and fixed in 70% ethanol for further identification [32].

Laboratory sample processing

A coprological and post-mortem examination was carried out at the veterinary parasitology laboratory at the Sokoine University of Agriculture for the detection of cestodes parasites. Cestode parasites were assessed quantitatively by using the floatation method [33]. Three milliliters of gastrointestinal contents were mixed with 42ml of saturated sodium chloride solution (400g of analytical sodium chloride with 1litre of distilled water to make an S.G of 1.2 which was measured by using a hydrometer), the suspension made was sieved using a tea strainer, the retained debris was discarded and the strained suspension were transferred into a clean container, a suspension made was filled in two compartments of Mc Master chamber (0.15ml × 2), and left to settle for 1minutes. Flotation fluid enabled debris to sink and eggs to float, making eggs more visible. Cestode eggs were identified according to their species levels based on general morphological features (shape, size, and shell structure) according to morphological keys using a compound microscope with a magnification power of 10x and 40x [34-36]. Finally, related morphotypes of eggs inside the grid were counted using an electronic tally counter in each of the positive samples and EPG (fecal eggs per gram) for each parasite was calculated [37]. Adult worms were identified based on the morphology of the cestode segments using guideline information [38]. After being stained with aceto-carmin staining solution (45% of glacial

acetic acid with 10 grams of carmine powder). Segments of cestodes (mature proglottids, immature proglottids, and the scolex) were first loaded in distilled water for 5 minutes to remove fixatives, dried with tissue paper by bloating, and immersed in acetocarmine solution for 20 minutes. Adult segments were examined under the compound microscope at 4x,10x, and 40x [32].

Data analysis

Prevalence was calculated by dividing the total number of silvery mole rats that tested positive for cestodes (represented as 'n') by the total number of animals that were sampled (represented as 'N'). In addition, the chi-square test was applied to assess the relationship between the prevalence of cestode infection and various body parameters of the animals, whereby a p-value of less than or equal to 0.05 was considered statistically significant. These associations were determined using the Epi-info version 7.2.4.0. Parasite intensity was calculated by counting the total number of adult worms recovered per sample and the total number of eggs per gram (EPG) in each positive sample [10,39,40]. The mean intensity (MI) for eggs and adult worms was computed by dividing the sum of eggs per gram (EPG) in feces for each cestode species divided by the number of hosts infected with that specific cestode species, and by dividing the sum of adult worms for each specific species divided by the number of hosts infected with that specific cestode species. The intensity range (RI) was the minimum and the maximum value of load (EPG) for cestode parasite eggs and adult cestode worms. The mean abundance (MA) is the total number of each isolated parasite egg or parasite adult divided by the number of total sampled animals.

Results

Gastrointestinal contents were obtained from 137 captured silvery mole rats. Of these sampled individuals 65.0% (89/137) were female, 86.7% (120/137) were adults and 12.4% (17/137) were sub-adults. Breeders were the most abundant animals covering 82.5% (113/137) of the collected silvery mole rats. The body weight of adult silvery mole rats ranged from 101.7gm to 331.6gm, and of sub-adult silvery mole rats ranged from 42.2gm to 98.7gm (Table 1). The overall prevalence of cestode infection in the study was 20.44% (28/137).

Table 1. Association of body parameters and prevalence of cestodes fauna in silvery mole-rats in Morogoro region (n=127)

Variable	Category	Collected animals	Infected animals	Prevalence (%)	Odds Ratio	95% CI	X ²	P-value
Age	Adult	120	20	16.7	0.23	0.08- 0.65	6.69	0.01
	Sub-adult	17	8	47.1				
Sex	Female	89	16	18.0	0.66	0.28-1.54	0.56	0.4
	Male	48	12	25.0				
Reproductive category	Breeder	113	18	15.9	0.27	0.1-0.69	6.56	0.01
	Non-breeder	24	10	41.7				

In this study, host age and reproductive status showed a significant association with the prevalence of cestode infection with P-values of < 0.05 (Table 1). Sub-adult silvery mole rats were more infected with cestodes compared to their counterpart adult silvery mole rats (p-value < 0.01), showing a high risk of acquiring cestodes infection with an odds ratio of 0.23 (95% C.

I= 0.08- 0.65). Likewise non- breeders were more infected with cestodes than their counterpart breeder's silvery mole rats (p-value < 0.01). Breeders were at lower risk of being infected with cestodes with an odds ratio of 0.27 (95% C.I =0.1-0.69). Host sex did not show a significant association with the prevalence of cestode infection where the p-value was > 0.05 (Table 1)

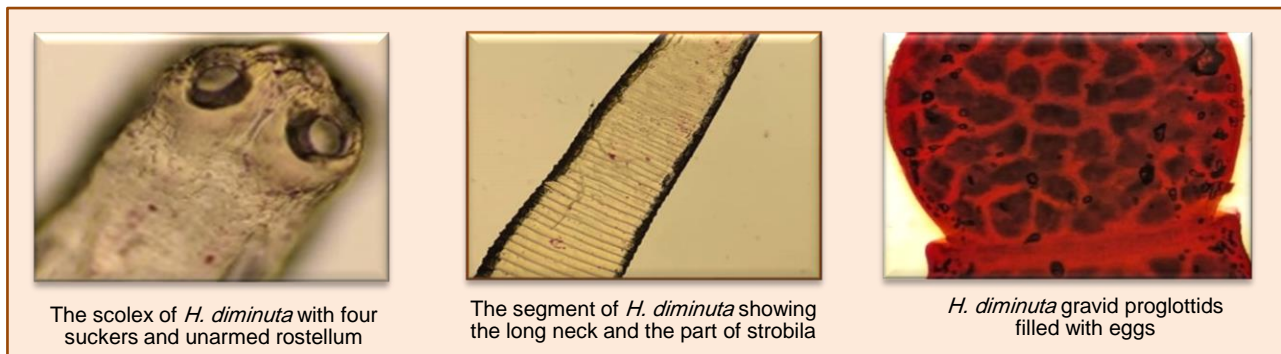


Figure 2. Hymenolepis diminuta adult stage (Source: author 'photos taken in SUA's Parasitology laboratory in examined silvery mole rats)

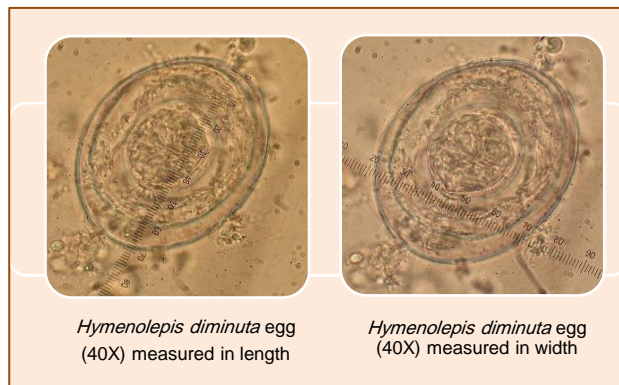


Figure 3. Eggs of Hymenolepis diminuta (Source: author's photo taken in SUA's parasitology laboratory in examined silvery mole rats)

One species of cestode helminth from the order Cyclophyllidae in the family Hymenolepididae namely “Hymenolepis diminuta” was detected by coprological examination and post-mortem examination (Figures 2 and 3). The *H. diminuta* eggs were oval-shaped with striated outer and inner membranes. Their sizes

ranged from 70µm to 78µm in length and 50µm to 57µm in width. The space between membranes appeared faint and granular. They did not possess filaments but they were embryonated with a 6-hooked oncosphere inside the shell (Figure 3). Adult *H. diminuta* appeared transparent with a long slender neck possessing segments that were wider than long, they were larger measuring from 35mm to 63mm long and 3mm to 4mm wider, the scolex was small and pear-shaped bearing four deep suckers without hooks and the rostellum was unarmed (Figure 3). Twenty-four of the examined silvery mole rats were found harboring adult worms only and four rats were harboring both cestode eggs and adult worms (Table 2). A total of 392 adult worms were collected including 385 from the intestines of silvery mole rats (363 from small intestines, and 22 from large intestines). Cestode proglottids were also collected from the large intestines of infected rats (Table 2). The total fecal eggs per gram (EPG) of these cestodes was 1450. Prevalence (P), mean intensity (MI), mean abundance (MA), intensity range (IR), and the predilection sites of *H. diminuta* among infected silvery mole rats are summarized in Table 2.

Table 2. Prevalence, mean abundance, mean intensity, mean range, and site of infection of each cestode species in silvery mole rats in Morogoro

Helminths spp	No. of host infected	Prevalence (%)	No. of collected Parasite or eggs	Mean abundance	Mean intensity	Intensity Range	Stomach	Small Intestines	Large Intestine
Hymenolepis diminuta adult	24	17.52	392	2.86	15.08	1-147	2 ^a (7) ^b	26 ^a (363) ^b	3 ^a (22) ^b
Hymenolepis diminuta eggs	4	2.92	1450	6.57	362.5	50-700	0	1 ^a (150) ^c	3 ^a (1300) ^c

Note: a: number of infected silvery mole rats; b: number of adult parasites; c: number of eggs collected

Discussion

The study provides the first baseline information on the prevalence of cestode infections (20.44%) in silvery mole rats in Tanzania. The parasite fauna found in silvery mole rats was limited to one cestode species namely *H. diminuta*. Similarly low species richness of macroparasites in African mole rats and other parts of Africa has been previously reported [16,41,42]. Low parasite fauna in our study may be attributed to the subterranean niche that may limit their exposure to parasites. Also, the herbivorous nature that includes feeding on bulbs and tubers which may have medicinal properties and result in self-medication can be an effective behavioral strategy to limit the amplification of parasites once an infection has occurred [22,43]. The findings of this study support Lutermann [44] who stated that subterranean rodents have a significantly impoverished macroparasites species richness.

Also, the presence of *H. diminuta* in silvery mole rats aligns with other previous studies conducted in subterranean rodents in the Nearctic, Northern Neotropical, and Palearctic regions [15]. Nevertheless, *H. diminuta* has been reported in terrestrial rodents [45]. This hypothesizes that rodents whether terrestrial or subterranean are natural mammalian hosts for *Hymenolepis diminuta*. The prevalence of cestode in this study is rather low when compared to cestode prevalence in the studies conducted in highveld mole rats from South Africa, mole rats from Georgia, and Tuco-tucos (Genus *Ctenomys*) from Argentina, the contrary is higher in comparison with the result documented in Ansell mole rats from Zambia [16,21,24]. The low prevalence of cestode infection in this study may be attributed to the subterranean lifestyle that limits these animals from parasite exposure. Coello-Peralta et al. [9] reported that *H. diminuta* is a common parasite

of rodents, their prevalence diversity may be attributed to their adaptability as well as the enormous capability of rodents to support parasites' behavioral, physiological or nutritive, and developmental needs, and the variation in ecology and climatic conditions in the study areas can also attribute the variation of parasites burden and prevalence [41,44]. The presence of *H. diminuta* in this study could be linked to the high exposure rate of their intermediate host within the burrow system and study area, favoring the transmission of parasites in these rats. Similar findings have been reported previously in terrestrial rodents [44]. A high abundance of adult *H. diminuta* in the small intestine of silvery mole rats suggests the predilection site of this parasite, scientifically small intestine is the right place for morphological building since an ileum portion of the small intestine is where digested food nutrients are absorbed into the circulatory system being the portion housing the villi [46]. *Hymenolepis diminuta* are common parasites of rodents, capable of infecting humans especially children and institutionalized groups of temperate regions. They have an indirect life cycle that usually depends on an intermediate host (various species of beetles, cockroaches, and fleas) during their larval stages for transmission. In their adult stage, *H. diminuta* dwells in the intestines of the definitive host where they lay eggs [9]. Eggs pass out of the definitive host through stool into the environment, where they are ingested by intermediate hosts and develop into cysticercoids, the stage that can infect both rodents and humans upon ingestion. Rarely, humans can acquire the intestinal stage by consuming uncooked corn, cereal, flour, dried fruits, and grains contaminated with infected intermediate hosts. Human infection is usually asymptomatic but can cause mild gastrointestinal symptoms including abdominal pain, irritability, and diarrhea. A significant host age variation with cestode infection was observed, sub-adult silvery mole rats were more infected with cestodes showing a higher chance of acquiring these parasites (odds ratio=0.23, p -value< 0.01) than their counterpart adult silvery mole rats. These may be linked to the innate immune response that is considered cheap and broadly targeting the foreign molecules in young developing animals making them more susceptible to parasitic infections [4,47]. The same observation has been previously reported in highveld mole rats [21]. Breeding status affected the prevalence of cestode infections, breeders of any sex were less infected in comparison to their counterpart non-breeders [odds ratio 0.27, p -value< 0.01] and these may be linked to the fact that reproductive individuals are likely to be older in age and the lower parasite abundance in these individuals may be an indicator of adaptive immunity which is probably the most important line of defense against parasites once transmission has occurred making adult silvery mole rats resistant to re-infections [47,48]. Also, breeders participate less to the extent of energetical costly foraging and locomotion activities than non-breeders making them less susceptible to parasite exposure [4]. Similarly, these findings have been reported in closely related species [21,27]. Regarding rats' sex, no difference was observed in cestode infection. A similar observation has been made for other bathyergids and it has been suggested that the sedentary lifestyle of these subterranean animals provides an equal chance of parasite exposure despite the sex [21,27,41,42]. However, males were at a higher risk of acquiring infections than their counterpart female rats. These may be supported by the fact that males become more infected due to the fact that males are more

vulnerable with increased home ranges and reduced immune function as a result of testosterone hormones [49,51].

Conclusion

The study reports the cestodes fauna of Emin's silvery mole rats of the family Bathyergidae in Tanzania at a prevalence of 20.44%. The identified cestode species was *H. diminuta*. The obtained cestodes have zoonotic potential in temperate regions, especially in developing countries including Tanzania. Therefore, more epidemiological surveillance should be carried out to assess Hymenolepiasis cases in community members of the respective study area (Mvomero and Morogoro districts).

Abbreviation

CDC: Center for Disease Control; CI: Confidence interval; CVMBS: College of Veterinary Medicine and Biomedical Science; EPG: Eggs per gram; GIT: Gastro-intestinal tract; MA: Mean Abundance; MI: Mean Intensity; MRP: Morogoro Region Profile; N: Total Number of Collected animals; n: number of infested/infected animals; OR: Odds ratio; RI: Range Intensity; SG: Specific Gravity; Spp: Species; SUA: Sokoine University of Agriculture; URT: United Republic of Tanzania; WHO: World Health Organization.

Declaration

Acknowledgment

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Availability of data and materials

Data will be available by emailing deboraeliphace@gmail.com

Authors' contributions

Debora Elias Shija (D.E.S) is the principal investigator who contributed to the conceptualization, data curation, formal analysis, and writing of the original manuscript draft. Jahashi Said Nzalawahe (J.S.N) and Mungo Kisinza Ngalameno (M.K.N) are the main supervisors, contributing to the manuscript's methodology, supervision, review, editing, and re-writing. Eliakunda M Mafie is the least supervisor contributing to the manuscript supervision and review. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Research Committee of the Sokoine University of Agriculture. All procedures in this study have been permitted and approved by the Institutional Ethics Committee of the Sokoine University of Agriculture (SUA/ DPRTC/ R/ 186/ Vol IV- 68 issued on 9/10/2023). The Ministry of Regional

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Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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