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SHORT COMMUNICATION

Occurrence of eggs and oocysts of intestinal parasites of pheasant (*Phasianus colchicus*) in droppings collected in differently managed protected areas of Tuscany (Italy)

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Abstract The releasing of farm-reared pheasant (*Phasianus colchicus*) is a very common practice in order to sustain high hunting pressures. However farm-reared birds may be carriers of parasites and diseases for the natural populations. We compared the parasite egg and oocyst prevalence and abundance of excretions found in faecal droppings collected in 13 different protected areas of Tuscany: seven areas where farm-reared pheasants are released every year to increase the reproduction and dispersion of the wild population (restocking areas) and in six areas where the production of pheasants is guaranteed only by the wild population (wild areas). *Eimeria* spp. oocysts were found in 33 of 129 (25.6%) samples collected in wild areas and in 59 of 119 (51.3%) of samples collected in restocking areas. Nematode eggs were found in 21 of 129 (16.3%) samples collected in wild areas and in 59 of 119 (49.6%) of samples collected in restocking areas. Significant differences were found for *Capillaria* spp. (31.9% of restocking areas vs. 9.3% of wild areas) and *Syngamus* spp. (10.1% of restocking areas vs. 3.1% of wild areas) but not for *Heterakis* or *Ascaridia* spp. (7.6% of restocking areas vs. 3.9% of wild areas). Parasitic excretion abundance was higher in the droppings collected in restocking areas compared to those collected in wild areas, but differences were significant only for *Eimeria* and *Capillaria* spp. In order to reduce the risk of spreading parasites and diseases, we suggest to interpose a strip (larger than the home range of the pheasants) where hunting is not forbidden between the restocking areas and the wild areas, and pheasant releases should not be allowed at least within a "pheasant home range distance" from the wild areas.

Keywords Game birds · Nematodes · Oocysts · Sanitary risk

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Introduction

The common pheasant (*Phasianus colchicus*) is one of the most important game species in many parts of Europe and North America. Management practices vary between and within countries and include habitat improvement, predator control, harvest management and release of reared or wild birds (Arroyo and Beja 2002; Draycott et al. 2008; Sokos et al. 2008). In Italy the release of wild and reared pheasants is quite common. Wild pheasants are captured in specific no hunting areas characterized by particularly favorable habitats and translocated in hunting territories after the end of the hunting season. Reared pheasants are released in summer in small open top pens bordered by no hunting areas (100–300 ha) where the pheasants are acclimatized. The birds spread outside these no hunting areas before and during the hunting season (Santilli and Bagliacca 2008).

Despite the fact that releasing reared Galliformes is a very common management option, there are some concerns about the sanitary risk connected with this practice (Villanúa et al. 2006a, 2008). As an example, the annual release of farmed ring-necked pheasant in the UK is believed to maintain or even increase *Heterakis gallinarum* burdens in the wild pheasant populations (Draycott and Sage 2005) which, in turn, could be one of the factors involved in grey partridge decline (Tompkins et al. 2001). In Spain it was observed that the releasing of farmed red-legged partridges (*Alectoris rufa*) may introduce new parasites into the wild population (Millán et al. 2004; Villanúa et al. 2007a). In addition the

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anthelmintic treatments, used normally in game bird farms, may be not effective enough to avoid the introduction of parasites into the field after release (Villanúa et al. 2007b).

The aim of the present study is to compare the intestinal parasites present in the areas with only natural pheasant populations with the intestinal parasites present in the areas where the resident natural pheasant populations are continuously increased by restocking with captive-reared animals. This can give useful information about the risks related to the two different managing choices.

Material and methods

Study areas, material collection and storage

Pheasant droppings were collected from 13 no hunting areas. Seven of them were areas where about 500–600 captive-reared young pheasants (60–90 days) are released per year in summer using open top pens (restocking areas); the birds which spread outside these no hunting areas are hunted during the hunting season. In the other six areas (wild areas), the wild resident pheasant populations are intensively managed by gamekeeping, through habitat improvement, supplemental feeding and predator control. After each hunting season, a part of the pheasant population of both areas are captured and relocated in the hunting territories (Santilli and Bagliacca 2008).

The areas were located in three different Tuscan hunting districts (provinces of Pisa, Livorno and Grosseto). Average surface of the wild areas was 757 ha (SD 140.3), and average surface of restocking areas was 202 ha (SD 92.6); droppings were collected simultaneously (same day) in wild and restocking areas located within the same hunting district and within the same month in every area.

Intestinal pheasant droppings were collected during March to May 2010 in areas (inside the protected areas around release pens and/or around feeders and/or at roosting sites) which were completely cleaned from every droppings 1–3 days before droppings collection.

All collected samples (n=248) were brought immediately to the laboratory, stored in a cold room at a constant temperature of 5°C and processed within 24 h (Thienpont et al. 1986).

Laboratory methods

The presence of parasite eggs in pheasant droppings was estimated using two different methods for prevalence and abundance (Bush et al. 1997).

1. Parasite reproductive form prevalence
   Faecal samples were examined by a qualitative flotation method (Seivwright et al. 2004): 2 g of faecal material was put into a container with 50 ml of saturated NaCl solution (40 g×l). Faeces and flotation fluid were thoroughly mixed using a fork. The faecal suspension was poured through a strainer into another container and then transferred into the test tube, leaving a convex meniscus at the top. A cover slip was carefully placed on top of the test tube. After 20 min the cover slip was carefully lifted off together with the droppings of fluid adhering to it. The slide was examined using a compound microscope at ×100 magnification. Eggs and oocysts on the slides were counted for approximate evaluation of the intensity of infestation. Since *Heterakis* spp. and *Ascaridia* spp. eggs are very similar and not easily distinguished, we considered these nematodes species in the same group. This examination was carried out on 238 samples.
2. Parasite reproductive form excretion abundance
   The count of eggs per gram (FEC) was carried out using the modified McMaster egg-counting technique (Permin and Hansen 1998). For this method, approximately 0.5 g of well-mixed faecal material was put into a shaker tube with 5 ml of the saturated NaCl solution. The tube was shaken until the faecal matter was suspended. Using a Pasteur pipette, a sample of the faecal suspension was extracted and carefully run into one chamber of a McMaster counting slide. The tube was shaken again and another sample extracted and run into the second section of the chamber. The saline suspension was left to settle for 2–3 min, allowing the eggs to float to the top of each chamber. Eggs were then counted beneath a marked grid on each chamber using a compound microscope with ×100 magnification. The number of eggs per gram of faeces was calculated by multiplying the total number of eggs counted under both grids by the total volume of faecal suspension contained in both chambers and then dividing this by the quantity of faeces used in the suspension. This examination was carried out on 124 samples.

Statistical analysis

We used χ^2 tests to evaluate the influence of management (restocking vs. wild areas) on parasite reproductive form excretion prevalence. Statistical analysis of egg counts was conducted using two different methods: the non-parametric Wilcoxon/Kruskal–Wallis test, on totally randomized data, and the nested ANOVA with the hunting districts as the blocking factor, on data transformed to approximate normal distribution, $\log_{10}$(egg concentrations+1). Significant differences were defined as p value of <0.0001, <0.001 and <0.05, respectively. All the analyses were conducted using JMP (SAS 2002).

Table 1 Relationship between different managed areas and parasite reproductive form prevalence in pheasant droppings with significant level of differences (χ^2 test)

	Wild (n=129)	Restocking (n=119)	χ^2	p value
Eimeria spp.	25.6	51.3	16.27	<0.0001
Capillaria spp.	9.3	31.9	18.31	<0.0001
Syngamus spp.	3.1	10.1	3.91	<0.05
Heterakis–Ascaridia spp.	3.9	7.6	0.96	ns

Results

In the faecal analysis, eggs of *Capillaria* spp., *Syngamus* spp., *Heterakis–Ascaridia* spp. and *Eimeria* oocysts were identified. Prevalence and mean abundance of all groups were constantly higher in the restocking areas, however only in the case of eggs of *Capillaria* spp., *Syngamus* spp. and *Eimeria* oocysts that these differences reached the significance level (Tables 1 and 2). In particular nematode eggs were found in 16.3% of samples collected in wild areas and in 49.6% of samples collected in restocking areas (p<0.0001). *Eimeria* oocysts were found in 25.6% of samples collected in wild areas and in 51.3% of samples collected in restocking areas (p<0.0001).

Discussion

Despite some limitations, faecal egg count may provide useful and reliable ways of measuring parasite prevalence and abundance in Galliformes (Goldova et al. 2006; Seivwright et al. 2004). In fact eggs are produced only by fertile adult female (or hermaphrodite) worms and will, therefore, be absent in immature or single sex infections. The daily output of eggs by fertile females is influenced by host physiological status (stress>immunity, egglaying, etc.); some natural feed stuffs may have effects similar to chemotherapy. The concentration of eggs is influenced by the daily volume of faeces being produced by the host, the rate of passage by the ingesta through the intestine and the distribution of eggs throughout the different faecal types (worm eggs are generally more abundant in intestinal than in caecal faeces, whereas the inverse pattern is found for coccidian oocysts) (Villanúa et al. 2006b).

The result confirms that prevalence and abundance of endoparasites in intestinal droppings (helminthes worms but also protozoa) is higher in the areas where farm-reared pheasants are regularly released than in the areas where the wild resident population is only managed through habitat improvements (Mani et al. 2001). This difference is probably one of the factors involved in the lower survival and reproductive success of the reared pheasant released in the no hunting areas (Woodburn 1995; Draycott et al. 2000; Millán et al. 2002).

Although the transmission of parasites from farmed birds to wild birds may be limited by natural and management factors (Villanúa et al. 2008), we suggest to interpose a strip (larger than the home range of the pheasants) where hunting is not forbidden between the restocking and the wild areas. This could reduce the risk of spreading parasites and diseases in the wild areas where the wild resident populations are only managed. In addition, the release of farming pheasants should not be allowed at least within a "pheasants home range distance" from the wild areas. The

Table 2 Relationship between different managed areas and parasite reproductive form excretion abundance in pheasant droppings (E, eggs×g^{-1}) with significant level of differences (Wilcoxon/Kruskal–Wallis test and nested ANOVA on $\log_{10}$ transformed concentrations)

	Wild (n=50)			Restocking (n=74)			
	Mean (least square mean)	Min–max	SD (standard error)	Mean (least square mean)	Min–max	SD (standard error)	p value
Eimeria spp.,	0.82	0–6	1.746	2.76	0–15	4.382	<0.01
$\log_{10}(E+1)$	(0.142)		(0.0484)	(0.344)		(0.0458)	(<0.01)
Capillaria spp.,	0.12	0–3	0.521	0.54	0–8	1.218	<0.05
$\log_{10}(E+1)$	(0.024)		(0.0260)	(0.138)		(0.0246)	(<0.01)
Syngamus spp.,	0.02	0–1	0.141	0.49	0–27	3.163	ns
$\log_{10}(E+1)$	(0.006)		(0.0191)	(0.046)		(0.0181)	(ns)
Heterakis+Ascaridia spp.,	0.10	0–3	0.463	0.16	0–5	0.663	ns
$\log_{10}(E+1)$	(0.025)		(0.0172)	(0.027)		(0.0163)	(ns)

Significant level of their differences by the Mann–Whitney U test

presence of a wide net of areas where the release of captive-reared bird is not allowed may be an important tool to safeguard wild populations preserved in the wild areas. Unlike what occurs in farm facilities, wild pheasant populations are subject to an intensive sexual selection which is probably an evolutionary adaptation to face parasite infestations or other diseases (Von Schantz et al. 1996). Sanitary conditions of reared game birds should be better evaluated before release, and prophylactic measures should be improved as observed for the red-legged partridge.

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