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ORIGINAL PAPER

Prevalence of *Escherichia coli, Salmonella* sp. and *Campylobacter* sp. in the intestinal flora of farm-reared, restocked and wild red-legged partridges (*Alectoris rufa*): is restocking using farm-reared birds a risk?

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Abstract For hunting purposes, several millions of red-legged partridges (*Alectoris rufa*) are released each year in Spain, and these releases have the potential to introduce new parasites and disease into wild populations. We studied the prevalence of *Escherichia coli*, *Campylobacter* sp. and *Salmonella* sp. in the intestinal flora of red-legged partridges from three different husbandry groups: farm-reared, restocked and natural populations. Prevalence of *E. coli* was significantly higher in farm-reared (45%, $p=0.01$) and restocked partridges (60%, $p<0.001$) than in wild ones (6%, $p>0.05$). The prevalence of *Campylobacter* sp. (23%, 100 out of 444) did not differ significantly between these three husbandry groups, and *Salmonella* sp. was only detected in a group of partridge chicks on one of the farms studied (0.9%, 5 out of 544). These results suggest that farm-reared and restocked partridges can act as carriers of these three enteropathogens and highlight a potential risk of transmission to natural populations via the releases of farm-reared partridges. However, future investigations are needed regarding the relation of the isolated bacteria with zoonotic strains and dissemination of antibiotic resistant microorganisms, especially *E. coli*, and to better evaluate the effect that these three enteropathogens have on partridge health and on the success of restocking with farm-reared birds.

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Introduction

The red-legged partridge (*Alectoris rufa*) is a gallinacean species whose global distribution is restricted to the Iberian Peninsula and southern regions of France (Cramp and Simmons 1980). This species is currently the most important gamebird in Spain, especially in farmland areas, and has a high socioeconomic value (Bernabeu 2000; Martínez et al. 2002). Due to a general decrease in red-legged partridge abundance in the field, numerous hunting estates have turned to releasing farm-reared partridges for hunting purposes and to reinforce natural populations (Beja et al. 2009; Duarte and Vargas 2004; Duarte et al. 2010). Overall, it is estimated that more than four million red-legged partridges are released annually in Spain (Gortázar et al. 2000; Garrido 2002; Millán et al. 2003). Wild and farmed red-legged partridges are host to different parasites (Millán et al. 2004). Parasites and diseases may have a negative effect on the success of the partridge releases, and can also be transmitted to and affect sympatric wildlife populations (Cunningham 1996).

In fact, the introduction of parasites into wild populations is one of the most serious concerns in restocking programmes (Viggers et al. 1993). Marked differences in the parasite community of wild and farm-reared red-legged partridges have been reported, as well as the transmission of farm-origin parasites to wild partridges (Millán et al. 2004; Villanúa et al. 2008) and to other sympatric species of birds such as the little bustard (*Tetrax tetrax*; Villanúa et al. 2007a) and pheasant (*Phasianus colchicus*; Madsen 1941; Draycott et al. 2006; Villanúa et al. 2006). The releases of farm-reared birds could thus potentially also introduce other new pathogens into the wild (Millán 2009; Villanúa et al. 2006). And, not only would negatively affect natural partridge populations, we have to considered the role of this naive populations as carriers of this enteric pathogens and as a potential source of infection for humans, domestic animals and other wildlife (Goodchild and Tucker 1968; Craven et al. 2000).

Poor post-release survival has been documented in restocking programmes with red-legged partridges (Gortázar et al. 2000). Parasites can reduce the condition of gamebirds, making them more vulnerable to predators (Tompkins et al. 2002). In the red-legged partridge, some nematode parasites reduce body condition, and may adversely affect survival after release (Millán et al. 2004). In pheasants, an increase of parasite burdens during restocking programmes has been described, and may reduce post-release survival and enhance the likeliness of new parasite introductions into the wild (Villanúa et al. 2008).

In contrast to the existing knowledge regarding parasites, there is little information on the gastrointestinal bacterial flora of wild birds (Glünder 1981; Benskin et al. 2009). Under natural conditions, Gram-negative bacteria, especially *Enterobacteriaceae*, are not considered part of the intestinal flora of granivorous birds (Glünder 2002). In this study, we focus on three groups of bacteria that have previously been associated with faecal contamination: *Escherichia coli, Campylobacter* sp. and *Salmonella* sp. (Vláhovic et al. 2004; Heuvelink et al. 2008).

Escherichia coli is part of the intestinal flora and has been isolated from a range of bird species, including healthy passerines and waterfowl; however, its presence in the digestive tract appears to be diet-related and is exceptional in healthy granivorous avian species (Brittingham et al. 1988; Damaré et al. 1979; Foster et al. 1998; Glünder 2002). Some gallinaceous birds have also been described as potential carriers of these bacteria, both in the intestine (Benskin et al. 2009), and also in meat (Paulsen et al. 2008; Arenas et al. 1999). Some studies suggest marked differences between the microbiological conditions in the small intestine and caeca of wild and captive birds (e.g. in willow grouse *Lagopus lagopus lagopus*: Hanssen 1979). In captive birds other than the domestic chicken, commercial diets and intensive management of farms have been found to generate an intestinal microflora similar to domestic chickens, thus, increasing the prevalence of aerobic bacteria such as *E. coli* (Barnes 1972; Hanssen 1979).

Avian colibacillosis is one of the main causes of mortality and economic losses to the poultry industry, and has been reported in chicken and captive turkeys (*Meleagris gallopavo*), pheasants and quail (*Coturnix coturnix*) (Creitz and Small 1967; Pierson and Barta 1996; Barnes and Gross 1997; Arenas et al. 1999). *E. coli* is also considered an important cause of enteritis and mortality in farmed red-legged partridges, but published information is limited to one case report (La Ragione et al. 2004).

Birds are considered the main reservoir of *Campylobacter* sp. (Newell and Fearnley 2003; Volkheimer and Wuthe 1986). It is a frequent commensal in the intestine of avian species, including wild birds, and also captive chicken, turkeys, quails, ducks, pheasants and even ostriches (Luechtefeld et al. 1980; Kapperud and Rosef 1983; Yogasundram et al. 1989; Stephens et al. 1998; Wallace et al. 1998; Waldeström et al. 2002). The presence of *Campylobacter* sp. is influenced by feeding behaviour and ecological guild of birds, with insectivores and granivorous rarely testing positive (Waldeström et al. 2010). *Campylobacter* sp. have, however, been detected in a wild gallinacean bird, the pheasant (Atanassova and Ring 1999), but information on the presence of *Campylobacter* sp. in wild or farmed red-legged partridges is lacking (Volkheimer and Wuthe 1986; Dipineto et al. 2009).

Finally, Salmonellosis is one of the most important bacterial diseases in the poultry industry, causing reduced production and increased mortality (Lutful Kabir 2010). *Salmonella* sp. has been found in both, healthy and diseased wild birds (Benskin et al. 2009). In gallinaceans, a Salmonellosis outbreak in a population of wild red-legged partridges has been described and related to partridge restocking (Lucientes 1998), but the prevalence of *Salmonella* sp. in this avian species is unknown.

The present study analyzes the prevalence of *E. coli, Campylobacter* sp. and *Salmonella* sp. in the intestinal flora of farm-reared, restocked and natural populations of red-legged partridges. We supposed that management of these birds influences the prevalence of these three enteropathogens. Taking into account that production on partridge farms has been intensified recently due to the increased demand of partridges for release, we hypothesized that farmed red-legged partridges should have higher enterobacteria prevalences. This would ultimately imply an increased infection pressure on the environment, by contaminated faeces, water and food (Lutful Kabir 2010).

Material and methods

We used samples from a total of 544 partridges. The partridges sampled were divided in three gropus, according to the husbandry/origin: farm-reared (N=184 from 6 farms), restocked (hunting estates with releases of farmed birds) (N=193 from 4 hunting estates) and natural population (hunting estates without partridge releases) (N= 167 from 4 hunting estates).The location of the sampling sites is described in Table 1. Their selection aimed to assess a broad geographical distribution and a wide representation of farm management practices. The samples included in the study were taken during the period 2007–2010. They were collected from the three husbandry groups in all seasons, except for restocked locations for which no samples were obtained in the breeding season, according to the hunting season.

For the detection of *E. coli* and *Salmonella* sp., cloacal samples (N=544) were collected with sterile cotton swabs into AMIES transport medium (Deltalab, Barcelona, Spain), maintained at 4°C until arrival at the laboratory and processed at a maximum of 24 h after sample collection. Isolation and identification of *E. coli* were performed by standard bacteriological methods. For detection of *Campylobacter* sp. cloacal samples (N=444) were collected sterilely, transferred into Cary Blair transport medium (Deltalab, Barcelona, Spain) and maintained at 4°C until arrival at the laboratory within 8 h of collection. The samples were cultured according to methods described previously (Arsenault et al. 2007). All isolates were stored at −80°C for further analyses. Isolation of *Salmonella* sp. was attempted according to the ISO 6579 (2002) standard method, culturing the swabs in the enrichment broth Rappaport Vassiliadis Soja (Scharlab, Spain) at 42°C 48 h, and as selective media, we used Agar Xilose Lysine Desoxicolato (Scharlab, Spain) at 37°C 24 h.

Differences in prevalences between husbandry groups, individual farms and hunting estates were tested using the chi-square test for homogeneity (SPSS 17.0 software). In order to test the effect of breeding, we analysed differences between husbandry groups separating natural populations in the breeding season out. In addition, we compared natural populations sampled in the breeding season with non-breeding populations.

Results

The overall prevalence of *E. coli* was 38% (0–93%; 209 out of 544 partridges, Table 1), while the prevalence of *Campylobacter* sp. was 23% (0–60%; 100 out of 444 partridges, Table 1) and that of *Salmonella* sp. was 0.9% (0–11%; 5 out of 544; Table 1). *Salmonella* sp. was only detected in a group of partridge chicks on one of the farms studied. In contrast, *E. coli* and *Campylobacter* sp. were isolated from partridges from all three sampling groups.

Table 1 Prevalence of *E. coli* and *Campylobacter* sp. in red-legged partridges of different origin

Partridge origin	Location (UTM)	(N^1)	Prevalence *E. coli*% (p^1)	*E. coli* prevalence (%) (p^1/n^2)	(N^1)	Prevalence *Campylobacter*% (p^1)	*Campylobacter* prevalence (%) (p^1/n^2)
Farm-reared	Albacete (539894.74 mE 4311410.27 mN)	47	40 (19)	45 (83/184)	47	36 (17)	20 (36/184)
	Albacete (610727.16 mE 4311410.27 mN)	30	57 (17)		30	0	
	Albacete (555998.23 mE 4311317.71 mN)	10	40 (4)		10	60 (6)	
	Ciudad Real (419690.22 mE 4315642.51 mN)	60	55 (33)		60	22 (13)	
	Toledo (425231.99 mE 4381663.24 mN)	27	37 (10)		27	0	
	Toledo (314251.36 mE 4421044.51 mN)	10	0		10	0	
Restocked	Ciudad Real (419690.22 mE 4315642.51 mN)	28	28 (11)	60 (116/193)	28	0	27 (46/172)
	Toledo (375624.75 mE 4416285.22 mN)	30	0		30	33 (10)	
	Toledo (383773.54 mE 4413602.09 mN)	92	71 (65)		92	39 (36)	
	Cádiz (247865.61 mE 4025640.35 mN	43	93 (40)		10	0	
Natural population	Ciudad Real 419690.22 mE 4315642.51 mN)	56	7 (4)	6 (10/167)	51	31 (16)	18 (18/100)
	Toledo (396863.39 mE 4434336.66 mN)	25	0		25	0	
	Ciudad Real (459345.31 mE 4277523.95 mN)	30	5 (3)		17	0	
	Navarra (608443.76 mE 4727839.07 mN)	56	10 (3)		7	0	
Total		544	38 (209)		444	22(98)	

N^1 number of samples taken in each location

P^1 number of isolates

n^2 total number of samples taken in each husbandry group

The prevalence of *E. coli* was significantly higher in farm-reared and restocked red-legged partridges than in wild ones ($p<0.001$, Fig. 1), while the prevalence of *Campylobacter* sp. did not differ significantly between groups ($p>0.05$; Fig. 1). *E. coli* prevalence differed significantly between farms, ranging from 0% to 57% ($p=0.01$). In contrast, *Campylobacter* sp. prevalence (0–60%) did not differ significantly ($p>0.05$) between farms. We found significant differences for both *E. coli* ($p<0.001$) and *Campylobacter* sp. prevalence ($p<0.001$) between individual hunting estates. In natural populations, the differences in prevalence for *E. coli* were not significant ($p>0.05$), while significant differences were observed for *Campylobacter* sp. prevalence ($p<0.001$; Table 1). We did not observe any significant differences between husbandry group prevalences when we tested the effect of season ($X^2=45.26$, 3 df, $p>0.05$).

Discussion

To the best of our knowledge, this is the first description of prevalences of *E. coli* and *Campylobacter* sp. in wild red-legged partridges. Based on our data, we confirmed our initial prediction that farmed partridges would have higher prevalences of *E. coli* and *Campylobacter* sp. than wild partridges.

The very low presence of *E. coli* in natural partridge populations in our study suggests that under natural conditions, this bacterium is not part of the digestive tract flora of red-legged partridges. This is in agreement with the findings in other wild birds (Fiennes 1959; Hanssen 1979; Brittinham et al. 1988; Glünder 1981, 2002). In contrast, *E. coli* is a common parasite of captive birds (Hanssen 1979; Naldo et al. 1998; Arenas et al. 1999), as found here in farmed partridges.

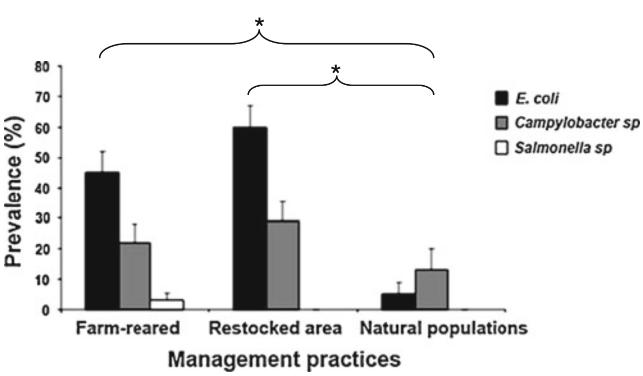

Fig. 1 Prevalence of *E. coli, Campylobacter* and *Salmonella* sp. in red-legged partridges from different management models. Significant differences in *E. coli* prevalence between management models (*asterisks*)

Unfortunately, in this study, it was not possible to distinguish released partridges from native ones on estates where partridge releases took place. So, we cannot evaluate if *E. coli* transmission between released and wild partridges occurred. Our results essentially document the effect of the massive release of infected farm-reared birds. Nevertheless, these results underline the importance of partridge releases as a source of introduction of *E. coli* into natural areas, with potential effects for partridges and other bird species (Dho-Moulin and Fairbrother 1999), as well as for game meat hygiene (Villanúa et al. 2007a; Gill 2007; Paulsen et al. 2008).

The high prevalence obtained for *Campylobacter* sp. in farmed and restocked red-legged partridges supports the hypothesis that game birds act as potential carriers of *Campylobacter* sp. (see also Dipineto et al. 2008). As an example, Campylobacteriosis has been reported in employees exposed to the pathogen at a pheasant farm (Heryford and Seys 2004). Also, *Campylobacter* sp. has been isolated from healthy wild birds in several countries (e.g. Japan, Italy or Sweden; Ito et al. 1988; Waldeström et al. 2002; Robino et al. 2010), including Spain, where prevalence in seagulls was related to the presence of refuse sites (Ramos et al. 2010). The low prevalence we observed in wild red-legged partridges coincides with other studies on free-living birds (Matsusaki et al. 1986; Atanassova and Ring 1999; Vázquez et al. 2010). Our data underlines the risk of *Campylobacter* sp. colonization during farm rearing as described by other authors in chicken and turkey flocks (Allos 2001; Gibbens et al. 2001; Arsenault et al. 2007) and the subsequent introduction into wild populations.

We found a very low prevalence for *Salmonella* sp. in the three study groups of red-legged partridges. This result is in concordance with previous studies describing low prevalences in healthy wild birds or the absence of *Salmonella* sp. in game birds and waterfowl (Brittingham et al. 1988; El-Ghareeb et al. 2009). The only isolate obtained was probably associated to reduced growth and mortality among juvenile red-legged partridges in an aviary. *Salmonella* sp. is an important pathogen in poultry farms (Arsenault et al. 2007) and represents a potential risk for wild birds after restocking of farm-reared birds. In fact, the only report of *Salmonella* sp. in a natural population of red-legged partridges was related to sharing of a feeding station with restocked red-legged partridges (Lucientes 1998).

The artificial environment and intensive management of game birds in farms has been shown to increase the risk of infection by numerous parasites (Gibbs et al. 2007; Villanúa et al. 2008; Lutful Kabir 2010). This could explain the high mean prevalences observed for *E. coli* and *Campylobacter* sp. in farm-reared partridges, as well as the detection of *Salmonella* sp. only in this husbandry group. The significantly higher prevalence of both *E. coli* and *Campylobacter* sp. in restocked partridges could be a consequence of the cease of antimicrobial treatments after release (Villanúa et al. 2007b; Draycott et al. 2006). Also, stress during transport, release and changes in the diet potentially alter the digestive tract flora (Glünder 2002; Shini et al. 2010). All these changes may provide commensal bacteria, the opening they need to proliferate in the avian intestine (Gross 1984; Durairaj and Dustan 2007). With our results, we have no evidence of a direct effect of *E. coli* on post-release survival of partridges. However, a previous study had shown low body condition in released partridges that was not related to parasite burden and could have had a bacterial origin (Putaala and Hissa 1995; Meriggi et al. 2002; Villanúa et al. 2008). In chickens and turkeys, stress has been shown to allow the colonization of tissues other than the intestine by pathogenic *E. coli* and the development of clinical disease (Leitner and Heller 1992). As an indirect effect, in red grouse (*Lagopus lagopus scoticus*), pheasants and red-legged partridges, parasites have been described to increase the risk of predation (Hudson et al. 1992; Tompkins et al. 2002; Villanúa et al. 2007a) and a similar situation could exist in partridges, in relation with an overgrowth of *E. coli* in the intestinal flora.

The striking difference in prevalence for *E. coli* and *Campylobacter* sp. between individual farms is also an interesting finding that indicates important variations in husbandry practices. This should be further investigated in the future. Likewise, the significant variation in prevalence among restocked partridges could reflect their diverse origin and management during transport and release, as well as differences in density and aggregation at feeders in the hunting estates (Lucientes 1998).

Future investigations about the influence of *E. coli*, *Campylobacter* sp. and *Salmonella* sp. on the success of restocking and on the health of farm-reared red-legged partridges will be of interest, in order to establish a base for the prevention of enteritis outbreaks on farms, increase of post-release survival and avoid the spread of these bacteria into naive populations (Millán et al. 2004; Tompkins et al. 2001). Also, molecular studies regarding the relation of the isolated bacteria with zoonotic strains, especially *Campylobacter coli* and *jejuni*, and the study of antibiotic resistances will be of importance.

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