A survey study on the helminth parasites of two wild jirds, Meriones shawi and M. libycus (Rodentia: Gerbillinae), in Tunisian desert areas

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(Received 27 November 2016; accepted 8 March 2017)

Abstract
A parasitological survey of 107 jirds, belonging to two species, Meriones shawi and M. libycus, was carried out. Rodents were trapped in three separate desert regions of Tunisia: Bouhedma (in the centre), Dghoumes (in the southwest) and Sidi Toui (in the southeast). The survey revealed infection with nine helminth species – six cestodes: Raillietina sp., Meggittina numida, Meggittina gerbilli and Inermicapsifer madagascariensis, and two Taeniidae species: Taenia endothoracicus and Taenia sp.; and three nematodes: Gongylonema neoplasticum, Physaloptera sp. and Trichuris gerbilli. The overall prevalence of helminth infection was 68.22% (73/107); the highest rate was in M. shawi (80.82%, 59 cases), whereas it was 41.17% (14 cases) in M. libycus. The most frequent helminth was Raillietina sp. (53.27%) followed by Meggittina numida (21.5%). Meriones shawi showed the highest helminth diversity with eight parasite species, with synchronous infections of two, three and four species of helminths in one rodent, compared to M. libycus in which only two cestode species were found, without synchronous infection. Moreover, significant differences in the overall infection prevalence between sexes (60.27 and 39.73% for males and females, respectively) and age were detected; only one juvenile specimen of M. shawi was found infected. Prevalence depended also on habitat: the highest infection rate was in Bouhedma (80%), followed by Dghoumes (58.33%), and the lowest was in Sidi Toui (47.82%).

Keywords: Meriones, cestodes, nematodes, desert areas, zoonoses

Introduction
Meriones species are gerbillin rodents distributed throughout the Palearctic region, especially dominant in its arid belt (Corbet 1978; Wilson & Reeder 2005). In Tunisia, there are three described species: M. shawi Duvernoy, 1842; M. libycus Lichtenstein, 1823; and M. crassus Sundevall, 1842. These three species live in desert areas (centre and south), with the exception of M. shawi, a ubiquitous species that also inhabits all the northern regions. Meriones crassus seems to be a more rare species since only two researchers, until now, have captured it in Tunisia (Choumowitch 1954; Bernard 1969). Meriones species interact with other animals sharing their biotope, especially carnivorous animals and raptors (Gharaibeh 1997; Isenmann et al. 2005). On one hand, these jirds are among the preferred prey of raptors and some carnivorous mammals; we have seen that directly in the field in the form of specimens torn up in traps, and a previous study reported that skulls of these species are most frequent in the rejection balls of raptors (Sekkour et al. 2010). On the other hand, Aulagnier et al. (2010) reported an omnivorous diet for these rodents, including insects in addition to plants. Furthermore, like many rodent species, acting as reservoirs and spreaders of zoonoses, is a major concern, and the study of parasites of wide geographic areas has medical and veterinary importance to prevent transmission of diseases to human and domestic animals. Many studies have reported the important role of these two Meriones species in cutaneous leishmaniasis (Ben Ismail et al. 1987; Rassi et al. 2006; Ghawar et al. 2011; Derbali et al. 2012; Lahmar et al. 2013; Mirzaei et al. 2013; Aou & Bouratbine 2014). However, little is known about the diversity of their internal parasites, and more information on the diversity and distribution of helminths is...
necessary. Previous studies on jirds are limited in Tunisia; Joyeux (1923) reported *Hymenolepis* sp. and *Hymenolepis diminuta* in *M. shawi*. Additionally, Jrijer and Neifar (2014) described *Meggittina numida* in *M. shawi*, a new tapeworm belonging to the genus *Meggittina* Lyndale, 1953 (family: Catenotaeniidae) of the order Cyclophyllidea. Recently, Jrijer et al. (2015) studied the diversity of nematode parasites of small mammals from Tunisia including *M. shawi* and *M. libycus*, and reported four nematode species parasites of these two rodent species: *Gongylonema neoplasticum* Fibiger & Ditllevsen, 1914; *Syphacia obvelata* Yamaguti, 1941; *Trichuris gerbilli* Bernard, 1969 and *Acanthocheilonema vitiae* Chabaud, 1952.

Therefore, and given that these two jirds, *M. shawi* and *M. libycus*, live close to humans in many arid regions in Tunisia, the aim of the current study is to shed some light on their common helminth parasites (cestodes and nematodes) in these regions, to evaluate their zoonotic potential and the risk of their transmission to humans and domestic animals.

### Materials and methods

Animals were captured alive using live rat traps; capture permits were given by the Tunisian Forest Office. Specimens were obtained after many separate campaigns in all seasons from July 2007 to March 2014, in three localities chosen to represent three different geographical regions: Bouhedma (34°47’N, 9°64’E) in the centre of Tunisia where only specimens of *M. shawi* were captured; Dghoumes (34°03’N, 8°27’E) in the southwest and Sidi Toui (32°39’N, 11°14’E) in the extreme southeast (Figure 1). The three regions were characterised by spontaneous vegetation and arid climate for Bouhedma and arid to saharan for Dghoumes and Sidi Toui.

After being euthanised by isoﬂurane inhalation (Gaertner et al. 2008), animals were dissected through the ventral surface, to expose the abdominal cavity and the gastrointestinal tract; the stomach, small intestine, large intestine and cecum were examined. Parasites were isolated, washed with water, fixed in 70% ethanol and then diagnosed. First, helminths were examined directly under microscope after being cleared in glycerin, then cestode parasites were stained with Semichon aceticarmine, dehydrated in a graded alcohol series (100, 90 and 70%), cleared in methyl salicylate and mounted in Canada balsam. All helminths were speciﬁcally identified under a microscope based on their morphology according to the literature (Khalil et al. 1994; Chervy 2002; Anderson et al. 2009).

Statistical tests were computed following Bush et al. (1997). We investigated the relations between prevalence of infection and host sex, maturity or localities by Chi-square test with Yates correction using the software R 3.3.2 (R Development Core Team 2016).

### Results

A total of 107 wild jirds belonging to the two species *Meriones shawi* (n = 73) and *M. libycus* (n = 34), including nine juveniles, were captured: 45 (42%) were female, of which seven (15.55%) were pregnant; and 62 (58%) were males. All the rodents were healthy and active and some even exhibited aggressive behaviours in captivity prior to dissection. Analysis of stomach contents shows the presence of many species of plants, especially grasses, which constitute the largest part of their food; we also noted the presence of fragments of elytra, antennae and feet of some species of insects such as ants and beetles.

Seventy-three jirds were infected (68.22%) and nine species of helminths were identified (Table I): six cestodes, *Raillietina* sp., *Meggittina numida*, *Meggittina gerbilli*, *Inernicapsifer madagascariensis*, in addition to larvae of two different Taniidae species: polycephalic cysticerci of *Taenia* sp. were found in the abdominal cavities of two individuals of *M. libycus* of Dghoumes and three cysts containing six, nine and 10 protoscolices of *T. endothermalicus* were located in the peritoneum attached to the large omentum in the abdominal cavity of one *M. shawi* from Bouhedma.

Three nematode species were found: *Gongylonema neoplasticum*, *Physaloptera* sp. and *Trichuris gerbilli*. The rate of infectivity within species was 80.82% for *M. shawi* and 41.17% for *M. libycus*. The most common helminth was *Raillietina* sp. (57/107, 53.27%) followed by *M. numida* (23/107, 21.5%), while the least common were *M. gerbilli*, *T. gerbelli* and *G. neoplasticum* (0.93%) (Figure 2). The three nematode species were found only in *M. shawi*: three individuals of *G. neoplasticum* (two males and one female) in the stomach mucosa of one specimens of Bouhedma, and two individuals of *Physaloptera* sp. in the cecum of two *M. shawi*, one from Bouhedma and one from Sidi Toui (2/107, 1.87%); finally, one *T. gerbelli* in the cecum of one *M. shawi* of Bouhedma. Other details of helminth infections are shown in Table I.

Synchronous infections occurred in up to four species of helminths in one rodent for *M. shawi* (23.72%), while no cases were found within *M. libycus*. The percentages of infectivity with one, two, three and four species of helminths in infected Shaw’s jirds were 76.27% (45 cases), 13.56% (eight cases), 8.47% (five cases) and 1.7% (one case), respectively.
The prevalence of overall helminth infection was investigated in relation to sex, maturity and localities. Infection prevalence differs between sexes, either when all specimens of the two species were pooled together (71 and 64.44% for males and females, respectively) or within each species (Table II), but statistical analysis showed a non-significant association between prevalence of helminth infection and host sex ($\chi^2 = 0.25, p = 0.61$). However, infection was highly significant between adults and juveniles ($\chi^2 = 12.05, p = 0.0005$): it increases with age – only one case of infection within juvenile jirds was detected, in M. shawi (Table II).

Infection rates were significantly different between localities ($\chi^2 = 9.3354, p = 0.009$): the highest prevalence was found in Bouhedma (48/60, 80%), followed by Dghoumes (14/24, 58.33%), and the lowest was in Sidi Toui (11/23, 47.82%). More results are shown in Table III.

**Discussion**

Our study reported nine helminth species within two *Meriones* species analysed; six tapeworms, including two larvae of two different Taeniidae species, and three nematode species. Results showed that *M.*
shawi harbours most of the parasites found, while the helminth fauna of M. libycus was very poor. Moreover, these results confirm that these jirds, like many other rodents, could act as definitive and intermediate hosts. Infection prevalence increases with host maturity; however, host sex does not influence it significantly.

In the south, the two host species M. shawi and M. libycus occur in sympatry in most regions sharing the same alimentation resources. Analysis of stomach contents revealed that these two species are to some degree omnivorous, even though plants constitute the largest part of their food as reported by Adamou-Djerbaouiz et al. (2013); these rodents, especially M. shawi, feed also on many insects, such as ants and beetles, as reported by Aulagnier et al. (2010). The main helminth parasite represented in our rodent samples was Raillietina sp. Among species of the genus Raillietina Fuhrman, 1920 the number of egg cells in each capsule is an identifying characteristic of each species (Khalil et al. 1994). Some studies (e.g. Fichet-Calvet 2003; Ben Faleh et al. 2012) consider that the Raillietina species parasites of rodents is R. trapezoïdes for which the number of egg cells in each capsule is known to be around 25; however, Raillietina specimens found in the current study showed different numbers of cells. This result requires further taxonomic study of these specimens to determine their parasitic entities. The high prevalence of the occurrence of this parasite in the two studied jirds indicates that these two host species feed on the intermediate host of this parasite, which is reported to be an arthropod such as ants and beetles, where the larvae (cysticercoids) grow with a direct life cycle of rodent–arthropod–rodent (Acha & Szyfres 2006; Baker 2008). Fragments of these insects were frequent in the stomach content of M. shawi and less frequent in that of M. libycus, whose stomach content showed fragments of plants including Chenopodiaceae while insect fragments were rare. The high infectivity rate indicates the susceptibility of these gerbils to this cestode. Raillietina species have been reported as parasites of Meriones spp. as well as of other gerbillin and Dipodidae rodents: Gerbillus spp., Psammomys obesus (Fichet-Calvet et al. 2003) and Jaculus jaculus (Wertheim et al. 1986; Ben Faleh et al. 2012); here we report two Meriones species as definitive hosts for this helminth.

The second prevalent helminth species found in the current study was M. numida. Species of the genus Meggittina are known to be parasites of African and Malagasy Muroïde rodents, Nesomyidae, Cricetidae

### Table I. Helminth infections in Tunisian specimens of Meriones shawi and M. libycus analysed.

| Host      | No. hosts | Helminthes species     | No. of positive hosts | Prevalence | Mean intensity | Mean Abundance | Range     |
|-----------|-----------|------------------------|-----------------------|------------|----------------|---------------|-----------|
| M. shawi  | 73        | Raillietina sp.         | 45                    | 61.64%     | 38.24          | 23.57         | 1–225     |
|           |           | Inermicapsifer madagascariensis | 3              | 4.1%       | 2              | 0.082         | 1–4       |
|           |           | Meggittina numida       | 23                    | 31.5%      | 33.21          | 10.46         | 1–228     |
|           |           | Meggittina gerbilli     | 1                     | 1.37%      | 1              | 0.013         | 1         |
|           |           | Physaloptera sp.        | 2                     | 4.1%       | 1              | 0.041         | 1–1       |
|           |           | Gonglyonema neoplasticum| 1                     | 1.37%      | 3              | 0.041         | 3         |
|           |           | Trichuris gerbilli      | 1                     | 1.37%      | 1              | 0.0137        | 1         |
|           |           | Larvae of Taeniidae     | 1                     | 1.37%      | 3              | 0.041         | 3         |
| M. libycus| 34        | Raillietina sp.         | 12                    | 35.3%      | 20.83          | 7.35          | 1–67      |
|           |           | Larvae of Taeniidae     | 2                     | 5.88%      | 39             | 2.3           | 18–60     |

### Table II. Prevalence of helminths in relation to sex and maturity of jirds collected in the three studied localities.

| Species | Rodent sex | M. shawi | M. libycus | Rodent maturity |
|---------|------------|----------|------------|----------------|
| Sex/age |            | M        | F          | M    | F    | Adults | Juveniles |
|         |            |          |            |      |     |        |            |
| No. examined |        | 43   | 30   | 19  | 15  | 98   | 9         |
| No. positive |      | 36   | 23   | 8   | 6   | 72   | 1         |
| Prevalence |      | 83.72% | 76.66% | 42.1% | 40% | 67.3% | 0.93%     |
and Muridae (Lynsdale 1953; Wertheim et al. 1986; Jrijer & Neifar 2014). While the newly recorded species *M. numida* (Jrijer & Neifar 2014) was very frequent in our samples of *M. shawi* of Bouhedma, a second species of the same genus, *M. gerbili*, was represented by only one case, which may be an accidental infection although the occurrence of this species in *Meriones* has been reported before, in *M. crassus*, and in many Gerbillus species (Wertheim et al. 1986). The fact that *M. numida* was very frequent in *M. shawi* of Bouhedma, but rare in specimens of the same species from the other two localities, Dghoumes and Sidi Toui (one case in each locality) and totally absent in *M. libycus*, can be explained by, on one hand, the fact that the intermediate host is not a part of the diet of *M. libycus*, which strengthens the difference already mentioned in the diet of these two species of rodents. On the other hand, this intermediate host cannot bear drier environments; Bouhedma is an arid region while Dghoumes is in the saharan habitat and the southern-most locality studied, Sidi Toui, is on the edge of the arid and saharan bioclimates.

*Inermicapsifer madagascariensis*, which we found only in three *M. shawi* specimens captured from the same colony in Bouhedma, was already reported in more than 20 species of rodents throughout the Ethiopian region, but also within humans in Africa, Cuba, Madagascar, Comoros and Reunion (Bailengé & Carcenac 1970; Hira 1975; González Núñez et al. 1996); it was known as a rodent parasite of the genus *Arvicanthis* in eastern Africa (González Núñez et al. 1996). In Tunisia, Ben Faleh et al. (2012) reported *Inermicapsifer* sp. in *faculus deserti*. Its life cycle is unknown, but by analogy with related parasites of the genus *Raillietina* (Herrera Valdes et al. 2007) and given the synchronous infection by these two parasites found in our specimens, we estimated that a coprophagous arthropod acts as intermediate host which ingests the eggs deposited with the excrement of the definitive host.

In the present study, new findings of immature-stage parasites are reported, that require carnivorous mammals or raptors to complete their life cycle; they are infrequent since they were found in a total of three jirds. The first one, in *M. shawi* of Bouhedma, was a larva of *T. endothoracicus* Kirchenblatt, 1948. Several authors (e.g. Abuladze 1964; Ryjikov et al. 1978) have reported this Taenia species from Georgia, Turkmenistan, Kazakhstan, Russia, Iran, and Morocco with members of Gerbillinae as intermediate hosts, specifically *G. cheesmani* (Khalil et al. 1979) and *Rhomboynys optimus* (Ganzorig et al. 1998; Kamranrashani et al. 2013). Within *Meriones* this larva has been previously described in *M. libycus* (Fashi-Harandi 1992; Mowlavi et al. 2004), *M. unguiculatus*, *M. meridianus* (Ganzorig et al. 1998) and *M. persicus* (Kia et al. 2010); therefore, occurrence of a *T. endothoracicus* larva in *M. shawi* in this study constitutes a record in a new intermediate host species. Khalil et al. (1979) reported the red fox (*Vulpes vulpes*) as a definitive host; this carnivorous animal lives in many regions in Tunisia including those we studied but is not very frequent, which could explain the rarity of this Taeniidae larva in our sample.

The second Taeniidae larva (*Cœnostrobilocercus*) of *Taenia* sp., found in the two *M. libycus* of Dghoumes, is similar to that of *T. parva* Baer, 1926, previously detected by Campana-Rouget (1950) and Dollfus and Saint-Girons (1958) in the abdominal cavity of the wood mouse (*Apodemus sylvaticus*) from the French Eastern Pyrenees; then Bernard (1963) and more recently Swiderski et al. (2007) found the same larval stages in the same rodent in Tunisia and the Iberian Peninsula, respectively. However, this parasite is known to be very specific in terms of both the definitive host and the intermediate host; the first being the common Genet (*Genetta genetta*) that lives in our study areas but seems to be very rare, and the second being the wood mouse. The low prevalence of the occurrence of *Taeniidae* larvae in our samples did

**Table III. Prevalence of helminths in *Meriones shawi* and *M. libycus* among three different localities in Tunisia.**

| Locality     | Bouhedma | Dghoumes | Sidi Toui |
|--------------|----------|----------|-----------|
| Host species | *M. shawi* | *M. libycus* | *M. shawi* | *M. libycus* |
| No. of specimens | 60 | 0 | 8 | 16 | 5 | 18 |
| No. positive | 48 | - | 6 | 8 | 5 | 6 |
| *I. madagascariensis* | 75% | - | 0% | 0% | 0% | 0% |
| *M. numida* | 6.25% | - | 100% | 75% | 60% | 100% |
| *M. gerbili* | 2.08% | - | 0% | 0% | 0% | 0% |
| *Physaloptera sp.* | 2.08% | - | 0% | 0% | 0% | 0% |
| *G. neoplasticum* | 2.08% | - | 0% | 0% | 0% | 0% |
| *T. gerbili* | 2.08% | - | 0% | 0% | 0% | 0% |
| Larvae of Taeniidae | 2.08% | - | 0% | 25% | 0% | 0% |
not allow us to exclude the possibility of accidental ingestion of eggs by the examined jirds.

The Nematoda are represented in our samples by three species. Species of the genus Gongylonema are known to be parasites of birds and mammals including man (Wilson et al. 2001), and are transmitted by beetles (Eira et al. 2006; Gibson 2007; Hallan 2007). They have been reported in rats (Hasegawa et al. 1994; Paramasvaran et al. 2009) and recently in M. shawi (Jrijer et al. 2015) with a high infection rate (50%); however, in the current study we found Gongylonema neoplasticum in the stomach mucosa of only one specimen of M. shawi.

Physaloptera sp. was found in the cecum of two M. shawi. Until now only one species, P. musayevi, was reported, by Mamedov (2012) in a Libyan jird. In Tunisia our result is the first report of a Physaloptera species within a rodent, since the only prior report was of P. clausa by Jrijer et al. (2015) with Atelerix algirus as host with high prevalence.

One individual of T. gerbilli Bernard, 1969 was found in the cecum of one specimen of M. shawi. This whipworm was previously described by Bernard (1969) from Gerbillus sp., and more recently Jrijer et al. (2015) reported this nematode in M. shawi and M. libycus in Tunisia.

A significant difference in infection prevalence was detected between localities. Bouhedma had the highest parasite diversity, with eight species, which may be explained in part by differences in sample size (Table III). Furthermore, as mentioned above, the important infection rate with M. numida alone or synchronous with Raillietina sp. in M. shawi of Bouhedma, but not found in specimens of the same rodent species from the two other localities, suggests that the purely saharan habitats of these two localities are not suitable for the intermediate host of M. numida, and probably not suitable for all intermediate hosts of other parasites.

Despite the fact that the majority of these species are infrequent, their occurrence in Meriones species, especially M. shawi which is ubiquitous in Tunisia and can be found in close proximity to humans, is a major concern. Among nine species of helminth parasites recovered from M. shawi and M. libycus in our study areas, the following species are considered to be zoonotic helminths: two cestode species, Raillietina sp. and I. madagascariensis, and the three nematode species. Possible infection by Raillietina species has been reported in humans after accidental ingestion of contaminated food by the intermediate host (Acha & Szyfres 2006). Inermicapsifer madagascariensis is considered to be a potential zoonotic agent; it was reported as a parasite of humans in many African countries (Bailenger & Carcenac 1970; Hira 1975; González Núñez et al. 1996). Among the nematode species discussed above, G. neoplasticum is also considered to be potentially zoonotic, based on its wide host spectrum and its high rates of transmission (Eira et al. 2006; Paramasvaran et al. 2009; Jrijer et al. 2015), although human infection is rare. Many species of Physaloptera are known to be zoonotic, although human infections have been reported on rare occasions after accidental ingestion of infected intermediate or paratenic hosts (Naem et al. 2006). Finally, the most potentially zoonotic nematode species are those of Trichurus; many authors have reported cases of infection in humans by Trichurus species (Hall & Sonnenberg 1956; Kagei et al. 1986; Crompton 1999; Brooker et al. 2000; Ashford & Crewe 2003).

In conclusion, the current study reveals remarkable differences in the prevalence of helminth infection between jirds examined herein, showing richness within M. shawi (five cestodes and three nematodes) in comparison with its congener M. libycus (two cestodes). It also gives evidence of new hosts for Physaloptera sp., M. gerbilli, I. madagascariensis and T. endothoracicus. Furthermore, the information presented here improves our understanding of the major parasitic infections that Meriones species harbour and can transmit to human and animal populations in Tunisia.

Acknowledgements

The authors are grateful to the Forest Office of the Tunisian Ministry of Agriculture for providing necessary permits for collection of specimens. Special thanks are addressed to all forest conservators and guards for their help during sampling. This work was supported by the Research Unit “Biodiversity and Population Biology 05/UR/09-10” which provided materials for the animal collection, and “Laboratoire de Biodiversité et Ecosystèmes Aquatiques, Faculté des Sciences de Sfax” which provided materials for analysis. We thank the anonymous referees for their useful comments and suggestions.

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