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Intestinal helminths of stray dogs from Tunisia with special reference to zoonotic infections

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Abstract

Sixteen intestinal helminth species were recovered from 271 necropsied stray dogs during a survey undertaken in Raoued and Soukra, two northeastern rural regions of Tunisia. Recorded parasites included trematodes (*Brachylaemus* sp., *Phagicola italica*, *Heterophyes heterophyes*), cestodes (*Echinococcus granulosus*, *Dipylidium caninum*, *Diplopylidium noelleri*, *Mesocestoides lineatus*, *Mesocestoides litteratus*, *Taenia hydatigena*, *Taenia pisiformis*, *Taenia multiceps*), nematodes (*Toxocara canis*, *Ancylostoma caninum*, *Uncinaria stenocephala*, *Trichuris vulpis*) and one acanthocephalan *Macracanthorhynchus hirudinaceus.* This is the first record of *Brachylaemus* sp., *P. italica*, *H. heterophyes*, *D. noelleri* and *M. hirudinaceus* in dogs from Tunisia. *Echinococcus granulosus* was found in 5·16% of dogs with a higher intensity of infection recorded in younger animals (303 worms/infected dog). Molecular analysis confirmed *E. granulosus* sensu stricto as the cause of canine echinococcosis. This epidemiological study investigating the status of intestinal helminths of dogs recorded a prevalence of 98·89% and a mean intensity of 87·62 worms per infected dog and confirmed the infection of 95·14% of dogs with helminths of potential zoonotic concern. Results of this study emphasize the need to interrupt parasite transmission using preventive approaches in zoonoses control programmes mainly against cystic echinococcosis, as well as reducing transmission to other animals by regular dog dosing treatments and proper management of dog populations.

Introduction

Stray dogs represent a significant reservoir for numerous intestinal helminth species, many of which are of zoonotic concern. The role of dogs in the spread of diseases that pose a potential risk to public health has been reported by many authors (Bentounsi *et al.* 2009; Jenkins *et al.* 2014; Ramos *et al.* 2015; Amissah-Reynolds *et al.* 2016; Geraili *et al.* 2016; Ilić *et al.* 2017; Dakkak *et al.* 2017). Dogs are known definitive hosts of several helminths and as such contaminate the environment with infective and/or parasitic stages that undergo maturation in the soil. To minimize human exposure to zoonotic parasites, it is generally recommended to humanely manage and control populations of roaming dogs (FAO, 2014). However, in developing countries, dog population management is still based on the mass killing of unowned dogs, particularly following epidemics of human rabies. In Tunisia, this traditional approach is still widely practised although it is strongly contested by the public. Stray dogs in Tunisia are estimated to constitute 80% of the 509 000 rural dog populations (DGSV, 2011) and easily have access to condemned organs and abandoned livestock carcasses (Deplazes *et al.* 2017). Several surveys on dog intestinal helminths have previously been carried out for the central west, northwestern and southern regions of Tunisia (Lahmar *et al.* 2001, 2008, 2009; Oudni-M'rad *et al.* 2017). To the best of our knowledge, this is the first study on the epidemiological parameters of intestinal helminths in stray dogs from two previously unexamined rural areas, Raoued and Soukra in northeastern Tunisia, with special reference to *Echinococcus granulosus* and other zoonotic helminths.

Materials and methods

Dogs and studied areas

Stray dogs (*Canis familiaris*) included in this study originated from Raoued and Soukra (Ariana Governorate) northeast of the capital Tunis, which have a population of 94 961 and 129 693 inhabitants, respectively (2014 census), and are located 20 and 6 km from the capital Tunis (Fig. 1). During the period between October and December 2014, a total of 271 stray dogs (130 males and 141 females) were shot as part of a rabies campaign conducted by the Ministry of Interior (MoI) to reduce stray dog numbers. During these campaigns, dog carcasses are normally disposed of in rubbish dumps and are subsequently burnt. Conscious of the relevance of such dog carcasses to scientific research and eager to implement strict

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Fig. 1. Map of Ariana Governorate, Tunisia.

carcass disposal measures, the Veterinary School of Sidi Thabet applied for and was granted special consent from the MoI to recover the animal carcasses directly following shooting. These were used for necropsy which, to date, remains the gold standard for exploring canine parasitic intestinal fauna in order to assess the potential risk of zoonotic infections. Carcasses were immediately transferred to the Veterinary School of Sidi Thabet where they were identified by place of origin, sex and age (estimated through the examination of teeth) (http://www.minpin.hu/health/teeth/teeth.htm). Dogs included in this study were between 3 months and 9 years old and were classified into four age groups, less than 1-year olds (<1), between 1 to less than 2 years (⩾1 to <2), from 2 years to less than 6 years (⩾2 to <6) and over ⩾6 years.

Parasitological procedures

Necropsies were carried out by qualified veterinarians at the Parasitology Department of Sidi Thabet Veterinary School. Ligatures were made at the cranial end of the duodenum and the end of the rectum thus allowing the removal of the entire intestinal tract. Intestines of necropsied animals were frozen at −80 °C for >15 days to inactivate infectious eggs of *Echinococcus* species. The intestine of each dog was defrosted, opened longitudinally and its contents and scrapings of the mucosa were washed with an isotonic saline solution (0·9% NaCl). The solution was allowed to stand and the sediment was carefully examined for the presence of helminth parasites. Differentiation of *Taenia* species and other species of cestodes was based on hook length and the size and morphology of the proglottids following staining with 1% acetocarmine solution (Khalil *et al.* 1994). The total number of *Echinococcus* tapeworms harboured by each dog was determined either directly by counting all the worms in the sediment (if worm burdens were small) or estimated by counting the worms in 20% of the sediment. Nematodes were clarified in chloral-lactophenol solution (44%) for morphological identification and enumeration (Anderson, 1992).

Molecular analysis

DNA-based molecular identification was carried out on retrieved *Echinococcus* adult tapeworms. Total genomic DNA was extracted using a commercial kit (Qiagen DNeasy Blood and Tissue DNA extraction Kit, Qiagen, Hilden, Germany) as per manufacturer's instructions. A fragment within the mitochondrial cytochrome *c* oxidase subunit 1 (*cox 1*) gene was amplified using published probes (Bowles *et al.* 1992). PCR products were viewed using UV illumination (Syngene G: Box gel documentation system, Cambridge Biosciences), purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and commercially sequenced in both directions (Macrogen EZ- Sequence, Amsterdam, The Netherlands). The generated nucleotide sequences were compared to the Bowles *et al.* (1992) G1-G3 genotype sequences as they appeared in the aforementioned publication.

Data analysis

The χ^2 test and Fisher's exact test were used to compare the prevalence of helminth species in the two studied regions, as well as prevalence according to host age and gender. All statistical analyses were performed using SPSS version 16. A *P*-value of ⩽0·05 denoted a statistically significant difference. The Student's *t*-test was also used to determine differences in prevalence and in abundance of each species according to host age and gender.

Results

Genotyping

DNA was extracted from *E. granulosus* adult tapeworms retrieved from 10 of the 14 infected dogs. A successful amplification of a 366 bp mitochondrial *cox 1* fragment to identify species/genotypes of *Echinococcus* was achieved for worms removed from nine dogs. The alignment of the generated nucleotide sequences against those reported by Bowles *et al.* (1992), showed that the nine Tunisian dogs were infected with *E. granulosus* sensu stricto (s.s.) (eight *E. granulosus* G1 and one *E. granulosus* G2 genotype).

General infection

Of the 271 examined stray dogs, 268 were infected with one or more helminth species, giving an overall prevalence of 98·89% (±0·006) and a mean intensity of 87·62 (95% CI 63·1–112·13) parasites per infected dog. Sixteen intestinal helminth species were identified, including three trematodes [*Brachylaemus* (*Brachylaima*) sp., *Phagicola* (*Ascocotyle*) *italica*, *Heterophyes heterophyes*], eight cestodes (*E. granulosus* s.s., *Dipylidium caninum*, *Diplopylidium noelleri*, *Mesocestoides lineatus*, *Mesocestoides litteratus*, *Taenia hydatigena*, *Taenia pisiformis*, *Taenia multiceps*), four nematodes (*Toxocara canis*, *Ancylostoma caninum*, *Uncinaria stenocephala*, *Trichuris vulpis*) and one acanthocephalan (*Macracanthorhynchus hirudinaceus*) (Table 1). Cestodes were the most prevalent parasites (97·04%), followed by nematodes (83·02%), trematodes (5·53%) and acanthocephalans (2·21%). The difference in the infection rate with each helminth group ($P < 0·001$) was highly significant. Nineteen dogs had a single infection, 61 had a double infection, 90 had a triple infection, 55 had four infections, 28 had five infections, 12 had six infections and three dogs had seven infections. A high percentage of infected dogs (95%) harboured zoonotic species including *E. granulosus* s.s., *D. caninum*, *T. multiceps*, *T. canis*, *A. caninum* and *H. heterophyes.*

Prevalence and intensity of intestinal helminths

Prevalence rates for each helminth species are presented in Table 1. The highest worm intensity (303·7 worms) was observed for *E. granulosus* s.s. with the total number ranging from 2 to 2540 tapeworms. Another high mean intensity was recorded for the trematode *P. italica* (136·6 parasites/infected dog) with the number of worms varying between 4 and 736. Lower mean intensities (⩽10 worms) were detected for *Brachylaemus* sp.,

Table 1. Prevalence, intensity and range of intestinal helminths of stray dogs from two northeastern areas in Tunisia

	Dogs							Total			
	Raoued (*N* = 210)		Soukra (*N* = 61)		χ2	Raoued (*N* = 210)	Soukra (*N* = 61)	(*N* = 271)			
Helminth species	*n*	P%	*n*	P%	(D.F. = 2)	I	I	*n*	P%	M. I.	Range
Trematoda											
Brachylaemus sp.	1	0·47	0	0	*F* (*P* = 1)	10	0	1	0·36	10	1–10
Phagicola italica	9	4·28	1	1·63	*F* (*P* = 0·46)	149·44	21	10	3·69	136·6	4–736
Heterophyes heterophyes	4	1·9	0	0	*F* (*P* = 0·57)	8·75	0	4	1·47	8·75	1–12
Cestoda											
Echinococcus granulosus	11	5·23	3	4·91	*F* (*P* = 1)	370·54	52	14	5·16	303·78	2–2540
Dipylidium caninum	168	80	50	81·96	χ^2 = 0·11; *P* = 0·73	27·58	10·32	218	80·44	23·62	1–659
Diplopylidium noelleri	60	2·85	15	24·59	χ^2 = 0·37; *P* = 0·54	8·21	8·86	75	27·67	8·34	1–65
Mesocestoides lineatus	98	46·66	25	40·98	χ^2 = 0·61; *P* = 0·43	32·93	23·36	123	38·45	30·99	1–677
Mesocestoides litteratus	35	16·66	6	9·83	χ^2 = 1·71; *P* = 0·19	11·45	11·33	41	15·12	11·43	1–134
Taenia hydatigena	55	26·19	5	8·19	χ^2 = 8·87; *P* = 0·002*	2·45	2·2	60	22·14	2·43	1–10
Taenia pisiformis	17	8·09	11	18·03	χ^2 = 5·03; *P* = 0·02*	3·35	1·27	28	10·33	2·53	1–32
Taenia multiceps	6	2·85	3	4·91	*F* (*P* = 0·42)	1·83	6·66	9	3·32	3·44	1–12
Nematoda											
Toxocara canis	58	27·61	14	22·95	χ^2 = 0·52; *P* = 0·46	8·24	3·35	72	26·56	7·29	1–181
Ancylostoma caninum	3	1·42	1	1·63	*F* (*P* = 1)	6·66	3	4	1·47	5·75	2–14
Uncinaria stenocephala	152	72·38	46	75·4	χ^2 = 0·22; *P* = 0·63	36·34	30·78	198	73·06	35·05	1–484
Trichuris vulpis	1	0·46	0	0	*F* (*P* = 1)	5	0	1	0·36	5	1–5
Acanthocephala											
Macracanthorhynchus hirudinaceus	3	1·42	3	4·91	*F* (*P* = 0·12)	5	1·33	6	2·21	3·16	1–9
All	208	99·04	60	98·36	χ^2 = 0·001; *P* = 0·97	98·4	50·23	268	98·89	87·62	

N, number of dogs examined; n, number of infected dogs; P, prevalence; I, intensity; M.I., mean intensity; *significant.

H. heterophyes, *D. noelleri*, *T. hydatigena*, *T. pisiformis*, *T. multiceps*, *T. canis*, *A. caninum*, *T. vulpis* and *M. hirudinaceus* (Table 1).

Prevalence and intensity of intestinal helminths in dogs by region

There was no significant difference in helminth prevalence observed between the two studied regions, Raoued (99·04% ± 0·007) and Soukra (98·36% ± 0·016) ($\chi^2 = 0{\cdot}001$; $P = 0{\cdot}97$). However, the mean intensity of infection was significantly higher in dogs from Raoued than in those from Soukra ($P = 0{\cdot}01$) (Table 1). Dogs from Raoued harboured all the helminth species described in this study while, *Brachylaemus* sp., *H. heterophyes* and *T. vulpis* were absent in dogs from Soukra. Multiple infections of dogs with each helminth species revealed no significant difference in prevalence between the two regions except for *T. hydatigena* ($P = 0{\cdot}002$) and *T. pisiformis* ($P = 0{\cdot}02$). *Dipylidium caninum* was the most prevalent helminth in the two regions with a higher intensity in Raoued (27·58 per dog) than in Soukra (10·32 per dog). Although *E. granulosus* s.s. infection rate was almost similar for the two regions, dogs from Raoued were more intensely infected (370·54 worms per infected dog), with one dog harbouring 2540 worms. However, in Soukra area, *E. granulosus* s.s. showed the highest helminth intensity followed by *U. stenocephala*. The trematode *P. italica* was present in dogs from Raoued with an intensity of 149·4 parasites per infected dog, while the lowest mean intensities of infection in the two regions were recorded for three *Taenia* species (*T. hydatigena*, *T. pisiformis*, *T. multiceps*) and *M. hirudinaceus* (Table 1).

Prevalence and intensity of intestinal helminths in relation to age and gender of dogs

Age and gender distribution of intestinal helminths are represented in Tables 2 and 3. In all age groups, dogs were highly infected although a reduced prevalence was observed in animals aged <1 year (96·92%) and those ⩾1 to <2 years (99·03%) ($P = 0{\cdot}48$). However, the abundance of infection was highest in dogs <2 years ($P = 0{\cdot}01$). There was no significant difference in the prevalence ($P = 1$) observed between male (99·23% ± 0·008) and female dogs (98·58% ± 0·01), although the mean abundance of infection was significantly higher in males ($P = 0{\cdot}049$). All helminth species were identified in both sexes except *Brachylaemus* sp. and *T. vulpis*, which were not found in any of the examined male dogs. There was no difference in prevalence between the sexes for any helminth species except for *T. canis* ($P = 0{\cdot}009$), *U. stenocephala* ($P = 0{\cdot}01$) and *H. heterophyes* ($P = 0{\cdot}01$)

Table 2. Prevalence of intestinal helminths of stray dogs from two northeastern areas in Tunisia by host age and gender

Helminth species	<1 year % (*n* = 65)	(⩾1 to <2) years % (*n* = 104)	(⩾2 to <6) years % (*n* = 70)	⩾6 years % (*n* = 32)	χ^2	Male % (*n* = 130)	Female % (*n* = 141)	χ^2
Trematoda								
Brachylaemus sp.	0	0	1·42	0	*F* (*P* = 0·61)	0	0·7	*F* (*P* = 1)
Phagicola italica	6·15	0·96	5·71	3·12	*F* (*P* = 0·17)	2·3	5·38	*F* (*P* = 0·3)
Heterophyes heterophyes	1·53	0·96	2·85	0	*F* (*P* = 0·88)	0·76	2·12	*F* (*P* = 0·01)*
Cestoda								
Echinococcus granulosus	1·53	6·73	4·28	9·37	*F* (*P* = 0·25)	3·07	7·09	*F* (*P* = 0·17)
Dipylidium caninum	80	81·73	80	78·12	$\chi^2 = 0{\cdot}23$; $P = 0{\cdot}97$	80·76	80·14	$\chi^2 = 0{\cdot}01$; $P = 0{\cdot}89$
Diplopylidium noelleri	35·38	25	34·28	6·25	*F* (*P* = 0·005)	25·38	29·78	$\chi^2 = 0{\cdot}65$; $P = 0{\cdot}41$
Mesocestoides lineatus	43·07	50	44·28	37·5	$\chi^2 = 1{\cdot}87$; $P = 0{\cdot}59$	41·53	48·93	$\chi^2 = 1{\cdot}49$; $P = 0{\cdot}22$
Mesocestoides litteratus	18·46	13·46	12·85	18·75	$\chi^2 = 1{\cdot}39$; $P = 0{\cdot}7$	16·92	13·47	$\chi^2 = 0{\cdot}62$; $P = 0{\cdot}42$
Taenia hydatigena	16·92	17·3	21·42	50	$\chi^2 = 16{\cdot}86$; $P = 0{\cdot}0007$*	20·76	23·4	$\chi^2 = 0{\cdot}27$; $P = 0{\cdot}6$
Taenia pisiformis	1·53	7·69	15·71	25	*F* (*P* = 0·0009)*	13·07	7·8	$\chi^2 = 1{\cdot}05$; $P = 0{\cdot}3$
Taenia multiceps	3·07	3·84	4·28	0	*F* (*P* = 0·89)	5·38	1·41	*F* (*P* = 0·09)
Nematoda								
Toxocara canis	35·38	29·8	21·42	9·37	*F* (*P* = 0·025)*	33·84	19·85	$\chi^2 = 6{\cdot}78$; $P = 0{\cdot}009$*
Ancylostoma caninum	0	2·88	0	3·12	*F* (*P* = 0·28)	1·53	1·41	*F* (*P* = 1)
Uncinaria stenocephala	73·84	74·03	71·42	71·87	$\chi^2 = 0{\cdot}18$; $P = 0{\cdot}97$	66·15	79·43	$\chi^2 = 6{\cdot}05$; $P = 0{\cdot}01$*
Trichuris vulpis	0	0·96	0	0	*F* (*P* = 1)	0	0·7	*F* (*P* = 1)
Acanthocephala								
Macracanthorhynchus hirudinaceus	1·53	0·96	5·71	0	*F* (*P* = 0·18)	2·3	2·12	*F* (*P* = 0·43)
All	96·92	99·03	100	100	*F* (*P* = 0·48)	99·23	98·58	*F* (*P* = 1)

n, number of dogs; *significant.

Table 3. Abundance and intensity of intestinal helminths of stray dogs from two northeastern areas in Tunisia by host age and gender

Helminth species	<1 year A	(*n* = 65) I	⩾1 to <2 years A	(*n* = 10) I	⩾2 to <6 years A	(*n* = 70) I	⩾6 years A	(*n* = 32) I	Student's *t*-test A	Male A	(*n* = 130) I	Female A	(*n* = 141) I	Student's *t*-test A
Trematoda														
Brachylaemus sp.	0	0	0	0	0·14	10	0	0	*P* = 0·39	0	0	0·07	10	*P* = 0·11
Phagicola italica	7·32	119	0·94	98	11·15	195·25	0·34	11	*P* = 0·15	6·01	260·66	1·41	83·42	*P* = 0·35
Heterophyes heterophyes	0·16	11	0·1	11	0·18	6·5	0	0	*P* = 0·07	0·09	12	0·16	7·66	*P* = 0·17
Cestoda														
Echinococcus granulosus	0·21	14	38·12	566·42	3·01	70·33	1·96	21	*P* = 0·32	29·26	951	3·18	44·9	*P* = 0·43
Dipylidium caninum	30	37·53	14·54	17·8	19·32	24·16	10·4	13·32	*P* = 0·02*	18·69	23·14	19·29	24·07	*P* = 0·01*
Diplopylidium noelleri	3·69	10·43	1·92	7·69	2·31	6·75	0·75	12	*P* = 0·36	2·83	11·18	1·82	6·11	*P* = 0·13
Mesocestoides lineatus	26·95	62·57	12·6	25·21	8·31	18·77	5·21	13·91	*P* = 0·07	11·5	27·68	16·43	33·57	*P* = 0·11
Mesocestoides litteratus	3·47	18·83	1·11	8·28	1·25	9·77	1·21	6·5	*P* = 0·05*	1·59	9·4	1·85	13·78	*P* = 0·48
Taenia hydatigena	0·26	1·54	0·3	1·77	0·41	1·93	2·12	4·25	*P* = 0·18	0·57	2·77	0·5	2·15	*P* = 0·42
Taenia pisiformis	0·01	1	0·38	5	0·24	1·54	0·4	1·62	*P* = 0·06	0·19	1·47	0·32	4·18	*P* = 0·15
Taenia multiceps	0·06	2	0·07	2	0·01	6·33	0	0	*P* = 0·14	0·13	2·42	0·09	7	*P* = 0·11
Nematoda														
Toxocara canis	4·6	13	1·12	3·77	1·42	6·66	0·28	3	*P* = 0·14	1·81	5·36	2·04	10·32	*P* = 0·38
Ancylostoma caninum	0	0	0·2	7	0	0	0·06	2	*P* = 0·26	0·03	2·5	0·12	9	*P* = 0·34
Uncinaria stenocephala	23·32	31·58	32·96	44·51	17·9	25·06	23·25	32·34	*P* = 0·004*	27·66	41·82	23·71	29·85	*P* = 0·49
Trichuris vulpis	0	0	0·04	5	0	0	0	0	*P* = 0·39	0	0	0·03	5	*P* = 0·5
Acanthocephala														
Macracanthorhynchus hirudinaceus	0·04	3	0·01	2	0·2	3·5	0	0	*P* = 0·27 *P* = 0·01	0·11	5	0·02	1·33	*P* = 0·38
All	100·16	31·45	104·49	33·03	66·17	20·31	46·03	14·73		100·53	101·31	73·85	74·92	*P* = 0·09

n, number of examined dogs; A, abundance; I, intensity; *significant.

(Table 2). For *A. caninum* there was no significant difference in prevalence between the sexes; however, females were more intensely infected (nine worms per infected dog) than males (2·5 parasites/infected dog). *Ancylostoma caninum* was found in two dog age groups (⩾1 to <2 and ⩾6 years) ($P = 0{\cdot}26$), whereas *U. stenocephala* was retrieved from dogs of all age groups with no significant difference in prevalence ($P = 0{\cdot}97$) but with a high difference in abundance ($P = 0{\cdot}004$) (Table 3).

The prevalence of *E. granulosus* s.s. was higher in female dogs (7·09%) than males (3·07%) ($P = 0{\cdot}17$), but the abundance of infection was lower in females (3·18) than in males (29·26) ($P = 0{\cdot}43$). The increase of *E. granulosus* s.s. infection rate with host age was not significant ($P = 0{\cdot}25$), although abundance and intensity were highest for the second (⩾1 to <2) dog age group (Table 3). The mean intensity of infection with *E. granulosus* s.s. varied from 14 to 566·42 worms/infected dog. Young dogs (⩾1 to <2 years) had the highest worm burdens including two massively infected dogs harbouring 1200 and 2540 worms each; the remaining 12 dogs had 2, 9, 12, 14, 16, 26, 38, 58, 65, 67, 86 and 120 *E. granulosus* s.s. adult tapeworms, respectively. The distribution of *E. granulosus* s.s. in dogs examined in this study was overdispersed and the data for the intensity of infection gave a significant fit to the negative binomial model. *Dipylidium caninum* was abundant in male and female dogs ($P = 0{\cdot}01$) with the highest abundance observed in young animals ($P = 0{\cdot}02$). *Brachylaemus* sp. and *T. vulpis* infection were only recorded in dogs aged ⩾2 to <6 and ⩾1 to <2 years, respectively. *Heterophyes heterophyes* and *T. multiceps* were not found in older dogs (⩾6 years) (Table 2).

Discussion

Stray dogs across all age groups were highly and intensely infected with intestinal helminths. A total of 23 423 helminths were retrieved from 268 infected dogs corresponding to 16 species. The majority of helminth species described in this study (11/16, 68·8%) were previously recorded in stray dogs, golden jackals and red foxes from the northwest and centre west of Tunisia (Lahmar *et al.* 2001, 2008, 2009). This epidemiological status is not surprising. To the best of our knowledge, no public or private measures and/or initiatives are known to have been undertaken in recent years to control zoonotic and veterinary parasites within the explored areas (Raoued and Soukra). Furthermore, significant levels of environmental contamination with canine helminth eggs, as indicated by a soil contamination index of 55% for 1270 dog faecal samples collected from different Tunisian regions, were recently reported (Oudni-M'rad *et al.* 2017). In the examined dogs, cestodes (97·04%) were more common than nematodes (83·02%), whereas trematodes (5·53%) and acanthocephalans (2·21%) were rare. The predominance of cestodes has previously been described (Bajalan, 2010; Geraili *et al.* 2016), and the prevalence of intestinal helminths observed in this study is similar to that reported for stray dogs from other parts of the world (Mateus *et al.* 2014; Emamapour *et al.* 2015; Ramos *et al.* 2015; Rehbein *et al.* 2016).

Dipylidium caninum, the most frequent cestode species identified in the present study (80·44%) was previously reported from necropsied stray dogs in Tunisia with prevalence rates ranging from 27·5 to 43·6% (Lahmar *et al.* 2001, 2009). Due to the absence of regular anthelminthic treatments and anti-ectoparasitic drugs for dogs, *D. caninum* can occasionally pose a threat to children through the accidental ingestion of flea intermediate hosts harbouring the infective cysticercoid stage (Szwaja *et al.* 2011). A report on the infection of a 17-month-old boy from China with *D. caninum* was recently published (Jiang *et al.* 2017).

The overall prevalence of *E. granulosus* s.s. seen in this study (5·16%) was lower than that reported from central and western parts of Tunisia (21%) (Lahmar *et al.* 2001). In Tunisia, *E. granulosus* s.s. is prevalent in domestic and wild intermediate hosts, golden jackals, stray dogs and humans (Boufana *et al.* 2014, 2015; Deplazes *et al.* 2017). Although three *Echinococcus* species namely, *E. granulosus* s.s., *Echinococcus canadensis* and *Echinococcus equinus*, have been identified from various intermediate hosts in Tunisia, to date, *E. granulosus* s.s. is the only species reported from definitive hosts (dogs and wild canids) (Lahmar *et al.* 2009, 2014; Boufana *et al.* 2014, 2015; Chaâbane-Banaoues *et al.* 2015). We speculate that stray dogs in the studied regions may have acquired infection through feeding on hydatid ridden-viscera from rural clandestine sheep slaughtering and abandoned ruminant carcasses in the environment following natural mortality of livestock; however, the variation in dog echinococcosis prevalence depends largely on human behaviour and hygiene (El Berbri *et al.* 2015). Interestingly, Raoued and Soukra are not important sheep raising regions, with no livestock transhumance. Cystic echinococcosis annual surgical rate for this region was estimated to be 4·8/100 000 inhabitants, while the mean annual surgical rate for the whole country is 12·6 cases/100 000 inhabitants (Chahed *et al.* 2015).

Our data showed that the age of dogs was not independent of *E. granulosus* s.s. infection ($P = 0{\cdot}25$). The non-linear age-prevalence profile observed in this study suggested that older dogs acquired immunity under the *E. granulosus* s.s. endemic steady-state equilibrium in Tunisia (Lahmar *et al.* 1999, 2013) where dogs, submitted to repeated numbers of challenge infections become resistant (Gemmell, 1990). Thus, the highest worm burdens were found in younger dogs compared with older ones that had lower parasite abundances. Similar findings were previously reported from Tunisia (Lahmar *et al.* 2001), Kazakhstan (Torgerson *et al.* 2003) and Morocco (Azlaf *et al.* 2007; Dakkak *et al.* 2017). In addition, this study revealed similarities in *E. granulosus* s.s. prevalence between male and female dogs ($P = 0{\cdot}17$), which may be due to equal free roaming opportunities in search of offal.

In this survey, *T. hydatigena* was the most frequently encountered taeniid (22·14%). Epidemiological studies on dogs, the definitive hosts, ruminants and wild boars, the intermediate hosts, indicate that *T. hydatigena* is endemic in Tunisia (Maâmouri, 2005; Lahmar *et al.* 2008; Lahmar, 2012; Lamouchi, 2009). However, no official data are available on the cost of condemnation of infected livers due to traumatic hepatitis caused by the metacestode larval stage, *Cysticercus tenuicollis*. Similar *T. hydatigena* infection rates to those seen here were reported in dogs from Albania (16·2%; Xhaxhiu *et al.* 2011) and Sardinia (11%; Scala *et al.* 2015), whereas higher prevalence in older dogs were recorded from Algeria (43%; Bentounsi *et al.* 2009), north-east (43%; Emamapour *et al.* 2015) and southeastern Iran (53·3%, Geraili *et al.* 2016). The second most frequently found taeniid was *T. pisiformis* (10·33%) whose prevalence increased with age from 1·53 to 25% ($P = 0{\cdot}0009$). This may be related to the greater ability of older dogs to hunt rabbits and hares in which the larval stage, *Cysticercus pisiformis*, develops. In a previous study from Tunisia, 6·36% of dogs eliminated *T. pisiformis* worms following arecoline purgation (Lahmar *et al.* 2008). Infection rates similar to those seen here for this parasite were reported from stray dogs in Spain (Benito *et al.* 2003) and Algeria (Bentounsi *et al.* 2009). *Taenia multiceps* was the least frequent taeniid (3·32%) observed in this study, which is consistent with the infection rates of 5·05 and 4·77% for this parasite previously reported from Tunisian dogs (Lahmar *et al.* 2001, 2008). *Taenia multiceps* eggs excreted into the environment by the definitive host are ingested by herbivorous, mainly sheep intermediate hosts, causing a coenurosis with neurological symptoms and death in young animals (Scala *et al.* 2007). Coenurosis is a rare zoonosis (Sabbatani *et al.* 2004; El-On *et al.* 2008); however, more than 100 human cases have been reported worldwide (Dhaliwal and Juyal, 2013). The occurrence of *E. granulosus* s.s., *T. multiceps*, and *T. hydatigena* in rural stray dogs confirms the role they play in the transmission of these taeniid infections. The persistence of these species in Tunisian dogs may be related to the large number of dogs, lack of control measures, extensive livestock husbandry, home slaughtering and the inadequate disposal of infected carcasses and offal.

This epidemiological investigation indicated that stray dogs are a potential source of *T. canis* infection to humans and other animals and are responsible for environmental contamination of rural areas. A contamination index of 27·08% for *T. canis* eggs in the environment was recently estimated (Chaâbane-Banaoues, 2016). The overall prevalence of *T. canis* in dogs observed in this study (26·56%) was higher than that previously reported from Tunisian dogs (Lahmar *et al.* 2001) but is comparable to that recorded from stray dogs in Iran (Emamapour *et al.* 2015). *Toxocara canis* is the causative agent of visceral and ocular *larva migrans* in humans (Fillaux and Magnaval, 2013; Chia-Kwung *et al.* 2015) who become infected through the inadvertent ingestion of eggs and/or larvae present in soil and shed in dog feces (Benito *et al.* 2003). Importantly, cases due to *T. canis larva migrans* have recently been diagnosed in Tunisian patients (Hamrouni *et al.* 2015; Lajmi *et al.* 2015).

Although *U. stenocephala* was identified as the most prevalent intestinal nematode in this study (198; 73·06%), *A. caninum* infection is highly pathogenic to dogs with evident zoonotic potential, as infective larvae can penetrate human skin and lead to eosinophilic enteritis and a possible sub-acute neuro-retinitis (Bowman *et al.* 2010). From a total of 271 examined dogs, only four (1·47%) were found to be positive for *A. caninum*, while several studies including those from northwestern Tunisia and other parts of the world, have shown *A. caninum* to be the most widespread of all hookworms (Lahmar *et al.* 2001; Bajalan, 2010; Mateus *et al.* 2014; Emamapour *et al.* 2015; Ramos *et al.* 2015; Ilić *et al.* 2017; Pumidonming *et al.* 2017). Few cases of presumed human infection with *T. vulpis* causing visceral *larva migrans* syndrome and/or intestinal infections have been reported (Díaz-Anaya *et al.* 2015; Ilić *et al.* 2017). However, the zoonotic potential of this parasite remains controversial (Traversa, 2011). Levels of dog infection with *T. vulpis* are very heterogeneous in several parts of the world and are influenced by host and environmental factors (Traversa, 2011). In the present study, *T. vulpis* infection was rare (0·36%), while in a previous Tunisian survey, a 10·6% infection rate in necropsied stray dogs was detected (Lahmar *et al.* 2001) and the environmental contamination index with whipworm eggs from rural dog feces was estimated to be 4·8% (Oudni-M'rad *et al.* 2017).

Three intestinal trematodes, *Brachylaemus* sp., *P. italica* and *H. heterophyes* were reported here as first records from Tunisian dogs. The three species were found in Raoued, whereas only *P. italica* was identified in Soukra region. The occurrence of *P. italica and H. heterophyes* in dogs is probably related to the presence of large bodies of brackish water within the studied regions. These two species are transmitted in marine lagoons and saline inland waters, where first (snails) and second intermediate hosts (fish: Mugilidae) are abundant (Simões *et al.* 2010). Dogs serve as the major definitive hosts. They become infected by consuming raw fish with muscle-encysted metacercariae. Euryhaline and marine fishes infected with *H. heterophyes* from Tunisia and Egypt (Chai, 2014; Hegazi *et al.* 2014), and with *H. heterophyes* and *P. italica* from Sardinia have previously been reported (Masala *et al.* 2016). Other studies reported the occurrence of *P. italica* in dogs and foxes from Italy (Nardi, 1959) and in dogs from Turkey (Tinar, 1976), while *H. heterophyes* was detected in dogs from Greece (Himonas, 1964) and western India (Sen, 1965). *Heterophyes heterophyes* is the causative agent of human heterophyiasis, an emerging fish-borne disease contracted through the consumption of raw mullet (Balozet and Callot, 1939; Collomb *et al.* 1960; Rousset and Pasticier, 1972; Taraschewski, 1984). The discovery of *P. italica* and *H. heterophyes* in Tunisia represents an indication of the potential impact of these helminths on public health. Further investigations are required to determine intermediate hosts and elucidate transmission modalities for these trematodes within the studied areas. *Brachylaemus* sp. is known to occur in dogs from northern Spain (Guisantes *et al.* 1994; Benito *et al.* 2003), red foxes from Italy (Fiocchi *et al.* 2016) and Tunisian wild boars (Lahmar, 2012). In these few reports, canids serving as definitive hosts of brachylaimid digeneans show consistently low burdens with very few parasites per host, which is similar to the prevalence rate (0·36%) reported in the present study.

The significant prevalence observed for *M. lineatus* (38·45%) and *M. litteratus* (15·12%) in the present survey could be due to predation by stray dogs on intermediate or paratenic hosts. These two cestodes are common taeniids of mammals and have previously been reported in dogs by several authors (Benito *et al.* 2003; Bentounsi *et al.* 2009; Bajalan, 2010; Nabavi *et al.* 2014; Emamapour *et al.* 2015; Geraili *et al.* 2016). An acanthocephalan species, *M. hirudinaceus*, was identified in this investigation (2·21%) as a first record in Tunisian dogs. It is common in red foxes, golden jackals and wild boars in Tunisia and elsewhere (Dalimi *et al.* 2006; Zare-Bidaki *et al.* 2010; Lahmar, 2012, Lahmar *et al.* 2014) and has also been reported in dogs from Iran (Dalimi *et al.* 2006; Zare-Bidaki *et al.* 2010). The presence of beetles and scarabs in the environment with probable encysted larval stages of these parasites could explain the infection seen in dogs in the current study. *Diplopylidium noelleri* recorded here for the first time in Tunisian dogs may represent an accidental infection as previous reports describe this parasite from stray cats (Schuster *et al.* 2009; Waap *et al.* 2014; El-Azazy *et al.* 2016) and wild canids (Lahmar *et al.* 2014). Definitive hosts become infected through the ingestion of the cysticercoid larvae encysting in reptiles, which serve as second intermediate hosts.

In conclusion, this study confirmed that stray dogs from the studied northeastern Tunisian regions may play an important role in contaminating the environment with various parasites some of which may potentially influence human health. To decrease the incidence of parasitic cestodes, intermediate hosts would need to be inspected at slaughter, and organs containing hydatid cysts of *Echinococcus*, *Coenurus cerebralis* of *T. multiceps* and *C. tenuicollis* of *T. hydatigena* should be removed and destroyed to prevent consumption by dogs. Canine echinococcosis could be detected by screening *E. granulosus* in dogs using arecoline purgation and/or molecular approaches (Craig *et al.* 2017). In addition, the combination of EG95 lambs' vaccination associated with regular praziquantel treatments of owned dogs could reduce the infection pressure of *E. granulosus* in the environment and decrease soil contamination (Torgerson and Heath, 2003).

To control helminth contamination in the environment, a close collaboration between sanitary and agriculture authorities is needed for public health education, screening of intestinal parasites in free-roaming owned dogs, regular dog dosing and dog population management. Unfortunately, dog management in many developing countries usually translates into indiscriminate population killing and/or poisoning of roaming dogs. This is ethically unacceptable as well as being ineffective (FAO, 2014). Other procedures such as humane euthanasia, fertility control and use of praziquantel baits could be efficient but require huge funds and are economically and logistically difficult to implement in developing countries (Kachani and Heath, 2014).

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