

First report of ectoparasites from black rats (*Rattus rattus* Linnaeus, 1758) in oasis regions from Algeria

Randa MLIK^{1*}, Salim MEDDOUR², Bilal DIK³,
Karim SOUTTOU⁴, Makhlouf SEKOUR¹

¹University of Ouargla, Faculty of Life and Natural Sciences, Department of Agronomic Sciences, Algeria; randa.mlik@yahoo.fr (*corresponding author); sekkmak@yahoo.fr

²University of Ghardaia, Faculty of Natural, Life, and Earth Sciences, Department of Agronomic Sciences, Algeria; meddour_salim@yahoo.fr

³University of Selçuk, Faculty of Veterinary Medicine, Department of Parasitology, Konya, Turkey; bdik2005@yahoo.com

⁴University of Djelfa, Faculty of Life and Natural Sciences, Department of Agronomy and Biology, Algeria; kasouttou@yahoo.fr

Abstract

Black rats are considered a carrier of arthropods that are vectors of diseases to animals and humans. No studies have ever been reported on ectoparasites of this rodent in Algeria. Where *Rattus rattus* was introduced recently in the south-eastern oasis and has taken refuge in palm groves. To identify the ectoparasites, richness, and prevalence of this species, we have collected 6237 arthropods from four species of lice, five mites, one tick, and flea's larvae, were collected from 462 infested rats of 484 (95.5%) total collected rats. The results showed that lice especially *Polyplax spinulosa* (n = 2888) and *P. serrata* (n = 1456) were the most trapped ectoparasites followed by *Ornithonyssus bacoti* (n = 1415). Otherwise, *Atricholaelaps* sp. (n = 10) was the least population. In addition, adult males of black rat were depicted as the most attacked category compared to females and other classes because of their mobility. Statistics confirm this ascertainment for sex (P = 0.0007) and age groups (P < 0.000). Hence, *R. rattus* is a favourable host target to parasites when transmission conditions allow it. All inventoried species were significantly greater (P < 0.0000) in summer than winter and decreased rainfall.

Keywords: Algerian Sahara; black rat; external parasite; human; infestation; *Polyplax spinulosa*; palm groves

Introduction

Commensal small mammals, such as rats and mice, generally expand their distributions in association with humans playing an important role as hosts of parasites and reservoirs of different zoonotic diseases transmitted by ectoparasites. World Health Organization (WHO) declared that 19% of the world's total human mortality and 53% of all deaths in Africa are due to parasitic and infectious diseases caused by these arthropods (WHO, 2004, 2018). Moreover, some species of rodents especially *Rattus rattus* has been classified as the most harmful invasive species in the world, as it has caused the extinction and displacement of several species of birds and mammals; it is also considered one of the main disease vectors for humans and wild animals (Townsend and Daugherty, 2006; Harris, 2009; Banks and Hughes, 2012). Clinton (1969) and Battersby *et al.*

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(2002) found that among all the animals collected in urban areas, wild rats (*Rattus rattus* spp.) are the most dangerous due to their high number (high reproductive capacity), zoonotic potential, and propensity towards close association with humans. Several rodent-associated Bartonella species have been linked to human diseases (Tsai *et al.*, 2010), where *B. elizabethae*, a recognized human pathogen, was isolated from *Rattus* spp. (Ying *et al.*, 2002; Li *et al.*, 2004; Inoue *et al.*, 2008). Whereas, some of the ectoparasites can biologically or mechanically transfer infectious agents to humans or animals and result in the spread of infection (Khachorian and Arakelian 1978; Bossi *et al.*, 2002; Torres-Mejia and Fuente, 2006). Meanwhile, one of the most known parasites are sucking lice which are obligate hematophagous ectoparasites of eutherian mammals where 550 species of Anoplura distributed in 16 families and 49 genera have been recorded worldwide (Durden and Musser, 1994; Light *et al.*, 2010). The need to take into account the ecology and evolution of infectious diseases to understand their dynamics is increasingly accepted (Schrag and Wiener, 1995), particularly in the case of zoonosis caused by ectoparasites of black rats. Previous studies focused on the ectoparasites associated with black rats (Gaaboub *et al.*, 1981; Soliman *et al.*, 2001; Abd El-Halim *et al.*, 2009; Bahgat, 2013; Nateghpour *et al.*, 2013; Solanki *et al.*, 2013; Asiry *et al.*, 2014; Khajeh *et al.*, 2017; Eslami *et al.*, 2018; Thille *et al.*, 2019) and zoonotic diseases attributed to these species as well as their involvement in its transmission to human and animals (Tijjani *et al.*, 2020; Panthawong *et al.*, 2020; Wang *et al.*, 2020). No studies have been reported in Algeria on ectoparasites of rodents. To make up for this deficiency and according to farmers declaration on the invasion of black rats and their damages in palm trees (Alia *et al.*, 2018), stock locations, and their farm animals, in palm groves of Algerian oasis (Mlik *et al.*, 2018; Mlik, 2019); the present study, provides the first data on the biodiversity of ectoparasites from *R. rattus*, we further explore the relationship between black rats and their ectoparasites in Algeria, which are suggested like vectors of borne-diseases provoking several zoonoses to these farmers.

The current study aims to determine the prevalence and frequency of ectoparasites on *R. rattus* in several oasis regions in Algeria. Our goal is to assess the community of external parasites that could potentially transmit infectious diseases to humans.

Materials and Methods

Study area

The present work was conducted in the region of Touggourt located in the south-eastern part of Algeria (33° 02' to 33° 12' N, 5° 59' to 6° 14' E) with an altitude of 75 m. It is limited to the north by Megarine palm groves, to the south and east by the great Oriental Erg, and to the west by sand dunes. This locality constitutes the upper part of Oued Righ valley.

Isolation and identification of ectoparasites

Rodents from palm trees and storage sites were trapped using BTS cages (Besonçon Technology System) measuring 26× 12× 14 cm (l x w x h). The sex and age of trapped animals were distinguished according to Aplin *et al.* (2003). After, they were transported to the laboratory and euthanized with chloroform and placed over a white tray, and brushed to recover ectoparasites. Also, ears were carefully examined by forceps. All removed ectoparasites were stored in ethanol (70%). Then, they were cleared by KOH (10%) and mounted on microscopic slides using 10x and 40x under an OPTIKA light microscope for identification. All lice, mites, and ticks were examined and identified by valid keys of Fain (1974) and Pajot (2000).

Ecological indices applied on ectoparasites

In order to study these ectoparasites, the obtained results were studied with two principal indices:

Prevalence Estimation (Pr %) is the number of individuals of a host species infected with a particular parasite species/number of hosts examined (Margolis *et al.*, 1982).

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

In order to run the analysis, we used Statistica v. 10.0 (Statsoft) software and used Analysis of Variance (ANOVA) for normal distribution data and Kruskal-Wallis for abnormal data. Noting that the variables of interest in the present experiment were the ectoparasites as dependent and the sex, age, and species as independent variables.

Results

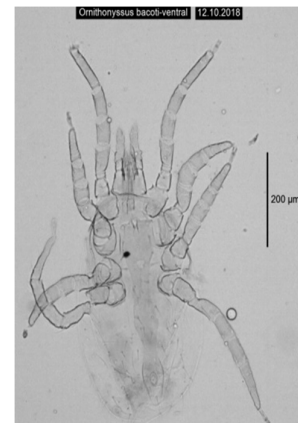
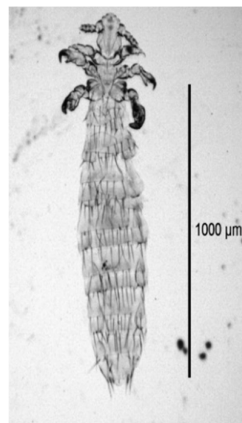
Diversity of black rats-associated arthropods

The sampling period of *Rattus rattus* was conducted from January to December 2017, and successfully collected a total of 484 individuals in which 462 rats (95.5%) were infested with 6237 individuals of ectoparasites. These were constituted by 4586 lice, 1551 mites, 30 ticks, and 70 flea's larvae with a mean intensity of 13.5. Systematically, these arthropods belong to eight families, eight genera, and 11 species (Table 1). Where the highest prevalence was recorded by *Polyplax spinulosa* (79.8%), *P. serrata* (56.4%) and *Ornithonyssus bacoti* (39.3%; Table 1, Figure 1). These species showed the highest number with 2888 individuals for *P. spinulosa*, 1456 for *P. serrata*, and 1415 for *O. bacoti*. Otherwise, *Atricholaelaps* sp. was the least population with ten individuals.

Table 1. Ectoparasites species collected from *R. rattus* caught in palm groves of Touggourt

Family	Genus	Species	N	Pr (%)	MI
Polyplacidae	<i>Polyplax</i>	<i>P. spinulosa</i>	2888	79.8	7.5
		<i>P. serrata</i>	1456	56.4	5.3
		<i>P. abyssinica</i>	175	14.7	2.5
Hoplopleuridae	<i>Hoplopleura</i>	<i>Hoplopleura</i> sp.	67	9.3	1.5
Macronyssidae	<i>Ornithonyssus</i>	<i>O. bacoti</i>	1415	39.3	7.5
Dermanyssidae	<i>Dermanyssus</i>	<i>D. gallinae</i>	61	7.6	1.7
		<i>Dermanyssus</i> sp.	47	6.4	1.5
Laelapidae	<i>Atricholaelaps</i>	<i>Atricholaelaps</i> sp.	10	2.1	1.0
Haemogamasidae	<i>Eulaelaps</i>	<i>Eulaelaps</i> sp.	18	3.3	1.1
Ixodidae	<i>Rhipicephalus</i>	<i>Rhipicephalus sanguineus</i>	30	4.8	1.3
Pulicidae	<i>Xenopsylla</i>	<i>X. cheopis</i>	70	6.2	2.3

N: individual's number; **Pr (%)**: parasitic prevalence; **MI**: mean intensity.



a) b) c)
Figure 1. Most captured ectoparasites in *R. rattus* (**a:** *Polyplax spinulosa*; **b:** *P. serrata*; **c:** *Ornithonyssus bacoti*)
Prevalence and mean intensity of ectoparasites

Regarding the rat ages, we document adults (N = 2475) as the most infested stage by these ectoparasites, followed by aged rats (N = 1785) and sub-adults (N = 1773), while juveniles (N = 204) come in the last position (Table 2). For the prevalence, the aged rats (99.1%), sub-adults (96.4%), and adults (93.1%) were the most infected classes. On the other hand, adults (MI = 16.7) and aged rats (MI = 16.2) have presented the most important mean intensity according to the other age classes.

Table 2. Prevalence (Pr %) and mean intensity (MI) of *R. rattus* ectoparasites depending on age

		P sp	P se	P ab	H sp.	O ba	D ga	D sp.	At sp.	E sp.	R hs	X ch	Total
Aged rats	N	868	373	63	21	362	32	18	3	5	15	25	1785
	Pr %	89.2	66.7	22.5	11.7	50.5	17.1	9.0	2.7	4.5	9.0	11.7	99.1
	MI	8.8	5.0	2.5	1.6	6.5	1.7	1.8	1.0	1.0	1.5	1.9	16.2
Adult	N	1089	585	77	27	601	15	15	5	8	12	41	2475
	Pr %	80.5	56.6	17.6	11.9	42.1	7.6	6.9	3.1	3.77	6.3	9.4	93.1
	MI	8.5	6.5	2.8	1.4	8.9	1.3	1.4	1	1.3	1.2	2.7	16.7
Sub-Adult	N	798	453	35	19	426	14	14	2	5	3	4	1773
	Pr %	75.5	61.2	12.9	10.1	41.0	6.5	7.2	1.4	3.6	2.2	1.4	96.4
	MI	7.6	5.3	1.9	1.4	7.5	1.6	1.4	1.0	1.0	1.0	2.0	13.2
Juvenile	N	133	45	0	0	26	0	0	0	0	0	0	204
	Pr %	72	32	0	0	13.3	0	0	0	0	0	0	2.9
	MI	2.5	1.9	0.0	0.0	2.6	0	0	0	0	0	0	2.2

P sp: *P. spinulosa*; **P se:** *P. serrata*; **P ab:** *P. abyssinica*; **H sp.:** *Hoplopleura* sp.; **O ba:** *O. bacoti*; **O sp.:** *Ornithonyssus* sp.; **D ga:** *D. gallinae*; **D sp.:** *Dermanyssus* sp.; **At sp.:** *Atricholaclaps* sp.; **E sp.:** *Eulaclaps* sp.; **R hs:** *Rhipicephalus sanguineus*; **X ch:** *X. cheopis*.

In relation to the species of collected ectoparasites, *P. spinulosa* is the most population collected from all the rat age stages, followed by *P. serrata* and *O. bacoti*. Additionally, the adult rodents record the highest number of *Hoplopleura* sp. and *Atricholaclaps* sp., while *Dermanyssus* sp. was the most isolated species in sub-adults.

According to the identified arthropod groups, lice were the most abundant group in black rat individuals with 4586 individuals followed by mites (1551) where adults constitute the most parasitized class by lice (1778), followed by aged rats (1325). On the other hand, mites were very common in adults (644) and sub-adults (461) compared to the other categories (Table 2).

With regard to the sex of *R. rattus*, it was observed that all ectoparasite species were noted in females and males with differences in density (Table 3). Indeed, the latter characterized by the highest prevalence (97.2%) and intensity (MI = 15) compared to females (P = 92.8%; MI = 11.1). Meanwhile, *P. spinulosa* was the most dominant species in both males (1835 individuals) and females (1053). Same for *P. serrata* (male = 1035; female = 421). Where lice were the most represented in both sexes (male = 3058; female = 1528) followed by mites (male = 1080; female = 471; Table 3).

Table 3. Prevalence (Pr %) and mean intensity (MI) of *R. rattus* ectoparasites depending on sex

		P sp	P se	P ab	H sp.	O ba	D ga	D sp.	At sp.	E sp.	R hs	X ch	Total
Male	N	1835	1035	136	52	979	43	37	6	15	21	69	4228
	Pr %	79.6	61.2	18	12.1	42.9	10	9	2.1	4.5	5.9	10	97.2
	MI	8	5.9	2.6	1.5	7.9	1.5	1.4	1	1.2	1.2	2.4	15.0
Female	N	1053	421	39	15	436	18	10	4	3	9	1	2009
	Pr %	80	49.2	9.7	5.6	33.8	5.6	2.6	2.1	1.5	3.1	0.5	92.8
	MI	6.8	4.4	2.1	1.4	6.6	1.6	2	1	1	1.5	1	11.1

Individuals number of ectoparasites depending on black rats' sex

The average number of collected ectoparasites in *R. rattus* allowed us to observe that males ($n = 9$ parasites/male) were more infested than females (7.7 parasites/female) with great variability, especially for the 25% of the most parasitized rat population. Males carry from 1 to 88 parasite/rat, whereas females have from 1 to 51 parasite/rat. Statistical analysis confirmed that there was a very highly significant difference (KW-H (3;1112) = 0.0941; $P = 0.0007$) between the number of ectoparasites collected from males than females.

Individuals number of ectoparasites depending on black rats' age

Depending on age categories of black rats, sampling of ectoparasites allowed us to note that adults ($N = 2475$) were the most infested class by these arthropods with an average of 16.7 parasite/rat (min = 1; max = 88), followed by aged rats ($N = 1785$) with 16.2 parasite/rat (min = 1; max = 86) and sub-adults ($N = 1773$), while juveniles ($N = 204$) arrived at the last position (Figure 2). This means that adults and aged rodents were the most mobile classes. Statistical data reveal the presence of a very highly significant difference between age groups ($P < 0.000$).

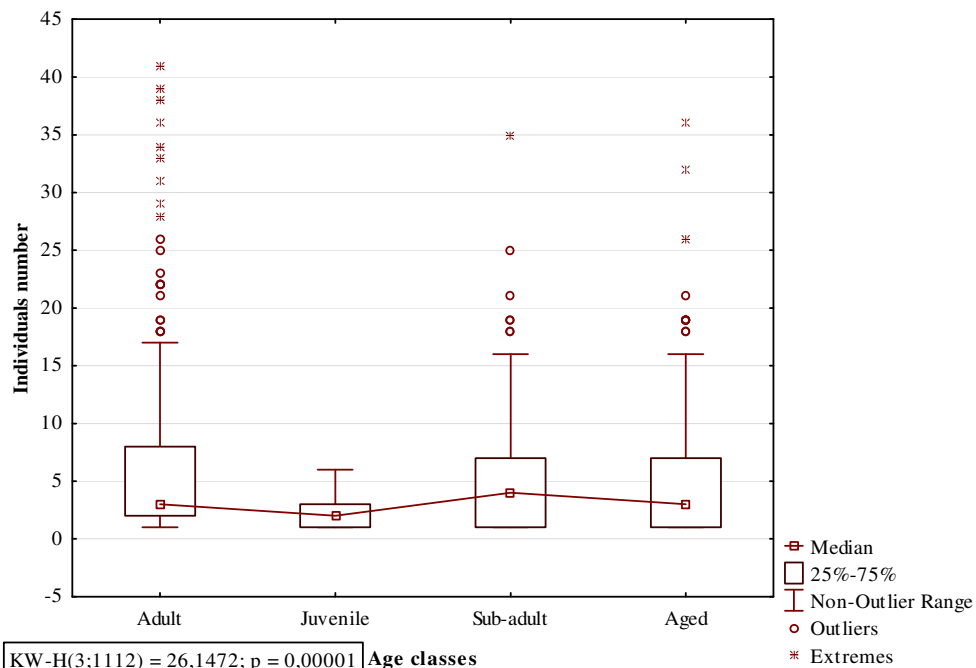


Figure 2. Individuals number of ectoparasites depending on black rats' age

Individuals number of ectoparasites depending on species

According to species of ectoparasites associated with black rats, *P. spinulosa* was the most abundant species in *R. rattus* with an average of 7.48 individuals/rat (min = 1; max = 41), followed by *O. bacoti* by 7.45 individuals/rat (min = 1; max = 36) and *P. serrata* with 5.33 individuals/rat (min = 1; max = 30) where they were represented with a great variability compared to the other species (Figure 3). *Atricholealaps* sp. arrived at the last position (1 individual/rat; Figure 3). This ascertainment was confirmed by statistical data revealing a very highly significant difference ($P < 0.000$) between individuals number collected in each species.

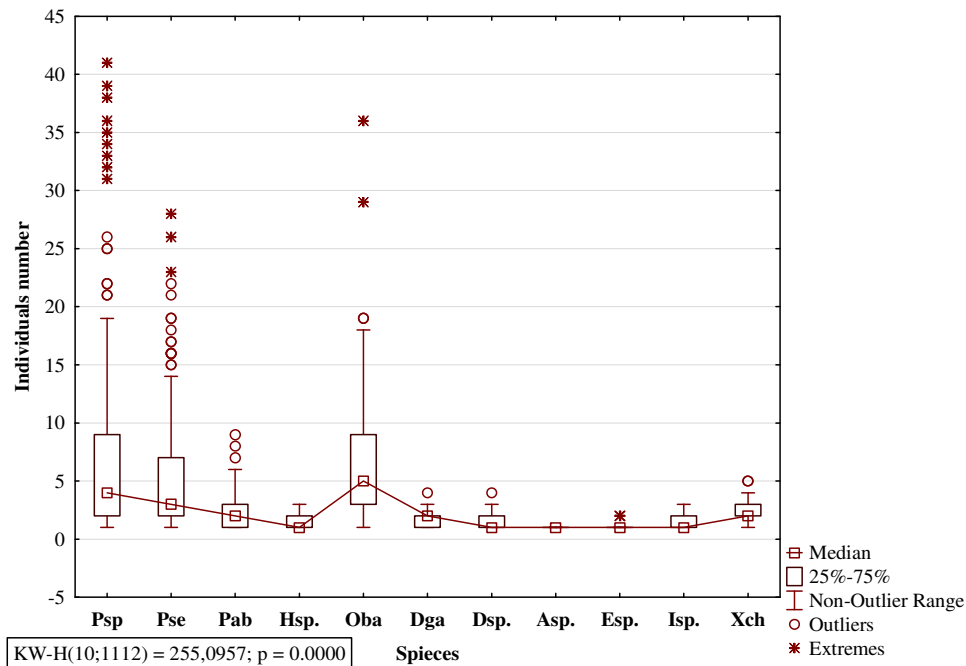


Figure 3. Individuals number of ectoparasites depending on species

Individuals number of ectoparasites depending on months

According to sampling months, the number of ectoparasites augmented from the beginning of spring until the beginning of summer of the year especially between May and July (Figure 4). In this period, temperatures were slightly increased, while in September and October, the number of parasites decreased, with a pure diminution of temperatures. Between November and February (cold period), ectoparasites were almost none. Concerning the influence of precipitations, the current work showed that the number of these arthropods decreased in rainy months (Figure 4). Statistical analyses showed that there was a very highly significant difference between the number of ectoparasites collected in hot and cold months ($P < 0.0000$) as well as in rainfall ($P < 0.0000$).

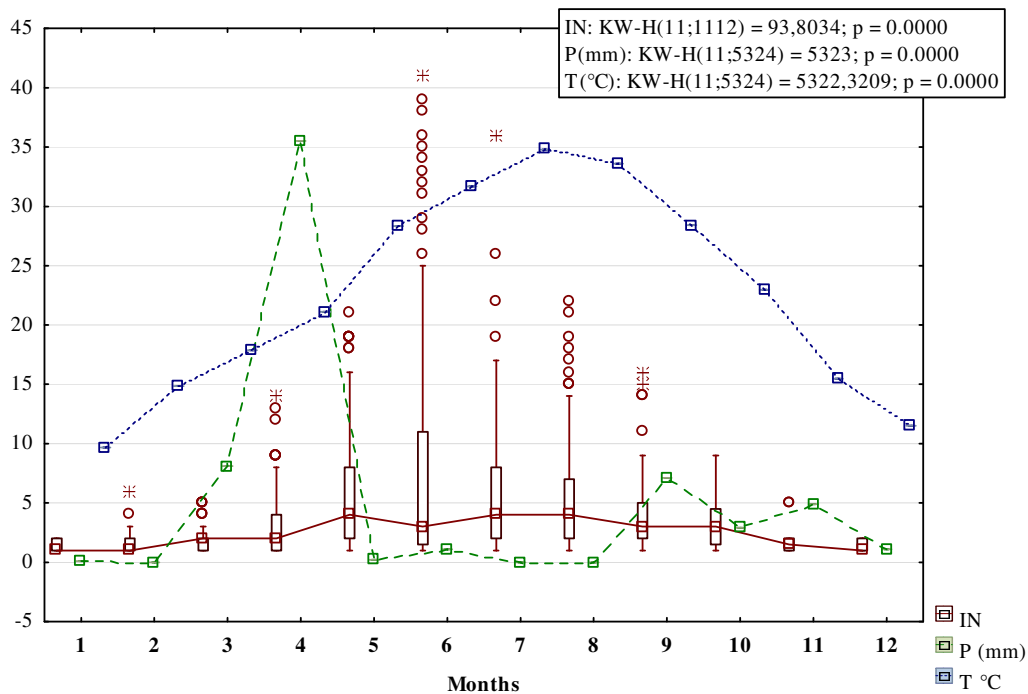


Figure 4. Individuals number of ectoparasites depending on months

Individuals number of ectoparasites in black rats' sexes depending on months

Concerning individuals' number of ectoparasites isolated from black rat's sex, males were more parasitized by these arthropods than females (Figure 5). In the winter period (November, December, January, and February), the number of parasites was almost the same in both sexes with an average of 1.5 to 2.67 parasites/male and 1 to 2.11 parasites/female. In autumn (March and April), females (2.54 to 8.39 parasites/female) were more infested than males (3.57 to 9.56 parasites/male). That coincides with the reproduction period where ectoparasites were transported by males to females. While in the beginning of summer, the number of ectoparasites increased with a peak (June) of 38.49 parasites/male (min = 3; max = 88) in males and of 26.15 parasites/female (min = 2; max = 51) in females, with great variability in both (Figure 5). After, the number of parasites gradually decreased during the rest of the months. Statistics confirmed the presence of a very highly significant monthly difference between the number of ectoparasites in males ($P < 0.00000$) and females ($P < 0.00000$).

Discussion

Diversity, prevalence, and intensity of black rats-associated arthropods

Due to the lack of studies of these parasites and the references herein Algeria, we had to discuss our results depending on the host (*R. rattus*) and its parasitic fauna in other countries. The present study revealed that 95.5% of captured rats were attacked by parasitic arthropods, where the highest prevalence was recorded with *Polyplax spinulosa* (Pr = 79.8%) followed by *P. serrata* (Pr = 56.4%). Previous literature has mentioned before the importance of black rats carrying a very large community of parasitic arthropods, where Solanki *et al.* (2013) and Nateghpour *et al.* (2013) found that this species was the most infested with a prevalence of 67% and 68.4% respectively. Meanwhile, *P. spinulosa* and *X. cheopis* were frequently registered in black rats with the highest prevalence (Taufelieb *et al.*, 1967; Soliman *et al.*, 2001; Schwam *et al.*, 2016; Khajeh *et al.*, 2017;

Eslami *et al.*, 2018; Mustapha *et al.*, 2019). Furthermore, other studies notified the presence of the other species on *Rattus* spp. (Roberts, 1991; Abd El-Halim *et al.*, 2009; Bahget, 2013; Winkel *et al.*, 2014).

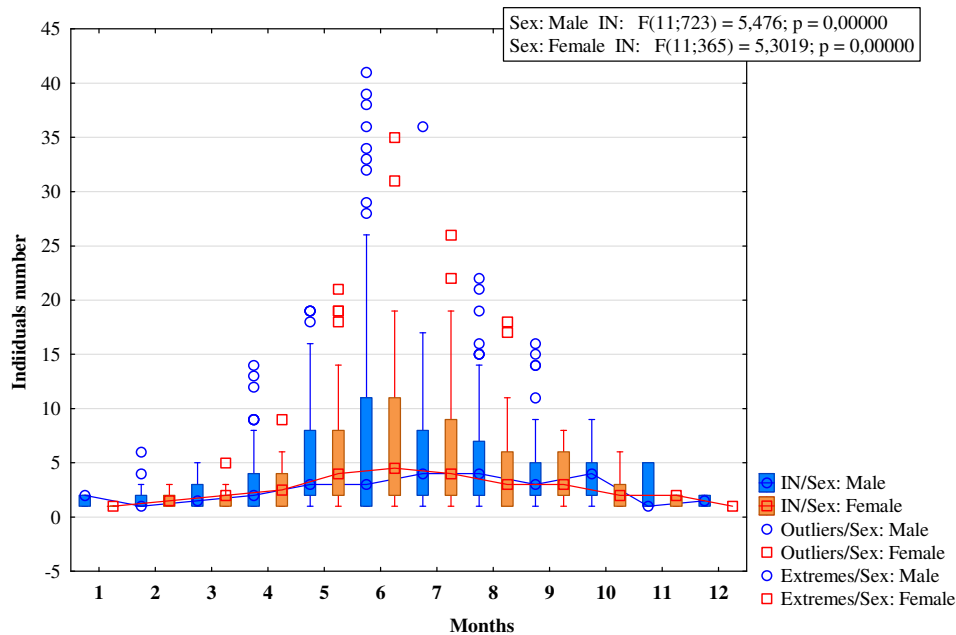


Figure 5. Individuals number of ectoparasites in black rats' sexes depending on months

Moreover, *Dermanyssus* spp. were probably transmitted to *R. rattus* from quail nests found in the study site (palm groves) where we have identified the same specimens in these nests showing cutaneous lesions and death of quails after 3 weeks of signs appearance. On the other hand, the species infesting house mice (*P. serrata* and *P. abyssinica*) were also transferred to the captured black rats by the coexistence of *M. musculus* in study sites. It is possible that these species were accidentally transferred to black rats because they are favourable host targets to parasites when transmission conditions allow it. It is quite clear that the parasitic diseases transmitted by these arthropods pose serious problems to medical and veterinary health. Where *R. rattus* was considered as the focus for a few species that constitute vectors of diseases such as the case of several species of sucking lice (*Polyplax* spp.) which are dangerous to human health, due to the fact that they participate in the distribution of various pathogens zoonoses like *Rickettsia* spp. and transferring pathogenic agents to hosts causing diseases to human beings (Crystal, 1958; Durden and Musser, 1994; Lehan, 2005; Badiaga and Brouqui, 2012; Chakma *et al.*, 2015; Thille *et al.*, 2019). Also, *X. cheopis* was considered the main epidemic and enzootic vector of murine typhus *Rickettsia typhi* (Soliman *et al.*, 2001). As well, Billeter *et al.* (2011) identified the DNA of *Bartonella rochalimae* and *B. tribocorum* in *X. cheopis*. In addition, this parasite (*X. cheopis*) was considered an important vector of the plague (*Yersinia pestis*) (Eisen *et al.*, 2007). Our study indicates the importance of the heavy infestation of ectoparasite's community associated with black rats, which highlights the degree of risks in terms of public health, especially farmers who are in direct contact with rodents carrying these arthropods.

In conclusion and according to black rat's age and sex, adult males were more susceptible to be attacked, than females and other age stages, by external parasites because of their mobility. Concerning ectoparasites' categories, lice were the main parasites in black rats' individuals.

Prevalence and mean intensity of ectoparasites

All the age classes of black rats were frequently infested by *P. spinulosa*, followed by *P. serrata*, and *O. bacoti*. Lice have been the most prevalent ectoparasite isolated from rats in most reports, which was consistent

with the results obtained in the present study. Whereas, Webster and Macdonald (1995) noted that heavy infestations of lice (*P. spinulosa*) in rats can cause irritation and anemia, and are also the most likely vector for the rat rickettsian parasite *Haemobartonella muris*. Obtained results in the present study were not similar to those found by Asiry and Fetoh (2014) who declared that ticks were the most abundant with 796 individuals (61.8%), followed by mites with 299 individuals (23.2%), lice with 152 individuals (11.8%), and fleas with 40 individuals (3.1%) in *R. rattus* samples. Also, Claveria *et al.* (2005) found that the mite *Echinolaclaps echidnius* (67%) was recorded in the first place in *Rattus* spp. followed by the louse *Polyplax spinulosa* (42%). On the other hand, Feldman and Easton (2006) found that *O. bacoti* can support the survival of *Borrelia burgdorferi* (Lyme disease).

So, we have concluded that these species were easily transmitted between the different categories without any preference. In contrast, adults presented the highest number of individuals of *Hoplopleura* sp. and *Atricholaclaps* sp., while *Dermanyssus* sp. was a very common species in sub-adults.

Individuals number of ectoparasites depending on black rats' sex and age

Mean individuals' number of collected ectoparasites from *R. rattus* allowed us to note that males were more infested than females with a great variability especially for the 25% of the most parasitized population of black rats. In contrast, in autumn (reproduction period) females were more infested than males, possible because of the suppressive testosterone effect (Mooring and Hart, 1995). This hormone leads to the high mobility of males where they are exposed to high infestation factors (Hughes and Randolph, 2001) as well as their multiple coupling. Our results confirmed those found by Soliman *et al.* (2001) who recorded a very high prevalence of external parasites in *R. norvegicus* males compared to females. Concerning age stages, our results were similar to those reported by Thille *et al.* (2019) who found that adults of *R. norvegicus* were the most infested by ectoparasites, with a prevalence of 90.7%, compared to juveniles (66.7%) with a significant difference ($P = 0.008$). Volf (1991) maintained that there is a relation between the level of infection of rats with the louse *Polyplax spinulosa* and the age and gender of rats. Likewise, Paul *et al.* (2016) remarked a high prevalence in adults of *R. norvegicus* (5.9%) compared to juveniles (3.5%). Also, Soliman *et al.* (2001) observed a high infestation rate in adults of *R. norvegicus*.

It is possible that males were more susceptible to be attacked by these parasites than females (especially in the lactation period) because of their mobility and coexistence with other animal species carrying these arthropods unlike in the reproduction period where ectoparasites were more detected in females than males.

Individuals number of ectoparasites depending on months

Depending on the sampling period, the number of ectoparasites increased from the beginning of spring until the beginning of summer, especially between May and July. In this period, temperatures were relatively high (between 35.9 °C and 41.8 °C), while in September and October, the number of parasites decreased, with a clear diminution in temperatures. On the other hand, between November and February (cold period), ectoparasites were almost none. Concerning the influence of precipitations, the study showed that the number of ectoparasites decreased during rainfall. Our results were similar to those found by Gaaboub *et al.* (1981) where the abundance of parasitic mites fluctuates throughout the year, reaching the max between May-July and the min between December-February. Thus, the peak of ectoparasites on black rat individuals was noted in the summer and decrease in winter and rainy months.

Conclusions

The current work investigating the external parasites associated with *Rattus rattus*, recorded in palm trees and storage sites from oasis regions in Algeria, showed the importance of ectoparasites' community infestation and highlights the degree of risks in terms of public health, especially farmers and domestic animals

who are in direct contact with rodents carrying these arthropods. This study revealed that adult males were more susceptible to be attacked than females and other age stages, by these external parasites because of their mobility. It should be noted that *Polyplax spinulosa* and *P. serrata*, considered as vectors of diseases, were the most trapped parasites followed by *Ornithonyssus bacoti*. Moreover, all the inventoried species were significantly greater in summer compared to winter, as well as they were decreased in rainfall. Therefore, to resolve a lot of problems, there is a need to promote awareness on prevention and control of black rats' population, not only in agriculture (to reduce damages) but also in medicine (to decrease the transmission of diseases).

As perspectives, detection, and identification of infectious microorganisms in these arthropods should be studied as well as their effect on human beings and animals.

Authors' Contributions

RM, MS and KS were involved in the conceptualization, design, execution, statistical analysis, draft and review of the manuscript. RM, BD and SM participated in the investigation, species identification, mounting and review of the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The experimental protocol was approved by the Animal Ethics Committee of the Pasteur Institute of Algeria.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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