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Preliminary Study on Ecto and Endoparasites of the Black Rats (*Rattus Rattus*) in Benghazi City

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Abstract

Forty-five adult trapped black rats (*Rattus rattus*), 24 males and 21 females, were dissected and examined in this study for ecto and endoparasites. Three ectoparasites were detected, namely, the flea (*Xenopsallya cheopis*), mites (*Ornithonyssus bacoti*), and tick (*Ixodes spp*) with 48.9%, 13.3%, and 2.2% respectively. Meanwhile, many species of endoparasites were: *Entamoeba histolytica/dispar* (73.3%), *Entamoeba coli* (31.3%), *Blastocystis hominus* (8.9%), *Giardia lamblia* (15.6), *Chilomastix sp.* (4.4%), a *coccidia* sp. (13.3%), *Hymenolips nana* (2.2%), *Hymenolips diminuta* (44.4%), and *Taenia taeniaformis* (6.7%), and *Moniliformis moniliformis* (2.2%). Of all examined rats, 97.8 % (44/45) were found to harbor at least one parasite species with no significant differences between body weight, body length, and gender of the host with the prevalence of parasite species. Multiple infections occurred at six species per rat with different combinations of parasite infections. These results are the first records of the parasitic fauna of *R. rattus* in the Benghazi area.

Keywords: *Rattus Rattus*, Black Rat, Ectoparasites, Endoparasites, Libya.

INTRODUCTION

Rats are distributed worldwide and are one of the most common rodents found in cities and their surrounding areas. In tropical and subtropical regions, Black rats (*Rattus rattus*) and brown rats (*Norwegians rattus*) are highly common in human-inhabited areas. Their origin is derived from the Fareast and distributed globally through ship trades. The breeding of rats has increased rapidly in recent years due to the abundance of food resources and the lack of environmental hygiene in urban areas (Abdel-Aal & Abou-Eisha, 1997; Mohd-Qawiem et al., 2022). Rats impose economic damages and significantly increase costs on the public health system. They can destroy food stuff, electrical equipment, and buildings by gnawing or contaminating with excreta leading to significant economic losses (Coomansingh et al., 2009). They also act as hosts for several ectoparasites and endoparasites.

In Libya, the black rat (*R. rattus*) is widely prevalent and frequently encountered among the rodent population, especially in coastal regions such as Benghazi city. It has been seen in the streets, between buildings, markets, and houses. Although there are several reports of ectoparasitic and endoparasitic infections in *R. rattus* from different parts of the world (Becir et al., 2012; Claveria et al., 2005; Daryani et al., 2023; Kataranovski et al., 2010; Menshawy et al., 2021; Ogunniyi et al., 2014; Porta et al., 2014; Shafiyyah et al., 2012; Sumangali et al., 2012), there were limited or no studies from Libya. Thus, this study aimed to conduct a preliminary survey of ectoparasites and endoparasites among the commonest rodent the black rat (*R. rattus*) in the city of Benghazi and to determine the risk of zoonotic parasites to humans.

MATERIALS AND METHODS

Study Area

This study was carried out in Benghazi city (32°10'N, 20°06'E), which is situated on the eastern southern coast of the Mediterranean Sea, north of Libya. It is the second-largest city in Libya, occupying an area of approximately 240 km². This city has a large port that serves general bulk cargoes and containers that imported from different parts of the world.

Samples Collection

All the rats were trapped alive using specially made wire box bait traps measuring 29 x 11 x 10.5 cm with a front spring door. Traps were distributed randomly indoors and outdoors of inhabited buildings just before sunset and collected by the following morning. The traps were cleaned with hot water and soap every time before reuse.

Anesthetization and killing of the study animals

The weight of each rat was obtained before anesthesia. Subsequently, each rat was placed individually in a glass flask and anesthetized using a cotton ball soaked in chloroform until it was euthanized. Additionally, the length of each rat was measured. The length of the rat was also measured.

Isolation and preservation of ectoparasites

The fur of each anesthetized rat was gently scraped and combed with a fine-tooth comb to dislodge any ectoparasite into a large white filter paper. Ectoparasites (mites, fleas, lice, and ticks) were then collected by forceps and transferred into 70% alcohol for preservation until used. A separate container was used for each animal. Preserved ectoparasites were transferred to10 % potassium hydroxide (KOH) for about 2-5 days until the exoskeleton dissolved, then washed with water to remove the excess of potassium hydroxide, transferred to serial grading of alcohol starting from 50% up to absolute alcohol, cleared in xylol, mounted with DPX on a glass slide, and examined under the microscope.

Animal dissection

In the post-mortem of rats, the skin was removed and the body cavity was slit open from the throat to the anus, revealing the esophagus, stomach, small intestine, large intestine, liver, and urinary bladder. The viscera were removed without damage, and dissected separately under the dissecting microscope and examined for helminths. All macro-parasites were washed with saline and fixed in warm 70% alcohol or 10% formalin. The contents of the small and large intestines were also examined carefully for microparasites (cysts, oocysts, trophozoites, and eggs) by direct wet mount smears and stained with 1% Lugol's iodine solution to be examined under the microscope.

Processing of cestode and acanthocephalan

Cestodes were collected individually in glass jars containing normal saline and washed many times to remove debris. The worm was then gently flattened between two glass slides that were held at the ends with rubber bands. It was then fixed in 10% formalin for 2-3 days. The specimen was dehydrated in an increasing series of alcohol starting from 30% to 100%, then transferred to carmine stain for 24 hrs. The excessive stain was removed with clove oil. The stained specimen was washed with distilled water and dehydrated with ascending grades of alcohol (70%, 80%, 90%, 100%), cleared in xylol, and mounted in DPX (Paniker & Ghosh, 2017). According to Paniker and Ghosh (2017), Priyanto et al. (2014), and Service (1996), the identification of ectoparasites and endoparasites followed established procedures outlined in previous studies.

Statistical analysis

A statistical analysis was carried out to determine the incidence and significance of the data. The logistic regression was used to find the relationships between parasitic infection, sex, length, weight, regions, and the presence or absence of the parasites (Dowdy et al., 2004). Chi-square $\chi 2$ was employed to detect the significance or non-significance of the relationships between length, body weight, sex, and parasitic infection. The accepted level of $P < 0.05$ was considered significant. The statistical program (SPSS) was used to compute all analyses within the Windows environment.

RESULTS

The examination of 45 black rats (*Rattus rattus*) in this study revealed that these rodents were host to a diverse range of parasites. The detected parasites fell into two categories: ectoparasites and endoparasites. The ectoparasites include fleas, mites and ticks, while the endoparasites include many species of protozoa, cestodes, and one species of acanthocephalan.

The protozoa parasites are *Entamoeba histolytica*, *Entamoeba Coli*, *Blastocystis hominus*, *Giardia lamblia*, *Chilomastix spp*, and one species of coccidia. Three species of cestodes are *Hymenolips nana*, *Hymenolips diminuta* and *Taenia taeniaformis*. *Acanthocephalans* are represented by only one species which is *Moniliformis moniliformis*. The overall prevalence of ectoparasite and endoparasites among the examined male and female black rats (*Rattus rattus*) collected in this study was 97.8 % (44/45) (Table 1). The ectoparasites (fleas, mites and ticks) showed a prevalence rate of 64% while endoparasites have 86.6% (Table 1).

The most common ectoparasite that has detected was the flea with a 48.9% prevalence rate, followed by mites and ticks with 13.3% and 2.2% prevalence in rats respectively. The study findings indicated that male black rats (*R. rattus*) exhibited a slightly higher rate of parasitic infection (24%) compared to females (20%), although no statistically significant differences were observed.

Table: (1). The prevalence of ecto and endoparasites in examined black rat *Ratus ratus*.

Parasite types	Number of examined rats	Number of infected	Prevalence of Infection%
Ectoparasites	45	29	64
Endoparasites	45	39	86.6
Both	45	44	97.8

On the other hand, *Entamoeba histolytica/ dispar* and *Hymenolepis diminuta* were the most dominant endoparasites with 73.3% and 44.4% respectively (table 2).

Both male (n=24) and females (n=20) of examined black rats, *R. rattus,* were infected with one or more species of parasites. Thirty-six out of forty-four rats (81.8%) have mixed parasites, and only eight (18.2%) have a single infection. There were no significant differences ($P \geq 0.05$) between male and female prevalence rats with their types of infections (Figure1).

Table: (2). The prevalence of ecto and endoparasites in examined black rat Ratus ratus.

Types of parasites	Prevalence of examined rats %	Number of infected
Flea (Xenopsally cheopis)	48.9	22
Mites (Ornithonyssus bacoti)	13.3	6
Ticks (Ixodes spp)	2.2	1
Hymenolepis nana	2.2	1
Hymenolepis diminuta	44.4	20
Moniliformes moniliformes	2.2	1
Cysticercus fasciolaris, Taenia taeniaformes	6.7	3
Entamoeba histolytica/dispar	73.3	33
Entamoeba coli	31.1	14
Coccidia spp	13.3	6
Blastocystis hominis	8.9	4
Giardia lamblia	15.6	7
Chilomastix spp	4.4	2

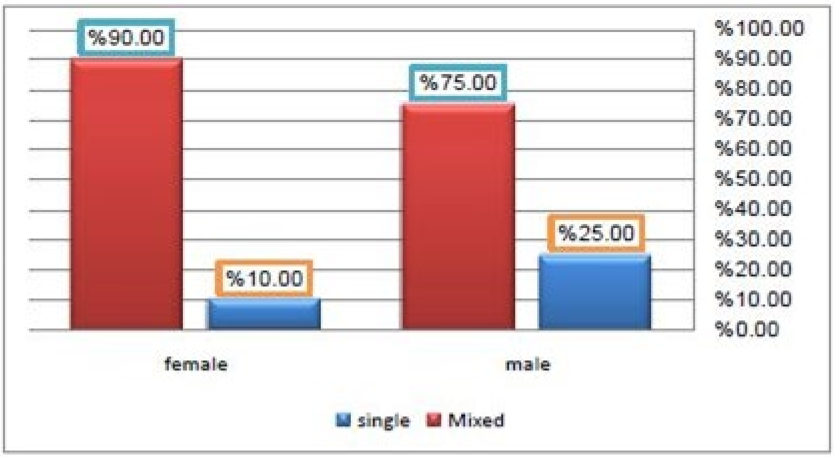

Figure: (1). Single and mixed parasitic infections among males and females of black rat (*R.rattus*).

The result also indicated that Twenty-five percent of examined rats (n = 11) were infected with three parasites, the highest level of infection, while some had more or fewer parasites. However, there were no significant differences between males (n = 24) and females (n = 20) about the intensity of parasites ($P \geq 0.05$). Male and female black rats were collected from urban (n = 33) and rural (n = 11) areas. This study showed there are no significant differences between parasitic infection rates ($P \geq 0.05$). In addition, the result showed no relationships between the examined rats' body weight, body length and parasitic infection.

DISCUSSION

In the present study, all the trapped rats belonged to only one species, which is the black rat (*R. rattus*). These rats are semi-domesticated and omnivorous and are often seen in streets, between buildings, in sewage channels, slaughterhouses, waste disposal sites, food storage, farms, and around houses in the city of Benghazi. The present investigation gives the first overview the ecto and endoparasitic infection of trapped black rats (*R. rattus*) in Benghazi, Libya of which 13 species of parasites were reported. These parasites are *Xenopslla cheopis*, *Orinthionyssus bacoti*, *Ioxodes sp*, *Hyminolepis nana*, *Hymenolepis diminuta*, *Cysticercus fasciolaris (Taenia taeniaformis), Acanthocephala*, *Entamoeba coli*, *Entamoeba histolytica/ dispar*, *Isospora sp*, *Blastocyst hominis*, *Giardia lamblia*, and *chilomastix sp*. All of them are zoonotic and have medical importance (Hernández et al., 2020; Paniker & Ghosh, 2017). The above parasites have been previously reported in black rats (*R. rattus*) from several parts of the world (Becir et al., 2012; Claveria et al., 2005; Kataranovski et al., 2010; Ogunniyi et al., 2014; Porta et al., 2014; Shafiyyah et al., 2012; Sumangali et al., 2012), and also many authors reported similar parasites in brown rats, *Rattus* norvegicus (Abu-Madi et al., 2001; Al-Bashan & Sabra, 2012; Coomansingh et al., 2009; Morsy et al., 2012; Pakdad et al., 2012; Porta et al., 2014; Priyanto et al., 2014; Stojcevic et al., 2004; Waugh et al., 2006).

The existence of rats, which act as reservoir hosts to different types of parasites in close association with human activities, may facilitate the transmission of zoonotic parasites (Amarasinghe & Premathilake, 2014; Guimarães et al., 2014; Sumangali et al., 2012). The results revealed a 97.8% overall prevalence rate of infected *R. rattus* with ectoparasites and endoparasites. Porta et al., 2014 recorded a lower incidence rate of 62.34 from the same species of rat in Brazil. Three ectoparasites were recorded on the trapped rats (*R. rattus*) with a prevalence rate of 64%. Solanki et al., 2013, reported a higher prevalence rate (67%) in black rats collected from India, while Kia et al., 2009, in Iran reported a lower prevalence rate (11.6%). The most abundant ectoparasites on trapped rats were fleas (*Xenopslla cheopis),* with an infection rate of 48.9%. This flea is the most important vector of plague, endemic typhus, and parasitic cestodes such as *H. diminuta* and *Dipylidium caninum* (Kia et al., 2009; Service, 1980). Many authors reported higher prevalence rates of fleas from different parts of the world (Becir et al., 2012; Zahedi et al., 1996), while others found lower infection rates (AL Hind & Abu-Haddaf, 2013; Ogunniyi et al., 2014; Porta et al., 2014; Sumangali et al., 2012). Tropical rat mites, *Ornithonyssus bacoti* were also collected from *R. rattus* in this study, with an incidence rate of 13.3%. This species is generally found on various animals, may temporarily also infest humans, and consequently, may be responsible for pruritic skin reactions (Baumstark et al., 2007). The same species of mites were reported from Iran (Daryani et al., 2023) and Hawaii (Yang et al., 2009) with, incidence rates of 73.9% and 14.7%, respectively. The third ectoparasite recorded in this study is a hard tick species (*Ioxodes spp.*) with an incident rate of 2.2%. Higher and lower infection rates of *R. rattus* with hard ticks were reported in different countries (Becir et al., 2012; Thanee et al., 2009; Zendehfili et al., 2015). Species of the hard tick may contribute to the spread of many viral, rickettsial, and bacterial diseases such as Russian spring-summer encephalitis, Rocky Mountain spotted fever, and Tularemia (Service, 1996).

The results of this study revealed that the overall prevalence rate of endoparasites in the examined rats was 86.6%. A high prevalence rate (58.5%) of endoparasitic infection was also recorded in black rats collected from Khan Younis and Jabalia in Palestine (AL Hind & Abu-Haddaf, 2013). The cestodes recorded in this study include *Hymenolepis nana*, *Hymenolepis diminuta*, and *cysticercus fasciolaris* (*Taenia taeniaformis*). *H. nana* is a common parasite of rodents as well as humans (Al-Bashan & Sabra, 2012). Infected persons are usually asymptomatic, but in massive infections, symptoms may include dizziness, abdominal pain, diarrhea, insomnia, and convulsions (Forbes et al., 2002). Only 2.2% of the total infected black rats (*R. rattus*) were infected with these parasites. Low prevalence rates of infection with *H. nana* were reported in rats from Ethiopia and Italy (Kassa & Assefa, 2000; Milazzo et al., 2010). On the other hand, Al-Bashan & Sabra, and Coello-Peralta two different studies, recorded a higher infection rate in black rats collected from Saudi Arabia Las, Pinas and Philippines (Al-Bashan & Sabra, 2012; Coello-Peralta et al., 2020). *Hymenolepis diminuta* is another intestinal cestode recovered from the examined black *R. rattus* with a high prevalence rate of 44.4%; this result is consistent with reports from Nigeria, Pakistan and Brazil (Guimarães et al., 2014; Okoye & Obiezue, 2008; Ramesh et al., 2021). However, many other studies showed lower infection rates (Al-Bashan & Sabra, 2012; AL Hind & Abu-Haddaf, 2013; C Milazzo et al., 2010). *H.diminuta* is a common and cosmopolitan parasite of rats and mice and is an occasional parasite of humans, with most cases reported in children (Amin, 2019; Mohd-Qawiem et al., 2022; Tena et al., 1998). The most important intermediate hosts of this parasite are rat fleas (*Xenopsylla cheopis*) and flour beetles *(Tribolium confusatum*). Therefore, the results of this study showed high infection rates of *H. diminuta* which correlated with the presence of rat fleas (*Xenopsylla cheopis*). The third cestode recovered from the examined black rats, *R. rattus,* was *Cysticercus fascioliasis* (*Taenia taeniaformis*) with a prevalence rate of 6.7%. Similar results but with higher prevalence rates were reported in the same species of rats from India (Sharma et al., 2017), Seri Lanka (Sumangali et al., 2012), and Nigeria (Ogunniyi et al., 2014). Human cases of this parasite were also recorded (Ashford & Crewe, 1998). In the current study, *Moniliformis moniliformis* was the only acanthocephalan isolated from the black rats, *R. rattus,* with an incidence rate of 2.2%. A wide range of mammals, including rats, are susceptible to *M. monilifomis* (Hernández et al., 2020). Human cases of this parasite have been reported in many countries (Paniker & Ghosh, 2017; Salehabadi et al., 2008). The result of this study is in agreement with those of many authors (AL Hind & Abu-Haddaf, 2013; C Milazzo et al., 2010; Okoye & Obiezue, 2008). The most abundant parasitic protozoa in the examined black rats *(R. rattus*) in this study were *Entamoeba histolytica/ dispar* with an infection rate 73.3%. This result is higher than those reported by Al-Bashan & Sabra, 2012, AL Hind & Abu-Haddaf, 2013 from Saudi Arabia and Palestine respectively. Non-pathogenic *Entamoeba coli* were found with a prevalence rate of 31.1%. Lower incident rats of *Entamoeba coli* in *R. rattus* were recorded by Guimarães et al., 2014 in Brazil, and Ogunniyi et al., 2014, in Nigeria. Another protozoan recorded in this study was *Giardia lamblia* with a prevalence rate of 15.6%. A similar result was reported by AL Hind & Abu-Haddaf, 2013, in Palestine black rats *R. rattus* while Al-Bashan & Sabra, 2012 found a lower incident rate (10.7%) in Saudi Arabia. *Chilomastix* spp was also detected in the examined rats with an incidence rate (4.4%). Ogunniyi et al., 2014, recorded two species of *chilomastix* namely *Chilomastix bettencourti* and *Chilomastix intestinalis* in Nigerian black rats. The results of this study showed that 13.3% of the examined black rats were infected with oocysts of unidentified species of Coccidia. This result is in agreement with that reported by Raharivololona, Rakotondravao & Ganzhorn, 2007, from Madagascar. *Blastocyst hominis* has been detected with an incident rate of 8.9%. Isolates resembling *B. hominis* have been described in a variety of mammals, birds, reptiles, and even insects (Al & Hökelek, 2007). The significance of this parasite to human infection is uncertain, but it is still one of the most common parasites isolated from stool specimens in symptomatic and asymptomatic individuals (Paniker & Ghosh, 2017).

The presence of this parasite in black rats may play a role in its epidemiology. The present study showed that males had a higher parasitic infection rate (24%) than females (20%) in black rat's *R. rattus* although no significant differences were observed. This result is consistent with those recorded by (AL HIND & Abu-Haddaf, 2013; Kataranovski et al., 2010; Porta et al., 2014). The reason behind the above situation could be attributed to males being more active than females and haveing larger home territories which could increase their exposure to infection while reproductive females show a stronger site-specific organization and also the male hormone testosterone harms the immune function (Grossman, 1989).

CONCLUSION

In conclusion, a lot of tension should be made to the role of black rats and other rodents in spreading parasitic diseases in Libya. So far, most of the recorded endoparasites in this study from black rats have been reported in humans from the city of Benghazi. Moreover, the increased population of rats in the city may increase the risk of infection with plague disease because they act as a reservoir host. Scattered foci of enzootic plague exist across the country. The last outbreak of plague was reported in the Mediterranean coastal town of Tobruk. To avoid unpleasant situations, a full program of rodent control should be implemented in the city of Benghazi and other parts of the country.

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ETHICS

Ethical approval was not sought for the present study because the number of forty-five animals was very low according to the huge amounts of rodents in the Libyan environment. The scientific procedures for killing animals were completely followed by the researchers during this research.

Duality of interest: There are no conflicts of interest regarding this research. We have no financial or non-financial relationships or affiliations with any organization or entity that could be perceived as a conflict of interest in connection with this work.

Author contributions: All authors contributed equally to this research project. They collectively conceptualized the study, designed the methodology, conducted data collection and analysis, interpreted the results, and jointly wrote and reviewed the manuscript. All authors have read and approved the final version of the manuscript.

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