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Prevalence of intestinal helminths of dogs and foxes from Jordan

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Abstract Necropsy of 340 stray and semi-stray dogs (*Canis familiaris*) and nine red foxes (*Vulpes vulpes*) from Jordan revealed that 239 dogs (70.3%) and all foxes were infected with at least one intestinal helminth species. No trematodes were found in the intestine of these hosts. The overall infection rates with cestodes, nematodes and acanthocephalans in dogs were 66.8%, 4.4% and 2.9%, respectively. The following cestodes were identified: *Echinococcus granulosus* (9.4%), *Taenia pisiformis* (11.8%), *T. hydatigena* (7.4%), *T. ovis* (4.4%), *T. multiceps* (3.8%), *T. taeniaeformis* (2.9%), *Dipylidium caninum* (19.4%), *Joyeuxiella* (3.2%), *Diplopylidium* (2.4%), and *Mesocestoides* (0.9%). Other intestinal worms in dogs were *Toxascaris* (2.6%), *Toxocara canis* (1.2%), and *Protospirura* (0.6%) nematodes, and gigantorhynchiid acanthocephalans (2.9%). Intestinal helminths found in foxes included cestodes (*D. caninum*, *Joyeuxiella*, *Diplopylidium*, *Mesocestoides*), nematodes (*Protospirura*, *Uncinaria stenocephala* and *Oxynema*) and an acanthocephalan (*Macracanthorhynchus*). In both hosts, most helminths were recovered from the second intestinal segment of four equally divided segments.

Introduction

Dogs and other canids act as definitive hosts for many intestinal parasites, some of which are responsible for several zoonotic diseases such as echinococcosis and toxocariosis (Dalimi and Mobedi 1992; Schantz 1994; Schantz and Kramer 1995; Eslami and Hosseini 1998). The close association between dogs and humans is responsible for the high endemicity of some of these zoonotic diseases. For example, in Ireland, garden soils were found to be more contaminated with *Toxocara* eggs than public park soils (Holland et al. 1991). Moreover, cystic echinococcosis (CE) is a major endemic disease of public health important in various Middle Eastern countries due to the close association between dogs, livestock and humans. The disease is endemic or highly endemic in Kuwait, Iraq, Syria, Lebanon, and Jordan (see reviews by Matossian et al. 1977; Al-Yaman et al. 1987; Kamhawi 1995; Abdel-Hafez and Kamhawi 1997). Cestodes, nematodes and acanthocephalans have been identified in the intestines of stray and pet dogs as well as foxes in various countries including Jordan (Ajlouni et al. 1984; Al-Tae et al. 1988; Abo-Shehada and Ziyadeh 1991; Shastri 1991; Vanparijs et al. 1991; Dalimi and Mobedi 1992; Deplazes et al. 1992; Jones and Walters 1992a, b; Epe et al. 1993; Saeki et al. 1997; Hoida et al. 1998).

In Jordan, the intestinal helminthic fauna of dogs and other canids has received little attention. In the present investigation, the intestinal helminthic fauna of stray and semi-stray dogs as well as foxes was studied in order to determine the current prevalence of intestinal helminths in these canids.

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Materials and methods

Three hundred and forty stray and semi-stray farm or shepherd dogs (*Canis familiaris*) from six governorates of Jordan (Fig. 1) and nine red foxes (*Vulpes vulpes*) from northern Jordan were shot in the field during the period May 1992–July 1995. An abdominal cut was made in each animal and the intestine was tied from the pyloric and anal ends and collected in a bag. Bags were stored in an icebox and carried to the laboratory within 3 h. The carcasses were burned in the field to ensure that there was no contamination of the environment.

In the laboratory, each intestine was divided into four pieces of equal length. Each piece was cut longitudinally and soaked in 0.15 M phosphate buffer saline (PBS, pH 7.2) for 5 min. The mucosal lining was gently scraped with a spatula into clean glass dishes and the collected intestinal contents were allowed to settle in 1000 ml conical Nalgene graduates (Nalge, Rochester, USA). Following several washes with PBS, aliquots were examined under a dissecting microscope.

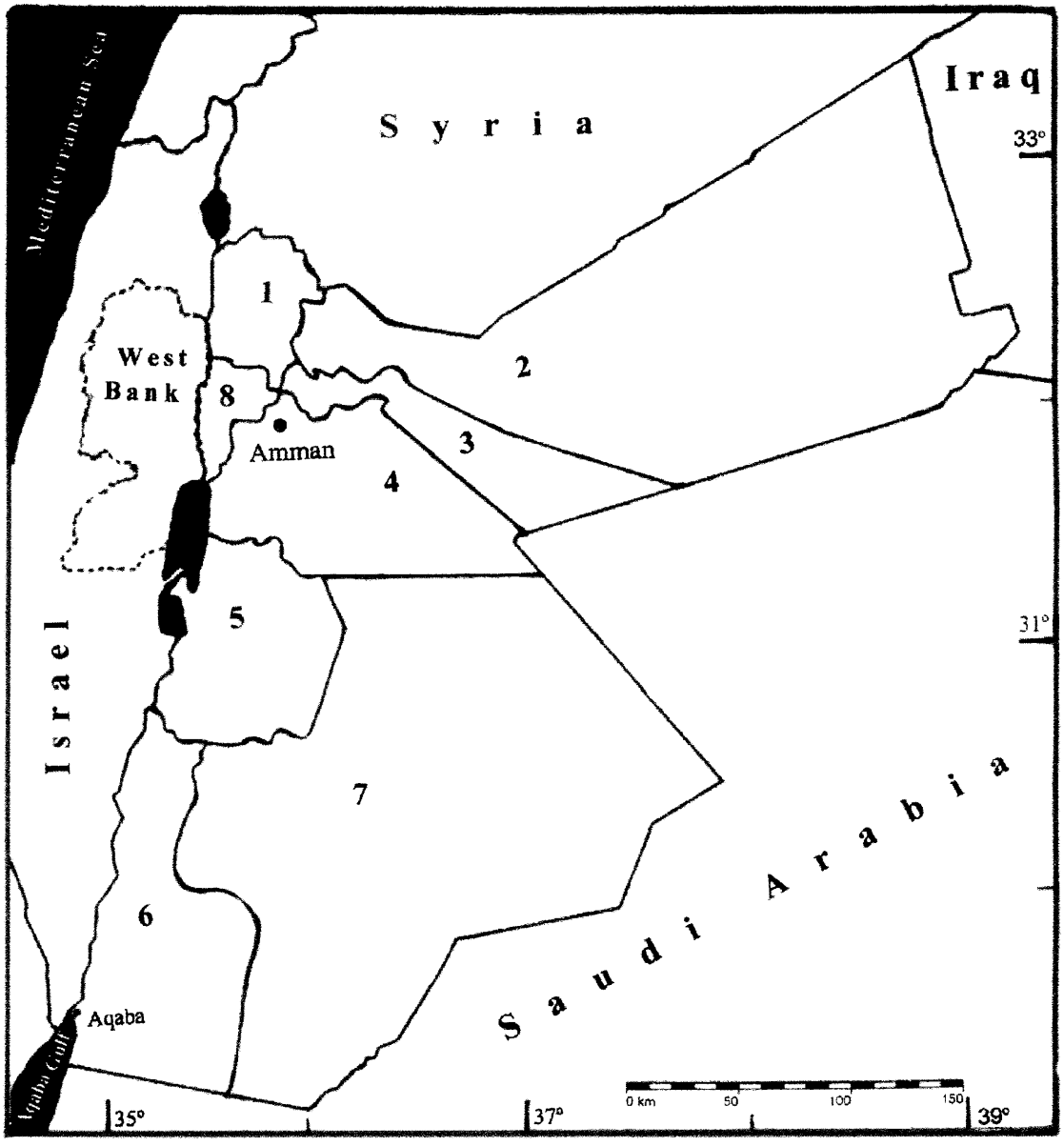

Fig. 1 Map of Jordan showing governorates. Dogs were collected from governorates of Irbid (1), Mafraq (2), Zarqa (3), Amman (4), Karak (5), and Aqaba (6). No dogs were collected from the other two governorates of Ma'an (7) and Balqa (8)

The preparation, staining and mounting of helminths were carried out as described by Meyer and Olsen (1980). Nematodes were killed and preserved in 10% formalin. Living cestodes and acanthocephalans were put in distilled water for 30 min for relaxation before they were killed and fixed using one of the following fixatives: 10% formalin, 70% ethanol or warm 85% ethyl alcohol/ formalin/ acetic acid (AFA) at a ratio of 85:10:5. The AFA was found to be better than 70% alcohol as a fixative for large cestodes.

Nematode parasites were prepared unstained. After washing 3–5 times (15 min each), in distilled water to remove any traces of fixative, they were cleared and mounted with either glycerin jelly or Berlese's medium (Meyer and Olsen 1980). The body wall of large nematodes was punctured with a fine needle to permit the passage of the fixative and mounting medium into the body. For staining of cestodes and acanthocephalans, worms were removed from the fixative, washed several times with distilled water (15–30 min depending on the size of the worms). The worms were dehydrated by soaking them in ascending series of ethyl alcohol solutions (10%, 35%, 50% and 70%) for 25 min each. The worms were subsequently stained either using Semichon's acetocarmine for 15–45 min or Grenacher's alcoholic borax carmine for 24 h. Overstained worms were differentiated with acidified alcohol (2 ml of concentrated HCl: 98 ml of 70% alcohol). Destaining was stopped when the cortical layer appeared free from the stain and the reproductive organs were pink. Subsequently, worms were soaked in another series of ascending alcohol solutions of 85%, 90%, 95% and 100% for 25 min each. After a second change in absolute alcohol, worms were cleared using a series of alcohol-xylene combinations (3:1, 2:2, 1:3) for 30 min each. Finally, the worms were soaked in pure xylene (2 changes, 30 min each) prior to mounting in either Canada Balsam (PARK, Northampton, UK) or D.P.X. medium (Gainland, UK).

Identification of intestinal helminths was based on keys and descriptions by Yamaguti, (1961), Benbrook, (1963), Yorke and Moplestone, (1969), Schmidt, (1970), Dunn, (1978), Anderson, (1992), and Khalil et al. (1994). Identification of various *Taenia* species was based on stained specimens and comparison of scoleces and various types of proglottids as well as on morphometric

measurements of small and large hooks of armed rostella drawn with the aid of a *camera lucida* (Soulsby 1982).

Statistical analysis was done by chi-square test using Epi-Info 6 Software (Centers for Disease Control and Prevention, Atlanta, Ga.). A *P*-value of ≤0.05 denoted a statistically significant difference. Rates of infection of dogs from the various governorates were compared in pairs.

Results

Prevalence of intestinal helminthes in dogs

Tables 1 and 2 show the overall infection rates with intestinal helminths (singly or concurrently) in dogs from various governorates of Jordan. Infection with at least one intestinal helminthic species was observed in 239 dogs (70.3%). No trematodes were encountered in any of the dogs inspected in this series. Infection with cestodes alone was very common, accounting for 62.9% of all dogs examined and it represented 89.5% of all helminthic infections (Table 1). As for nematodes, concurrent infections with cestodes were more common than single infections. The overall prevalence with cestodes, nematodes and acanthocephalans was 66.8%, 4.4% and 2.9%, respectively (Table 2). No significant difference in infection rates with helminths was observed between male and female dogs of any governorate.

No statistically significant difference was noted between the overall infection rate of dogs with cestodes compared to that of dogs from each separate governorate (Table 2). Cestode infections in dogs were consistently high in all governorates, ranging between 58.8–72.2%. Dogs from Irbid and Amman governorates showed the highest nematode infection rates (6.3% and 5.9%, respectively). None of the 23 dogs examined from Mafraq governorate had a nematode infection. Acanthocephalan worms were encountered mostly in dogs from Mafraq and Aqaba (8.7% and 7.7%, respectively) and to a lesser degree in those from Karak governorate (4.0%). None of the 38 dogs from Zarqa governorate showed any acanthocephalan infection (Table 2).

At the generic level, *Taenia* species were the most prevalent in dogs with an overall infection rate of 49.1%, while at the species level *Dipylidium caninum* was the most prevalent species with an overall rate of infection of 19.4% (Table 2). Dogs from Irbid governorate showed a significantly higher infection rate with *D. caninum* than dogs from any other governorate. Among Dipylididae species, the rates of infection with *Joyeuxiella* and *Diplopylidium* were significantly lower

Table 1 Single and concurrent infection of dogs with intestinal helminthic parasites from various governorates of Jordan (number in parentheses indicates number of dogs examined)

Parasites	Percentage of infected dogs in governorates						
	Irbid (126)	Mafraq (23)	Amman (51)	Zarqa (38)	Karak (50)	Aqaba (52)	All (340)
Cestodes alone	66.7	65.2	56.9	63.2	62.0	59.6	62.9
Nematodes alone	1.6	0.0	3.9	0.0	2.0	1.9	1.8
Acanthocephalans alone	0.0	4.3	2.0	0.0	4.0	3.8	1.8
Cestodes and nematodes	4.8	0.0	2.0	2.6	0.0	1.9	2.6
Cestodes and acanthocephalans	0.8	4.3	0.0	0.0	0.0	3.8	1.2
Total	73.8	73.9	64.7	65.8	68.0	71.2	70.3

Table 2 Percentage of dogs infected with different helminths in various governorates of Jordan (number in parentheses indicates number of dogs examined)

Parasites	Percentage of infected dogs in governorates						
	Irbid (126)	Mafraq (23)	Amman (51)	Zarqa (38)	Karak (50)	Aqaba (52)	All (340)
Cestodes							
Echinococcus granulosus	15.1	8.7	3.9	2.6	10.0	5.8	9.4
Taenia spp.	42.1	47.8	56.9	47.4	52.0	57.7	49.1
Dipylidium caninum	42.1	8.7	3.9	13.2	2.0	5.8	19.4
Joyeuxiella sp.	6.4	4.4	0.0	2.6	0.0	1.9	3.2
Diplopylidium sp.	5.6	0.0	0.0	0.0	2.0	0.0	2.4
Mesocestoides sp.	0.8	0.0	0.0	2.6	2.0	0.0	0.9
Diphyllobothriidae sp.	0.8	0.0	0.0	0.0	0.0	0.0	0.3
Total	72.2	69.6	58.8	65.8	62.0	65.4	66.8
Nematodes							
Toxocara sp.	1.6	0.0	2.0	0.0	2.0	0.0	1.2
Toxascaris sp.	4.0	0.0	3.9	2.6	0.0	1.9	2.6
Protospirura sp.	0.8	0.0	0.0	0.0	0.0	1.9	0.6
Total	6.3	0.0	5.9	2.6	2.0	3.9	4.4
Acanthocephalans							
Macracanthorhynchus sp.	0.8	0.0	0.0	0.0	0.0	3.8	0.9
Gigantorhynchiidea sp.	0.0	8.7	2.0	0.0	4.0	3.8	2.1
Total	0.8	8.7	2.0	0.0	4.0	7.7	2.9

Table 3 Percentage of dogs infected with different Taeniidae species in various governorates of Jordan (number in parentheses indicates number of dogs examined)

Species	Percentage of infected dogs in governorates						
	Irbid (126)	Mafraq (23)	Amman (51)	Zarqa (38)	Karak (50)	Aqaba (52)	All (340)
E. granulosus	15.1	8.7	3.9	2.6	10.0	5.8	9.4
Taenia pisiformis	8.7	8.7	19.6	18.4	8.0	11.5	11.8
T. hydatigena	7.9	8.7	5.9	2.6	8.0	9.6	7.4
T. ovis	0.8	4.4	2.0	0.0	8.0	15.4	4.4
T. multiceps	4.8	8.7	2.0	2.6	0.0	5.8	3.8
T. taeniaeformis	4.8	4.4	2.0	2.6	0.0	1.9	2.9
Unidentified *Taenia* species[a]	19.8	13.0	27.5	23.7	30.0	21.2	22.6

[a] Species were unidentified due to loss of distinctive identifying features during preparation

than that with *D. caninum*. It was also observed that the worm burden with *D. caninum* was much greater than with the other two dipylidid species. Among taeniid species, *T. pisiformis*, *E. granulosus* and *T. hydatigena* were the most prevalent worms found in dogs, with overall infection rates of 11.8%, 9.4% and 7.4%, respectively (Table 3). The highest *E. granulosus* infections were seen in dogs from Irbid, Karak and Mafraq governorates at rates of 15.1%, 10.0 and 8.7%, respectively. More than one third of *E. granulosus* infections occurred in dogs that had no other intestinal helminthic parasites. *E. granulosus* infection rate in dogs from Irbid governorate was significantly higher than that in dogs from Amman governorate. In contrast, infection rate with *T. pisiformis* was significantly lower in dogs from Irbid governorate than those from Amman governorate (Table 3).

Nematode infections in dogs from Jordan included *Toxascaris*, *Toxocara* and *Protospirura* worms that occurred at rates of 2.6%, 1.2% and 0.6%, respectively (Table 2). The worm burden with these parasites was very low, ranging between 1–4 worms per dog.

Two acanthocephalan species belonging to the order Gigantorhychidea were found in dogs. One was identified as *Macracanthorhynchus* sp., but the other did not show the characteristic features to allow further identification beyond the order level. *Macracanthorhynchus* sp. was encountered in only three (0.9%) of the dogs examined.

Spatial distribution and relative intensity

Figure 2 shows the spatial distribution and relative intensity of the most common helminths in 73 infected dogs whose intestines were separated into four equally divided segments. Evidently, most helminths were found inhabiting segment 2. Fewer worms were found in segments 3 and 1, in decreasing order. Only detached and often disintegrating proglottids of cestodes were seen in the last segment.

The location of *E. granulosus* depended on the intensity of infection. In light infections (<500 worms per dog), most of the worms were found in segment 1 and to a lesser degree in segment 2. In moderate infections (500–1000 worms per dog), parasites occupied segment 2

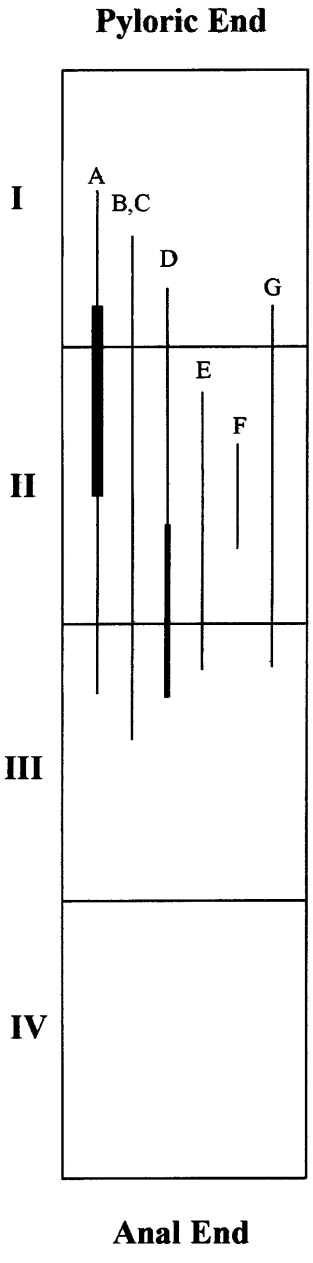

Fig. 2 Spatial distribution of common helminths and their relative intensity (as reflected by line thickness) in four equally divided segments of dog intestine. (*A*) *E. granulosus*, (*B*) *Joyeuxiella* sp., (*C*) *Diplopylidium* sp., (*D*) *D. caninum*, (*E*) *Taenia* spp., (*F*) *Mesocestoides* sp., (*G*) *Macracanthorhynchus* and other acanthocephalan species

more than segment 1. In heavy infections (> 1000 worms per dog), *E. granulosus* was collected from the first three segments, but mainly from segments 1 and 2. As for *D. caninum* and acanthocephalans, a greater number of worms occupied segment 2 and to a lesser degree segment 3 (Fig. 2).

Prevalence and spatial distribution of intestinal helminths in foxes

Nine red foxes were killed in northern Jordan and nine different species of cestodes, nematodes and acanthocephalans were found in these animals (Table 4). All foxes were found to be infected with a minimum of a single worm species and three of them had multiple infections with 4–7 different species per animal (Table 5). Among cestode species, *Mesocestoides*, *Joyeuxiella* and *Diplopylidium* were the most common helminths found in foxes. Infection with *Joyeuxiella* and *Diplopylidium* was more common than that with *Dipylidium*. Moreover, heavier worm loads with the former two species than the latter were noted. Among the three different nematode species identified, *Protospirura* species was the most common, as it was encountered in five of the nine foxes (Table 4). *Uncinaria stenocephala* was recovered from three foxes with a worm burden between 2 and 8 worms per animal. *Macracanthorhynchus* sp. was identified in four foxes with a maximum worm burden of 8 worms per animal. The spatial distribution of worms in the intestines of infected foxes was similar to that in dogs for the various worms that were shared by both hosts.

Table 4 Intestinal helminthic parasites of nine red foxes (*Vulpes vulpes*; 7 males and 2 females) shot in Irbid governorate, north Jordan

Parasite	No. of infected foxes		
	Male	Female	Both
Cestodes			
D. caninum	1	0	1
Joyeuxiella sp.	3	1	4
Diplopylidium sp.	3	0	3
Mesocestoides sp.	3	1	4
Diphyllobothriidae sp.[a]	0	1	1
Nematodes			
Uncinaria stenocephala	2	1	3
Protospirura sp.	4	1	5
Oxynema sp.	1	0	1
Acanthocephalans			
Macracanthorhynchus sp.	2	2	4

[a] Only proglottids were recovered from one fox

Table 5 Single and concurrent intestinal helminthic infections in nine red foxes (*V. vulpes*) from Irbid governorate, north Jordan. Intestinal helminthes identified in foxes were: (a) *D. caninum*, (b) *Joyeuxiella* sp., (c) *Diplopylidium* sp., (d) *Mesocestoides* sp., (e) Diphyllobothriidae sp., (f) *Uncinaria stenocephala*, (g) *Protospirura* sp., (h) *Oxynema* sp., (i) *Macracanthorhynchus* sp.

Type of infection	No. of infected foxes			Parasites
	Male	Female	Both	
Single	3	0	3	(a), (b) or (g)
Double	2	0	2	(c + d) or (g + i)
Triple	0	1	1	(b + f + i)
Multiple	2	1	3	(d + e + g + i), (b + c + d + f + g), (b + c + d + f + g + h + i)
Total	7	2	9	

Discussion

The present study shows that intestinal helminthosis is very common in both male and female stray dogs throughout Jordan. The lack of dog ownership registration, accessibility of stray dogs to intermediate hosts and the absence of periodic anthelmintic dosing all contribute to the high overall prevalence of intestinal helminths (70.3%) and particularly cestode infections (62.9%) (Table 1). The rate of cestode infections was slightly lower than that reported 15 years ago by Ajlouni et al. (1984), but significantly higher than that reported in dogs from the Moslem town of Tamra (42.9%) in northern Israel (Hoida et al.1998). As the population of Jordan is predominantly Moslem, a major contributing factor in obtaining higher infection rates in Jordan compared to those in Tamra is the method of sampling. In the present series, dogs were necropsied while in the study carried out by Hoida et al. (1998), dogs were sampled for intestinal helminthes by purging with arecoline hydrobromide and by coproantigen ELISA for *E. granulosus*. The latter methods are not as sensitive as the necropsy method particularly when dogs harbor light worm burdens (Deplazes et al. 1992; Craig et al. 1995).

Several of the intestinal helminthic parasites of dogs recorded here can cause significant human disease. Of particular importance are *E. granulosus*, *T. multiceps* and *T. canis*. Jordan is known for its high endemicity for CE (Al-Yaman et al. 1987; Abdel-Hafez and Kamhawi 1997). The overall prevalence of *E. granulosus* in dogs (9.4%) was lower than that reported earlier (14.0%) by Ajlouni et al. (1984) but similar to the infection rate of dogs in Tamra (10.7%) (Hoida et al. 1998). Higher infection rates with *E. granulosus* were observed in dogs sampled from Irbid, Karak and Mafraq compared to those from other governorates (Tables 2, 3). Agriculture and animal husbandry are more widely practiced in these governorates than in the others. The high infection rate of *E. granulosus* in dogs can explain the high mean annual surgical incidence in Jordan and particularly in Karak governorate, which was reported to be 8.2 per 100,000 for the period 1985–1993 (Kamhawi 1995). The identification of *T. multiceps* in dogs from Jordan for the first time, with an overall infection rate of 3.8% and up to 8.7% in Mafraq governorate, points to the potential

danger of human infection with coenurosis, which warrants further investigation. The rate of *Toxocara* infection was relatively low in the present study. However, Abo-Shehada (1989) found *Toxocara* eggs in 15.5% of school playgrounds and public places in northern and central Jordan, and as many as 10.9% of individuals aged 5–24 years from Irbid were seropositive for *T. canis* (Abo-Shehada et al. 1992). Also, as many as 19.0% of dog fecal deposits tested in Jordan contained *T. canis* ova (Abo-Shehada and Ziyadeh 1991). *D. caninum*, which may also infect humans, was the most predominant cestode species in dogs from Jordan (Table 2). Such a high infection rate is consistent with previous observations made by Ajlouni et al. (1984) and Abo-Shehada and Ziyadeh (1991) in Jordan as well as by Hoida et al. (1998) in a Moslem community in Israel.

T. pisiformis and *T. hydatigena* were the most prevalent (11.8% and 7.4%, respectively) *Taenia* species in dogs from Jordan (Table 3). In contrast, Ajlouni et al. (1984) reported infection rates of 8.0% and 46.0% for these two species, respectively. It appears that the stray dogs examined in the present study depended more on rodents (the intermediate hosts for *T. pisiformis*) for food than on other sources. Indeed, rodent hair was frequently seen in the intestinal contents of the dogs studied here.

Although only a few foxes were examined for their intestinal helminthic infections in the present study, several observations were made:

1. None of the foxes was free from worm infection. Instead, mixed concurrent infection with two or more species was seen in six of the nine foxes examined. This observation reflected the wilder behavior of foxes compared to dogs.
2. Neither *E. granulosus* nor *Taenia* species were found, which indicates that foxes do not rely on livestock meat as food. The foxes in this study originated from hilly villages in northern Jordan, where livestock husbandry is rarely practiced. The major source of food for foxes appeared to be chicken or other birds. Both feathers and bird bones were commonly found in the intestinal contents of necropsied foxes.
3. *Joyeuxiella* and *Diplopylidium* species were more frequently encountered than *Dipylidium* species. The present findings in foxes are preliminary, as a larger number of foxes have to be studied before any conclusive remarks can be made.

The second quarter of the small intestine appeared to offer the most suitable habitat for tapeworms and other intestinal helminthes of dogs and foxes. This portion of the intestine appears to contain the digested food that can easily be absorbed through the tegument of cestodes and acanthocephalans.

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