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Gastroenteric parasite of wild Galliformes in the Italian Alps: implication for conservation management

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1	Gastroenteric parasite of wild Galliformes in the Italian Alps: implication for conservation
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24 Summary page

Abstract 25

This study provides insights about the diversity, prevalence and distribution of alpine wild 26 galliforms gastrointestinal parasite community, trying to fill a gap in the scientific information 27 currently available in scientific literature. The analysis included 3 host species: 77 rock partridge 28 (Alectoris graeca saxatilis), 83 black grouse (Tetrao tetrix tetrix) and 26 rock ptarmigan (Lagopus 29 mutus helveticus) shot during the hunting seasons 2008–2015. Parasites isolated were Ascaridia 30 compar, Capillaria caudinflata and cestodes. The rock ptarmigan was free from gastrointestinal 31 parasites, whereas the most prevalent helminth (37%) was A. compar in both black grouse and rock 32 partridge. *C.caudinflata* occurrence was significantly higher in black grouse (prevalence=10%, 33 mean abundance=0.6 parasites/sampled animal) than in rock partridge (prevalence=1.20%, mean 34 abundance=0.01 parasites/sampled animal). Significant differences were detected among hunting 35 districts. A. compar was found with significant higher degree of infestation in the hunting districts in 36 the northern part of the study area whereas cestodes abundance was higher in Lanzo Valley. 37 Quantitative analysis of risk factors was carried out using a generalized linear model (GLM) only 38 on the most common parasite (A.compar). Latitude was the only factors associated with infestation 39 risk (OR= 52.4). This study provides information on the composition and variability of the parasite 40 community in the alpine Galliformes species.

41

43 Keywords: Galliformes, parasites, Alps, Ascaridia, conservation

45 Key findings

- Three different parasites were detected in Alpine Galliformes: *Ascaridia compar, Capillaria caudinflata* and cestodes.
- 48 *A. compar* was the most prevalent parasite
- No parasites were detected in the rock ptarmigan
- A significant difference in infestation risk was found among the different hunting districts

52 **1. Introduction**

The Alps are recognized as one of the major hotspots of biodiversity in Europe. Specifically, they 53 54 host different species belonging to Galliformes order, and among them the rock partridge (Alectoris 55 graeca saxatilis), the black grouse (Tetrao tetrix tetrix) and the rock ptarmigan (Lagopus mutus 56 helveticus). These three species are listed in the Directive 2009/147/EC of the European Parliament for the conservation of wild birds in Europe (European Parliament and European Council, 2009). 57 Moreover, according to the National Red Data Book of Italy, both rock partridge and rock 58 ptarmigan are classified as "vulnerable", whereas the black grouse as "least concern" (Rondinini et 59 al., 2013). More in general, the wild Galliformes population in some areas of the Alps has globally 60 reduced in the last years (Giordano, 2017). Considering the critical status of conservation of some 61 62 species, a correct management of these population is of pivotal importance. Several factors are negatively affecting wild Galliformes population density, including habitat loss and degradation 63 (Pearce-Higgins et al. 2007; Ludwig et al. 2009; Signorell et al. 2010). Some studies have also 64 highlighted the harmful effects of climate changes on alpine environments (Anfodillo 2007; Tinner 65 66 and Vescovi 2007), and some typical alpine fauna, such as the rock ptarmigan, living between 1800 and 3000 meters above sea level (a.s.l) with fresh north-facing slopes (Lasagna, 2009), has been 67 proved to be particular sensitive to climate changes (Watson and Moss, 2004). 68

69 In a context of wildlife conservation, there is a great interest to assess the role of pathogens in 70 influencing population dynamics (Smith et al., 2006; Delogu et al., 2013). This is partially due to 71 the fact that the number of pathogens to which wildlife is exposed has recently increased, for 72 several reasons. In particular for wild Galliformes there is an increased risk of disease transmission as a consequence of the restocking of games species (Gortazar et al., 2014). Every year the red 73 partridge, the ring neck pheasant and other wild species are introduced for hunting purpose in the 74 Italian Alps (Regione Piemonte, 2018). These game animals, not always raised with adequate 75 hygienic and sanitary conditions, can potentially shed pathogens in the environment, which might 76 be hazardous for wildlife population. Based on scientific literature in fact, different factors make 77 captive-born animals particularly susceptible to infectious diseases (Lafferty and Gerber, 2002). For 78 79 this reason, they can potentially have heavy parasite loads that can be transmitted to wild 80 populations (Power and Mitchell, 2004).

Taking into account that pathogens might have a negative impact on welfare and population
dynamics (Hudson 1986; Holmstad *et al.* 2005; Citterio *et al.* 2006), the studies on the parasite

83 fauna hosted by endangered species can provide a better understanding of such interactions

84 (Formenti *et al.*, 2013). However, the health status of wild Galliformes populations is poorly

85 known. This is in partly due to the complexity of sampling animals in the alpine environments, to

the low density of targeted species and to the few ongoing projects focusing on health issues of

87 Galliformes.

With the objective of improving the knowledge on wild Galliformes populations pathogens, we
evaluated the helminth community parasitizing the rock partridge, the black grouse and the rock
ptarmigan in the Italian Alps.

91 **2.** Material and Methods

92 <u>2.1 Sample collection</u>

During the hunting seasons (October-November) 2008 to 2015, 77 rock patridge, 83 black grouse 93 94 and 26 rock ptarmigans were collected in 6 different hunting grounds in the Italian Alps. 95 Specifically, the following districts were investigated: Comprensorio alpino Sondrio (C.A. 96 Sondrio), Aosta valley, Comprensorio Alpino Biella valley (C.A. BI1), Comprensorio Alpino 97 Lanzo valleys (C.A. TO4), Comprensorio Alpino Varaita valley (C.A. CN2), Comprensorio Alpino 98 Maira valley (C.A. CN3). This work has been carried out in accordance with the hunting activity laid down by regional laws (Regione Piemonte, 2018). Figure 1 shows the provinces sampled 99 100 during the study. The sample composition, divided by species and district, is presented in table 1. 101 [Figure 1] 102 [Table 1] 103 104 2.2 Parasitological analysis

Gastrointestinal tracts were examined and processed. Parasite collection was done with the support
of a stereomicroscope. The identification was carried out consulting the available identification
keys (Skrjabin, 1954; Kalil *et al.*, 1994). Nematodes were stored in 70% ethanol while cestodes
were fixed in AFA solution (acetic acid 3%, formaldehyde 15%, alcohol 50 degrees 82%). Cestodes
identification at genera and species levels was not possible due to the non-optimal conservation of
the samples.

111

112 <u>2.3 Statistical analysis</u>

113 Prevalence (positive/total animals), abundance (number parasites / total animals) intensity (number

parasites / positive animals) were computed for each parasite in each host species and hunting

district. The "number of parasites" refers to the number of individuals found for each parasite taxa.

116 Prevalence data were compared using the Fisher's exact test, frequency distribution of parasites

intensity and abundance using the Kruskal-Wallis test (Rozsa *et al.*, 2000). Considering the
unbalanced sample size for some areas, Aosta valley and Sondrio samples were excluded from the
statistical comparison, while Varaita and Maira valleys were aggregated in a unique sample area, as
for geographic location they can be consider as a part of a common population sharing the same risk
factors. In case of significant difference between groups, pairwise comparisons with correction for
multiple testing were computed between groups (Benjamini and Hochberg, 1995). Rock ptarmigan
samples were excluded from the statistical analysis as no parasite was detected in this species.

Nematode distribution can be highly influenced by environmental variable (Sanchis-Monsonís et al, 124 2019), considering that they can influence the survival of infective stages in the environment. With 125 126 the purpose to better understand the geographic distribution of nematodes, the influence of 127 environmental factors on parasite presence was evaluated through a generalized linear model (GLM). Taking into account that a low prevalence value might lead to a loss in accuracy and 128 129 precision of risk estimates (Doerken et al., 2019), the GLM model was applied on A. compar only, because it was the only parasite with a adequate number of presence data (72 geo-referenced 130 131 records). Spatial analysis was carried out with QGIS software version 3.6 (QGIS Development Team, 2017). Environmental covariates were computed considering a buffer area around the 132 sampled location, using species specific home ranges according to the available literature: 686 133 meters radius for the black grouse (Storch, 1994) and 829 meters radius for the rock partridge 134 (Asters, 2012). Covariates were log transformed and tested for multicollinearity through VIF 135 approach (Heiberger, 2018). The ones whose VIF was greater than 5 were excluded from the 136 analysis. The most parsimonious models were selected computing the Akaike Information Criterion 137 (AIC) (Akaike, 1973). Model performance was assessed by computing the area under the curve 138 value (AUC). All statistical analyses were performed using R (R Core Team, 2018). GLM was built 139 140 using glmulti package (Calcagno, 2019). Table 2 shows the environmental variables used, a brief description and the original source. 141

142

[Table 2]

143 **3. Results**

144 *3.1 <u>Parasitological analysis</u>*

Seventy-five out of 186 animals hosted gastrointestinal helminths (prevalence: 40.3%). Parasites
were found in 42 black grouse out of 83 (prevalence: 50.6%) and 33 rock partridges out of 77
(prevalence: 42.8%) whereas no worm was detected in the rock ptarmigan. Three different parasites
were identified; *Ascaridia compar, Capillaria caudinflata* and cestodes. Parasites were mostly

found in the small intestine (40%) and cecum (13.7%), while the gizzard was parasitized in only
three cases (1.9%)

151 *3.2 <u>Analysis by host species</u>*

A. *compar* was recorded with the highest prevalence in both species (37%), while *C. caudinflata*was more prevalent in the black grouse (10%) than in the rock partridge (1.2%). Finally, cestodes
prevalence was similar for both species (7% in black grouse and 5% in rock partridge). Prevalence,
abundance and intensity for each parasite and in each host species are presented in table 3.

156

[Table 3]

Fisher test shows that the proportion of birds infested by *C. caudinflata* was significantly different
between host species (p value=0.017). No significant difference was found for the remaining
parasites groups. In accordance with the Kruskal-Wallis test, the frequency distributions of *C. caudinflata* abundance indicates that also the level of infection is significantly different between the
two host species (p value=0.02).

162

163 No difference was detected for the abundance of the other parasite and in general no difference was164 highlighted for parasite intensity.

165 *3.3 <u>Analysis by hunting district</u>*

A clear spatial agregation of the parasites has been found. *A. compar* was found mainly in Biella (59%) and Lanzo valleys (54%). Biella valley shows also the highest prevalence of *C. caudinflata* infestation (12%). Cestodes were the only parasite not showing a specific geographic distribution of prevalence. Table 4 shows the prevalence, abundance and intensity values for each parasite in each hunting district. Considering the small sample size in Aosta and Sondrio district, the differences detected in these two hunting districts have not be considered for statistical comparison but just for descriptive purposes, as the different sampling intensity could reduce the robustness of the analysis.

173

[Table 4]

Significant differences for prevalence among the areas were found only for *A. compar* Biella and Lanzo Valleys prevalence was in fact significantly higher than the hunting district of Maira and Varaita valleys together. Prevalence in Aosta valley was also significantly higher than in Sondrio and in Varaita valley. Detailed information on areas comparison, and statistical differences for the relevant hunting districts are provided in the supplementary material.

- The Kruskal-Wallis test showed significant differences for *A. compar* abundance; Biella valley and
 Lanzo valley were significantly higher than Maira and Varaita valleys together.
- 181 Finally, cestodes abundance was significantly higher in Lanzo valleys compared to Biella valley.

182 *3.2.3. Generalized linear model for A. compar infestation*

The reduced GLM model for *A. compar* (AIC=53.7) included latitude and aspect (western exposition)as independent variables. The only significant factor retained in the model was the latitude (p value=0.002) The estimated odds ratio of latitude (OR=52.4) highlights that there is a elear risk of parasite infestation in animals living at higher latitude in our study areas. The model fitted the data very well (AUC=0.91), and the amount of deviance accounted was 0.42.

188 4. Discussion

This paper provides important information on the parasite community of wild Galliformes in the 189 Italian Alps. Despite the objective difficulties in sampling wild animals in the alpine environment, 190 this study was able to include a sample of 186 wild Galliformes, collected in six different hunting 191 192 areas from 2009 to 2015. Apart from the relevant number of animals that have been sampled, another important aspect of the paper is the description of the parasite community in 3 different 193 species with different biology, ecological needs, conservation status and population densities. Only 194 three helminths were detected in the black grouse and rock partridge, whereas no gastrointestinal 195 parasite was found in the rock ptarmigan. The epidemiological indexes of prevalence, intensity and 196 distribution highlighted differences among infected species and sampled areas. In particular, the 197 most infected hunting districts were localized in the northern part of the study areas, and C. 198 caudinflata showed a specific host predilection for the black grouse. The presence of a spatial 199 aggregation of some parasite species, has been also confirmed by the results of the GLM model. 200

The low parasite richness recorded in this work is in accordance with previous studies carried out in 201 Eastern Italian Alps (Viganò et al., 2012a,b; Formenti et al., 2013). It is particularly worthy to 202 highlight the completely absence of parasite in the rock partridge, living at high altitude, in areas 203 with more extreme climatic conditions that probably do not allow the development of parasite 204 cycles. On the contrary, some parasites extensively detected in previous studies like Heterakis 205 206 gallinarum (in the rock patridge) (Florio and Gamba 1993; Milani 2010; Viganò et al., 2012b; 207 Viganò and Giacomelli, 2014), were not detected in our survey. The absence of this parasite might 208 be due to either a reduction of the host population density, which is no longer able to maintain the parasite cycle, or to a host-parasite equilibrium reached in the ecosystem. In a context of low host 209

density, the parasite fitness can in fact declines with a lower ability to infest next generations and apotential local extinction in a long-term perspective.

212 Compared with similar surveys carried out in northern European countries, the parasite richness

found in our study is significantly lower. Several parasite species found in northern Europe have

never been recorded on the Alpine Galliformes. These include *Heterakis bonasae* (Kalla *et al.*,

215 1997), Trycostrongilus tenuis (Holmstad et al., 2005), Syngamus trachea (Wissler and Halvorsen,

216 1977). A possible explanation to these findings may be provided by the work of Altzier *et al.*

217 (2007). These authors in fact have demonstrated how an isolated and low-density host population

218 might have fewer parasites. Hence Alpine Galliformes may harbour fewer parasite species, as a

consequence of restricted and isolated geographical ranges. This is particularly true for species like

the rock ptarmigan that is a glacial relict, whose population remained isolated at the end of the lastglaciation.

Regarding the prevalence of the parasite in the other two host species, *A. compar* was detected in

the black grouse at lower prevalence (37%) in comparison with other studies (62.3-82.4%) (Viganò

et al., 2012a), as well as *C. caudinflata* (10% vs 48.4-79.3%) (Viganò *et al.*, 2012a). On the

contrary, in rock patridge, *A. compar* prevalence (37%) was three times higher than the one found

in Viganò *et al.* (2012b), while *C. caudinflata* prevalence was lower (1.2% vs 19.2%) (Viganò *et al.*, 2012b).

Despite there were no statistically significant differences in prevalence among the areas, *C. caudinflata* was found mainly in Biella valley, providing evidences of a heterogeneous sanitary
 status of the different alpine populations.

Additionally, *Capillaria* shows a clear host preference: 9 over 83 black grouse were found

parasitized versus 1 over 77 rock partridge. This difference might be due to the fact that the parasite

host-specificity increases with higher host densities (Forbes *et al.*, 2017). In the Italian Alps in fact,

the black grouse is the wild Galliformes with the highest population density (Giordano et al. 2017).

235 Moreover, considering that *Capillaria* have an indirect cycle, with earthworms as intermediate host,

the different risk of infestation can also be due to different feeding behavior and diet of the two host

species. The black grouse in fact feed more on earthworms while the rock partridge on insects. Yet

238 data on *C. caudinflata* infestation in wild Galliformes are relatively limited, this worm has been

recorded in a wide range of bird species causing severe enteritis and anemia leading to poor general

conditions and weight loss even in case of mild infestation (Villanúa *et al.*, 2007, Pinto *et al.*, 2008,

241 McCain, 2015). The impact of this parasite on black grouse population dynamics should be then

242 further evaluated.

Host specificity has not been recorded in the case of *Ascaridia* or Cestodes which have been foundto equally infest black grouse and rock partridge.

In addition to density and diet preferences, other specific variables related to behaviour, phenology,
and nest structure can influence the predisposition to parasitism. Indeed, the quality of being a
competent host for parasites depends on host-specific variation in parasite reproductive success
(Stokke *et al.*, 2018). However, more studies about parasites of Galliformes on the Alps are needed
to better evaluate the factors explaining this variation in host selection.

As regards *A. compar* infestation, the prevalence, abundance and intensity values found in the hunting districts in the northern part of the study area (Lanzo and Biella Valleys) were significantly higher than in the southern part. This different degree of parasitism is probably due to different climatic conditions influencing the development and survival of both eggs, larvae and intermediate hosts of the parasites.

255 The spatial variation of parasitism degree might also reflect the sanitary management and sanitary 256 status of game birds released in the different hunting districts. Restocking with farmed game birds, above all for red-legged partridge and ring-necked pheasant, is a practice still quite common in 257 258 Italy. Specific articles of the Regional laws (Regione Piemonte, 2018) do not allow to release animals in the areas where alpine Galliformes lives; however, these interdictions are not always 259 260 fully respected. Game birds are kept in aviaries were proper hygienic conditions cannot be 261 maintained, thus infestation by parasites are likely (Stadler and Carpenter, 1996). Moreover, sanitary check before releasing the animals are always lacking. Once released, these animals can 262 shed their parasites in the environment which becomes a potential source of infestation for free-263 ranging wild birds. This might be an additional problem for wild Galliformes 264 conservation(Tompkins et al., 2015). A study carried out in Spain have clearly demonstrated that 265 266 areas where restocking is a common practice, the sanitary status of the wild Galliformes can significantly deteriorate (Villanúa et al., 2008). Under this perspective, the sanitary status of the 267 different areas could be considered an indirect index of the quality of the wildlife management 268 activity carried out in the different hunting districts. 269

270 In addition, various environmental risk factors might play an important role in parasite infestation.

271 The GLM model built for Ascaridia compar, highlighted that, among all the environmental factors

assessed, latitude was the only one with a clear influence on parasite distribution. Latitude in this

273 case can be seen as a proxy of the climatic and environmental conditions required for the

development of free-living stages of nematodes. These parameters in fact, can vary according to the

275 latitude. In particular according to climatic data from the Regional Agency for the protection of the

- environment (ARPA Piedmont <u>http://www.arpa.piemonte.it/</u>, accessed August 2019) the northern
- hunting districts are characterized by annual average precipitation values higher than the southern
- 278 ones, thus creating more favorable condition for parasites development (humidity and dense
- 279 vegetation cover). Latitude, as factor explaining parasite distribution and parasite richness has been
- found also in other works, modelling the spatial distribution of parasite, at local (Sanchis-Monsonís
- et al, 2019) and regional scale (Nunn et a., 2005).

282 **5.** Conclusion

- This work increases the limited knowledge on the sanitary status of Galliformes in the Alps. Our results show that the level of parasitic infestation in the black grouse and rock partridge varies across the study areas. This insight is of interest considering that the parasite load might affect the conservation of endangered population. On the other hand, our sample was not homogeneously distributed across the whole study area and an increase in the number of animals sampled from
- some hunting districts would help to clarify further the host and environmental requirements of the
- 289 parasites. Further epidemiological studies about parasite species and their impact on wild
- 290 Galliformes populations are needed to correctly plan conservation measures, considering that some
- 291 parasite can have a huge direct (mortality) or indirect (reduce population fitness) effect at
- 292 population level.

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434 Figure

435

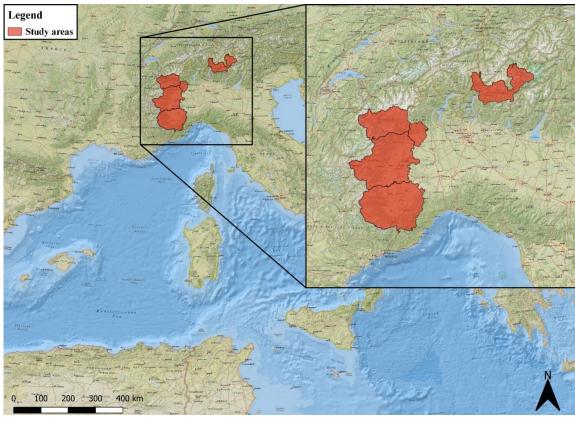




Figure I: Italian provinces where the study was undertaken

Species	Sondrio (C.A. Sondrio)	Aosta Valley	Biella Valley (C.A. BI1)	Lanzo Valleys (C.A .TO4)	Varaita and Maira Valley (C.A. CN2 and C.A. CN3)
Rock partridge	1	2	34	8	32
Black grouse	1	5	25	24	28
Rock ptarmigan	17	1	0	3	5

Table 1: Number of animals sampled, divided per species and hunting district

Variable	Description	Source
Altitude*	Average altitude	DEM from SRTM
North exposition	Area exposed to North (%)	http://srtm.csi.cgiar.or
South exposition*	Area exposed to South (%)	<u>g/</u>
East Exposition	Area exposed to East (%)	
West exposition	Area exposed to West (%)	
Artificial lands	Areas covered by Artificial lands (%)	Corine Land Cover
Permanent pastures	Areas covered by Permanent pastures	2006
	(%)	https://land.copernicus
Extensive agriculture	Areas covered by Extensive	<u>.eu/pan-</u>
	agriculture (%)	european/corine-land-
Shrubs and grass*	Areas covered by Shrubs and grass	cover
	(%)	
Sparse and absent	Areas covered by Sparse and absent	
vegetation	vegetation (%)	
Coniferous and	Areas covered by Coniferous and	
mixed forests	mixed forests (%)	
Deciduous forest	Areas covered by Deciduous forest(%)	
Latitude	Latitude of the sample location	NA

- 443 *variables with collinearity (VIF>5)
- 444 Table 2: Overall listing of variables taken into account in the study

Host	Parasite	Prevalence	Mean	Median	Mean	Median
species		(CI95%)	abundan	abundanc	intensit	intensity
			ce (sd)	e (IQR)	y (sd)	(IQR)
Black	Ascaridia	37%	3.27	0 (2)	8.74	3 (10.5)
grouse	compar	(0.27 0.47)	(7.95)		(11.1)	
	Capillaria	10%	0.61(3.6	0 (0)	5.67(10.	1(3)
	caudinflata	(0.04-0.16)	2)		1)	
	Cestodes	7%	0.08(0.3	0 (0)	1.17(0.4	1(0)
		(0.02-0.12)	2)		1)	
Rock	Ascaridia	37%	3.75	0 (2)	9.97	3(14)
patridge	compar	(0.26 -	(8.97)		(12.4)	
		0.48)				
	Capillaria	1.20%	0.01(0.1	0 (0)	1(NaN)	1(0)
	caudinflata	(-0.01-0.03)	1)			
	Cestodes	5%	0.13(0.6	0 (0)	2.5(1.73	2(1)
		(0 - 0.10)	6))	

Table 3:Prevalence, abundance and intensity for each helminth and host species

			Mean	Median	Mean	Median
District	Parasite	Prevalence	abundance(s	abundanc	intensity	intensit
		(CI95%)	d)	e (IQR)	(sd)	y (IQR)
	Ascaridia	0.38 (0.04-				
	compar	0.71)	3 (6.27)	0 (2)	7(8.72)	3 (8)
	Capillaria					
Aosta Valley	caundinflat	0	0(0)	0 (0)	-	-
	а					
		0.25 (-0.05-	0.42(0.00)	0 (0, 5)	1 5 (0 5 1)	1 7 (0 7)
	Cestodes	0.55)	0.43(0.80)	0(0.5)	1.5(0.71)	1.5(0.5)
	Ascaridia	0.54 (0.38-	4.28(10.3)	1 (2)	6.85(12.5	2(4.25)
	compar	0.70)	4.28(10.5)	1 (2))	2(4.23)
	Capillaria					
C.A. TO4	caundinflat	0	0(0)	0(0)	-	-
	а					
	Cestodes	0.11 (0.06-	0.16(0.45)	0(0.5)	1.25(0.5)	1(0.25)
		0.21)				
	Ascaridia	0.01 (-0.01-	0.01(0.12)	0(0)	1(NaN)	1(0)
	compar	0.04)				
C.A. CN2 and C.A.	Capillaria	0.05 (-	0.07(0.41)	0(0)	17(115)	1(1)
CN3	caundinflat	0.004-0.09)			1.7(1.15)	1(1)
	<i>a</i>	0.06 (0-			2 25(1 80	
	Cestodes	0.12)	0.14(0.68)	0(0)	2.25(1.89	2(2)
		0.12))	
	Ascaridia	0.59 (0.46-			11.1(11.5	
	compar	0.71)	6.8(10.5)	2 (10.5))	7.5(14)
	Capillaria	0.12 (0.04		0(0)		
C.A. BI1	caundinflat	0.12 (0.04-	0.80(4.27)		6.71(11.4	-
	а	0.20))	
	Cestodes	0	0(0)	0(0)	-	-

	Ascaridia	0	0(0)	0(0)	-	-
	compar	-	0(0)	0(0)		
C.A. SONDRIO	Capillaria					
C.A. SONDRIO	caundinflat	0	0(0)	0(0)	-	-
	а					
	Cestodes	0	0(0)	0(0)	-	-

448 Table 4:Prevalence, abundance and intensity for each helminth in each hunting district