

1 Parasitofauna survey of thrushes (*Turdus philomelos*) from eastern Spain

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26 **Abstract**

27 Thrushes (*Turdus* spp.) are migratory passerine birds found in northern Europe during
28 the summer months and in southern Europe and north of Africa during the winter. They
29 constitute an important small game bird group very appreciated by Spanish hunters.
30 Between October 2013 and February 2014, 90 thrushes were collected and submitted to
31 the Veterinary Faculty of the University CEU-Cardenal Herrera (Valencia, Spain). After
32 necropsies, three species of helminths were macroscopically recovered from 15 animals
33 (16.7%): *Morishitium polonicum* (16.7%), *Splendidofilaria* spp. (6.7%) and
34 *Raillietina* spp. (7.8%). Moreover, twelve of the positive thrushes (80%) harboured
35 microfilaria in pulmonary blood vessels and three of them (20%) were infected by
36 *Sarcocystis* spp. on skeletal musculature. All parasitised birds showed lesions, ranging
37 from mild to moderate tracheitis, aerosaculitis, enteritis and hepatitis, being the first
38 report of severe enteric and hepatic lesions associated to *M. polonicum* infection in
39 Turdidae. Furthermore, this is the first description of *Raillietina* spp. and *Sarcocystis*
40 spp. in thrushes from Spain. The results obtained in this survey reveal the need for
41 further studies to evaluate the epidemiological role of thrushes as spreaders of several
42 parasites during their annual migration.

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44 **Keywords:** *Turdus*; parasites; *Morishitium polonicum*; *Sarcocystis*; *Splendidofilaria*;
45 *Raillietina*.

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51 **Introduction**

52 Thrushes (*Turdus* spp.) are one of the most popular group of game birds in Europe.
53 [1,2]. These migratory birds can be found in northern latitudes during the summer
54 months, although they return to southern ones, including the Iberian Peninsula, for
55 wintering, coinciding with the hunting season in Spain [2].

56 Different helminths (trematodes, cestodes, nematodes, and acanthocephalan) have been
57 previously isolated from several anatomical regions of *Turdus* spp. [3–5]. These birds
58 have been widely studied as reservoirs of some viral pathogens (e.g., highly pathogenic
59 influenza aviar and usutu virus; [6,7]), but it is still unclear their role as reservoir of
60 parasites for domestic poultry, humans or other game bird species. In this sense, factors
61 like seasonal migration, habitat changes or thrushes' diet (mainly based on insects,
62 earthworms, snails, etc.), could enhance the role of thrushes as pathogen dispersers
63 among other possible hosts [3,8,9].

64 The aim of this study was to deepen in the knowledge of *Turdus philomelos*
65 parasitofauna and its ability to cause injury to different host tissues, as well as the
66 effects of theses parasites on birds' weight as a determining factor to carried out a
67 successful migration.

68 **Material and methods**

69 Ninety Turdidae birds (*Turdus philomelos*) were sampled during the hunting season
70 (October 2013-February 2014) in Castellón province (eastern Spain). Thrushes
71 carcasses were sent to the Veterinary Faculty (University CEU-Cardenal Herrera
72 (Valencia, Spain)) in refrigerated and hermetic containers (4-6°C), and necropsies were
73 carried out before 24 hours from animals' death.

74 Each bird was weighted before the necropsy and a detailed external assessment was
75 carried out in order to evidence any kind of lesion or morphological alteration of the

76 corpses. Subsequently, a thorough macroscopic examination of corporal cavities and air
77 sacs was performed before removing the digestive and respiratory tracts. These parts
78 were assessed separately to collect parasites and tissues showing associated lesions.
79 Parasite specimens were counted and preserved in 70% alcohol for their subsequent
80 morphometrical identification attending to Anderson (1961) [10], Schmidt (1986) [11],
81 Dubey et al. 1989 [12], Gibson et al. (2002) [13] and Machalska (1980) [14].

82 Tissue biopsies from skeleton, cardiac muscle, air sacs, trachea, lungs, liver, small and
83 large intestine, spleen and kidney were fixed in 10% formalin, processed and embedded
84 in paraffin. The paraffin block was cut into 5 μ M slices with a rotary microtome
85 and stained with hematoxylin and eosin [15].

86 Weight data showed a normal distribution according to the valuation of skewness and
87 kurtosis tests. A parametric test (Student's t-test) was used to evaluate the relationship
88 between variables (weight and presence of parasites). Significance was taken for alpha
89 = 5% ($P < 0.05$). R software v3.6.0 (<http://cran.r-project.org/>) was employed for
90 statistical analysis.

91 **Results and discussion**

92 A total of 15 thrushes (16.7%) were infected with one or more parasite species,
93 including trematodes (*Morishitium polonicum*), nematodes (*Splendidofilaria* spp.)
94 cestodes (*Railletina* spp.) and cysts of the Protozoa (*Sarcocystis* spp.). Ten (66.7%) of
95 the parasitized animals presented co-infection. One of them (10%) hosted four different
96 parasitic species, while five thrushes (50%) showed three different species and the
97 remaining three birds (30%) harbored two species. The most common association was
98 formed by trematodes and nematodes.

99 The registered weight values (mean = 67 g) were lower for infected birds (mean = 65.9
100 g \pm 1.4; n = 15) than for non-infected ones (mean = 67.2 g \pm 2.3, n = 75); however,

101 although a negative trend was detected, difference between the weight of both groups of
102 thrushes was not statistically significant (p-value: 0.1). This result suggests a long-term
103 coevolution between thrushes and parasites in the natural environment [16]. This close
104 adaptation allows the birds to develop a good corporal condition despite the presence of
105 the parasites, and therefore to complete the annual migration, avoiding bird mortality
106 during this energy-intensive process and simultaneously allowing the birds to act as
107 parasite spreaders during their migration [17].

108 The trematode *Morishitium polonicum* was recovered from the coelomic cavity of nine
109 birds (10%), with a median parasitic intensity of 24 (range 4-25) (Fig. 1). Although mild
110 associated lesions were showed in most infected animals in this study, only one bird
111 revealed a massive infection (72 adult trematodes in the coelomic cavity) with severe
112 damage in several organs (tracheitis, airsacculitis, enteritis and hepatitis). This
113 trematode has been previously described on Turdidae from different European countries
114 such as Spain [1], Poland [14] or Italy [4]. Birds get infected through the consumption
115 of wetland-associated mollusks. Although there is scarce information about the
116 pathology related to *M. polonicum* infection, airsacculitis, bronchitis and peribronchitis
117 have been recently described in *Turdus merula* [4]. However, this is the first report of
118 severe enteric and hepatic lesions associated to *M. polonicum* infection in Turdidae.

119 Six thrushes (6.7%) showed adults of *Splendidofilaria* spp. in blood vessels with a
120 median intensity of 4 (range 1-4). The histopathological study revealed microfilaria in
121 pulmonary vessels of twelve animals (13.3%), however, lesions associated to these
122 parasites were not found. The absence of lesions could be derived from the low intensity
123 of this parasitism found in our study, although nodules of *Splendidofilaria* spp. have
124 been reported in leg joints, subcutaneous tissues, artery walls and myocardium of wild
125 birds in previously surveys [18].

126 The cestode *Raillietina* spp. was collected from the small intestine of seven thrushes
127 (7.8%). Although the median intensity was low (2) (range 1-4), lesions of moderate
128 enteritis were found in all parasitized birds. *Raillietina* spp. causes a negative impact on
129 infected hosts, and the transmission of this cestode between wild reservoirs and
130 domestic poultry has been already documented, considering its presence quite harmful
131 for production birds [19]. The consumption of ants, flies, coleoptera and molluscs has
132 been described as the commonest transmission way of this cestode among birds [20].
133 In addition, cysts of *Sarcocystis* spp. were identified in skeletal muscles of three animals
134 (3.3%) during the histological evaluation (Fig. 2), also affecting the myocardium in one
135 individual. Nutrition peculiarities of thrushes (consumption of insects, grubs,
136 earthworms and molluscs) could favour the presence of this protozoa since they could
137 ingest oocysts or sporocysts excreted by sparrowhawks and red foxes, the definitive
138 hosts [21]. Furthermore, weakness and extensive damage on muscular tissues has
139 previously been reported in massive infections due to this protozoa cyst proliferation
140 [22].
141 To our knowledge, this is the first report of *Raillietina* spp. and *Sarcocystis* spp. from
142 thrushes in Spain, which highlights the importance of these wild birds in the
143 epidemiology of parasitism in their migration areas
144 The epidemiological role of migratory birds as reservoirs of parasitic agents must be
145 considered. Parasites described in this study were recovered from tissues directly
146 involved in the migration (i.e., muscle, respiratory and digestive systems). Although
147 corporal condition of evaluated thrushes was not affected, probably due to the low
148 intensity of parasitism derived from an evolutionary adaptation [16], under stressful
149 conditions (migration) the consecutive immunosuppression can compromise the health
150 status of *Turdus* when pathogens are present, making it difficult for birds to travel long

151 migratory distances [23]. There is scarce information related to thrushes parasitofauna
152 both in Europe and concretely in Spain, since most of studies have been traditionally
153 focused on viral processes [6,7] or only in one parasitic species, mainly *M. polonicum*
154 [1,4]. Because the parasitic richness of migratory birds could be higher than the one of
155 resident specimens [24], it is important to consider the results in the present study to
156 evaluate their consequences on the thrushes' migration [24–26].

157 In conclusion, this is the first report of *Raillietina* spp. and *Sarcocystis* spp. in thrushes
158 from Spain, as well as the first description of severe enteric and hepatic lesions
159 associated to *M. polonicum* infection in Turdidae. Even though moderate prevalences
160 and parasitic intensities have been described in this study (16.6%), these findings have
161 relevant implications for thrushes' health specially under stress-induced
162 immunosuppression conditions. The lack of data about parasitic infection in thrushes, as
163 well as about the role of these birds as spreaders of parasites during their migration,
164 makes it necessary to promote epidemiological studies in Turdidae species with a
165 multifactorial approach.

166

167 **Declaration of interest**

168 The authors declare that there is no conflict of interest.

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275 **Figures**

276

277 Fig. 1. a: adults of *Morishitium polonicum* in coelomic cavity. b: detail of the anterior
278 end of an adult trematode. c: longitudinal section of an adult trematode, HE staining. d:
279 detail of eggs on pulmonary tissues, HE staining.

280

281 Fig. 2. Microcysts of *Sarcocystis* spp in skeletal muscle tissue, HE staining.

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