



Parasitofauna survey of song thrushes (*Turdus philomelos*) from the eastern part of Spain

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ABSTRACT

Thrushes (*Turdus* spp.) are migratory passerine birds found in northern Europe during the summer months and in southern Europe and north of Africa during the winter. They constitute an important small game bird group very appreciated by Spanish hunters. Between October 2013 and February 2014, 90 adult song thrushes were collected for their exam. After necropsies, three species of helminths were macroscopically recovered from 15 birds (16.7%): *Morishitium* sp. (16.7%), *Splendidofilaria mavis* (6.7%) and *Dilepis undula* (7.8%). One of them showed an adult cestode in the lung. Moreover, 12 of the positive thrushes (80%) harboured microfilaria in pulmonary blood vessels and three of them (20%) were infected by *Sarcocystis* sp. on skeletal musculature. All parasitized birds showed lesions, ranging from mild to moderate airsacculitis, bronchitis and coelomitis associated to *Morishitium* sp. infection. To the authors' knowledge, this is the first description of *Sarcocystis* spp. in song thrushes from Spain. Our results reveal the need for further studies to evaluate the epidemiological role of song thrushes as spreaders of parasites during their annual migration.

1. Introduction

Thrushes (*Turdus* spp.) are one of the most popular groups of game birds in Europe. [1,2]. They can be found in northern latitudes during the summer months, although they return to southern ones, including the Iberian Peninsula, for wintering, coinciding with the hunting season in Spain [2].

Different helminths (trematodes, cestodes, nematodes, and acanthocephalans) have been previously isolated from several anatomical regions of *Turdus* spp. [3–6]. These birds have been widely studied as reservoirs of some viral pathogens (e.g. zoonotic usutu virus [7]), but it is still unclear their role as reservoir of parasites for domestic poultry, humans or other game bird species. In this sense, factors like seasonal migration, habitat changes or thrushes' diet (mainly based on insects, earthworms, snails, etc.), could enhance their role as pathogen dispersers among other possible hosts [8].

The aim of this study was to deepen in the knowledge of *Turdus philomelos* parasitofauna and its ability to cause injury to different

host tissues, as well as to evaluate the effects of these parasites on birds' weight as a determining factor to carried out a successful migration.

2. Material and methods

Ninety (61 males and 29 females) adult song thrushes (*Turdus philomelos*) were sampled during the hunting season (October 2013–February 2014) in the forest of Castellón province (eastern of Spain). Birds' carcasses were sent to the Veterinary Faculty (University CEU-Cardenal Herrera (Valencia, Spain)) in refrigerated containers (4–6 °C), and necropsies were carried out within 24 h of the bird's death.

Each bird was weighted before the necropsy and a detailed external assessment was performed in order to evidence any kind of lesion or morphological alteration of the corpses. The body condition was evaluated by calculating the ratio of sternum musculature to body weight and scored on a four-point scale (1–4) as previously described [9]. Subsequently, a thorough macroscopic examination of carcasses was performed. Digestive and respiratory tracts were assessed separately to collect parasites and to evidence tissues lesions associated to the para-

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sitism [10]. Helminths were preserved in 10% buffered formalin until identification. Nematodes were cleared with lactophenol of Amman, while trematodes and cestodes were flattened, stained with acetic carmine, dehydrated, cleared in clove oil, mounted using DPX and observed under the microscope once dry. Morphometrical identification of all parasitic specimens was performed attending to Anderson (1976) [11], Machalska (1980) [12], Schmidt (1986) [13], Dubey et al. (1989) [14], Dronen and Trach (2015) [15].

Tissue biopsies from cardiac muscle, air sacs, trachea, lungs, liver, small and large intestine, spleen and kidney were fixed in 10% formalin, processed and embedded in paraffin. Blocks were cut into 5 µM slices with a rotary microtome and stained with hematoxylin and eosin.

Weight data showed a normal distribution according to the evaluation of skewness (D'Agostino skewness test) and kurtosis (Bonett-Seier test); also, data distribution was confirmed using the Shapiro-Wilk normality test. A Student's *t*-test was used to evaluate the relationship between variables (weight and presence of parasites). Significance was taken for alpha = 5% (*P* < 0.05). R software v3.6.0 (<http://cran.r-project.org/>) was employed for statistical analysis.

3. Results and discussion

A total of 15 song thrushes (16.7%) were infected with one or more adult or larvae parasite species, including trematodes (*Morishitium* sp.), nematodes (*Splendidofilaria mavis*) and cestodes (*Dilepis undula*) (Table 1), as well as with cysts of the protozoa (*Sarcocystis* sp.). Nine (60%) of the parasitized birds presented co-infection: one of them (11.1%) hosted four different parasitic species, while five (55.6%) showed three species, and the remaining three birds (33.3%) harboured two. The most common association was trematodes and nematodes, present in the 70% of cases (7/10).

The registered weight values (mean = 67 g) were lower for infected birds (mean = 65.9 g ± 1.4; *n* = 15) than for non-infected ones (mean = 67.2 g ± 2.3, *n* = 75), although difference was not statistically significant (*p*-value (*t*-test) = 0.1). The body condition was assigned as 4 (normal birds, with rounded pectoral muscles, and a light dip on either side of the sternum) in all cases, which suggests a long-term coevolution between song thrushes and these parasites in the natural environment [16]. This adaptation enable the birds to develop a good body condition despite the presence of the parasites, and therefore to complete the annual migration avoiding mortality during this energy-intensive process but, simultaneously, allowing the birds to act as parasite spreaders during their migration [17].

Trematodes were recovered from the air sacs of nine birds (10%), with a median intensity of 16.6 parasites per infected bird (range 4–25) (Fig. 1A). The morphometric identification matches *M. polonicum* (Table 1). However, given the similarity between this species and *M. turdia*, authors decided to identify it as *Morishitium* sp. pending future researches. Mild airsacculitis was observed in all birds. In some cases, parasites were found into the coelomic cavity or even the bronchi (Fig. 1B), causing coelomitis or bronchitis. Histological examination of the air sacs showed epithelial ulceration and mild to moderate heterophilic and lymphoplasmacytic inflammation (Fig. 1C). Large number of variable shaped, yellowish to brown eggs were observed inside the parasite (Fig. 1D). This trematode was previously described in Turdididae from European countries such as Spain [1], Poland [12] or Italy [4]. Birds get infected through the consumption of wetland-associated molluscs. Although there is scarce information about the pathology related to *M. polonicum* infection, airsacculitis, bronchitis and peribronchitis have been recently described in *Turdus merula* [4].

Six song thrushes (6.7%) showed adults of *S. mavis*, with a median intensity of 3.2 parasites per infected bird (range 1–4) (Fig. 1E). Four males and 15 females were extracted directly from coelomic cavity or by dissection from blood vessels. Besides, the histopathological study revealed microfilaria in pulmonary vessels of 12 birds (13.3%). However, nor gross or histological lesions associated to these parasites were observed (Fig. 1F). Prevalence and intensity in this study are in con-

Table 1
Measurements of helminths according to previous references and our findings.

Parameter (µm, except as indicated)	<i>Morishitium polonicum</i> (Machalska, 1980)	Our findings average (10 trematodes)	
		min-max	Average
Body length (mm)	7.138–13.109	8.231–12.824	10.324
Body width (mm)	2.088–2.314	2.033–2.283	2.125
Oral sucker width	208–323	188–283	233
Pharynx width	208–323	216–310	258
Cirrus sac length	219–474	225–482	359
Ovary width	202–377	211–362	281
Testes width	340–1118	35–1143	724
Egg length	81–139	86–147	119
Egg width	58–90	53–98	68
<i>Splendidofilaria mavis</i> (Leiper, 1909)		Our findings average (4 males)	
		min-max	Average
Total body length (mm)	8.4–22.7	7.2–9.8	8.42
Maximum body width	132–180	191–241	219
Length of esophagus	505–710	595–893	768
Nerve ganglion-anterior body end	125–183	116–170	149
Length of spicules	78–84	59–90	68
Length of tail	38–50	43–64	52
<i>Splendidofilaria mavis</i> (Leiper, 1909)		Our findings average (10 females)	
		min-max	Average
Total body length (mm)	28–49	15.5–22	18.62
Maximum body width	235–392	215–391	306
Length of esophagus	490–889	713–984	857
Nerve ganglion-anterior body end	119–204	125–216	163
Vulva-anterior body end	407–914	394–873	647
Length of tail	42–56	67–101	95
<i>Dilepis undula</i> (Schranck, 1788)		Our findings average (10 cestodes)	
		min-max	Average
Total length (mm)	70	25–71	43
Total width (mm)	3.5	2.1–3.4	2.6
Scolex width	450–900	487–925	670
No. of rostellar hooks	49–64	40–59	54.2
Length of hooks	18–20	16–24	20.2
No. of testes	28–36	25–39	32
Cirrus sac length	28–42	23–39	35.8
Cirrus sac width	3.2	2.8–3.3	3.2

cordance with other avian filariases in European thrushes [5]. Black flies and biting midges, known vectors for *Splendidofilaria* spp., are highly widespread in Spain.

The cestode *Dilepis undula* was collected from the small intestine of seven thrushes (7.8%), showing one of them one specimen in the bronchus (1.11%) (Fig. 1G, H). The median intensity of this cestode was low (2, range 1–4), being all cases in the intestine associated with mild to moderate enteritis. The location of this cestode in the lung is quite unusual and it is unclear how it reached the bronchus. It could have moved from the intestine as a result of a shot, although no haemorrhage around was observed, as it might be expected in that case. However, histological exam revealed lesions consisting on bronchial ulceration with lymphoplasmacytic infiltration, which indicates that

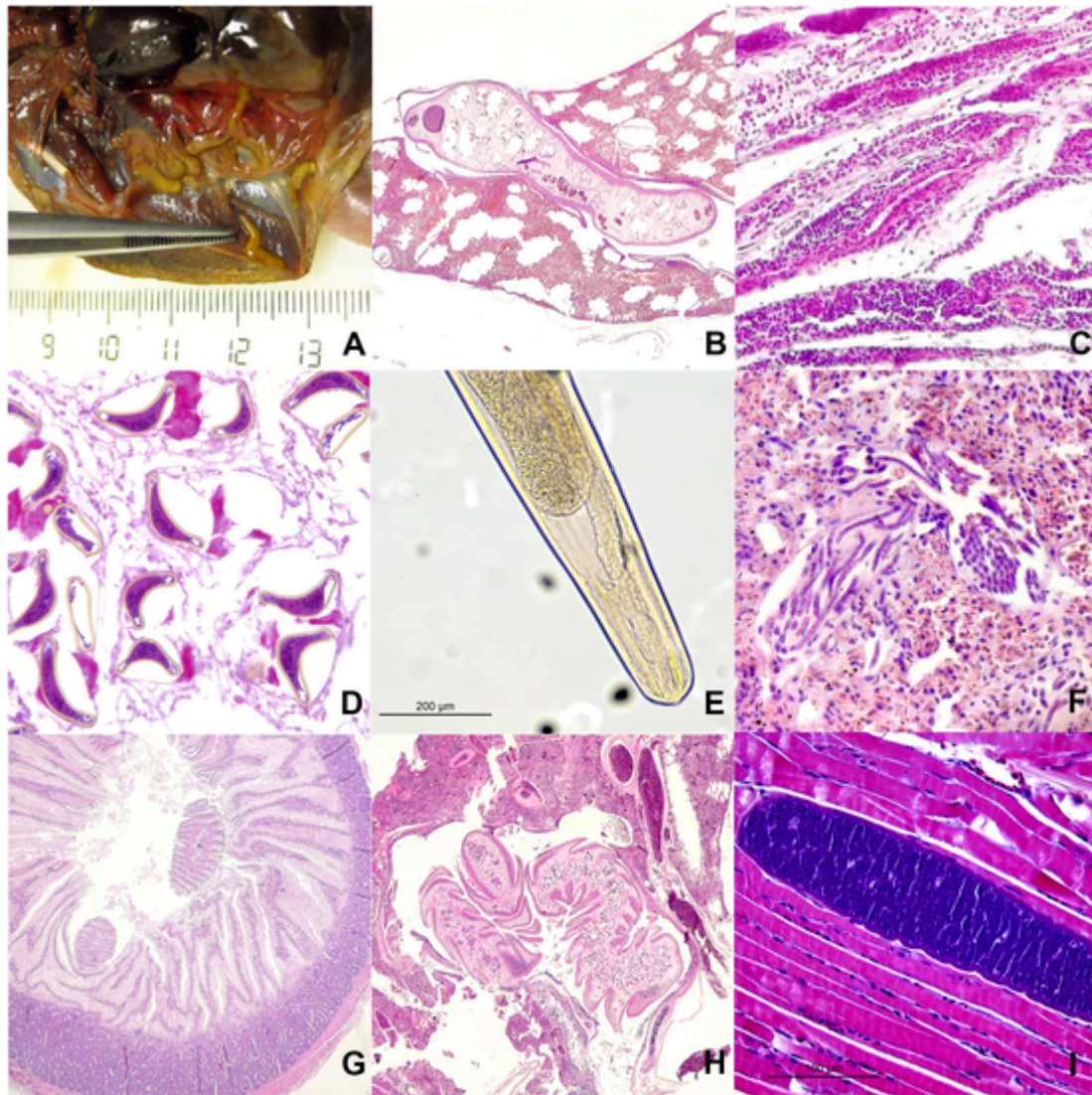


Fig. 1. A: adults of *Morishitium* sp. in coelomic cavity. B: adult of *Morishitium* sp. in bronchus. C: air sac showing epithelial ulceration and heterophilic and lymphoplasmacytic inflammation, HE staining. D: detail of *Morishitium* sp. eggs, HE staining. E: anterior end of an adult *Splendidofilara mavis*. F: microfilaria in pulmonary vessels, HE staining. G: adult *Dilepis undula* in the small intestine, HE staining. H: adult cestode in lung, HE staining. I: cyst of *Sarcocystis* sp. in skeletal muscle, HE staining.

the cestode had been residing there for some time. It is well documented erratic larval migration in tapeworms, but authors did not found references about adult cestodes in this location. *D. undula* has been previously described as a dominant species in forests and urban thrushes from Europe, with a higher prevalence and intensity of infection that those found by us [5,6]. The consumption of annelids, molluscs and arthropods has been described as the commonest transmission way of this cestode among birds.

Cysts of *Sarcocystis* sp. were identified in skeletal muscles of three birds (3.3%) during the histological evaluation (Fig. 1I), also affecting the myocardium in one individual. To the authors' knowledge, this is the first report of *Sarcocystis* spp. in thrushes from Spain. The presence of this protozoan highlights the great importance of these hosts in the epidemiology of this parasite in other birds, both domestic and wild, in the migration area of song thrushes. This parasitism should be considered a severe risk in the case of song thrushes, since the weakness and extensive damage on muscular tissues have been previously reported in massive infections due to this protozoa cyst proliferation and could compromise their migrations [18]. Nutrition peculiarities of thrushes (consumption of insects, grubs, earthworms and molluscs) may

favour the presence of this protozoa since they could ingest oocysts or sporocysts excreted by predatory birds, the definitive hosts [19].

Only four species of parasites with a low prevalence were detected in our study. This low richness is in concordance with the three species found in the same host in Czech Republic in 2012 [20]. Other surveys carried out in blackbirds (*T. merula*) from Central Europe reported a prevalence of up to 98.7% [3] and the identification of 30 and 24 different species were recorded also in *T. merula* from the Czech Republic [6] and Poland [5], respectively. Also, these authors reported a higher parasitic richness in thrushes captured in forest populations than in those coming from urban environments. In contrast, our study was carried out with birds hunted in forest areas, but both prevalence and the parasitic richness were low. Low parasitic prevalence and intensity found by us could be then attributed to the different geographical location. However, even among studies carried out in one single country (Czech Republic), findings differed considerably [6,20]. Thus, other factors such as season, presence of intermediate hosts, as well as sex and age of birds may be involved in prevalence, parasitic abundance or parasitic richness. Regarding season, it seems that spring is the most adequate for birds to be infected, since the presence of intermediate hosts in the environment increases [5]. However, this study was car-

ried out during autumn and winter months, when is less likely to find these intermediate hosts. Considering sex, five males and 10 females (8.2% and 34.5% of the total examined) were found to be parasitized in our study. Sex determines the diet of birds, and females have more risk for being infected with trematodes, as they prefer molluscs and crustaceans (intermediate hosts for these parasites) to build the shell of the eggs [6,20]. This evidence agrees with our findings, although the low number of females examined may contribute to explain that only one trematode species was identified. In previous studies from north and Central Europe, trematodes *Leucochloridium perturbatum* or *Lutztrema attenuatum* were found to be the most frequent species in thrushes [5,6,20]. Although all thrushes in this study were adults, and we cannot compare with other age groups, host age is considered other biological feature that influences the presence of particular parasite species. According to other authors, age is related to diet and to differences in the digestive tract between adults and juveniles, where most of helminths reside [6].

Parasites described in this study were recovered from tissues directly involved in the migration (i.e., muscle, respiratory and digestive systems). Although body condition of evaluated thrushes was not affected, probably due to the low parasitic intensity of parasitism derived from an evolutionary adaptation [16], punctual moderate lesions have been also described. In this sense, under stressful conditions (migration), the consecutive immunosuppression can compromise the health status of thrushes when pathogens are present, making it difficult for birds to travel long migratory distances [21,22]. In fact, even a small number of parasites large-sized (as those found in this study), may cause even death in passerines [20].

In conclusion, there is scarce information related to thrushes parasitofauna both in southern Europe and concretely in Spain, where most studies have been focused on one parasitic species [1,4,23]. The lack of data, as well as the relevant role of these birds as spreaders of parasites during their migration, makes it necessary to promote epidemiological studies in Turdidae species with a multifactorial approach, considering information related to different geographical locations and, subsequently, climatic conditions. Other factors, such as age or effect of urbanisation should be analysed, since previous studies showed their influence on the results [5,6]. Finally, in future researches it would be interesting to deepen in the knowledge of song thrushes parasitofauna evaluating the presence of digestive protozoa and blood parasites.

Ethics approval

No ethics permit was awarded since birds were legally hunted by the Hunting and Fishing Federation of the Valencian Community.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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