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FIRST DATA ON BACTERIAL, FUNGAL AND PARASITIC INFECTIONS OF BLACK RATS (*RATTUS RATTUS*) FROM THE PALM GROVES OF THE ALGERIAN SAHARA

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ABSTRACT

The present study aimed to detect the parasitic fauna associated with black rats (*Rattus rattus*) from southeastern Algeria. It showed the presence of seven species of parasitic fungi namely *Penicillium* sp. (Prevalence Pr=91.3%), *Aspergillus niger* (Pr=91.3%), *Alternaria* sp. (Pr=58.7%), *Cladosporium* sp. (Pr=87%), *Microsporum* sp. (Pr=19.6%), *Trichophyton* sp. (Pr=21.7%) and *Chrysosporium* sp. (Pr=10.9%), noting that saprophytic fungi were the most recorded. On the other hand, according to the richness (S), adults (S = 7) and sub-adults (S = 7) of black rats were the most infested, with leaning for males compared to females, considering all the isolated species as satellites except the *Chrysosporium* sp. (2.9%) which is presented as a rare species. Concerning parasitic bacteria, aged rats were the most infected followed by adults and sub-adults where total coliforms were present in all individuals of the three classes tested. However, fecal streptococci were noted with a similar infestation rate in all age groups. Unlike this, clostridium sulfite-reducer (CSR) was mostly recorded on aged rats. Concerning the endoparasites found in the intestines of black rats, the pinworms (*Syphacia muris*, *Syphacia obvelata*, and *Aspiculuris tetraptera*) were more abundant than the other species. Hence, the current study allowed us to demonstrate that black rats can be considered an important reservoir of several microorganisms that can hold germs and represent a threat to biomedical and veterinary public health.

Keywords: *Rattus rattus*, dermatophyte fungi, parasitic bacteria, nematode, Algeria

INTRODUCTION

Rodents are considered among the most frequent and important mammals because they can adapt to different locations and environmental changes (**Seifollahi *et al*., 2016**). They act as a vital component in various ecosystems either acting as prey to their predator or as a carrier and reservoir of the diseases (**Okoye and Obiezue 2008**). It is well recognized that they harbor several ecto and endoparasites thus posing threats to the health of human beings who live close to rodent populations (**Namue and Wongsawad, 1997; Zain *et al*. 2012**). For this, in isolated ecosystems, most of these studies have been targeting the parasite helminth fauna and the potential role of rodents as reservoirs of parasitic zoonoses (**Casanova *et al*. 1996, Miquel *et al*. 1996, Waugh *et al*. 2006, Milazzo *et al*. 2010**). Knowing that rodents share their food and habitat with humans, secures the transmission of zoonotic pathogens to humans via their urine, feces, hair, and saliva (**Meerburg, 2010**). Among these dangerous animals, we have chosen to study the associated parasites (intern and extern) of black rats (*Rattus rattus*), known for sharing their habitat and food with humans. Hence, we have been interested in analyzing the presence of protozoa, helminths, bacteria, and dermatophyte fungi that could be transmitted to humans and cause infectious diseases. The present work is unprecedented in Algeria and provides the first data on the parasite biodiversity in *R. rattus*.

MATERIAL AND METHODS

Study area

This study was conducted in palm groves (Fig 1) and stocks (date hangars located in different palm groves) (Fig 2) from the region of Touggourt (33° 02' to 33° 12' N; 5° 59 'to 6° 14' E) which is located in southeastern Algeria at an altitude of 75 m. It is bordered to the north by palm groves, to the south and east by the Great Eastern Erg, and to the west by dunes of sand. To detect and confirm the presence of parasites (fungi, protozoans, and helminths) associated with black rats, 46 individuals of four age classes were examined (12 adult, 10 old, 12 juvenile, and 12 sub-adult).

Figure 1 Palm grove stations

Figure 2 Hangars stations

Sampling method and identification of rodents

For the trapping of rats, Besancon technology system (BTS) traps were used (eight traps/station) (**Mlik, 2019**). They were gridded meshes of 26 cm × 12 cm × 14 cm, which were triggered by a hook when the animal touched the bait hooked in the trap. These are very lightweight devices, easy to store and transport. They permit the capture of live individuals which allows very good exploitation of captured animals. Several baits were used including toast, dates, and cheese. Each specimen of *R. rattus* captured was kept in a numbered jar containing alcohol until manipulation. Once in the laboratory, each individual was examined and identified based on several criteria, notably morphology (coat coloration, soles palmar, and plantar) and craniometry (upper and lower molars). Age and confirmation of species were made through examination of the shape and wear of the molar rows of each individual (**Barreau *et al*., 1991**). The farmers capture all the individuals used in the present study within the control framework against these pests. In addition, the current experiment has the approval of the ethical committee of the University of Kasdi-Merbah, Ouargla, Algeria.

External parasites

Hair specimens of four parts of the body (back, belly, tail, and vibrissae) were recovered and preserved aseptically in sterile paper. They were rinsed with alcohol, disinfected with 2% sodium hypochlorite (to eliminate saprophytic fungi), rinsed again with distilled water, and then they were dried near a benzene beak. After drying, they were seeded in Petri dishes containing PDA medium and incubated in an oven at a temperature of 37° c. After obtaining fungal colonies in the Petri dishes, fungi were purified and identified by their macro and microscopic aspects with an identification key of **Dufresne (2014)**.

Internal parasites

All captured individuals were dissected, and their digestive tube was retrieved to examine the internal parasitic fauna (bacteria, nematodes, cestodes, etc.). The contents of these tubes were emptied, using two pincers, in a bottle containing 180 ml of distilled water, then agitated for 20 min to allow the separation of the stomach contents. For fecal coliforms, 5 ml was added in a vial (with a bell) containing 50 ml of bromoCresol purple liquid (BCPL) medium and 1ml in the tubes (with bell) containing 5 ml of BCPL medium (single and double concentration with five replicates for each). After, the gas present in the bells was emptied before incubation at 45° C from 24 to 48 h. For streptococci, the same protocol was adopted but with a Roth medium. The presence of these bacteria is recorded as positive when there is acidification of the medium (change of color from purple to yellow) plus the production of gas reported by the bells. For the clostridium sulfite-reducer (CSR), introduce in a sterile tube a volume of 20 ml of the crude solution and put in a water bath at 80° C (10 min), then cool them rapidly under cold water (to eliminate the vegetative forms and to keep only sporulated forms. After that, they were inoculated in a vial, containing liver agar (la) with additives (iron alum and sulfite of sodium), and incubated at 45° C for 48 h. The presence of these microorganisms appeared in the form of colonies surrounded by a black halo.
Coproscopies enriched by flotation with water saturated with salt were carried out for each rodent captured: the feces collected in the terminal part of the colon (2 cm) were mixed with the flotation solution and then filtered. The filtrate was then centrifuged at 3500 rpm for 5 min. Then, a few drops of the flotation solution were added to the centrifuge tube until a meniscus formed on which a cover-slid was placed for 20 min, before being fixed on a microscope slide for observation (**Dryden *et al*., 2005; Ballweber *et al*., 2014**).

Parasitic prevalence (Pr%)

The results of this study were exploited by the parasitic prevalence (Pr%) as well as the mean intensity (MI). According to **Valtonen *et al*. (1997)**, the prevalence (Pr%) is the number of individuals infected with a particular parasite species (np)/number of hosts examined (n).

$$Pr(\%)=np/n\times 100$$

Depending on the value of the prevalence, the following categories are distinguished:

Dominant species, if Pr% > 50% ;
Satellite species, if 10% ≤ Pr% ≤ 50% ;
Rare species, if Pr% <10%.

Mean intensity (MI) is the total number of individuals of a particular parasite species in a sample of a host species/number of infected individuals of the host species in the sample (**Margolis *et al*., 1982**).

Statistical analysis

Statistical analysis was performed using STATISTICA software (v.0.20.) and R software (v.4.2.3). We have used parametric tests for normal results, unlike; the non-normal data were treated with non-parametric tests (e.g. ANOVA test for the normal data and Kruskal-Wallis for the non-normal).

RESULTS

Diversity of black rats-associated parasites

The sampling period of *R. rattus*, from January to July 2017, allowed us to collect 46 individuals (23 rats for each site). This rodent species was infected with seven species of dermatophytes fungi, three bacterial genera, and six species of helminths (Tab 1).

Table 1 Different types of parasites found in black rats from the region of Touggourt

Infected site	Parasite species	Colony/Individual number of parasites	Individual number of infected rats	Mean Intensity	Prevalence%
Hair	*Alternaria* sp.	89	34	2.62	73.91
	Cladosporium sp.	95	46	2.07	100.00
	Penicillium sp.	41	45	0.91	97.83
	Aspergillus niger	80	45	1.78	97.83
	Microsporum sp.	15	15	1.00	32.61
	Trichophyton sp.	21	17	1.24	36.96
	Chrysosporium sp.	10	10	1.00	21.74
Intestines	Coliforms	34	34	1.00	73.91
	Streptococcus	19	19	1.00	41.30
	Clostridium S/R	19	19	1.00	41.30
	Syphacia muris	18	16	1.13	34.78
	Syphacia obvelata	56	29	1.93	63.04
	Aspiculuris tetraptera	15	09	1.67	19.57
	Ascaris lumbricoides	14	11	1.27	23.91
	Schistosoma sp.	35	21	1.67	45.65
	Hymenolepis sp.	15	10	1.50	21.74

Seven species of pathogenic fungi were isolated in the present study (Tab 1). The most isolated were saprophytes, namely *Cladosporium* sp. with a prevalence of 100% followed by *Penicillium* sp. and *Aspergillus niger* (97.83%) and *Alternaria* sp. (73.91%). While dermatophyte fungi accounted for 36.96%, 32.61% and 21.74% of *Trichophyton* sp., *Microsporum* sp. and *Chrysosporium* sp., respectively (Tab 1). Concerning the three bacterial genera examined, coliforms (34 infected rats) were most frequently detected in the feces of *R. rattus*, followed by *Streptococcus* and CSR with the same number (19 infected rats). Although, the internal parasites, *S. obvelata* (63.04%) was the most frequently detected species, followed by the *Schistosoma* sp. (45.65%), while *A. tetraptera* (19.57%) comes last (Tab 1).
Concerning the recorded mean intensity, *Alternaria* sp., and *Cladosporium* sp. have presented the most important mean intensities according to the other species, unlike *Penicillium* sp. which showed the lowest intensity for fungi. On the other hand, *A. lumbricoides* and *Schistosoma* sp. have recorded the highest values for nematodes (Tab 1).

Parasitic infection of black rats

The variety of parasites in *R. rattus* allowed us to observe that individuals caught in palm groves were infected with 290 parasites and 285 parasites were found in stock rats (Fig 4). Statistical analysis showed that there was no difference (p=0.5271) between the number of parasites collected in the two locations. Regarding the sex of the black rats, when sampling parasites, no remarkable difference between the two sexes whereas the number of parasites appearing in infected males (mean = 8.2 ind./rat) was more than in females (mean = 5.4 ind./rat). On the other hand, the statistical analysis ascertained that there was no significant difference between the two sexes (p=0.7495). On the other hand and according to the age classes, the old rats were more infested with parasites than the others, unlike the juveniles. Statistical data ascertain this with a very highly significant difference (p=0.0008) between the number of parasites collected from all the age categories. These results ascertain that whatever the age or sex of black rats, they will be infected with these parasites either fungi, bacteria, or other parasites.

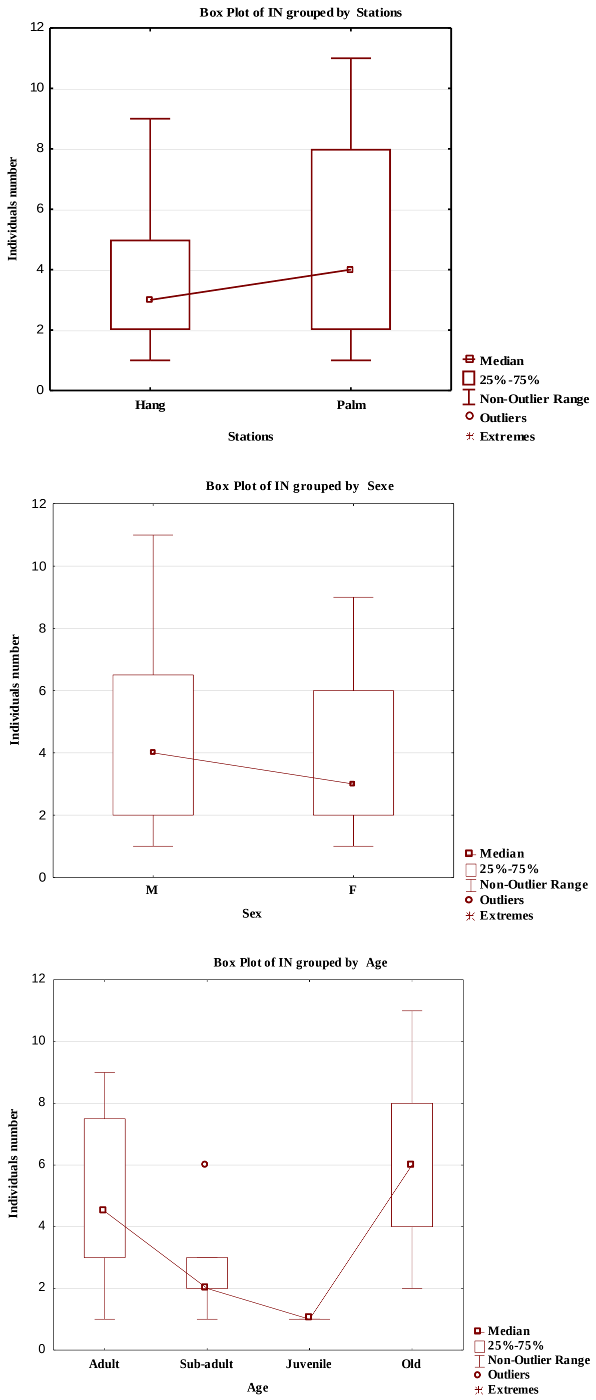

Figure 4 Number of individuals infected with parasites depending on station, sex, and age

Internal parasites infection in black rats

The results obtained indicated that *S. obvelata* comes in first position with 56 individuals, followed by *Schistosoma* sp. (35 ind.). While *A. lumbricoides* comes last with 14 individuals (Fig 5). Statistically, there was a significant difference (p=0.0120) between the number of black rats infected by these species.

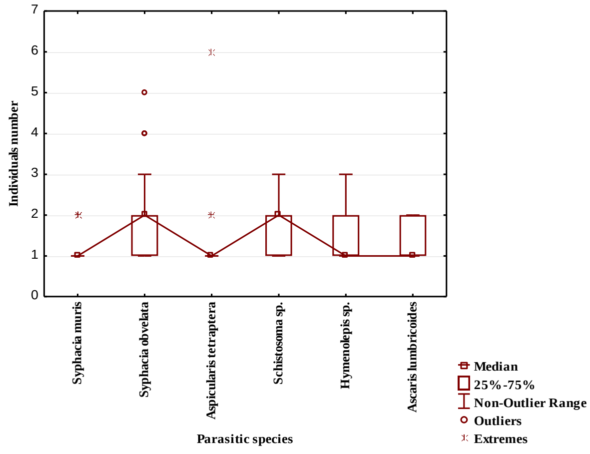

Figure 5 Internal parasites infection in black rats

Concerning the study stations, the black rats captured in the palm groves were the most attacked by the parasites whose number of *Syphacia muris*, *S. obvelata*, *Schistosoma* sp., and *Hymenolepis* sp. was higher in individuals caught in palm groves than stocks (Fig 6). On the other hand, the number of individuals of *Aspicularis tetraptera* and *Ascaris lumbricoides* identified in the black rats from stocks was higher than those collected from palm groves. Statistical analysis showed that there was a highly significant difference (p=0.0029) between individuals from the two sampling stations.

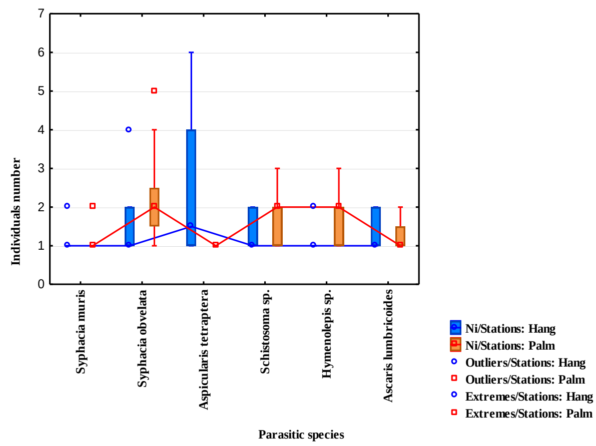

Figure 6 Internal parasite infection in black rats depending on stations

While depending on the sex of *R. rattus*, the results obtained showed that males were more infected than females. Hence, the continuous movement of male rats over females may explain it. Statistical data showed that there was a highly significant difference between the two sexes (Fig 7).

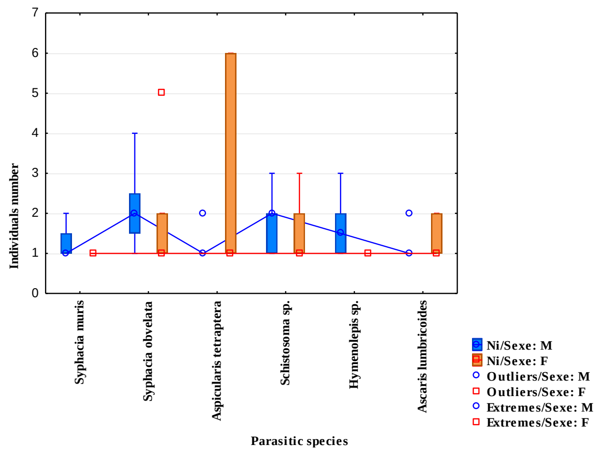

Figure 7 Internal parasite infection in black rats depending on sex

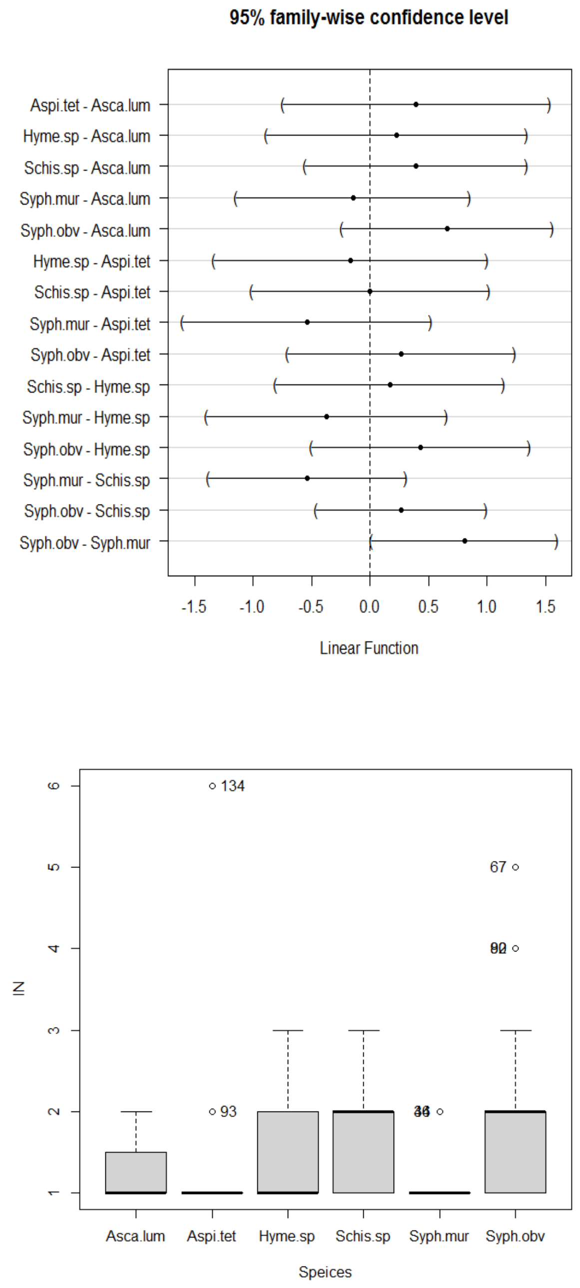

Figure 8 Difference of infection between the captured individuals of black rats

This figure presents the boxplot of the infection of black rats with the six parasites found in their intestines. It is important to note that *A. tetraptera* and *S. muris* were less recorded in these animals. All these infections allowed us to note that *S. obvelata* presents higher values than the infections caused by *Schistosoma* sp., which in turn, presents higher values than those of *Hymenolepis* sp. (Fig 8).

Dermatophyte fungi infection in black rats

The present experiment, with the isolation of different species of fungi from different parts of black rats' bodies, allowed us to observe the dominance of saprophytic fungi in the four parts. This type of fungi was highly recorded in the tails of these animals, unlike the dermatophytic fungi that were mostly isolated from the belly of black rats tested (Fig 9). Statistical data revealed that there was a very high significant difference between the isolated fungi (p=0.0010).
Depending on the body parts examined, the belly was the most part carrying these fungi (31.7%) followed by the tail (28%). Depending on the statistics, there was no difference recorded between these parts (p=0.4232).

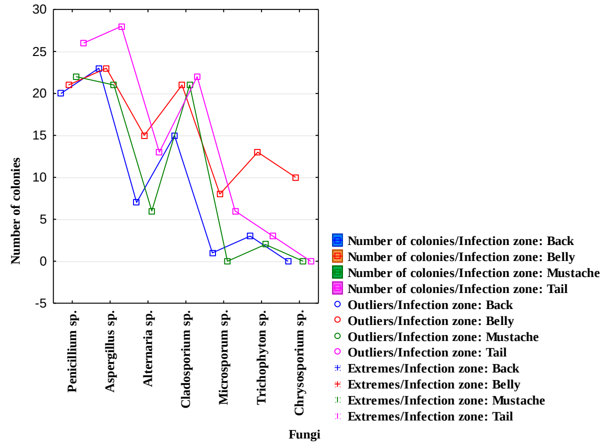

Figure 9 Fungi infection recorded in different parts of black rats

Bacterial infection in black rats

The current study showed that the coliforms were more detected in the black rats examined whereas the prevalence of these bacteria was 73.9%, followed by streptococcus and CSR with 41.3% (Fig 10).

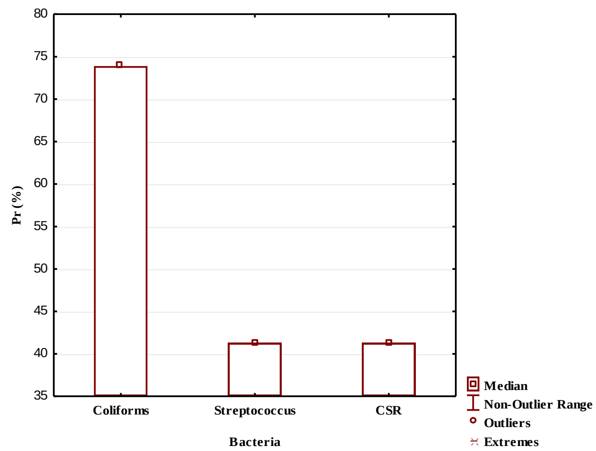

Figure 10 Prevalence of bacterial infection in black rats

DISCUSSION

Among the main vectors of disease contamination, not only humans, but also domestic animals (dogs, cats), rodents (rats and mice), reptiles (lizards, margouillat), and insects (flies) can constitute reservoirs for various germs (staphylococci, streptococci, salmonella) (**Gwenzi *et al.*, 2021**). *Rattus rattus* is a known carrier of bacteria, viruses, and parasites of zoonotic and veterinary importance (**Meerburg *et al.*, 2009; Reperant *et al.*, 2009**). In addition, rats transmit diseases directly or indirectly where they are incriminated for deaths more than any other causes (**Mohammed Ayyal *et al.*, 2019**). Threat to human health is well recognized when potentially life-threatening diseases currently have no specific treatment, cure, or vaccine (**Desvars-Larrive *et al.*, 2017**). On the other hand, rats are capable of carrying and shedding *Escherichia coli* (**Burriel et al, 2008; Guenther et al., 2010; Nkogwe *et al.*, 2011**). *Rattus rattus* is a serious pest in urban and rural environments. It is the cause of extensive economic damage to crops, stored food, farms, industries and households (**Pimentel *et al.*, 2005**). Black rat populations also harbor and spread zoonotic pathogens, such as viruses (e.g., Seoul hantavirus), bacteria (e.g., *Leptospira interrogans*), protozoa (e.g., *Toxoplasma gondii*) and helminths (e.g., *Hymenolepis* spp.) (**Himsworth et al., 2013**).
The diversity of internal parasites (bacteria and helminths) could be probably due to the diversity of the arthropods-vector-disease community collected from the same individuals of black rats from southeastern Algeria (same region of study) (**Mlik *et al.*, 2022**). The same authors have isolated several lice and acari species that were identified on this species are among the main disease vectors.
Concerning our finding on helminths, **River (2015)** has confirmed that the three pinworms found in rats and mice are *Syphacia muris*, *S. obvelata* and *Aspicularis tetraptera*. In addition, **Panti-May *et al.* (2017)** found that *Hymenolepis diminuta* was the most prevalent pathogen, particularly in black rats (14.2%). **Meshkekar *et al.* (2014)** declared that *R. rattus* was the predominant rodent species infected with five different parasites, in Iran, two of which are zoonotic (*H. diminuta* and *H. nana*). Furthermore, **Mahmood Amin (2019)** noted that 43.63% of black rats

were infected with these two helminths. Noting that human hymenolepiasis is a zoonosis caused by the cestodes *H. nana* and *H. diminuta* (**Nkouawa *et al*., 2016**). It is often asymptomatic but can cause chronic diarrhea, abdominal pain, irritability and itching (**Martínez-Barbabosa et al., 2012; Chero et al., 2016**). On the other hand, **Franssen *et al*. (2016)** declared that *S. muris* gravid females actively leave their host's intestine and deposit the eggs around their host's anus, after which (auto) infection and transmission between rats take place through grooming and social behavior.

Hymenolepis spp. are frequently transmitted to humans and reported as zoonotically important (**Khan *et al*., 2021**), whereas *H. diminuta* is transmitted by ingestion of *Tribolium confusum* (flour beetle, intermediate host) with contaminated cereals, or by the fecal–oral route. *Hymenolepis nana* is transmitted through fecal–oral contact (eggs), or by accidental ingestion of intermediate hosts harboring cysticercoids (**Franssen *et al*., 2016**). The infections of these species in humans are mostly asymptomatic, although weakness, headache, abdominal pain, and diarrhea may occur in artificial digestion of the diaphragm and both hind legs (**CDC, 2016**).

The presence of infected black rats around animal facilities poses high risks not only for animals but also for farmers and their families. Hence, the possibility of these rats contaminating the environment, food, and water source with their parasites poses a public health threat since these rats live in close association with humans. Hence, these parasite infections in the urban rats in this study poses a health risk to human.

The current study allowed us to detect considerable differences in parasites, especially in helminth species. In consequence, black rats could be considered an important vector of several microorganisms that can hold germs and represent a threat to biomedical and veterinary public health. Based on these findings, it is necessary to perform a control way aimed at full rat eradication to prevent zooanthroponosis.

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