

First Data on Gastrointestinal Parasitic Infection in the Red-Legged Partridge (*Alectoris rufa*) in Italy

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Abstract: The Red-legged partridge (*Alectoris rufa*) is a Mediterranean Galliformes, recently classified as Near Threatened by the International Union for the Conservation of Nature, due to the constant and significant decline of its global population. While the gastrointestinal parasites of the species are well studied in some part of its range (Iberian peninsula), limited information is available for the Italian populations, that represent the eastern limit of the species range. This study was conducted to fill this gap of knowledge, determining the composition, richness, prevalence, intensity and abundance of *A. rufa* gastrointestinal parasite community in two populations in Italy. During the autumn seasons 2008–2009 and 2009–2010, necropsies were conducted on 18 Red-legged partridge from the southern part of Alessandria province (Piedmont, northwestern Italy) and 34 from the Parma province (Emilia Romagna, northern Italy). All the animals were examined for the presence of gastrointestinal parasites. Additionally, 229 fecal samples were collected from live animals in Alessandria province. Prevalence, abundance and intensity of infection were calculated for each parasite species, except for coccidia for which only the prevalence was determined. The following parasites were observed: *Heterakis gallinarum*, *Ascaridia* spp., *Ascaridia columbae*, *Raillietina* spp., *Eimeria* spp. The highest parasite prevalence was found in Alessandria province for *Eimeria* sp., infecting almost half of the sampled animals (P: 45%, CI_{95%}: 39–51). *Eimeria* sp. was also the most prevalent parasite in Parma province but with much lower prevalence (P: 19%, CI_{95%}: 5–32). Intestinal helminths prevalence ranged from 3% (CI_{95%}: 0–9) for *A. columbae* and *Raillietina* spp. (Parma Province) to 9% for *H. gallinarum* in both Parma (CI_{95%}: 0–19), and Alessandria province (CI_{95%}: 0–22). To our knowledge, this is the first study providing information on the gastrointestinal parasites of the Red-legged partridge in Italy. *Ascaridia columbae*, a parasite typical of the domestic pigeon, was reported for the first time in *A. rufa*. The epidemiological descriptors reported herein could serve as a basis for future studies, allowing for epidemiological comparison across countries, locations, and time periods.

Keywords: parasite community; biodiversity; Galliformes; restocking; wildlife management



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The Red-legged partridge (*Alectoris rufa*) is a medium-sized Phasianidae (Galliformes) whose range includes Spain (including the Balearic Islands), Portugal, France (including Corsica) and northwestern Italy [1]. Introduced populations are reported in the United Kingdom, Ireland and Greece. Over the last ten years, the species has experienced a dramatic decline at a rate of 40–45% of the global population, and it is currently classified as Near Threatened by the International Union for the Conservation of Nature [2]. The species is listed in Annex I of the Directive 2009/147/EC EU Birds Directive, which aims to

preserve wild birds and their habitats [3]. The main threats for the Red-legged partridge are represented by agricultural intensification with consequent habitat loss, over-hunting and the restocking operations using farm-reared birds, many of which are hybrid *Alectoris chukar* × *A. rufa* [4–6].

While the socio-economic and conservation relevance of the species is widely recognized, there is scarce knowledge on its health status and on the main pathogens that can threaten its long-term conservation, specifically in some part of its natural range [7]. In particular, few studies are available on the pathogens affecting the Red-legged partridge in Italy, including a recent study that described the occurrence of *Haemoproteus* spp., a blood parasite, for the first time in the species in Italy [8].

In numerous articles, it has been demonstrated that infectious diseases may impact significantly on wildlife population dynamics, with parasites playing a critical role in the conservation of threatened species [9,10]. Additionally, it is worth highlighting that the free roaming population of *A. rufa* are historically managed in several countries through the artificial introduction of large numbers of animals, released for restocking purposes, but without proper health monitoring in place. These practices, in addition to the already mentioned risk of hybridization, represent a significant risk for the introduction and spread of pathogens [11,12].

Given the above, the objective of this study is to describe the gastrointestinal parasite community of the Italian Red-legged partridge, using gastrointestinal tracts and fecal samples collected from two populations. The populations were selected based on: (i) the availability of a local network of field operators and hunters able to provide samples and (ii) the different management of the population (hunted population with introduction of animals for restocking purposes versus a not hunted population).

During the autumn seasons 2008–2009 and 2009–2010 18 Red-legged partridge from the southern part of Alessandria province (Piedmont, northwestern Italy) and 34 from the province of Parma (Emilia Romagna, northern Italy) were analysed following the common parasitological standard techniques described below [13]. In Alessandria province no hunting or restocking operation was allowed, while in Parma province the population was actively hunted, and introduction of animals for restocking purposes was a common practice. The location of the two study areas is represented in Figure 1. Samples from Alessandria province included animals found dead while all the samples from Parma were animals harvested during hunting activity. For each bird, the gastrointestinal tract, including the glandular stomach, gizzard and intestine, were extracted, sealed in plastic bags and frozen until processing. In the laboratory the gastrointestinal tract was opened with a longitudinal incision and the content of the individual sections (proventriculum, gizzard, small and large intestine) was analyzed. The complete content of each gastrointestinal tract was filtered several times using a sieve (mesh size 75 µm), and emptied onto a Petri dish to detect adult parasites (nematodes and cestodes). All helminths were collected and counted under a stereoscope and conserved in 70% ethyl alcohol until identification. Moreover, fecal samples from 229 birds live trapped in Alessandria province were collected, frozen and subsequently examined for ova and oocyst presence by light microscopy after concentration by sedimentation and flotation with a 33% zinc sulfate solution (ZnSO₄ 33%) [13]. Parasites were identified using the keys suggested by Skryabin [14] Molecular identification by PCR amplification and sequencing was carried out as previously described by Gasser and Hoste [15]. One worm, morphologically identified as *A. columbae* was used for the molecular analyses. Genomic DNA was isolated with a commercial kit (NucleoSpin Tissue Macherey-Nagel, Düren, Germany), and the internal transcribed spacers (ITS1 and ITS2) as well as the 5.8S rDNA gene were amplified using conserved oligonucleotide primers NC5, 5'-GTAG GTGAACCTGCGGAAGGATCATT-3" (forward) and NC2, 5'-TTAGTTTCTTTTCCTCCGCT-3" (reverse). PCR was performed with 10–20 ng of DNA in 100 µL volumes, 25 pmol primer and 0.5 U Taq polymerase (HotStarTaq DNA Polymerase, Qiagen, Venlo, The Netherlands) under the following PCR conditions: an initial denaturation at 95 °C for 15 min, followed by 40 cycles at 94 °C for 45 s (denatura-

tion), 53 °C for 30 s (annealing) and 72 °C for 1 min (extension), and a final extension cycle at 72 °C for 10 min. Templates of the PCR were analyzed by MacroGene Europe Laboratories (EZ-Seq service, Amsterdam, The Netherlands) for sequencing. After generating a sequence type, the sample was inferred to species according to the data available on the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI). Parasite community composition and richness were evaluated for the two study areas.



Figure 1. Location of the study areas in which the parasitological investigations were carried out (grey areas on the map): Alessandria province (grey area on the left) and Parma province (grey area on the right).

Prevalence ($(\text{number of infected birds}/\text{number of samples } N) \times 100$), intensity of infection (total number of parasites found/number of infected birds), and abundance (total

number of parasites found/number of birds) were calculated for each parasite species. *Eimeria* sp. identification was done only at the genus level, as all the samples were frozen before the analysis, not allowing for the evaluation of the sporulate form of the parasite (needed for identification at the species level). Cestode identification was also done only at genus level due to the scarce preservation status of the parasites.

The species of parasites (parasite richness) recorded were: three in the province of Alessandria (*Heterakis gallinarum*, *Ascaridia* spp. and *Eimeria* spp.), and four in the province of Parma (*H. gallinarum*, *Ascaridia columbae*, *Raillietina* spp., *Eimeria* spp.). Identification of *A. columbae* was confirmed by PCR molecular identification, as previously described. No coinfection among helminth species was detected and usually only one parasite species was detected in the infected birds. The only exception was represented by one animal in Parma province with the presence of both *Ascaridia* spp. and *H. gallinarum*. *Eimeria* spp. oocysts were found as well in the faeces of the 229 birds. No other parasite eggs were found in the faeces, probably due to the freezing process of the sampled material (faeces congelation may damage the eggs and prevent the diagnosis through flotation). Prevalence, mean abundance and mean intensity values, with 95% confidence interval (CI) and standard deviation (S.D.) are presented in Table 1.

Table 1. Gastrointestinal parasite species from Red-legged partridge in Italy. Alessandria province: 18 animals and 229 fecal samples; Parma province 34 animals.

Source	Species	Prevalence (CI 95%)		Mean Abundance (S.D.)		Mean Intensity (S.D.)	
		Alessandria Province	Parma Province	Alessandria Province	Parma Province	Alessandria Province	Parma Province
Gastrointestinal tract	<i>Heterakis gallinarum</i> (Phylum: Nematoda)	9 (0–22)	9 (0–19)	0.06 (0.24)	0.41 (1.56)	1 (0)	4.33 (3.51)
Gastrointestinal tract	<i>Ascaridia</i> spp. (Phylum: Nematoda)	6 (0–17)	NA	0.06 (0.24)	NA	1 (0)	NA
Gastrointestinal tract	<i>Ascaridia columbae</i> (Phylum: Nematoda)	NA	3 (0–9)	NA	0.72 (4.18)	NA	24 (0)
Gastrointestinal tract	<i>Raillietina</i> spp. (Phylum: Platyhelminthes)	NA	3 (0–9)	NA	0.09 (0.52)	NA	3 (0)
Faeces	<i>Eimeria</i> spp. (Phylum: Apicomplexa)	6 (0–17)	19 (5–32)	NA	NA	NA	NA
Faeces	<i>Eimeria</i> spp. (Phylum: Apicomplexa)	45 (39–51)	NA	NA	NA	NA	NA

NA = Not available.

The results of this study provide an important contribution to the literature on parasite communities in wild Galliformes, an order that is less studied than others. While for Alpine Galliformes (Black grouse *Lyrurus tetrix*; Hazel grouse *Tetrastes bonasia*; Rock partridge *Alectoris graeca saxatilis*; Rock ptarmigan *Lagopus muta*) the information has improved in the last few years [8,16–19], the data for other European Galliformes such as *A. rufa*, *Phasianus colchicus* and *Perdix perdix* is still scarce.

No data on gastrointestinal parasites were available until now for the Italian Red-legged partridge populations, and the description of *Raillietina* spp. and *H. gallinarum* represents the first report for the species in Italy. Additionally, *A. columbae* is reported for the very first time in *A. rufa*. Molecular analysis shows that the ITS1–5.8rRNA–ITS2

sequence obtained is 100% homologous with *A. columbae* sequences present in the GenBank database. This finding is of particular interest being *A. columbae* a parasite typical of pigeons, described for the first time in 1920 [20], and never reported in the Red-legged partridge. This nematode appears to be highly pathogenic for its host, causing diarrhoea, growth reduction, enteritis, and so raising questions on its impact on *A. rufa* populations [21]. Regarding *Eimeria*, the oocysts observed in our samples had a morphology similar to the two species described so far in Italy in the Red-legged partridge, namely *Eimeria kofoidi* and *Eimeria legionensis* [22]. However, as previously mentioned, we did not identify *Eimeria* at species level.

While in Italy only a few parasite species have been described in the Red-legged partridge, a larger number have been found in Europe, including: nine species of *Eimeria*, five of *Raillietina*, three of *Capillaria*, three of *Heterakis*, three of *Ascaridia*, one of *Cyrnea*, one of *Trychostrongylus*, one of *Rhabdometra* and one of *Conspicuum* [23–29]. The reason for the lower parasite richness observed in our study may be due to the small sample size and to the limited geographical range sampled, with consequent reduced probability of finding parasites at low prevalence. As reported by other authors in fact, several factors may have a major effect on the estimation of the structure and parasite community richness, such as the season, the size of the area, and the number of hosts sampled [27]. As an example Calvete et al. [27] analyzed 537 Red-legged partridge, finding 16 different parasite species, Calvete et al. [28] sampled 235 partridge, describing 15 different parasites.

The same authors observed a very low repeatability of parasite community and structure among different sites, mainly due to different habitats and environmental conditions. The difference in parasite community structure is in fact a quite common pattern detected in other surveys. Variations in characteristics of host populations and/or their habitat seem to determine the composition and richness of communities at a fine scale [27]. On another side, a low parasite richness is commonly found in host species living at the edge of their distribution range and at low density [17].

Almost all the data referring to the parasite community in *A. rufa* are currently based on studies carried out in the Iberian Peninsula [23,28,29]. In the Red-legged partridge from Spain most of the worms reported are ubiquitous in poultry and gamebird farms, such as ascarids or *H. gallinarum*, and restocking practices seems to play a major role in introducing this parasite in wild populations. Also *Eimeria* spp. is in general introduced in wild birds through the release of captive partridges (in which the infection is fairly common) for hunting purposes [28,29]. Few other data on *A. rufa* parasites derive from studies conducted in France in experimental infections [30] and in Czech Republic in farmed animals [31].

Restocking with farmed game birds, without a proper sanitary monitoring, is a common practice also in Italy. The data reported in our study represents a baseline for future surveillance activities and investigations aiming to evaluate the effect of restocking also in this country. It was not the objective of this research to evaluate the influence of restocking on the parasite community, but as a preliminary finding it may be useful to highlight that no particular differences, apart from a higher parasite richness in Parma province, was detected between the two study areas.

Despite some limitations, this work provides valuable findings and baseline data for comparison and to better understand the parasite gastrointestinal community of *A. rufa* in Italy.

Our data collection lacks of seasonality, being all the samples collected in autumn. This is a common problem in studies that rely on animals harvested during the hunting season [27–29]. Parasite presence and dynamics may present large variation along the seasons [32], and future works should focus on parasite monitoring during the whole year, to better understand how the parasite community composition, richness, and prevalence is influenced by seasonality.

Targeted parasite surveillance has proven to be a useful tool to investigate and understand parasite and host dynamics, especially at multi-host population level [32,33]. Even if

the objective of our work was not to investigate the role of parasite as population dynamics drivers, the detection of species with demonstrated pathogenic effect as *A. columbae* [21] highlights the importance of including pathogens surveillance in ecological studies [9]. Thus, we encourage further surveys, involving a larger number of samples collected over a wider period of time and seasons, to evaluate the sanitary status of *A. rufa*, the potential presence of risk factors that could influence the structure of the helminths community, and the role of parasites in the Red-legged partridge wider population dynamics and ecology.

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