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 (*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 (Okeye and Obieze, 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 (Namne and Wongswad, 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 Tougourt (33° 02’ to 33° 12’ N, 6° 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).

**Sampling method and identification of rodents**

For the trapping of rats, Besançon 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 (Barreae 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 beam. 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 pickers, 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. Cropscopties 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 Vallonen et al. (1997), the prevalence (Pr%) is the number of individuals infected with a particular parasite species (np/number of hosts examined (n)).

\[
\text{Pr(\%)} = \frac{\text{np}}{n} \times 100
\]

Depening 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

<table>
<thead>
<tr>
<th>Infected site</th>
<th>Parasite species</th>
<th>Colony/Individual number of parasites</th>
<th>Individual number of infected rats</th>
<th>Mean Intensity</th>
<th>Prevalence%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hair</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alternaria sp.</td>
<td>89</td>
<td>34</td>
<td>2.62</td>
<td>73.91</td>
</tr>
<tr>
<td></td>
<td>Cladosporium sp.</td>
<td>95</td>
<td>46</td>
<td>2.07</td>
<td>100.00</td>
</tr>
<tr>
<td></td>
<td>Penicillium sp.</td>
<td>41</td>
<td>45</td>
<td>0.91</td>
<td>97.83</td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>80</td>
<td>45</td>
<td>1.78</td>
<td>97.83</td>
</tr>
<tr>
<td></td>
<td>Mirosporum sp.</td>
<td>15</td>
<td>15</td>
<td>1.00</td>
<td>32.61</td>
</tr>
<tr>
<td></td>
<td>Streptococcus sp.</td>
<td>21</td>
<td>17</td>
<td>1.24</td>
<td>36.96</td>
</tr>
<tr>
<td></td>
<td>Chrysosporium sp.</td>
<td>10</td>
<td>10</td>
<td>1.00</td>
<td>21.74</td>
</tr>
<tr>
<td><strong>Intestines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coliforms</td>
<td>34</td>
<td>34</td>
<td>1.00</td>
<td>73.91</td>
</tr>
<tr>
<td></td>
<td>Streptococcus sp.</td>
<td>19</td>
<td>19</td>
<td>1.00</td>
<td>41.30</td>
</tr>
<tr>
<td></td>
<td>Clostridium sp.</td>
<td>19</td>
<td>19</td>
<td>1.00</td>
<td>41.30</td>
</tr>
<tr>
<td></td>
<td>Syphacia muris</td>
<td>18</td>
<td>16</td>
<td>1.13</td>
<td>34.78</td>
</tr>
<tr>
<td></td>
<td>Syphacia obvelata</td>
<td>56</td>
<td>29</td>
<td>1.93</td>
<td>63.04</td>
</tr>
<tr>
<td></td>
<td>Aspicularis tetraperta</td>
<td>15</td>
<td>09</td>
<td>1.67</td>
<td>19.57</td>
</tr>
<tr>
<td></td>
<td>Acsartis lumbricoides</td>
<td>14</td>
<td>11</td>
<td>1.27</td>
<td>23.91</td>
</tr>
<tr>
<td></td>
<td>Schistosomus sp.</td>
<td>35</td>
<td>21</td>
<td>1.67</td>
<td>45.65</td>
</tr>
<tr>
<td></td>
<td>Hymenoptes sp.</td>
<td>15</td>
<td>10</td>
<td>1.50</td>
<td>21.74</td>
</tr>
</tbody>
</table>

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. tetraperta* (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. lumbrioides* 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 infected 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.
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 tetrapiera* 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.

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 5** Internal parasites infection in black rats

**Figure 6** Internal parasite infection in black rats depending on stations

**Figure 7** Internal parasite infection in black rats depending on sex

**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.

**Internal parasites infection in black rats depending on station, sex, and age**

**Figure 4** Number of individuals infected with parasites depending on station, sex, and age
This figure presents the boxplot of the infection of black rats with the six parasites examined whereas the prevalence of these bacteria was 73.9%, followed by streptococcus and CSR with 41.3% (Fig 10).

**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).

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

This figure presents the boxplot between the captured individuals 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).

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).

**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 (Desvirs-Larriue 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 arthropod-vertebrate disease community collected from the same individuals of black rats from southeastern Algeria (same region of study) (Milk 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, Pantig-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 43.65% of black rats...
were infected with these two helminths. Noting that human hemonoeleopiasis 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-Barbosa et al., 2012; Chero et al., 2016). On the other hand, Frison et al. (2018) declared that *M. musculus* gravid females actively leaves 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.

**Hymenoeleopis spp.** are frequently transmitted to humans and reported as zoonotic agents (Khan et al., 2021), whereas *H. diminuta* is transmitted by ingestion of *Trichobilium confusum* (flour beetle, intermediate host) with contaminated cereals, or by the fecal–oral route. **Hymenoelepis 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 duodenum 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 zoonotic reservoir of pathogens that can be transmitted 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 zoonanthroposcopy.

**REFERENCES**


