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Gastrointestinal parasitic infection in the hazel grouse *Tetrastes bonasia* in France

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The hazel grouse *Tetrastes bonasia* is a Galliformes species of major conservation interest and threatened throughout Europe. However, little information is currently available on its sanitary status both in the Alps and worldwide. Considering that gastrointestinal parasites are one of the factors negatively impacting on Galliformes population dynamics, the objective of this study is to fill the gaps of knowledge on the parasite-fauna of the Alpine hazel grouse. Twenty-nine hazel grouse were collected in the French Alps during the period 1987–2019 (no samples collected during 1997–1999). All birds were searched for gastrointestinal parasites utilizing conventional parasitological techniques. Prevalence, intensity of infection and abundance (mean number of parasites) were calculated for each parasite species. In addition, parasite richness was evaluated. Forty-one percent ($CI_{95\%}$: 23.4–59.3) of birds harboured parasites, belonging to seven parasite species. *Capillaria caudinflata* was the most common with a prevalence of 27.6% ($CI_{95\%}$: 11.3–43.8), whereas *Eimeria* spp. was found with a prevalence of 10.3% ($CI_{95\%}$: 0–21.4). For both parasites, the majority of the infected animals show low parasite load. A low prevalence (3.4% – $CI_{95\%}$: 0–10.1) was detected for *Capillaria anatis*, *Heterakis gallinarum*, *Raillietina* sp., *Dispharynx nasuta* and Trematodes. To our knowledge, this is the first report of *C. anatis* and *D. nasuta* in the hazel grouse, and in general one of the few article providing information on hazel grouse parasite community. Overall, the parasite richness was very low, with 75% of the infected birds carrying one parasite species, 17% two species and only one bird (8%) with three species. This study increases the scarce literature available on the hazel grouse parasites. The epidemiological descriptors reported are particularly useful to compare the parasite-fauna of the hazel grouse across locations and time periods.

Keywords: Alps, Galliformes, Hazel grouse, parasite community, parasite richness

The hazel grouse *Tetrastes bonasia* is a forest-specialist bird, inhabiting coniferous and mixed forests of Eurasia (Bergmann et al. 1996). This Galliformes found optimal conditions in dense forests, in both plain and mountain areas, with varied undergrowth and clearings (Madge et al. 2002). The species is highly sedentary. Its diet is vegetarian and varies significantly according to the season, while chicks feed almost exclusively on arthropods during the first two weeks of life (Birdlife International 2016). It is also the smallest and less known European Galliformes. Figure 1 shows the global geographic range of the species. Hazel grouse is classified as least concern (LC) by the IUCN red list (Birdlife International 2016), meaning that at global level the conservation status of the species is considered satisfactory. However, on the Alps the situation is different. Nowadays, this Galliformes is threated by forestry practices, causing a significant interference with the species ecology and a loss and fragmentation of its habitat. For these reasons, it has been listed in Annex I of the Directive 2009/147/EC of the European Parliament (European Parliament and European Council 2009), which aims to preserve species and their habitats. While the species has been deeply studied as indicator of the richness and biodiversity in the mountain forests (Montadert 2005), data on its sanitary status is rare in France and at global level. Indeed, the only information available for the French population comes from birds monitored under the national surveillance programme on wildlife diseases and poisoning (ONCFS, Accessed on 19/03/2020).

Parasites have been proved to regulate wildlife population dynamics (Iacopelli et al. 2020). In particular, several authors have described this phenomenon in wild grouse populations (Dobson and Hudson 1992, Isomursu et al. 2017). Considering that, it is of pivotal importance to characterise infectious agents circulating in wildlife, by improving wildlife sanitary surveillance and consequently the conservation actions. Surveillance of parasite is a useful tool to investigate

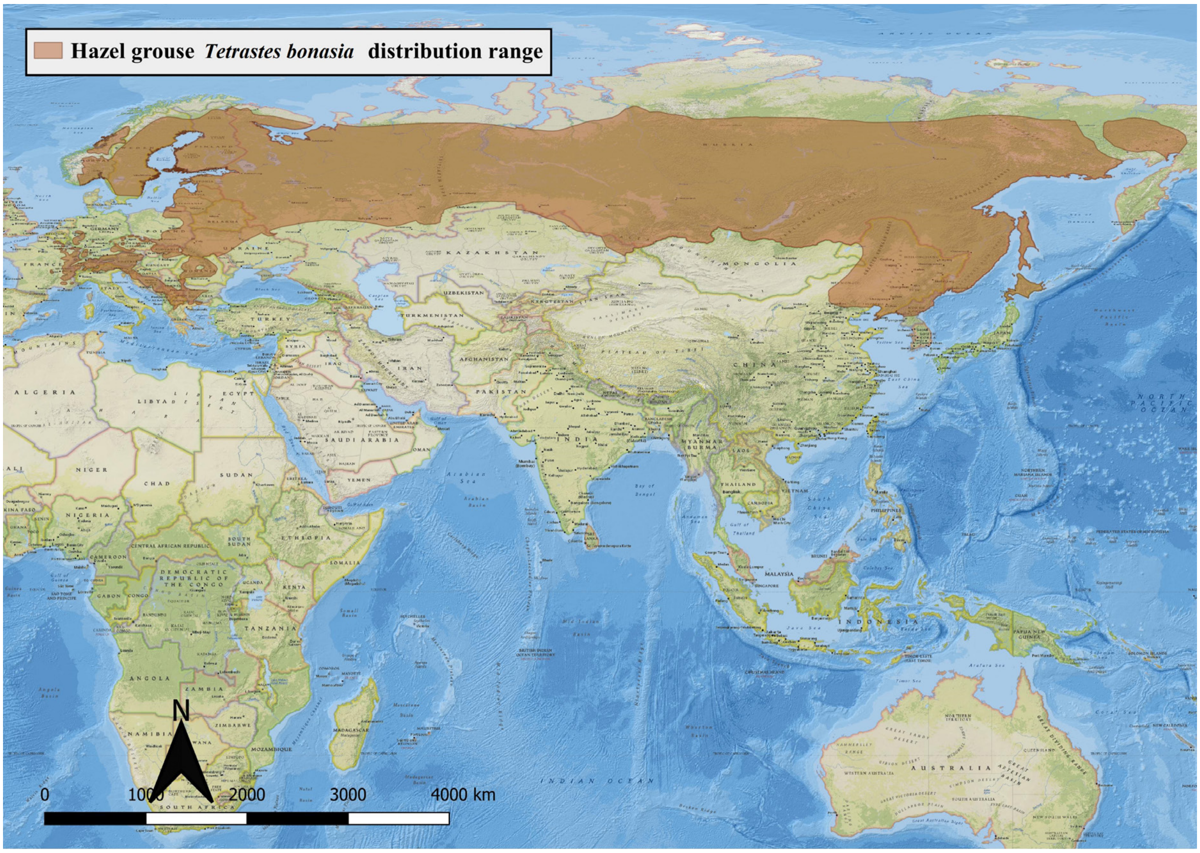

Figure 1. Hazel grouse distribution range (data retrieved from: BirdLife International 2016).

parasite dynamics at multi-host population level. Unfortunately, monitoring of parasitic disease in wildlife is sometimes a neglected field, and parasite presence and occurrence can be often undetected. This lack of surveillance has been well documented by some researchers in the last years. An example of this situation is well represented by the detection of alien parasites in some countries more than 40 years after their probable introduction (Gontero et al. 2020). Few projects focused on health issues of some species of wild Galliformes. In particular few information is available for the ones living at high altitude in mountain ranges, and with low population density, like the hazel grouse, the Pyrenean rock ptarmigan and others.

Recently, the literature available on parasites of the Alpine Galliformes has increased for the Italian and French Alpine populations (Fanelli et al. 2020a, b, c, Tizzani et al. 2020), however, little is still known and published on the hazel grouse in the Alps.

In general, scarce and obsolete literature is available on the parasitic infection of this wild species worldwide, and most of it is of difficult consultation as either unpublished (grey literature) or published in a language other than English (Belopolskaya 1963, Couturier 1964, Gromova 1972, Andreev 1983, Isomursu et al. 2006, 2017, Ito et al. 2012, Isomursu 2014, Bednarski et al. 2018).

For these reasons, we carried out a long-term epidemiological survey to describe the Alpine parasite community, and assess the prevalence, intensity and abundance of the gastrointestinal parasites harboured by the hazel grouse in the French Alps.

Material and methods

Study area and sample

Twenty-nine hazel grouse were collected in the French Alps during the period 1987–2019 (no sample collected during 1997–1999), from 21 municipalities. Some of the bird were killed during hunting activity (in September–November – n = 12), and others (n = 17) found dead for other reasons (impact with cables, predation or diseases). The cause of death (different from hunting activity) was determined by necropsy, through the evaluation of anatomopathological lesions.

It was not possible to collect sex and age information for all the hosts (for some animals only the gastrointestinal tract was available), however, this information was collected for 17 birds. Based on this data, the sample was equally represented in term of sex classes (47% females/53% males), while most of the animals sampled were adults (75%).

Figure 2 shows the municipalities from which originated the samples included in the study. Animals were sampled through the French Alps, from eight different massifs and from the Jura. Sampling locations were characterized by quite variable climatic conditions, in particular regarding

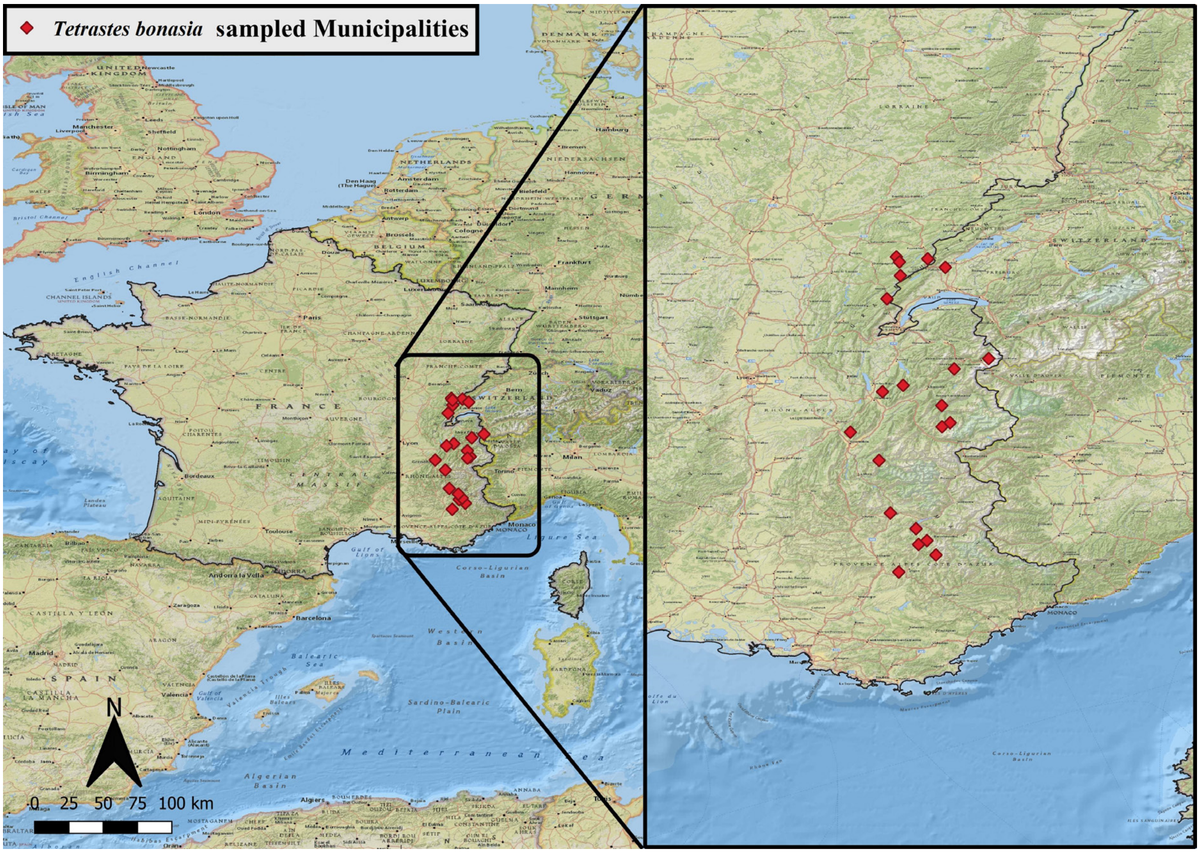

Figure 2. French municipalities sampled from 1987 to 2019 (1997–1999 excluded).

humidity. In fact, the northern part of the Alpine range receives higher precipitations through the year than the rest of the Alps (Durand et al. 2009).

Parasitological analysis

For each animal, we opened the gastrointestinal tract with a longitudinal incision, and the content of the individual sections (proventriculum, gizzard, small and large intestine) was analyzed following the common parasitological standard techniques. The complete content of each gastrointestinal tract was washed and thinned in water, and the sediments analyzed in a petri dish to detected adult parasites. Adult worms were counted under a stereoscope (MAFF 1986). We identified parasites with a light microscope and using the identification key suggested by Euzeby (1981, 1982). It was not possible to identify the *Trematoda* worm, due to the reduced preservation status of the samples. To evaluate *Eimeria* spp infection, faecal samples collected from the rectum were examined using saturated sodium chloride flotation and formol ether sedimentation techniques. Oocysts were identified using a light microscope at ×40 (MAFF 1986). Since samples were frozen before the analysis, we could not evaluate the sporulate form of *Eimeria* spp, which is needed for the identification at species level.

Epidemiological descriptors

Epidemiological characteristics including prevalence (percentage of infected host individuals in each sample), intensity of infection (mean number of parasites per infected host) and abundance (mean number of parasites per host) were calculated for each parasite species. The number of parasite species within the host population (parasite richness) was quantified as well. For *Eimeria* spp., the epidemiological indexes of intensity and abundance refer to the number of oocystis per gram of feces (OPG). The analysis was carried out with the software R ver. 3.5.2 (<www.r-project.org>).

Results

Seven parasite species were identified during the survey (at either species, genus or class level). A total of 12 birds were parasitized (prevalence [P]: 41%, $CI_{95\%}$: 23–59). *Capillaria caudinflata* was recorded with the highest prevalence (27.6% – confidence interval $[CI]_{95\%}$: 11.3–43.8), followed by *Eimeria* spp. (10.3% – $CI_{95\%}$: 0–21.4). *Capillaria anatis*, *Heterakis gallinarum*, *Raillietina* sp., *Dispharynx nasuta* and *Trematoda* were found with a prevalence of 3.4% ($CI_{95\%}$: 0–10.1). Details on the prevalence, mean abundance and mean intensity values for each parasite are reported in Table 1.

Considering the most prevalent parasites, *C. caudinflata* and *Eimeria* spp., the majority of the infected animals show no or low density of parasite, even if the low sample size does not allow any further consideration on the distribution of parasite density at population level. The maximum intensity of infectionwas 20 parasites for *C. caudinflata* and 100 OPG for *Eimeria* spp. As regards the remaining parasite species, we

Table 1. Prevalence (percentage of infected hosts/examined hosts), abundance (number of parasites/examined hosts) and intensity (number of parasites/infected hosts) of gastrointestinal parasites found in the hazel grouses *Tetrastes bonasia* in the French Alps (n=29). For *Eimeria* spp., the epidemiological indexes of intensity and abundance refer to the number of oocystis per gram of feces (OPG).

Total (n=29)					
Parasite	Number of positive animals	Prevalence % ($CI_{95\%}$)	Total parasites	Mean abundance (SD)	Mean intensity (SD)
Capillaria anatis	1	3.4 (0–10.1)	30	1.03 (5.57)	30.00 (na)
Capillaria caudinflata	8	27.6 (11.3–43.8)	59	2.03 (4.56)	7.37 (6.18)
Dispharynx nasuta	1	3.4 (0–10.1)	6	0.20 (1.11)	6.00 (na)
Heterakis gallinarum	1	3.4 (0–10.1)	50	1.72 (9.28)	50.00 (na)
Raillietina sp.	1	3.4 (0–10.1)	12	0.41 (2.22)	12.00 (na)
Trematodes	1	3.4 (0–10.1)	1	0.034 (0.18)	1.00 (na)
Eimeria spp.	3	10.3 (0–21.4)	250	8.62 (26.95)	83.33 (28.86)

found: 1) one bird harbouring 30 *C. anatis* and 50 *H. gallinarum* worms; 2) one bird with 6 *D. nasuta*, and 12 *Raillietina* sp.; 3) one bird with 1 *Trematoda*.

Considering the parasite richness, 75% of the infected birds harboured one parasite species, 17% harboured two species and one bird (8%) three species. No geographic cluster in parasite distribution or richness were highlighted in the study area, but the limited sample size did not allow any further considerations.

Discussion

This study was conducted to fill a gap of knowledge on the sanitary status of the hazel grouse and to determine the composition, prevalence and intensity of its gastrointestinal parasites community in the Alpine environment. Information regarding the parasite community, as well as prevalence and diversity of pathogens in this wild bird is very limited. The hazel grouse is present in 25 countries, however only in few of them some studies have been carried out (Belopolskaya 1963, Gromova 1972, Andreev 1983, Isomursu et al. 2006, 2017, Ito et al. 2012, Isomursu 2014, Bednarski et al. 2018). Moreover, most of these studies have been conducted in hazel grouse population from northern or eastern Europe and Russia. This fact highlights even more the necessity to collect sanitary data from the Alpine region.

Overall, our results show a high prevalence of gastrointestinal parasites (41% of the birds), being the most common *C. caudinflata* and *Eimeria* spp. No influence of the source of animals (hunted versus other reason of death), sex or age classes of the animals were detected, but the low sample size did not allow any accurate evaluation of the influence of these risk factors. The prevalence of *C. caudinflata* is similar to the one of *Capillaria* sp. (30.77%) found in the hazel grouse in Poland (Bednarski et al. 2018). The high prevalence detected for *C. caudinflata* in our study is quite surprising, due to the low population density and the grouse's arboreal lifestyle. Both these factors should be unfavorable to the completion of the Capillaria cycle.

The parasite frequency distribution highlighted in *C. caudinflata* and *Eimeria* spp., with few hosts infected by a high number of parasites, is a common finding in parasitological surveys. These type of frequency distribution is common not only for gastrointestinal parasites but also in parasite external infestations (Alexander 2012, Sanchis-Monsonís et al. 2019). Additionally, the finding of three carcasses severely infected by *C. caudinflata* suggests a potential impact on the population dynamics.

Ascaridia compar, a frequent parasite in the hazel grouse in Finland (Isomursu et al. 2006, 2017, Isomursu 2014), was not found in this study. However, it is worth to mention that this parasite is the most prevalent helminth in the black grouse *Tetrao tetrix tetrix* and rock partridge *Alectoris graeca saxatilis* in the Alps, as recently demonstrated by Fanelli et al. (2020a). The same authors identified *C. caudinflata* with a prevalence of 10% and 1.2%, respectively in black grouse and rock partridge (Fanelli et al. 2020a). Another study identified *C. caudinflata* as the most common parasite in the Alpine rock ptarmigan *Lagopus muta helvetica* whereas *A. compar* was detected only in few animals (Fanelli et al. 2020b). These studies highlighted that both *A. compar* and *C. caudinflata* are widely present in the wild Galliformes in the Alpine region. The different prevalence among wild Galliformes species suggest local species-specific host–parasite dynamics. However, this hypothesis should be confirmed with further studies involving a comparable number of animals for each species,and investigating a larger sample area. Parasitic infection seems to have been the primary cause of death for six birds, found with congestive lesions of the internal organs. In particular, three of these animals were highly infected by *C. caudinflata*.

As regards *Eimeria* spp., we found a lower prevalence (10%) compared to the rock ptarmigan (38.9%) in the Alpine region (Fanelli et al. 2020b), however, as previously mentioned, the limited number of samples does not allow further comparisons.

Overall, we detected a low parasite loads and richness, with mono-infections in most birds. This finding is in line with the study of Bednarski et al. (2018) who identified a scarce parasite richness and few parasite species in the hazel grouse in Poland.

Several species found in this study have never been recorded in the hazel grouse. Indeed, while *Raillietina* sp. (Isomursu et al. 2006) and *H. gallinarum* (Ito et al. 2012) have already been described by other authors, to our knowledge this is the first report of *C. anatis* and *D. nasuta* in this host species.

The finding of *D. nasuta* is particularly important as it has been proved to produce severe lesions, even in the case of light infections (Davidson et al. 1977). This parasite is responsible of cyclical fluctuations in the ruffed grouse *Bonasa umbellus* in the United States (Davidson et al. 1977). Future evaluations on the prevalence and distribution of this parasite should be made in the Alpine region, in order to understand its impact on the hazel grouse population. The other parasite species detected, raise no specific conservation issues, at the light of the current knowledge and low prevalence reported.

Data from this study provides an important contribution to the literature on the parasite community of the hazel grouse, representing the first information published for the Alpine population, after the pioneering work carried out by Couturier in the sixties, that described Eimeria and cestodes as main parasites affecting the species (Couturier 1964). Baseline parasitological data presented herein will be useful for comparative purposes in future studies. Further long-term surveys, involving a larger number of samples, should be carried out to explore the sanitary status of this species, and to evaluate the potential presence of risk factors that could determine geographic cluster of parasites in the Alpine region. This information would be of pivotal importance to apply proper conservation plans and management measures. This kind of sanitary surveillance is of particular interest, considering the low density of hazel grouse population and the recent Finnish work (Isomursu 2014) that suggests an unfavorable effect of inbreeding on grouse resistance to digestive parasites, particularly in populations that have suffered drops in numbers (and consequent low heterozygosity). Sanitary surveillance, and genetic analyses carried out in parallel would be particularly interesting for a conservative management of this species.

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