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

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Running title: Parasite community of wild alpine Galliformes.

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Summary page

Abstract

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

Keywords: Galliformes, parasites, Alps, Ascaridia, conservation

Key findings

- Three different parasites were detected in Alpine Galliformes: *Ascaridia compar, Capillaria caudinflata* and cestodes.
- *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

1. Introduction

The Alps are recognized as one of the major hotspots of biodiversity in Europe. Specifically, they host different species belonging to Galliformes order, and among them the rock partridge (*Alectoris graeca saxatilis*), the black grouse (*Tetrao tetrix tetrix*) and the rock ptarmigan (*Lagopus mutus 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). Moreover, according to the National Red Data Book of Italy, both rock partridge and rock ptarmigan are classified as "vulnerable", whereas the black grouse as "least concern" (Rondinini *et al.*, 2013). More in general, the wild Galliformes population in some areas of the Alps has globally reduced in the last years (Giordano, 2017). Considering the critical status of conservation of some 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 (Pearce-Higgins *et al.* 2007; Ludwig *et al.* 2009; Signorell *et al.* 2010). Some studies have also highlighted the harmful effects of climate changes on alpine environments (Anfodillo 2007; Tinner 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 proved to be particular sensitive to climate changes (Watson and Moss, 2004).

In a context of wildlife conservation, there is a great interest to assess the role of pathogens in influencing population dynamics (Smith *et al.*, 2006; Delogu *et al.*, 2013). This is partially due to the fact that the number of pathogens to which wildlife is exposed has recently increased, for 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 partridge, the ring neck pheasant and other wild species are introduced for hunting purpose in the Italian Alps (Regione Piemonte, 2018). These game animals, not always raised with adequate hygienic and sanitary conditions, can potentially shed pathogens in the environment, which might be hazardous for wildlife population. Based on scientific literature in fact, different factors make captive-born animals particularly susceptible to infectious diseases (Lafferty and Gerber, 2002). For this reason, they can potentially have heavy parasite loads that can be transmitted to wild 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 fauna hosted by endangered species can provide a better understanding of such interactions (Formenti *et al.*, 2013). However, the health status of wild Galliformes populations is poorly 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 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.

2. Material and Methods

2.1 Sample collection

During the hunting seasons (October-November) 2008 to 2015, 77 rock patridge, 83 black grouse and 26 rock ptarmigans were collected in 6 different hunting grounds in the Italian Alps. Specifically, the following districts were investigated: Comprensorio alpino Sondrio (C.A. Sondrio), Aosta valley, Comprensorio Alpino Biella valley (C.A. BI1), Comprensorio Alpino Lanzo valleys (C.A. TO4), Comprensorio Alpino Varaita valley (C.A. CN2), Comprensorio Alpino 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 during the study. The sample composition, divided by species and district, is presented in table 1.

[Figure 1]

[Table 1]

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.

2.3 Statistical analysis

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. 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, 2019), considering that they can influence the survival of infective stages in the environment. With the purpose to better understand the geographic distribution of nematodes, the influence of 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 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 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 sampled location, using species specific home ranges according to the available literature: 686 meters radius for the black grouse (Storch, 1994) and 829 meters radius for the rock partridge (Asters, 2012).Covariates were log transformed and tested for multicollinearity through VIF approach (Heiberger, 2018). The ones whose VIF was greater than 5 were excluded from the analysis. The most parsimonious models were selected computing the Akaike Information Criterion (AIC) (Akaike, 1973). Model performance was assessed by computing the area under the curve value (AUC). All statistical analyses were performed using R (R Core Team, 2018). GLM was built using *glmulti package* (Calcagno, 2019). Table 2 shows the environmental variables used, a brief description and the original source.

[Table 2]

3. Results

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%)

3.2 Analysis by host species

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.

[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).

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

3.3 Analysis by hunting district

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.

[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.

Finally, cestodes abundance was significantly higher in Lanzo valleys compared to Biella valley.

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 ~~clear~~ 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.

4. Discussion

This paper provides important information on the parasite community of wild Galliformes in the Italian Alps. Despite the objective difficulties in sampling wild animals in the alpine environment, this study was able to include a sample of 186 wild Galliformes, collected in six different hunting 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 species with different biology, ecological needs, conservation status and population densities. Only three helminths were detected in the black grouse and rock partridge, whereas no gastrointestinal parasite was found in the rock ptarmigan. The epidemiological indexes of prevalence, intensity and distribution highlighted differences among infected species and sampled areas. In particular, the most infected hunting districts were localized in the northern part of the study areas, and *C. caudinflata* showed a specific host predilection for the black grouse. The presence of a spatial aggregation of some parasite species, has been also confirmed by the results of the GLM model.

The low parasite richness recorded in this work is in accordance with previous studies carried out in Eastern Italian Alps (Viganò *et al.*, 2012a,b; Formenti *et al.*, 2013). It is particularly worthy to highlight the completely absence of parasite in the rock partridge, living at high altitude, in areas with more extreme climatic conditions that probably do not allow the development of parasite cycles. On the contrary, some parasites extensively detected in previous studies like *Heterakis gallinarum* (in the rock patridge) (Florio and Gamba 1993; Milani 2010;Viganò *et al.*, 2012b; Viganò and Giacomelli, 2014), were not detected in our survey. The absence of this parasite might 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 density, the parasite fitness can in fact declines with a lower ability to infest next generations and a potential local extinction in a long-term perspective.

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.*, 1997), *Trycostrongilus tenuis* (Holmstad *et al.*, 2005), *Syngamus trachea* (Wissler and Halvorsen, 1977). A possible explanation to these findings may be provided by the work of Altzier *et al.* (2007). These authors in fact have demonstrated how an isolated and low-density host population 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 last glaciation.

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

Host specificity has not been recorded in the case of *Ascaridia* or Cestodes which have been found to 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.

The spatial variation of parasitism degree might also reflect the sanitary management and sanitary 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 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 fully respected. Game birds are kept in aviaries were proper hygienic conditions cannot be 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 shed their parasites in the environment which becomes a potential source of infestation for free-ranging wild birds. This might be an additional problem for wild Galliformes conservation(Tompkins *et al.*, 2015). A study carried out in Spain have clearly demonstrated that 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 different areas could be considered an indirect index of the quality of the wildlife management activity carried out in the different hunting districts.

In addition, various environmental risk factors might play an important role in parasite infestation. 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 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 latitude. In particular according to climatic data from the Regional Agency for the protection of the environment (ARPA Piedmont - http://www.arpa.piemonte.it/, accessed August 2019) the northern hunting districts are characterized by annual average precipitation values higher than the southern ones, thus creating more favorable condition for parasites development (humidity and dense 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).

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 parasites. Further epidemiological studies about parasite species and their impact on wild Galliformes populations are needed to correctly plan conservation measures, considering that some parasite can have a huge direct (mortality) or indirect (reduce population fitness) effect at population level.

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Figure

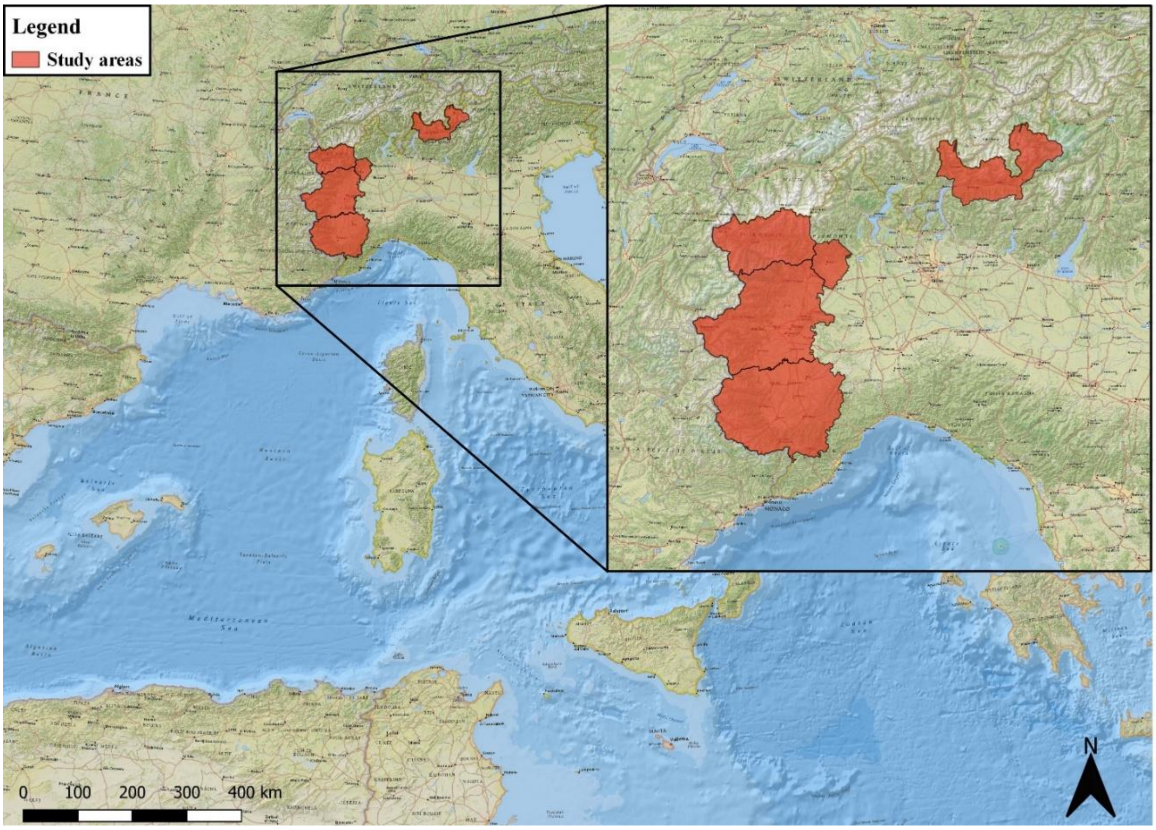

Figure I: Italian provinces where the study was undertaken

Tables

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 http://srtm.csi.cgiar.org/
North exposition	Area exposed to North (%)	
South exposition*	Area exposed to South (%)	
East Exposition	Area exposed to East (%)	
West exposition	Area exposed to West (%)	
Artificial lands	Areas covered by Artificial lands (%)	Corine Land Cover 2006 https://land.copernicus.eu/pan-european/corine-land-cover
Permanent pastures	Areas covered by Permanent pastures (%)	
Extensive agriculture	Areas covered by Extensive agriculture (%)	
Shrubs and grass*	Areas covered by Shrubs and grass (%)	
Sparse and absent vegetation	Areas covered by Sparse and absent vegetation (%)	
Coniferous and mixed forests	Areas covered by Coniferous and mixed forests (%)	
Deciduous forest	Areas covered by Deciduous forest(%)	
Latitude	Latitude of the sample location	NA

*variables with collinearity (VIF>5)

Table 2: Overall listing of variables taken into account in the study

Host species	Parasite	Prevalence ($CI_{95\%}$)	Mean abundance (sd)	Median abundance (IQR)	Mean intensity (sd)	Median intensity (IQR)
Black grouse	*Ascaridia compar*	37% (0.27 0.47)	3.27 (7.95)	0 (2)	8.74 (11.1)	3 (10.5)
	Capillaria caudinflata	10% (0.04-0.16)	0.61(3.62)	0 (0)	5.67(10.1)	1(3)
	Cestodes	7% (0.02-0.12)	0.08(0.32)	0 (0)	1.17(0.41)	1(0)
Rock patridge	*Ascaridia compar*	37% (0.26 - 0.48)	3.75 (8.97)	0 (2)	9.97 (12.4)	3(14)
	Capillaria caudinflata	1.20% (-0.01-0.03)	0.01(0.11)	0 (0)	1(NaN)	1(0)
	Cestodes	5% (0 - 0.10)	0.13(0.66)	0 (0)	2.5(1.73)	2(1)

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

District	Parasite	Prevalence (CI95%)	Mean abundance(sd)	Median abundance (IQR)	Mean intensity (sd)	Median intensity (IQR)
Aosta Valley	*Ascaridia compar*	0.38 (0.04-0.71)	3 (6.27)	0 (2)	7(8.72)	3 (8)
	Capillaria caundinflata	0	0(0)	0 (0)	-	-
	Cestodes	0.25 (-0.05-0.55)	0.43(0.80)	0(0.5)	1.5(0.71)	1.5(0.5)
C.A. TO4	*Ascaridia compar*	0.54 (0.38-0.70)	4.28(10.3)	1 (2)	6.85(12.5)	2(4.25)
	Capillaria caundinflata	0	0(0)	0(0)	-	-
	Cestodes	0.11 (0.06-0.21)	0.16(0.45)	0(0.5)	1.25(0.5)	1(0.25)
C.A. CN2 and C.A. CN3	*Ascaridia compar*	0.01 (-0.01-0.04)	0.01(0.12)	0(0)	1(NaN)	1(0)
	Capillaria caundinflata	0.05 (-0.004-0.09)	0.07(0.41)	0(0)	1.7(1.15)	1(1)
	Cestodes	0.06 (0-0.12)	0.14(0.68)	0(0)	2.25(1.89)	2(2)
C.A. BI1	*Ascaridia compar*	0.59 (0.46-0.71)	6.8(10.5)	2 (10.5)	11.1(11.5)	7.5(14)
	Capillaria caundinflata	0.12 (0.04-0.20)	0.80(4.27)	0(0)	6.71(11.4)	-
	Cestodes	0	0(0)	0(0)	-	-

C.A. SONDRIO	*Ascaridia compar*	0	0(0)	0(0)	-	-
	Capillaria caundinflata	0	0(0)	0(0)	-	-
	Cestodes	0	0(0)	0(0)	-	-

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