

Enteric Bacteria and Helminths in *Rattus rattus* in Tema Community One Market: Potential for Zoonotic Disease Transmission

E. Abrefi¹, H. Arthur¹, F. G. Acheampong¹, J. E. Futse² and G. Futagbi^{1*}

¹ Department of Animal Biology and Conservation Science, College of Basic and Applied Sciences, University of Ghana, Accra, Ghana

² Department of Animal Science, College of Basic and Applied Sciences, University of Ghana, Accra, Ghana

*Corresponding Author: gfutagbi@ug.edu.gh

Abstract

Rattus rattus is a significant pest in urban settings and also acts as reservoir host for zoonotic diseases, posing public health challenges. The abundance of *R. rattus* in the urban environment, due to ample food and availability of shelter, makes them significant potential reservoir hosts and vectors of diseases. This study aimed to investigate the prevalence of enteric helminth and bacteria in *R. rattus* in Tema Community One market, Ghana, and to assess the potential for transmission of zoonotic diseases. Zinc Sulphate flotation method was used for the parasitological examination. Conventional technique of bacterial culture and standard biochemical tests were used for the identification and characterization of bacterial isolates. Out of the 30 rats examined, 93.3% (28/30) were infected with at least one helminth. Helminth genera identified were *Hymenolepis*, *Strongyloides*, *Taenia*, *Moniezia*, *Toxocara*, *Enterobius*, *Ascaris*, *Capillaria*, *Fasciola*, and *Trichostrongyle*-type. The most common helminth was *Hymenolepis* spp. (80%), including *H. nana* (33.3%) and *H. diminuta* (50.0%). Additionally, 76.7% (23/30) of the rats harboured three or more parasite genera, and 50.0% (15/30) were infected with at least four different parasite species. Generally, infections were of low to moderate intensity, except for *Hymenolepis* spp., which showed high (501-2000 EPG) to very high (> 2000 EPG) intensity in a significant proportion of the rats. The most common enteric bacterium identified in the rats was *Escherichia coli* (86.7%), followed by *Serratia marcescens* (83.3%), *Proteus mirabilis* (76.7%), and *Salmonella* spp. (66.6%). Variations in the frequency of helminth and bacterial infections were observed among the rats. The study highlights the significant prevalence of pathogenic enteric bacteria and helminths in *R. rattus* populations in the Tema Community One market and indicates a potential for zoonotic disease transmission.

Keywords: Rat, helminth, parasite, enteric bacteria, zoonotic

Introduction

Helminthic parasites and pathogenic enteric bacteria are significant health concerns for both animals and humans due to their ability to cause a range of diseases (Murray et al., 1996; Strand & Lundkvist, 2019; Dahmana et al., 2020). Helminthic infections, such as those caused by various species of cestodes, nematodes, and trematodes, contribute to malnutrition, anaemia, organ damage, cognitive impairments, reproductive health issues, and gastrointestinal issues like diarrhoea and dysentery, significantly affecting the quality of life of affected individuals (Murray et al., 1996; Sartorius et al., 2021; Hotez et al., 2008; Fauziah et al.,

2022). Common pathogenic enteric bacteria include *Escherichia coli*, *Salmonella* spp., and *Shigella* spp. These pathogens are prevalent in wildlife, cattle, sheep, poultry, and swine, causing severe diseases such as enteritis and septicaemia in livestock, as well as diarrhoea, dysentery, and food poisoning in humans (WHO, 2015; Rahman et al., 2020).

Studies have demonstrated the carriage and spread of parasites and pathogenic bacteria by wildlife, livestock, pets, and pests (Gratz, 1994; Rahman et al., 2020; Oduro et al., 2024). This study focuses on helminths and pathogenic enteric bacteria carriage by *Rattus rattus*. Commonly known as the black rat or house rat, *R. rattus* is a highly adaptable and abundant rodent species found in various

environments, including natural, built, and urban ecosystems (Stenseth et al., 2003; Feng & Himsworth, 2014). The abundance of the black rat is particularly high in urban centres such as markets, residential areas, and poorly managed dump sites, due to the continuous availability of food and shelter (Stenseth et al., 2003).

While *R. rattus* play important roles in the ecosystem, their interactions with humans often result in significant health and economic challenges. These rats are considered major pests in agricultural and urban settings and are potential reservoir hosts and vectors of zoonotic diseases (Stenseth et al., 2003; Meerburg et al., 2009). They acquire pathogens through exposure to human waste and, through their feeding activities in the urban sprawl, contaminate food supplies with pathogens in their excrements and spread diseases (Gratz, 1994; Himsworth et al., 2013). Some important enteric zoonotic pathogens found in *R. rattus* and other *Rattus* species include *Cyclospora cayetanensis*, *Cryptosporidium* spp. and *Giardia lamblia* (protozoans), *Trichinella* spp., *Strongyloides* spp., *Trichuris muris*, *Hymenolepis* spp. and *Taenia* spp. (helminths), *Salmonella* spp. and *Escherichia coli* (bacteria), and rat hepatitis E virus (ratHEV) (virus) (Mafiana, et al., 1997; Meshkekar et al., 2014; Batista et al., 2016; Coello Peralta et al., 2023; de Cock et al., 2023; Oduro et al., 2024). Non-enteric pathogens of zoonotic importance associated with *Rattus* species include *Toxoplasma gondii*, *Babesia* spp., *Bartonella* spp., *Leptospira* spp., *Borrelia* spp., *Rickettsia* spp., *Anaplasma phagocytophilum*, *Streptobacillus moniliformis* and *Coxiella burnetii* (bacteria), as well as cowpox virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (viruses) (Fitte et al., 2021, de Cock et al., 2023).

Recent studies indicate that enteric bacterial and helminth infections in *R. rattus* are prevalent in urban environments, where rats are in close contact with human populations (Fitte et al., 2021; de Cock et al., 2023). The synanthropy of *R. rattus* increases the risk of

disease transmission. Tema Community One market, a bustling commercial hub where food and other goods are traded daily, faces challenges, particularly in terms of sanitation and infrastructure. The sanitation situation in the Tema Community One market, just as in many markets in Accra, provides ideal environment for the growth of the rat population. Poor waste management systems were observed in the market. This resulted in accumulation of garbage containing abundance of food waste in certain areas (G. Futagbi, personal communication, June 18, 2023). Efforts have been made to minimize rat infestation in the market and to prevent their destructive activities, but they still pose challenges in the market (GSS, 2019; Lee et al., 2022; Arthur, 2023). The presence of *R. rattus* in this market area presents a potential public health challenge.

Research on the prevalence of enteric bacteria and helminths in *R. rattus* in Ghana is limited, particularly, in the Tema Community One market. However, available studies indicate significant prevalence of pathogens in rat populations in Ghana (Bimi et al., 2021; Oduro et al., 2024). Information about the prevalence and types of helminth and bacterial infections in *R. rattus* is crucial for understanding the problem and developing effective control measures. Therefore, this study aimed to add to the body of knowledge about infectious pathogens in *R. rattus*. The prevalence of helminth and enteric bacterial species in *R. rattus* within the Tema Community One market in Ghana was investigated and the potential public health threats associated with the house rat-human interaction was assessed.

Materials and Methods

Study area

The sampling of *R. rattus* was done at the Tema community One market Accra (5.6455° N, 0.0003° E). It is the central market in the industrial city of Tema, Ghana. It is a bustling and vibrant commercial hub, renowned for its diverse trade activities, and serves as a crucial

centre for trading activities. Goods traded range from fresh produce, meats, and fish to textiles, electronics, and household items. Beyond its economic role, Tema Community One Market serves as a social hub where people gather to exchange and discuss news, and to strengthen community ties. The market boasts of mix of permanent stalls, temporary stands, and open-air vending spaces. While some areas are well developed with permanent structures, others remain less developed, featuring makeshift arrangements that accommodate a high volume of traders and customers.

Sanitation remains a significant challenge in Ghanaian markets and Tema Community One Market is not an exception. Waste disposal systems are often inadequate, leading to the accumulation of garbage in some areas. These insanitary conditions and abundance of food remnants attract many animals, particularly rodents, to the market areas.

Sample collection

A total of 30 rats were trapped at night, using fish baits, at various locations in the market. They were transported to the laboratory for identification and sampling of faeces. Those that were alive were euthanized with chloroform. They were then dissected to remove the intestines. Faecal samples were collected from intestine and placed immediately into sterile Eppendorf tubes. The samples were stored in the fridge at 4°C for analyses.

Analysis of Samples

Parasitological examination

The Zinc Sulphate flotation method was used for processing the samples for parasitological examination as described in our previous article (Owusu et al., 2023). Briefly, two grams of faecal matter were weighed and pressed through a strainer into a dish. Subsequently, 15 ml of saline (0.85%) was added to the faecal matter, and the faecal-saline mixture was sieved into a centrifuge tube. The capped tube was centrifuged at 2000 rpm for two minutes. The supernatant was carefully discarded. Zinc Sulphate flotation solution (specific gravity

1.18) was then added to the tube. The tube was gently shaken to resuspend the sediments in the flotation fluid and centrifuged again at 1500 rpm for two minutes. The surface solution was carefully removed using a Pasteur pipette and transferred into a tube. A drop of this solution was then placed on a glass slide, stained with iodine solution, and covered with a coverslip for microscopic examination. The slides were examined under the 10× and 40× objective lenses of a light microscope to identify and estimate helminth eggs. The identification of helminths, based on morphological features, was performed using standard practical manuals (Ballweber, 2001).

Bacteria culture

Conventional technique of bacterial culture was conducted on the faecal samples. Briefly, nutrient agar medium for culturing bacteria was prepared and sterilized through autoclaving at 121°C for 15 minutes to eliminate any pre-existing contaminants. The agar was then cooled to approximately 45°C to remain liquid, but not hot enough to harm the bacteria being cultured. One gram of the rat faecal sample was weighed and mixed with 2 ml of saline, after which serial dilution was prepared to lower the density of cell culture to a more workable concentration. The cooled sterile agar was poured into the Petri dish, thoroughly mixed with the faecal sample-saline mixture by swirling, left to solidify, and incubated at 37°C for 48 hours. Distinct bacterial colonies were observed and counted. The bacterial isolates were subcultured on nutrient agar to obtain pure colonies. After gram-staining, identification and characterization of isolates were achieved through motility and standard biochemical tests (triple sugar iron (TSI), indole, motility, oxidase, catalase, citrate and urease tests).

Statistical Analysis

The charts were drawn using either Microsoft excel 2016 software or GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA). The prevalence of helminth eggs and bacteria coliforms in the faecal matter

and multiple infections were calculated as percentage of the samples. Mean intensity of infection was estimated as the mean of number of eggs per gram of faecal matter. A Z-test for proportions was employed to evaluate differences in prevalence among the different species of parasites.

Results

Diversity and Prevalence of Helminthic Parasites

Faecal samples of 30 *R. rattus*, trapped in the Tema Community One market, were examined for parasites. At least ten genera of helminths, belonging to classes Cestoda and Trematoda, and the phylum Nematoda, were

identified in the faecal samples examined. Out of the thirty rats examined, 93.3 (28/30) were infected with at least one helminth. The most common helminth shed by the rats was *Hymenolepis* spp. (80.0%), comprising both *H. nana* (33.3%) and *H. diminuta* (50.0%). Other parasites detected were Trichostrongyle-type (46.7%), *Strongyloides* sp. (36.7%), *Taenia* sp. (36.7%), *Moniezia* sp. (23.3%), *Toxocara* sp. (20.0%), *Enterobius* sp. (20.0%), *Ascaris* sp. (13.3%), *Capillaria* sp. (10.0%), and *Fasciola* sp. (13.3%) (Figure 1). The prevalence differed significantly among the various parasites identified ($p < 0.05$).

Intensity of Infection

The intensity of helminthic parasite infections in the sampled *R. rattus* was quantified in terms of eggs per gram (EPG) of faecal matter.

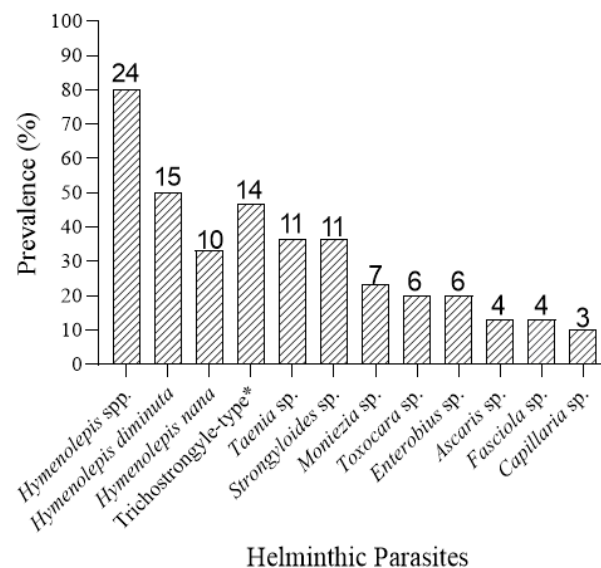


Figure 1 Proportion of *R. rattus* infected with helminths in Tema Community One market

TABLE 1
Helminth infection intensity based on eggs per gram (EPG) of rat faeces

Parasite	Load (EPG)			Intensity of infection (% of all rats examined)			
	Mean	SEM	Range	Low	Moderate	High	Very high
<i>Hymenolepis</i> spp.	1153	263.4	0 – 4350	16.7	6.7	40.0	20.0
<i>Taenia</i> sp.	276.9	53.0	0 – 550	16.7	23.3	16.7	0
Trichostrongyle-type	245.0	35.3	0 – 400	10.0	40.0	0	0
<i>Moniezia</i> sp.	87.5	12.5	0 – 100	20.0	0	0	0
<i>Ascaris</i> sp.	175.0	125.0	0 – 550	10.0	0	6.7	0
<i>Strongyloides</i> sp.	281.8	29.6	0 – 450	6.7	33.3	0	0
<i>Toxocara</i> sp.	137.5	87.5	0 – 400	16.7	6.7	0	0
<i>Capillaria</i> sp.	50.0	0	0 – 50	10.0	0	0	0
<i>Enterobius</i> sp.	237.5	31.5	0 – 300	0	20.0	0	0
<i>Fasciola</i> sp.	137.5	71.8	0 – 350	16.7	6.7	0	0

SEM: standard error of the mean; Low infection (1- 100 EPG); moderate infection (101- 500 EPG); high infection (501-2000 EPG); very high (> 2000 EPG) as described by Nwafor (2019)

Infections were generally of low (1- 100 EPG) to moderate (101- 500 EPG) intensity, except *Hymenolepis* spp., which showed high (501-2000 EPG) to very high (> 2000 EPG) intensity in a significant proportion (60%) of the rats (Table 1).

Multiple Infections

Out of the 30 *R. rattus* samples examined, 93.3% (28/30) were infected with at least one parasite genus. Furthermore, 76.7% (23/30) of the samples harboured three or more parasite genera, and as many as 50.0% (15/30) were found to be infected with at least four different parasite species (Figure 2). This high prevalence of multiparasitism indicates significant parasite richness and co-infection within the sampled rat population.

Frequency of bacteria isolates from the rats

Several enteric bacterial species were identified in the rat faecal samples, consisting of *Escherichia coli*, *Salmonella* spp., *Klebsiella pneumoniae*, *Citrobacter freundii*, *Enterobacter* spp., *Serratia marcescens* and *Proteus mirabilis*.

The infection rates for these bacteria were notable, with each species infecting 40% or more of the rats examined. The most common bacterium was *E. coli*, detected in 86.7% of the rats. This was followed by *Serratia marcescens* (83.3%), *Proteus mirabilis* (76.7%), and *Salmonella* spp. (66.6%). Variations in the frequency of infection with each bacterial species were observed among the rats (Figure 3).

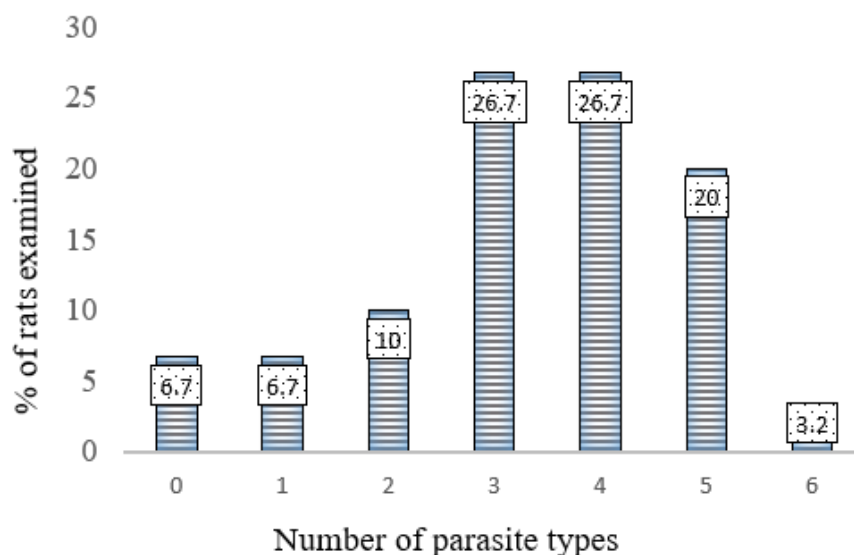


Figure 2 Frequency of multiparasitism in *R. rattus* in Tema Community One market

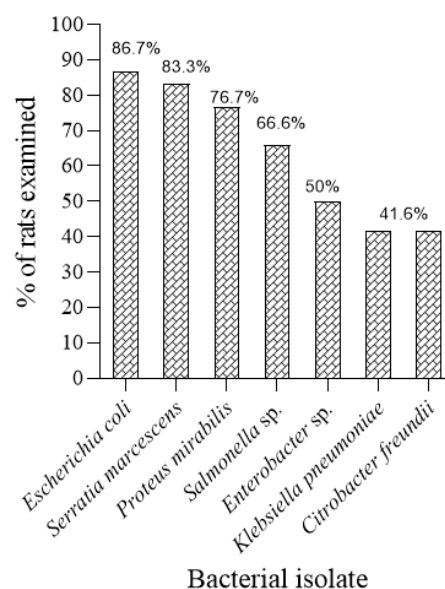


Figure 3 Prevalence of enterobacteria in *R. rattus* in Tema Community One market

Discussions

Wildlife plays a significant role as reservoirs for emerging, and re-emerging infections and their interactions with humans are known to promote development and transmission of zoonoses (Cunningham et al., 2017). The intensity and frequency of interactions between humans and nonhuman animals have been heightened by urbanization and inadequate sanitation (Battersby et al., 2002; Soulsbury and White 2015; Kuddus et al., 2020). It is not surprising that rodents such as *Rattus rattus* are abundant in the urban sprawl because ideal conditions are created for them to thrive. The abundance of *R. rattus* in the urban environment, particularly in markets in relation to their disease reservoir and vector status, makes them potential threat to public health (Meerburg et al., 2009; Antoniou et al., 2010; Himsworth et al., 2013; Tijjani et al., 2020; Bimi et al., 2021). In this study, we investigate helminthic parasites and enteric bacteria carriage in *R. rattus* within the Tema Community One market in Ghana.

This study revealed at least 12 genera of helminthic parasites in the rats and almost all the rats (93.3%) were infected with at least one helminth genus, indicating high parasite species richness and infection rates, respectively. The most common helminth eggs shed by the rats was those of *Hymenolepis* spp. (80%). The high prevalence of *Hymenolepis* spp. might be due to the small home range of the population of rats in the market area, promoting the maintenance of infection in the population (Meshkekar et al., 2014). Both *Hymenolepis nana* and *Hymenolepis diminuta* were identified. This is not the first time *H. nana* has been reported in wildlife. It is a common parasite in *R. rattus* (Siti Shafiyyah et al., 2012; Gliga et al., 2020; Coello Peralta et al., 2023). The presence of *H. nana* in the rats is particularly significant, as they can contaminate the environment and infect humans directly without an intermediate host (Schantz, 1996; CDC, 2024). *H. diminuta* (rat tapeworm) is one of the parasites to expect in the rats and no wonder it was

higher in prevalence (50.0%) compared to *H. nana* (33.3%). Though *H. diminuta* is less common in humans, accidental infections are known (Ahmad et al., 2017; Galoş et al., 2022). Hymenolepiasis, a disease caused by *Hymenolepis* spp., is characterized by gastrointestinal distress, weakness, headaches, diarrhoea, eosinophilia, and anaemia, among other health issues (Coello Peralta et al., 2023; CDC, 2024).

Other parasites identified include *Ascaris*, *Strongyloides*, *Taenia*, *Moniezia*, *Toxocara*, *Enterobius*, *Capillaria*, *Fasciola*, and *Trichostrongyle*-type. Some *Ascaris* spp. (*A. lumbricoides* and *A. suum*, primarily found in humans and pigs, respectively) are zoonotic and have been detected in wildlife (Okulewicz et al., 2002). But we are not sure the species infecting these rats were any of the above or zoonotic. Further study is required to identify the species infecting the rats. Species of *Strongyloides* are zoonotic. *S. stercoralis*, although primarily a human parasite, has been reported in dogs and cats, which have now become reservoir hosts for the parasite (Mafiana et al., 1997; Jaleta et al., 2017; Wulcan et al., 2019). Its cousin species, *Strongyloides fuelleborni*, *S. ratti*, and other parasites such as *Toxocara* spp., *Capillaria* spp., and *Fasciola* spp. are parasites of non-human animals, but have assumed zoonotic status (Beknazarova et al., 2016; Delahoy et al., 2018; White et al., 2019). Conversely, the human pinworm, *Enterobius vermicularis*, which is primarily a human parasite, has been reported in other animals (Medkour et al., 2020; Owusu et al., 2023), but not in rodents (Knopp et al., 2012). This is the first time *E. vermicularis* has been identified in the *R. rattus*. This implies that non-human animals have become reservoir hosts of the human pinworm. Many *Taenia* species (tapeworms), are also known to infect rats, which usually serve as intermediate hosts. The *Taenia* species seen in this study have the rats as definitive host and, therefore, are less likely to be *T. saginata* or *T. solium*. Moreover, though zoonotic, *T. saginata* and *T. solium* have not been reported in rodents. But *T. taeniaeformis*

and *T. crassiceps*, which are also zoonotic, have been detected in *R. rattus* (Mafiana, et al., 1997; Mohd Zain et al., 2012). The presence of *Taenia* spp. in these rats is significant because *Taenia* spp. are zoonotic. In humans, infection with *Taenia* spp. can lead to severe health conditions, including organ damage and allergic reactions (Holland & Smith, 2006). *Moniezia* spp. was another tapeworm identified in the *R. rattus*. They are tapeworms of ruminants and not commonly associated with rats. Since zoonotic infection of *Moniezia* was detected in an Egyptian shepherd (el-Shazly et al., 2004), no other human infections have been reported, indicating very low risk for zoonotic transmission. However, their presence in the rats reveals the potential for zoonotic spillover (Ellwanger & Chies, 2021). Their transmission to domesticated animals may indirectly negatively affect humans through treatment costs and loss of farm animals (Hamid et al., 2023). Another group of helminths identified in this study is the Trichostrongyle-type nematodes. They are a diverse group of gastrointestinal parasites that can infect a wide range of hosts, including humans and non-human animals. Members of this group are not reliably distinguishable by eggs. Members of Trichostrongyle-type nematodes that are zoonotic are hookworms (*Bunostomum* spp., commonly found in cattle, *Ancylostoma duodenale* and *Ancylostoma caninum*, helminthic parasites of humans and dogs, respectively and are the commonly reported species in Africa) (Sugathan & Bhagyanathan, 2016; Lauwers et al., 2018; Stracke et al., 2020). Human infections with hookworms are associated with dermatitis, eosinophilic enteritis, cutaneous larva migrans, and lesions (Sugathan & Bhagyanathan, 2016). Other Trichostrongyle-type nematodes such as *Haemonchus* spp., *Ostertagia* spp., *Nematodirus* spp., and *Cooperia* spp. are known to infect domesticated ruminants like sheep and goats, causing significant economic losses in livestock production (Kultscher et al., 2019). Reliably identifying these species will be required to fully understand the potential danger of zoonotic transmission at the human-

wildlife interface.

A significant proportion (50.0%) of the *R. rattus* in this study harboured at least four parasite genera, indicating a high prevalence of multiparasitism. Wide variation in parasite richness was also observed. These findings are consistent with previous studies that have reported high levels of multiparasitism and variation in richness of parasite species in rodent populations, particularly, in urban *Rattus* spp. in developed countries (Rendón-Franco et al., 2013; Vaumourin et al., 2015). The high prevalence of multiparasitism observed in the rats may be attributed to various factors, including their ability to inhabit diverse environments, particularly poorly managed dump sites, omnivorous nature of their feeding, and their role as reservoirs for various zoonotic diseases (Meerburg et al., 2009; Himsforth, 2013). The co-infection with multiple parasite species may also have implications for public health, as it can increase the risk of transmission of diseases to humans (Kajero et al., 2022).

The intensities of helminth infection varied, but were generally of low (1-100 EPG) to moderate. However, *Hymenolepis* spp. had high intensity, with some infections reaching very high levels (>2000 EPG), heightening the potential for transmission of infection.

The study also identified several enteric bacterial species with infection rates above 40%. *Escherichia coli*, *Serratia marcescens*, *Proteus mirabilis*, *Salmonella* spp. *E. coli*, and *Salmonella* spp. are well-documented pathogens responsible for foodborne illnesses in humans, causing symptoms ranging from mild gastroenteritis to severe systemic infections (Todd, 2014). The high prevalence of *Salmonella* spp. is particularly of interest given its role in salmonellosis, a common and serious foodborne disease. The presence of *Klebsiella pneumoniae*, *Citrobacter freundii*, *Enterobacter* spp., and *Proteus mirabilis* also highlights the potential for these rats to act as reservoirs for bacterial pathogens that can lead to significant infections in humans, particularly in immunocompromised individuals (Podschun & Ullmann, 1998).

Conclusion

This study identified some important pathogenic enteric bacteria and helminths in *R. rattus*. These include *Hymenolepis* spp. which was the most prevalent helminth (80%), with *H. diminuta* (50.0%) and *H. nana* (33.3%) being the species identified. Notably, 76.7% of the rats were co-infected with three or more parasite genera, and 50.0% harboured at least four different parasite species. While most infections were of low to moderate intensity, *Hymenolepis* spp. infections were often high to very high in majority of rats. The most common enteric bacteria were *E. coli* (86.7%), followed closely by *S. marcescens* (83.3%), *P. mirabilis* (76.7%), and *Salmonella* spp. (66.6%). These findings underscore the potential public health threats posed by *R. rattus* in urban environments, particularly in markets where human-rat interactions are frequent. The high prevalence and intensity of parasitic and bacterial infections in these rats indicate a potential for the spread of zoonotic diseases that can pose challenges to human health. Effective control measures, including improved sanitation and rodent control programmes, are needed to mitigate the threat of zoonotic disease transmission.

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