

Factors Affecting Diversity and Distribution of Haemoparasites and Ectoparasites of Rodents and Shrews In Iringa District

Faktor yang Mempengaruhi Keanekaragaman dan Persebaran Hemoparasit-Ektoparasit Rodensia dan Curut Distrik Iringa

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ABSTRACT

Background: Rodents and shrews serve as a reservoirs and final hosts of various parasitic agents. Different factors are known to affect the diversity and distribution of the parasites harbored by them. Little is known on the factors affecting the diversity and distribution of parasites in rodents and shrews found at different elevations in Iringa District. Understanding these factors is crucial to comprehending how parasitic populations among small mammals spread. **Purpose:** This study aimed to investigate the factors associated with the diversity and distribution of ectoparasites and hemoparasites in rodents and shrews in Iringa. **Method:** A cross-sectional study was conducted in selected sites between March and June 2023. Rodents and shrews were captured using Sherman and wire traps, euthanized, and then their sex and species were identified. After that, ectoparasites, blood and tissue sample were collected and processed. The diversity and distribution of parasites at different sites were calculated using the Shannon-Wiener Index formula. **Results:** The overall prevalence of hemoparasites infection was 24.2 % (50/207), dominated by *Anaplasma centrale* (17.4%:36/207) followed by *Plasmodium spp.* (8.7%:18/207) and *Anaplasma marginale* (4.4%:9/207). A total of 713 ectoparasites belonging to five taxa were recovered from small mammals with an overall prevalence of 55.1% (114/207), dominated by mites; *Echinolaclaps echidninus* 46.4% (96/207), followed by lice; *Polyplax spinulosa* 16.9% (35/207), tick *Hemaphysalis spp.* 7.3% (15/207), and two flea taxa; *Dinopsylla lypusus* 2.9% (6/207) and *Xenopsylla cheopis* 1% (2/207). **Conclusion:** The high occurrence of *Anaplasma centrale* and *Echinolaclaps echidninus* suggests potential ecological and public health significance.

ARTICLE INFO

Received: 20 December 2024

Revised: 10 April 2025

Accepted: 29 April 2025

Online: 30 April 2025

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Keywords: Ectoparasites; Hemoparasites; Rodents; Shrews

ABSTRAK

Latar Belakang: Hewan pengerat dan curut merupakan reservoir dan inang akhir berbagai agen parasit. Berbagai faktor diketahui mempengaruhi keanekaragaman dan distribusi parasit yang ditampungnya. Sedikit yang diketahui tentang faktor-faktor yang mempengaruhi keanekaragaman dan distribusi parasit pada hewan pengerat dan tikus yang ditemukannya pada ketinggian berbeda di Kabupaten Iringa. Memahami faktor-faktor ini sangat penting untuk memahami bagaimana populasi parasit di antara mamalia kecil menyebar. **Tujuan:** Penelitian ini bertujuan untuk mengetahui faktor-faktor yang berhubungan dengan keanekaragaman dan sebaran ektoparasit dan hemoparasit pada hewan pengerat dan tikus di Kecamatan Iringa. **Metode:** Studi cross-sectional dilakukan di lokasi terpilih antara bulan Maret dan Juni 2023. Tikus dan tikus ditangkap menggunakan perangkap Sherman dan kawat, di-eutanasia, lalu diidentifikasi jenis kelamin dan spesiesnya. Setelah itu, sampel ektoparasit, darah dan jaringan dikumpulkan dan diproses. Model Linier Umum dalam perangkat lunak R dilakukan untuk menunjukkan hubungan antara parasit dan parameter lainnya. Keanekaragaman dan distribusi parasit di berbagai lokasi dihitung menggunakan rumus Indeks Shannon-Wiener. **Hasil:** Prevalensi infeksi hemoparasit secara keseluruhan adalah 24.2% (50/207), didominasi oleh *Anaplasma centrale* (17.4%:36/207) diikuti oleh *Plasmodium spp.* (8.7%:18/207) dan *Anaplasma marginale* (4.4%:9/207). Sebanyak 713 ektoparasit yang termasuk dalam lima taksa ditemukan dari mamalia kecil dengan prevalensi keseluruhan 55.1% (114/207), didominasi oleh tungau; *Echinolaclaps echidninus* 46.4% (96/207), diikuti kutu; *Polyplax spinulosa* 16.9% (35/207), kutu *Haemaphysalis spp.* 7.3% (15/207), dan dua taksa kutu; *Dinopsylla lypusus* 2.9% (6/207) dan *Xenopsylla cheopis* 1% (2/207). **Kesimpulan:** Tingginya tingkat kemunculan *Anaplasma centrale* dan *Echinolaclaps echidninus* mengindikasikan potensi dampak yang signifikan terhadap ekologi dan kesehatan masyarakat.

Cite This Article:

Njogela, N., Nzalawahe, Mafie, E., 2025. Factors Affecting Diversity and Distribution of Haemoparasites and Ectoparasites of Rodents and Shrews In Iringa District. Journal of Applied Veterinary Science and Technology. 6(1): 54-63. <https://doi.org/10.20473/javest.V6.I1.2025.54-63>

Kata kunci: Ektoparasites; Hemoparasites; Rodensia; Shrews

INTRODUCTION

Rodents are small mammals distributed globally, they belong to the order Rodentia which is the largest and most diverse mammalian order comprising more than 40% of all mammalian species with approximately 30 families (Stevens, et al., 2022). On the other hand, shrews are small mammals belonging to the order Eulipotyphla and the family Soricidae. About 40% of shrew species are found in Africa, and the rest are found in the Northern hemisphere except in Australia and some parts of South America (Encyclopedia Britannica, 2021). A total of 385 shrew species are reported and grouped into 26 genera and three subfamilies which are Crocidurinae, Soricinae, and Myosoricinae (Wilson and Reeder, 2011). Rodents and shrews are reservoirs of both ectoparasites and hemoparasites which are sources of disease transmission.

Ectoparasites are organisms that live permanently or intermittently on the body surface of their hosts, they are well-adapted and diverse ranging from facultative to obligatory (Mullen and O'Connor, 2019). They are vectors of disease-causative agents that can be transmitted from one host to another (Dada, 2016). The most common ectoparasites reported in rodents and shrews are ticks, mites, lice, and fleas (Philip Samuel, et al., 2021; López-Pérez, et al., 2022). Mites and lice spend all their life stages on host bodies while only some stages of fleas, ticks, and some species of mites are temporarily associated with the host (off-host), they are only found in host bodies for feeding and copulation purposes (Pakdad, et al., 2012). Ectoparasites species reported in small mammals are *Xenopsylla cheopis*, *Echinophaga gallinaea*, *Echinolaepus echidninus*, *Laelaps* spp., *Varroa* spp., *Haplopleura* spp., *Hemaphysalis* spp., *Ixodes* spp., *Rhipicephalus* spp. and others (Theonest, et al., 2019; Mawanda, et al., 2020; Gebrezgiher, et al., 2023; Mhamphi, et al., 2024; Musese, et al., 2024). In Tanzania, ectoparasites of rodents and shrews have been identified to be vectors of different pathogens such as *Bartonella* spp., *Yersinia pestis*, *Anaplasma* spp., and *Babesia* spp. which cause disease to both humans and animals (McCauley, et al., 2015; Katakweba, 2018; Mhamphi et al., 2024).

Hemoparasites are pathogens that inhabit the bloodstream of the host. These parasites can be transmitted from one host to another through vectors either biologically or mechanically (Solanki, et al., 2013). The most common blood parasites in vertebrates are *Trypanosoma* spp., *Babesia* spp., *Plasmodium* spp., *Anaplasma* spp., *Toxoplasma gondii*, and *Leishmania* spp. (Chen, et al., 2016). Rodents and shrews are reservoir hosts of hemoparasites and humans acquire infection either directly from animal bites or indirectly through arthropods vector infected with a particular parasite. Studies conducted by Katakweba, (2018), Materu, (2023), and Mhamphi, et al., (2024) in different parts of Tanzania reported hemoparasites of rodents and shrews which are *Trypanosoma lewis*, *Babesia* spp., *Plasmodium* spp., *Bartonella* spp., and *Anaplasma* spp. Small mammal (rodents and shrews) population distribution and density may directly affect the ectoparasite population which in turn accelerates the spread of pathogens like hemo-

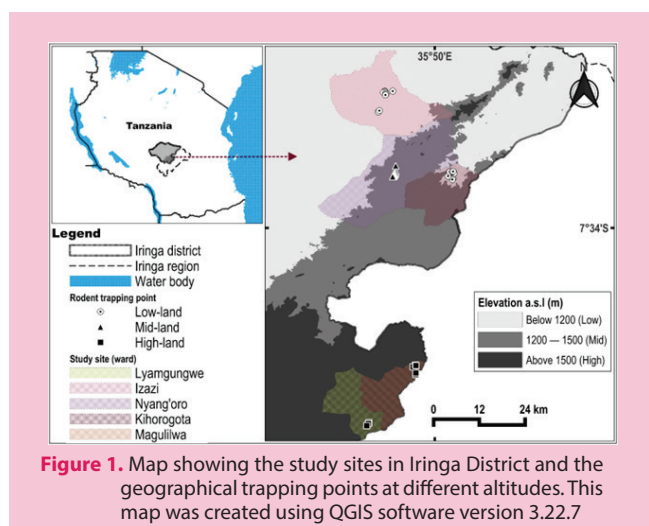
parasites which are carried by arthropods (Obiegala, et al., 2021). Different factors which are host-related and environmental-related are known to affect the abundance and population of small mammals, altitude is one of them. Altitude differences may result in a high or low population of small mammals over an area due to changes in environmental conditions which may influence the abundance of parasites and increase the chance of zoonotic infection due to interaction between humans, livestock animals, and small mammals. Different studies have been conducted to evaluate the effects of altitudes and other factors on the diversity and distribution of rodents and shrews and revealed the influence of altitude in the distribution and diversity of rodents and shrews' population (Stanley, et al., 2014; Sabuni, et al., 2018). Distribution of parasites both ectoparasites and hemoparasites, among their host within a population is uneven due to factors that are related to hosts (sex, hormone levels, body weight, host immunity) and environment (geographic features, vegetation, elevation, climate, or season) these factors influence the exposure and susceptibility of the host to parasites (Kramm III, et al., 2017; De Fuentes-Vicente, et al., 2018; Gebrezgiher, et al., 2023). The distribution of ectoparasites in their hosts depends on the interaction between hosts and ectoparasites, and the co-existence between ectoparasites; in contrast, hemoparasites distribution is mostly related to the habitat selection of their host which includes both small mammals and ectoparasites (Obiegala, et al., 2021). Understanding the mechanisms influencing the diversity, distribution, and species richness of parasites may be greatly expanded by studying different factors including elevation gradients since in both higher and lower elevations there is variability in biological historical, geographical, and anthropogenic factors, or human aspects that influence the availability of host and their parasites (McCain and Grytnes, 2010).

Different studies have been conducted on hemoparasites and ectoparasites of rodents and shrews in various parts of the world. The studies are based on the prevalence of these parasites, their distribution, land use effects, and their importance in disease transmission (Katakweba, 2018; Mawanda, et al., 2020a; Votýpka, et al., 2022); however, the information on the diversity and distribution of ectoparasites and hemoparasites based on altitude variation at different places especially in Iringa District is not well-understood. This study aims to investigate the influence of altitude and other factors on the diversity and distribution of hemoparasites and ectoparasites of rodents and shrews in Iringa District. The information from the current study will provide baseline information on the presence and distribution of ectoparasites and hemoparasites of rodents and shrews found in the Iringa District and help the responsible authority in control of the small mammal population to reduce the risk of zoonotic diseases transmission.

MATERIAL and METHOD

Description of the Study Area

This study was carried out in Iringa District, the district borders Mpwapwa District (Dodoma region) to the north,



Kilolo District to the east, Mufindi District to the south, Chunya District (Mbeya Region) to the southwest and Manyoni District (Singida Region) to the northwest. The study area is located between latitude 806.0°S to 7012.0°S, and longitude 3506.0°E to 3600.0°E at an elevation between 744m and 1809m above sea level in five villages within five wards of Iringa District (Figure 1). The district is divided into three ecological zones, highland zone, lowland zone, and midland zone. The highland areas receive annual rainfall ranging from 500mm to 2700mm whereas the lowland areas receive less than 600mm rainfall annually (Iringa Rural District, 2015). The soil is relatively acidic due to high rainfall, which is suitable for agricultural activities. The main activity in this area is agriculture and livestock playing an important role in the economy of this district.

Study Design and Sampling Strategies

A cross-sectional study was conducted in five wards of Iringa District between March and June 2023, where rodents and shrews were captured for collection of ectoparasites, tissue and blood sample. Rodents and shrews were captured across three geographical zones, high, low, and midland. The location of traps and altitudes in each site where trapping was conducted were recorded using a global positioning system device (GPS). Five villages in five wards were selected purposively based on altitude variation, two villages were selected from low altitude (Izazi and Igula), one village from mid-altitude (Chamdindi), and two villages from high altitude (Ndiwili and Lupembelwasenga). Trapping units were farms, peri domestic areas, inside houses, and livestock boma, and at least 20 traps were set per site. Inside houses the traps were set according to the house dimensions and in the areas where rodents are expected to be found.

Rodents and Shrew Trapping and Identification

Rodents and shrews were captured live by using Sherman trap (H.B. Sher-man Traps Inc., Tallahassee, FL, USA) and wire traps. Traps were baited with a mixture of maize flour and peanut butter, sardines, and tomatoes (Mulungu, et al., 2008). Inside houses, the traps were set according to the dimensions of the house and the areas where rodents are expected to be found such as in the kitchens, on top of

shelves, and in storage facilities used for storage of maize and sunflowers. Traps were set in the evening and then inspected in the morning and late afternoon, empty traps were rebaited and left at the site. Captured rodents and shrews were removed from the traps using an animal-handling cloth bag. Before identification, animals were euthanized by placing them inside a bottle soaked with cotton wool containing diethyl ether. After being euthanized, animal body parameters were recorded by following morphometric measurements whereby body weight was measured in grams using a digital weighing balance, tail length, hind foot, ear length, and head body length were measured using a Vernier caliper. The identification of rodents and shrews was done using morphological, taxonomic identification keys and illustrations developed by Hapold and Hapold (2013).

Ectoparasites and Blood Sample

After euthanization, rodents and shrews were placed in the basin and ectoparasites were collected from animals' bodies by using a fine shoe brush. Rodents and shrews' fur were combed from the base of the ear to the base of the tail on both dorsal and ventral sides to allow parasites to dislodge from their bodies to the enamel tray as recommended (Ibrahim, 2020). Each cloth bag where rodents and shrews were housed was also carefully examined by turning upside down to the enamel tray to collect any ectoparasite present. The contents in the enamel tray were carefully examined with the aid of a hand lens to allow visualization of the parasite present. Ectoparasites present were retrieved using Camel brush and placed in vials containing 70% alcohol. Blood sample from small mammals was drawn from the supraorbital vein by using glass capillaries; this procedure was carried out before collection of ectoparasites and identification of small mammals. Immediately after blood collection, thin blood smears were prepared by placing a drop of blood onto the center of glass slides directly from capillary tubes and spread throughout the center using a spreader, the slides were air dried, fixed in absolute methanol for three minutes then placed in slide boxes for preservation. Viscera were collected after dissection of study animal bodies in the dissection board using scissors, impression smears were prepared by lightening a fresh cut of viscera (liver, kidney, and spleen) onto the microscopic slide. The prepared impression smears were waved into the air for about three minutes and stored inside the slide box. The collected ectoparasites, prepared blood and impression smears were transported to the Parasitology laboratory at Department of Microbiology, Parasitology and Biotechnology of Sokoine University of Agriculture for laboratory processing and identification.

Laboratory Sample Processing and Identification of Ectoparasites and Hemoparasites.

Ectoparasites were removed from vials, ticks and mites were allowed to dry on the surface and placed on a microscopic slide for identification. Ticks and mites were allowed to dry before observation on a microscope. Ticks were identified based on key morphological features such as mouth parts, scutum, festoons, and the presence or absence of eyes. The key observational features for identification of mites were the size

Table 1. Prevalence Of Hemoparasites Infection And Ectoparasites Infestation In Rodents and Shrew Species.

Host Species	Ectoparasites					Hemoparasites		
	<i>Echinolaelaps echidninus</i>	<i>Hemaphysalis spp.</i>	<i>Dinopsyllus spp.</i>	<i>Xenopsylla cheopis</i>	<i>Polyplax spinulosa</i>	<i>Anaplasma centrale</i>	<i>Anaplasma marginale</i>	<i>Plasmodium</i>
<i>Arvicanthis niloticus</i> (53)	23(43.4)	6(11.32)	2(3.77)	1(1.89)	15(28.3)	8(15.09)	2(3.77)	2(3.77)
<i>Acomys spp.</i> (1)	0 (0)	0 (0)	0(0)	0 (0)	0 (0)	0(0)	0(0)	0(0)
<i>Praomys spp.</i> (1)	1 (100)	0 (0)	0(0)	0 (0)	0 (0)	0(0)	0(0)	0(0)
<i>Rattus rattus</i> (23)	6(26.09)	0 (0)	0(0)	1(4.35)	0 (0)	0(0)	1(4.35)	0(0)
<i>Petrodromus spp.</i> (4)	0 (0)	0 (0)	0(0)	0 (0)	3 (75)	0(0)	1(25)	0(0)
<i>Gerbilliscus spp.</i> (1)	1 (100)	0 (0)	0(0)	0 (0)	0 (0)	0(0)	0(0)	0(0)
<i>Mastomys natalensis</i> (96)	54(56.25)	5(5.21)	4(4.17)	0 (0)	12(12.5)	23(23.96)	4(4.17)	14(14.58)
<i>Otomys spp.</i> (7)	3(42.86)	1(14.29)	0(0)	0 (0)	1(14.29)	0(0)	0(0)	0(0)
<i>Tatera spp.</i> (4)	2(50)	2 (50)	0(0)	0 (0)	0 (0)	1 (25)	1(25)	0(0)
<i>Graphiurus murinus</i> (1)	0 (0)	0 (0)	0(0)	0 (0)	0 (0)	0(0)	0(0)	0(0)
<i>Mus musculus</i> (7)	5(71.43)	0 (0)	0(0)	0 (0)	3 (42.85)	1(14.29)	0(0)	0(0)
<i>Grammomys spp.</i> (1)	1(100)	0 (0)	0(0)	0 (0)	0 (0)	1 (100)	0(0)	1(100)
<i>Crocidura spp.</i> (3)	0 (0)	0 (0)	0(0)	0 (0)	0 (0)	2(66.67)	0(0)	1(33.33)
<i>Beamys spp.</i> (1)	0 (0)	1(100)	0(0)	0 (0)	1 (100)	0(0)	0(0)	0(0)
<i>Lemniscomys striatus</i> (1)	0 (0)	0 (0)	0(0)	0 (0)	0 (0)	0(0)	0(0)	0(0)
Total (207)	96(46.38)	15(7.25)	6(2.89)	2(0.97)	35(16.91)	36(17.39)	9(4.35)	18(8.7)

Table 2. Prevalence of Hemoparasites Infection and Ectoparasites Infestation In Rodents and Shrews at Different Habitats, Altitudes, Sex and Age Groups.

Parameters	Categories	Examined Hosts	Hemoparasites Prevalence			Ectoparasites Infestation				
			<i>Plasmodium</i>	<i>Anaplasma centrale</i>	<i>Anaplasma marginale</i>	<i>Echinolaelaps echidninus</i>	<i>Hemaphysalis spp.</i>	<i>Dinopsyllus spp.</i>	<i>Xenopsylla cheopis</i>	<i>Polyplax spinulosa</i>
Altitude	Low altitude	104	3 (2.88)	19(18.27)	2(1.92)	46(44.23)	8(7.69)	0(0)	1(0.96)	31(29.81)
	Mid altitude	40	6 (15)	6(15)	4(10)	21(52.5)	0(0)	2(5)	0(0)	5(12.5)
	High altitude	63	9 (14.29)	11(17.46)	3(4.76)	29(46.03)	7(11.11)	4(6.35)	1(1.59)	0(0)
	Total	207	18 (8.7)	36(17.39)	9(4.35)	96(46.38)	15(7.25)	6(2.9)	2(0.97)	36(17.39)
Habitats	Farmland	134	14(10.45)	26(19.4)	5(3.73)	66(49.25)	12(8.96)	5(3.73)	1(0.75)	22(16.42)
	Peri domestic	22	2(9.09)	6(27.27)	2(9.09)	11(50)	2(9.09)	1(4.55)	0(0)	0(0)
	Livestock boma	25	0(0)	4(16)	1(4)	11(44)	1(4)	0(0)	0(0)	12(48)
	Indoor	26	2(7.69)	0(0)	1(3.85)	8(30.77)	0(0)	0(0)	1(4.35)	2(7.69)
	Total	207	18(8.7)	36(17.39)	9(4.35)	96(46.38)	15(7.25)	6(2.9)	2(0.97)	36(17.39)
Age group	Adult	166	12(7.23)	30(18.07)	9(5.42)	81(48.8)	15(9.04)	6(3.61)	2(1.2)	33(19.88)
	Juvenile	41	6(14.63)	6(14.63)	0(0)	15(36.59)	0(0)	0(0)	0(0)	3(7.32)
	Total	207	18(8.7)	36(17.39)	9(4.35)	96(46.38)	15(7.25)	6(2.9)	2(0.97)	36(17.39)
Sex	Female	114	13(11.4)	27(23.68)	8(7.02)	51(44.74)	6(5.26)	5(4.39)	1(0.88)	20(17.54)
	Male	93	5(5.38)	9(9.68)	1(1.08)	45(48.39)	9(9.68)	1(1.08)	1(1.08)	16(17.2)
	Total	207	18(8.7)	36(17.39)	9(4.35)	96(46.38)	15(7.25)	6(2.9)	2(0.97)	36(17.39)

of the dorsal plate, anal plate, and genital pore. Fleas and lice were cleared in a series of reagents to make their features clear for easy identification. They were soaked in 10% potassium hydroxide for 24 hours for decolorization purposes/-clearing, then in normal saline for 30 minutes to remove potassium hydroxide, followed by dehydrating in a series of ethanol (10% ethanol, 70% ethanol, 80% ethanol, and absolute ethanol, each for 30 minutes) then dried and fixed in methyl salicylate for 20 minutes for clarification of exoskeleton and thereafter cleared again in xylene for an hour and finally the DPX (Dibutyl phthalate Polystyrene Xylene) mordant was used to mount fleas and lice on microscopic slides for examination. OPTA-TECH® Stereo Microscope with an objective lens of 45x was used to observe different features of ectoparasites. Fleas, ticks, mites, and lice were identified at the species level according to published morphological keys (Harimalala, et al., 2021). Thin blood and organ

impression smears were stained with 10% Giemsa stain for 30 minutes and 25 minutes respectively. The slides were flushed with water to remove excess stains and allowed to air dry before examination under a microscope. OPTA-TECH® compound microscope was used to observe the stained blood smear and approximately 200 fields of vision in each smear were examined at 100x objective lens with the aid of oil immersion (Siński, et al., 2006; Thane, et al., 2009). Identification of blood parasites was based on morphological features of parasites and their blood stages in parasitized red blood cells using protocols explained by Thane, et al., (2009).

Data Analysis

Collected data were statistically analyzed using generalized linear models with logistic regression in R software version 4.2.2 to show the association between hemoparasites infection/ectoparasite infestations in rodents and shrews with

Table 3. Logistic Regression Table (An Association of Hemoparasites Infection)

Variable	Term	Estimate	Odds Ratio	Standard Error	z value	P Value
	(Intercept)	-16.776	-0.06	2662.86	-0.006	0.9949
Altitude	Low altitude	-0.3398	-2.943	0.35433	-0.959	0.3376
	Mid altitude	0.52006	1.923	0.39058	1.332	0.1830
Habitat	Indoor habitat	-0.1846	-5.417	0.74571	-0.248	0.8044
	Livestock boma	-0.0448	-22.32	0.60053	-0.075	0.9405
	Peri domestic	0.01553	64.392	0.4032	0.039	0.9692
Sex	Male	-0.9614	-1.04	0.31054	-3.096	0.0019*
Age	Juvenile	-0.5042	-1.983	0.42878	-1.176	0.2396

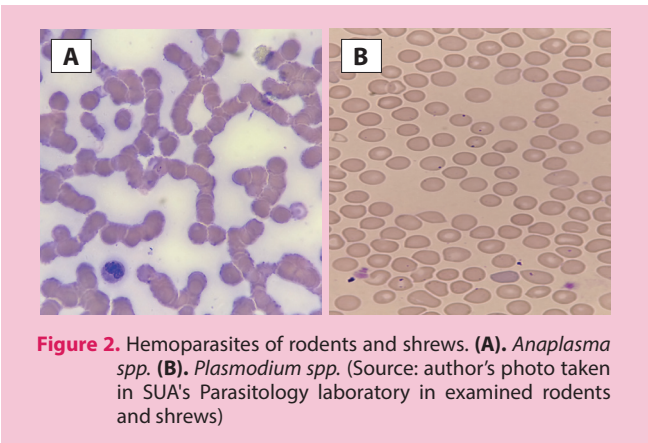
Note: CV= Crop vegetation, H= House, PD= Peridomestic

Table 4. Logistic Regression Table (An Association of Ectoparasites Infection)

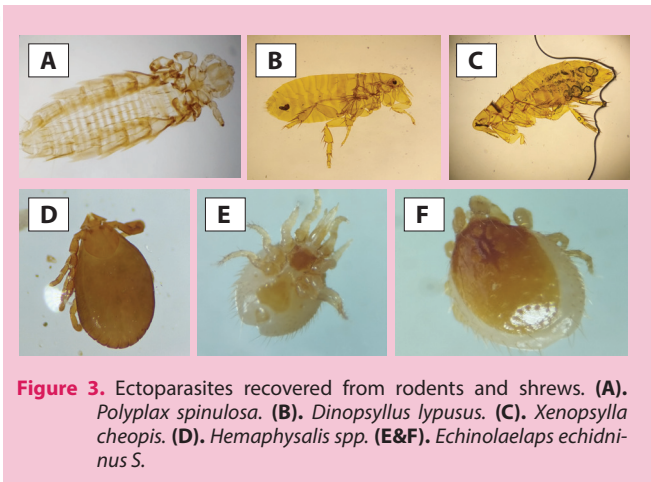
Variable	Term	Estimate	Standard Error	t value	P value
	(Intercept)	-0.3873	0.59252	-0.654	0.5135
Altitude	Low altitude	0.5794	0.24553	2.36	0.0185*
	Mid altitude	0.05859	0.33179	0.177	0.8599
Habitat	Indoor	-0.5808	0.38542	-1.507	0.1322
Age	Juvenile	0.17591	0.57032	0.308	0.7578
	Weight	0.00712	0.00604	1.179	0.2388

Table 5. Diversity of Ectoparasites and Hemoparasites Infection

Parameters	Category	Shannon Wiener Diversity Index (H)	
		Ectoparasites	Hemoparasites
Altitude	High altitude	0.864	0.986
	Low altitude	0.923	0.652
	Mid altitude	0.801	1.082
Habitat	Indoor	0.639	0.637
	Peri domestic	0.656	1.012
	livestock boma	0.837	0.5
	Farmland	1.056	0.808



body parameters, altitude and habitat as explanatory variables. The findings were considered statistically significant at $p \text{ value} < 0.05$. Diversity and distribution of hemoparasites and ectoparasites at different altitudes and habitats were computed by using Shannon-Weiner diversity in R software whereby a $H = -\sum (p_i) \ln(p_i)$ whereby $p_i = n_i/N$, n_i = a number of individuals of species, N = a total number of individuals of all species p_i = relative abundance of species, H = Shannon Diversity Index (Shannon and Weaver, 1949), using Vegan Package in R software.



RESULTS

Distribution of Ectoparasites Infestation

A total of 713 ectoparasites belonging to five taxa (Figure 3) were obtained from 114 infested rodents and shrews. Of which *Echinolaelaps echidninus* were 483 (67.74%), followed by *Polyplax spinulosa* 198 (27.77%), *Hemaphysalis spp.* 24 (33.66%), *Dinopsyllus lypusus* 6 (0.84%), and *Xenopsylla cheopis* (Figure 3). The overall prevalence of ectoparasites infestation in rodents and shrews was 55.07% (114/207). About 33.33% (38/114) of infested hosts harbor at least two or more ectoparasites species. *Echinolaelaps echidninus* was the most dominant species with a prevalence of 46.38% (96/207), followed by *Polyplax spinulosa*, *Hemaphysalis spp.*, *Dinopsyllus lypusus*, and *Xenopsylla cheopis* (Table 1). Among the captured rodents, *Beamys spp.*, *Grammomys spp.*, *Praomys spp.*, and *Gerbilliscus spp.* both had a 100% prevalence of ectoparasite infestation followed by *Mus musculus*, *Mastomys natalensis*, *Arvicanthis niloticus*, *Otomys spp.*, and *Rattus rattus* (Table 1). Shrews (*Petrodromus spp.*) were infested with lice (*Polyplax spinulosa*) only and no infestation was observed in *Crocidura spp.* The distribution of ectoparasites at different habitats, altitude zones, age, and sex of rodents and shrew species is shown in Table 2. There was a significant association of ectoparasites infestation with altitudes where more rodents and shrews found at low altitudes were likely to be infested with ectoparasites as compared to other altitudes ($p \text{ value} = 0.0185$, odds ratio = 0.5794) (Table 4). Moreover, ectoparasites infestation in rodents and shrews found at low altitudes and farmlands was found to be more diverse with a diversity of 0.923 and 1.056, respectively, as compared to other altitudes and habitats (Table 5).

Distribution of Hemoparasites Infection

Rodents and shrews were found to be infected with three species of hemoparasites identified as *Anaplasma centrale* (*A. centrale*), *Anaplasma marginale* (*A. marginale*), and *Plasmodium spp.* (Figure 2). The overall prevalence of hemoparasites infection was 24.15% (50/207), dominated by *A. centrale* with a prevalence of 16.43% (34/207) followed by *Plasmodium spp.* and *A. marginale* 8.21% and 4.35%, respectively. Out of 50 infected rodents and shrews, only 20% were infected with two hemoparasites species, most commonly multiple infections

were between *A. centrale* and *Plasmodium spp.* seven cases compared to three cases of *A. centrale* and *A. marginale*. Among the captured rodents *Grammomys spp.* had a prevalence of 100% (1/1), followed by *Tatera spp.*, *Mastomys natalensis*, *Arvicanthis niloticus*, *Mus musculus*, and *Rattus rattus* (Table 1). *Mastomys natalensis* and *Arvicanthis niloticus* were found to be infected with all hemoparasites species obtained in this study and for shrews hemoparasites infection was dominant in *Crocidura spp.* with a prevalence of 66.67% (2/3), followed by *Petrodromus spp.* 25% (1/4) and they were infected with *Anaplasma spp.* and *Plasmodium spp.* but the infection was low as compared to rodents (Table 1) The distribution of hemoparasites infection at different altitude zones, ecological habitats, sex and age is shown in the Table 2. There was a significant association of hemoparasites infection with host sex whereby male individuals had a lower chance of infection as compared to female individuals (p value=0.0019, odds ratio=-1.04), other factors had no significant effect on hemoparasites infection (Table 3). Moreover, hemoparasites infection in rodents and shrews found at mid-altitude and peri domestic was found to be more diverse with a diversity index of 1.082 and 1.012 respectively, as compared to other altitudes and habitats (Table 5).

DISCUSSION

The present study highlights the basic information on the diversity and distribution of ectoparasites and hemoparasites in rodents and shrews collected in selected wards in the Iringa District attributed to altitude variations and other factors. Thirteen species of rodents were captured and two species of shrews were collected. The collected parasites comprised five species of ectoparasites and three species of hemoparasites. The overall prevalence of ectoparasites and hemoparasites were 55.07% and 24.15%, respectively. Five ectoparasites species were collected which are *Echinolaelaps echidninus*, *Polyplax spinulosa*, *Hemaphysalis spp.*, *Dinopsyllus lypusus* and *Xenopsylla cheopis*; hemoparasites species obtained were *Anaplasma marginale*, *Anaplasma centrale*, and *Plasmodium spp.*

The higher prevalence of ectoparasites was also reported in other studies done elsewhere (Hamidi and Bueno-Marí, 2021; Shilereyo, et al., 2022; Babyesiza, et al., 2023; Gebrezgiher, et al., 2023). The presence of hemoparasites and ectoparasites in rodents and shrews suggests that these small mammals are reservoir hosts of these parasites. Among collected ectoparasites mites were most dominant followed by lice, ticks, and fleas. The higher prevalence of mites in rodents may be because they can infect a wide number of host species, enabling them to maintain their population in a particular habitat or given ecosystem (Mawanda, et al., 2020). The dominance of mite species in this study is similar to other studies conducted by Mawanda, et al., (2020) and Babyesiza, et al., (2023) in Uganda. Members of the genus *Echinolaelaps* are known to be vectors of leptospirosis and dermatitis-causing pathogens (Babolin, et al., 2016). Lice (*Polyplax spinulosa*) was the second most common ectoparasite in the present study. It is known as spine rat lice and blood-sucking lice, heavy infestation results in dermatitis and anemia (Vignesh-

war, et al., 2021). *Polyplax spinulosa* serves as a vector of *Mycoplasma muris*, *Brucella brucei*, *Borrelia duttoni*, and *Rickettsia typhi* (Baker, 2007). *Xenopsylla cheopis*, *Dinopsyllus lypusus*, and *Hemaphysalis spp.* were least observed in the current study this may be due to their behaviors as they live temporarily to the host as compared to mites and lice. *Dinopsyllus lypusus* was most abundant flea than *Xenopsylla cheopis*; this result is in line with the study conducted by Kessy, et al., (2024) in Mbulu who reported a higher number of this flea in *Mastomys natalensis*. *Dinopsyllus lypusus* was most observed in *Mastomys natalensis* and *Arvicanthis niloticus* whereas *Xenopsylla cheopis* was observed in *Rattus rattus* and *Arvicanthis niloticus*. *Dinopsyllus lypusus* is a principal flea vector of wild rodents and zoonotic vector of *Bartonella spp.* and *Rickettsia spp.* whereas *Xenopsylla cheopis* is the principal vector of *Yersinia pestis* (Babyesiza, et al., 2023). In this study, the ectoparasites infestation was more highly observed in rodents than shrews; this may be attributed to few number of shrews collected in comparison to rodents, also differences in ecology and behaviors of the two groups of small mammals (Babyesiza, et al., 2023). Rodents are considered herbivores while shrews are primary insectivores; therefore, it can be argued that shrews can potentially prey on their ectoparasite unlike rodents, thereby contributing to their low infestation (Hagenah, et al., 2009; Babyesiza, et al., 2023). Of the two species of shrews collected, only *Petrodromus spp.* was infested with lice known as *Polyplax spinulosa*. These findings differ from other studies conducted by Islam, et al., (2020) in Bangladesh, Paulraj, et al., (2022) in India, and Babyesiza, et al., (2023) in Uganda who reported ectoparasites in other shrews species like *Crocidura spp.*, and *Suncus murinus*. *Mastomys natalensis* was the most infested rodent with different species of ectoparasites as compared to other species; this could be attributed to the ability of this species to occupy a wide range of environments which increases their chance to encounter parasites (Mlyashimbi, et al., 2019).

This study has established that rodents and shrews collected at low-altitude villages were more infested with ectoparasites compared to other villages; this could be due to different eco-climatic conditions at both mid and highland areas, as at lowland there is high temperature which provides favorable conditions for the development of parasites but also low numbers of rodents and shrews hosts at high altitudes areas which contribute less movement of hosts and hence the low abundance of ectoparasites (Gebrezgiher, et al., 2023). But this was different from flea species, in our present study more fleas were collected from high and mid-altitude only, and these results are similar to other studies conducted by (Eisen, et al., 2012; Meliyo, et al., 2014). Other factors such as sex were observed to have no significant association with ectoparasite infestation in rodents and shrews in the present study, this is different from Shilereyo, et al., (2022) and Gebrezgiher, et al., (2023) which reported the impact of sex on ectoparasite infestation of which male rodents were more infested than female. Ectoparasites of rodents and shrews found at farmland and low altitude were more diverse than those found in other habitats and altitudes. The higher diversity of ectoparasites in farms and lowland areas may have

contributed to a high number of rodents and shrews collected in those areas, as hosts are the habitat of a particular parasite. Farmlands and lowland areas provide favorable environmental conditions and food resources for small mammals which influences breeding and foraging, which leads to the increase of their diversity and abundance, hence an increase in the diversity of ectoparasites due to the availability of different host species. But also, agriculture activities provide food and habitat resources for small mammals, hence ectoparasites thrive in these environments due to the presence of suitable hosts. Land use which includes agriculture activities causes changes in the microclimate and habitat quality which in turn leads to resource availability for small mammals, and consequently shapes the structure and abundance of their parasite assemblages, hence higher ectoparasites load (Shilereyo, et al., 2022). But also increased parasitic abundance in intensively used habitats by humans may be influenced by low immunity status in small mammals due to higher stress levels which are caused by increased pressure on habitats by humans (Yin, et al., 2020). Furthermore, this study revealed that hemoparasites infections were prevalent among rodents and shrews. The detected hemoparasites were *Anaplasma marginale*, *Anaplasma centrale*, and *Plasmodium* spp. These results are similar to other studies conducted by Katakweba, et al., (2012) and Islam, et al., (2020) in Tanzania and Bangladesh, respectively, which reported the presence of these parasites in rodents and shrews. However, Plasmodium infection was lower than Anaplasma infection. Low Plasmodium infection in this study may be due to the availability and behavior of mosquito parasites which live temporarily in their host as they visit the host for blood feeding only, but also environmental conditions such as habitats, humidity, temperature, and rainfall which affect biting rates, development of parasites within host and mosquito abundance (Ngowo, et al., 2017). These results align with a study conducted by Makokha, et al., (2011) in Kenya who reported low Plasmodium infection in rodents but different from Alias, et al., (2014) who reported higher infection of Plasmodium. On the other hand, the infection with Anaplasma species was high in rodents and shrews. The higher level of Anaplasma infection in small mammals may be attributed to their nesting and foraging behavior in different habitats such as grasslands, and dense vegetation which expose them to ticks (Pfaffle, et al., 2013). Of the captured rodents and shrews, *Mastomys natalensis* was most infected followed by *Arvicanthis niloticus* and *Cricetura*. The difference in the level of infection between rodents and shrew species may be attributed to environmental factors which support the development of parasites but also the presence of parasites in the host depends on the availability of a suitable host (Krasnov, et al., 2008; Mize, et al., 2011).

Sex was found to have a significant effect on hemoparasites infection in rodents and shrews, whereby males were less infected than females. The higher infection in females than males is because of more females collected in the present study. This is different from other studies which reported higher infection in males than females which is due to their behavioral and ecological conditions whereby males have a

wider home range than females increasing the likelihood of encountering new parasites; furthermore, the physiological composition of males having high testosterone immunosuppressive characteristics tends to decrease the body's defenses against parasites (Krasnov, et al., 2021). Blood parasites of rodents and shrews found at mid altitudes and peri domestic habitats were found to have more diverse as compared to those found at other habitats and altitudes. More hemoparasites species were found in these areas due to the favorable environmental conditions but also a close interaction with human which increased their chance of getting infection and also the availability of food and habitat resources.

CONCLUSION

The findings from this study provide baseline information on the influence of various factors in the distribution and diversity of hemoparasites and ectoparasites infestation in rodents and shrews and open the door for further investigations and research. This study provides information on parasites that are present in rodents and shrews which may help responsible authorities to develop methods that can be used to control rodent populations.

ACKNOWLEDGEMENT

The authors extend their sincere appreciation to the Iringa Regional and District Authorities for granting the necessary permissions to conduct this research within their jurisdiction. The authors also gratefully acknowledge the contributions of laboratory technicians Mr. Salim Bwata and Mr. Mabula Kashindy, as well as Ms. Rukia Amiry, Mr. Lusajo, and Mr. Joshua Jakonico, for their valuable assistance during field data collection.

CONFLICT of INTEREST

The authors declare no conflict of interest

FUNDING INFORMATION

The African Centre of Excellence for Innovative Rodent Pest Management and Biosensor Technology Development project (ACE-II-IRPM&BTDP project number 5799/TZ), Sokoine University of Agriculture, provided funds for this research

ETHICAL APPROVAL

The procedures to conduct this research were performed based on approval by the Institutional Ethical review board of the Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy of Sokoine University of Agriculture with Reference number SUA/DPRTC/R/186 VOL IV 67.

AUTHORS' CONTRIBUTIONS

NN is the principal investigator in proposal writing, data collection, data analysis interpretation, and manuscript writing. JN and EM were responsible for drafting and reviewing the paper.

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