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Influence of phylogeny, migration and type of diet on the presence of intestinal parasites in the faeces of European passerine birds (*Passeriformes*)

Petra Bandelj, Rok Blagus, Tomi Trilar, Modest Vengust and Aleksandra Vergles Rataj

P. Bandelj (petra.bandelj@vf.uni-lj.si), M. Vengust and A. Vergles Rataj, Veterinary Faculty, Univ. of Ljubljana, Ljubljana SI-1115, Slovenia. – R. Blagus, Inst. for Biostatistics and Medical Informatics, Univ. of Ljubljana, Vrazov trg 2, SI-1000 Ljubljana, Slovenia. – T. Trilar, Slovenian Museum of Natural History, Prešernova 20, SI-1000 Ljubljana, Slovenia

Migratory and non-migratory passerine birds can carry several pathogens, including parasites, which may cause significant diseases in birds, other animal species and humans. Parasites have been shown to negatively impact many populations of wildlife, and this may become more significant with global temperature changes. This study was performed to investigate the prevalence of intestinal parasites in faecal samples of European passerines. Intestinal parasites identified were statistically associated with passerines phylogenetic classification, migratory habits (migratory, non-migratory) and the type of diet (omnivorous, insectivorous and granivorous). A total of 385 passerines of 42 species were captured and their droppings collected. The prevalence of parasites in faecal samples of passerines was 15.6%. Intestinal parasites were identified in 50/309 (16.2%) migratory passerines and 10/76 (13.2%) non-migratory passerines using the faecal flotation method. *Coccidia* were most often identified parasites; they were more likely to be present in an omnivorous bird species ($p = 0.02$). *Syngamus* spp. was more likely to be detected in omnivorous passerines ($p = 0.04$). Tits ($p = 0.01$) and finches ($p = 0.006$) were less likely to have intestinal parasites present in their faecal samples than passerines classified in other phylogenetic clades. Tits ($p = 0.02$) and finches ($p = 0.008$) were also less likely to have coccidia present in their faecal samples. Phylogeny was associated with the presence of parasites in faecal samples of passerines ($p = 0.03$). The prevalence of parasites, however, was not associated with the migration habit of passerines, but to the type of diet ($p = 0.04$). Our analysis suggests that the diversity of feeding sources of omnivore passerines exposes them to infection with intestinal parasites to a greater extent than granivore or insectivore passerines.

Several bird species migrate continental or intercontinental distances to survive seasonal climate changes and/or to be able to breed. In Europe birds migrate from north and central Europe to southern parts of Europe, the Middle East and sub-Saharan Africa, or they roam across the European continent (Hahn et al. 2009). Such migration provides a mean for circulation of parasitic, bacterial and viral pathogens along macro-ecological niches present in Europe, Asia and Africa (Hubálek 2004, Jellison et al. 2007, Keawcharoen et al. 2008, Fuller et al. 2012).

A vast body of research has been published defining the epidemiology of different pathogens in migratory passerine bird species (passerines; *Passeriformes*) that are, or are potentially harmful for human or other animal species health (Bengis et al. 2004, Hubálek 2004). Less research, however, has been directed towards studying emerging pathogens, and those that were not considered highly virulent for passerines and are now re-emerging. Recent disease outbreaks in wild bird populations can or are expected to become more significant as a consequence of climate changes (Robinson et al. 2010, Kilpatrick 2011, Fuller et al. 2012). Besides their lethal effect, parasite emergence

can change host population dynamics and modify co-evolution relationships between hosts and their parasites. The evolutionary pressure of the host and the parasite contributed to the position of species on the phylogenetic tree (Best et al. 2010).

Studies in European passerines investigated their arthropod ectoparasites (Trilar 2004, Sychra et al. 2008), blood parasites including *Plasmodium* (Scheuerlein and Ricklefs 2004, Levin et al. 2009) and intestinal parasites (Svobodová 1994, Zinke et al. 2004, Dolnik 2006, López et al. 2007, 2011, Brown et al. 2010, Dolnik et al. 2010). However, the prevalence and influence of the overall presence of intestinal parasites has not been investigated. Intestinal parasitic infections are present globally and are a significant cause of illness and disease in animals and humans (Thomas et al. 2005, Haque 2007, Benchaoui 2010). They may become a more significant problem in temperate climates because global temperature patterns may change established parasite–host relationships with an increased parasite development rate and a larger parasite pressure on the host (Tompkins and Begon 1999, Read and Taylor 2001, Hudson et al. 2006).

The purpose of this study was, therefore, to evaluate the prevalence of intestinal parasites in faecal droppings of different species of European migratory and non-migratory passerines, and to associate specific parasite prevalence with the birds' phylogenetic classification and, more specifically, with their migratory habit and the type of diet.

Material and methods

Animals and samples

Wild passerines were ringed and sampled at the bird observatory near Vrhnika town (45°58'07"N, 14°18'27"E) and at the lake Cerknica (45°45'24"N, 14°22'11"E) in the central part of Slovenia (Bandelj et al. 2011) (Fig. 1). The study was carried out with animal ethics approval by the Ministry of the Environment and Spatial Planning (document no. 35601-10/2010-6).

Birds were classified into eight clades according to their morphological and molecular characteristics (Table 1) (Cramp 1988, 1992, Cramp and Perrins 1993, 1994a, b, Jancar et al. 1999, Barker et al. 2002, Fregin et al. 2012, McCormack et al. 2013, BirdLife International 2014). They were further classified according to their migrating habits (migratory, non-migratory) and the type of diet (omnivorous, granivorous and insectivorous) (Table 2) (Cramp 1988, 1992, Cramp and Perrins 1993, 1994a, b).

Birds were captured in the afternoon with mist nets and placed individually in clean cloth bags for no more than 30 min. After the bird was removed from the bag to be ringed, weighed, measured and their species and age determined, the faecal sample was collected from the bag if present. Droppings that corresponded to urine (i.e. lacked faecal material) were rejected (López et al. 2007). Faecal samples were placed in clean 2 ml vials and stored immediately at

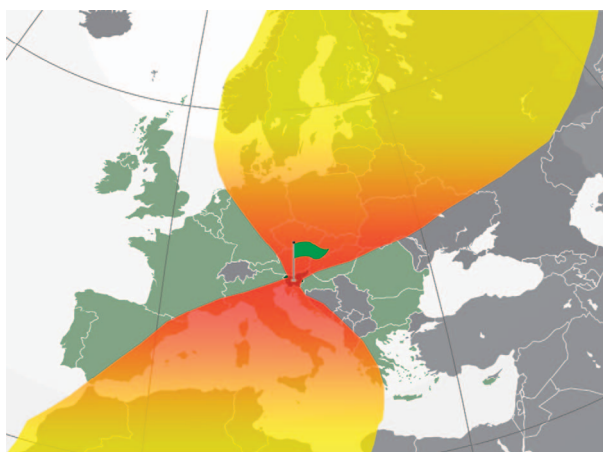


Figure 1. The map shows the summer breeding grounds of migrating passerines from north Europe (yellow and red coloured areas north of the green flag) that migrate through Slovenia (green flag) to their non-breeding grounds (red and yellow coloured areas south of the green flag) in winter. The yellow to red coloured areas above the green flag indicate increase density (congregation) of migrating passerines, whereas the red to yellow coloured areas below the green flag indicate decrease density (dispensation) of migrating passerines.

4°C to preserve diagnostic parasitic stages. Samples were transported to the laboratory for parasitological analysis on the morning following the sampling.

The flotation method was used (with saline solution density: 1.20) as a diagnostic concentration method to separate parasite particles from faecal debris. The solution was left standing for a minimum of 15 min before making the wet mount for the microscopic examination. The whole amount of the concentrate from the surface of the solution was placed on a microscope slide and evaluated at 100× magnification (Maizels and Yazdanbakhsh 2003).

Statistical analysis

Fisher's exact test was used to univariately associate phylogenetic characteristics, the type of diet and migration to the presence of parasites. The association between phylogenetic characteristics and the presence of parasites was also analysed with the logistic regression using the Firth's correction (Heinze and Schemper 2002). Briefly, Firth's correction was proposed to solve the problem of separation that can occur when studying rare events. Logistic regression was used also to estimate the multivariate association between the type of diet, migratory habits and the presence of parasites. In order to account for the possible confounding effect of species and phylogeny, both effects were included in separate models as a random effect; separate models for both effects were estimated as a simultaneous inclusion of both factors was not possible due to scarce presence of parasites. A p-value equal to or less than 0.05 was considered as statistically significant. The analysis was performed using the R language for statistical computing (< www.r-project.org >).

Results

Droppings (n = 385) were collected from migratory (n = 309, 26 species) and non-migratory (also expressing horizontal migration: n = 76, 17 species) European passerines (Table 2). Parasites were isolated from the faeces of 60 of these 385 birds (15.6%; 95% CI: 12–19.2%): 50/309 (16.2%; 95% CI: 12.2–20.8%) migratory and 10/76 (13.1%; 95% confidence interval (CI): 6.4–22.9) non-migratory passerines had parasites in their faeces (Supplementary material Appendix 1 Table A1).

Granivorous (6.3%; 2/32), insectivorous (14.5%; 40/276) and omnivorous (23.4%; 18/77) passerines had parasites present in their faeces (Fig. 2). Phylogenetic characteristics ($p = 0.03$) and the type of diet were univariately associated with the presence of intestinal parasites ($p = 0.04$). Passerines from clades 4 (OR: 0.2, 95% CI: 0.02–0.7, $p = 0.01$) and 7 (OR: 0.2, 95% CI: 0.04–0.6, $p = 0.006$) were less likely to have intestinal parasites in their faeces. It was less likely for the insectivorous and granivorous (borderline significant) passerines to have intestinal parasites in their faeces than omnivorous passerines (OR adjusted for migration and species: 0.3, 95% CI: 0.1–0.8, $p = 0.01$ and 0.2, 95% CI: 0.05–1.1, $p = 0.06$ for insectivorous and granivorous passerines, respectively).

Coccidia (*Eimeria* spp. and *Isoospora* spp.) were present in migratory (14.9%) and non-migratory (10.5%) passerines

Table 1. Phylogenetic classification of passerines in this study.

Phylogeny	Family	Species
1 clade	Hirundinidae	<i>Hirundo rustica</i> , <i>Riparia riparia</i>
2 clade	Motacillidae	<i>Motacilla cinerea</i>
3 clade	Muscicapidae	<i>Erithacus rubecula</i> , <i>Luscinia luscinia</i> , <i>Luscinia megarhynchos</i> , <i>Phoenicurus phoenicurus</i> , <i>Saxicola rubetra</i> , <i>Saxicola torquata</i>
	Turdidae	<i>Turdus merula</i> , <i>Turdus philomelos</i>
	Prunellidae	<i>Prunella modularis</i>
4 clade	Certhiidae	<i>Certhia familiaris</i>
	Paridae	<i>Cyanistes caeruleus</i> , <i>Parus major</i> , <i>Periparus ater</i> , <i>Remiz pendulinus</i>
	Aegithalidae	<i>Aegithalos caudatus</i>
5 clade	Muscicapidae	<i>Ficedula albicollis</i> , <i>Ficedula hypoleuca</i> , <i>Muscicapa striata</i>
6 clade	Acrocephalidae	<i>Acrocephalus arundinaceus</i> , <i>Acrocephalus schoenobaenus</i> , <i>Acrocephalus scirpaceus</i> , <i>Hippolais icterina</i>
	Locustellidae	<i>Locustella naevia</i>
	Phylloscopidae	<i>Phylloscopus collybita</i> , <i>Phylloscopus sibilatrix</i> , <i>Phylloscopus trochilus</i>
	Sylviidae	<i>Sylvia atricapilla</i> , <i>Sylvia borin</i> , <i>Sylvia communis</i> , <i>Sylvia curruca</i> , <i>Sylvia nisoria</i>
7 clade	Fringillidae	<i>Carduelis carduelis</i> , <i>Carduelis chloris</i> , <i>Coccothraustes coccothraustes</i> , <i>Emberiza citrinella</i> , <i>Emberiza schoeniclus</i> , <i>Fringilla coelebs</i> , <i>Passer montanus</i> , <i>Serinus serinus</i>
8 clade	Troglodytidae	<i>Troglodytes troglodytes</i>

Table 2. Passerine species in alphabetical order (n = 385) with their migratory and feeding habit.

	Passerine species	No.	Migration*	Type of diet*
1	<i>Acrocephalus arundinaceus</i> (great reed warbler)	1	migratory	insectivorous
2	<i>Acrocephalus schoenobaenus</i> (sedge warbler)	15	migratory	insectivorous
3	<i>Acrocephalus scirpaceus</i> (reed warbler)	15	migratory	insectivorous
4	<i>Aegithalos caudatus</i> (long-tailed tit)	1	non-migratory	insectivorous
5	<i>Carduelis carduelis</i> (European goldfinch)	12	non-migratory	granivorous
6	<i>Carduelis chloris</i> (European greenfinch)	4	non-migratory	granivorous
7	<i>Certhia familiaris</i> (Eurasian treecreeper)	1	non-migratory	insectivorous
8	<i>Coccothraustes coccothraustes</i> (hawfinch)	1	non-migratory	omnivorous
9	<i>Cyanistes caeruleus</i> (blue tit)	12	non-migratory	insectivorous
10	<i>Emberiza citrinella</i> (yellowhammer)	1	non-migratory	granivorous
11	<i>Emberiza schoeniclus</i> (reed bunting)	8	non-migratory	granivorous
12	<i>Erithacus rubecula</i> (European robin)	46	migratory	omnivorous
13	<i>Ficedula albicollis</i> (collared flycatcher)	1	migratory	insectivorous
14	<i>Ficedula hypoleuca</i> (pied flycatcher)	6	migratory	insectivorous
15	<i>Fringilla coelebs</i> (common chaffinch)	4	non-migratory	omnivorous
16	<i>Hippolais icterina</i> (icterine warbler)	3	migratory	insectivorous
17	<i>Hirundo rustica</i> (barn swallow)	19	migratory	insectivorous
18	<i>Locustella naevia</i> (common grasshopper warbler)	1	migratory	insectivorous
19	<i>Luscinia luscinia</i> (thrush nightingale)	1	migratory	insectivorous
20	<i>Luscinia megarhynchos</i> (common nightingale)	2	migratory	insectivorous
21	<i>Motacilla cinerea</i> (grey wagtail)	1	non-migratory	insectivorous
22	<i>Muscicapa striata</i> (spotted flycatcher)	1	migratory	insectivorous
23	<i>Parus major</i> (great tit)	4	non-migratory	insectivorous
24	<i>Passer montanus</i> (Eurasian tree sparrow)	7	non-migratory	omnivorous
25	<i>Periparus ater</i> (coal tit)	2	non-migratory	insectivorous
26	<i>Phoenicurus phoenicurus</i> (common redstart)	1	migratory	insectivorous
27	<i>Phylloscopus collybita</i> (common chiffchaff)	13	migratory	insectivorous
28	<i>Phylloscopus sibilatrix</i> (wood warbler)	3	migratory	insectivorous
29	<i>Phylloscopus trochilus</i> (willow warbler)	1	migratory	insectivorous
30	<i>Prunella modularis</i> (dunnock)	52	migratory	insectivorous
31	<i>Remiz pendulinus</i> (European penduline tit)	11	migratory	insectivorous
32	<i>Riparia riparia</i> (sand martin)	1	migratory	insectivorous
33	<i>Saxicola rubetra</i> (whinchat)	1	migratory	omnivorous
34	<i>Saxicola torquata</i> (common stonechat)	1	migratory	insectivorous
35	<i>Serinus serinus</i> (European serin)	7	non-migratory	granivorous
36	<i>Sylvia atricapilla</i> (Eurasian blackcap)	56	migratory	insectivorous
37	<i>Sylvia borin</i> (garden warbler)	44	migratory	insectivorous
38	<i>Sylvia communis</i> (common whitethroat)	2	migratory	insectivorous
39	<i>Sylvia curruca</i> (lesser whitethroat)	3	migratory	insectivorous
40	<i>Sylvia nisoria</i> (barred warbler)	1	migratory	insectivorous
41	<i>Troglodytes troglodytes</i> (Eurasian wren)	1	non-migratory	insectivorous
42	<i>Turdus merula</i> (common blackbird)	8	non-migratory	omnivorous
43	<i>Turdus philomelos</i> (song thrush)	10	non-migratory	omnivorous

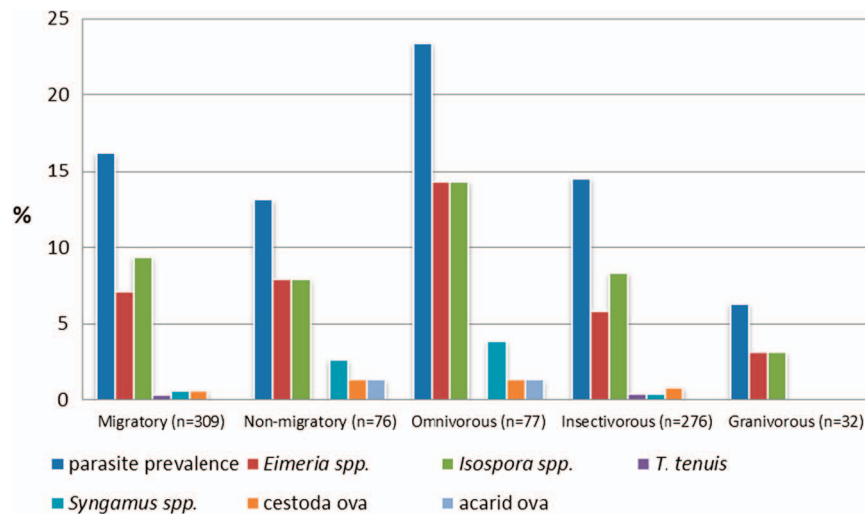


Figure 2. Parasite burden in passerines according to their migratory and feeding habit. Results presented in Supplementary material Appendix 1 Table A1.

and were the most frequently detected parasites. Phylogenetic characteristics were univariately associated with the presence of intestinal parasites ($p = 0.04$). Passerines from clades 4 (OR: 0.2, 95% CI: 0.02–0.7, $p = 0.02$) and 7 (OR: 0.2, 95% CI: 0.04–0.7, $p = 0.008$) were less likely to have coccidia in their faeces. Insectivorous passerines were less likely to have coccidia in their faeces than omnivorous passerines (OR adjusted for migration and species: 0.3, 95% CI: 0.1–0.8, $p = 0.02$). *Eimeria* spp. were identified in 7.1% of migratory and 7.9% of non-migratory passerines. The type of diet was univariately associated with the presence of *Eimeria* spp. in faeces ($p = 0.03$). Insectivorous passerines were less likely to have *Eimeria* spp. in their faeces than omnivorous passerines (OR adjusted for migration and species: 0.2, 95% CI: 0.04–0.5, $p = 0.003$). *Isospora* spp. were identified in 9.4% of migratory and 7.9% of non-migratory passerines. Passerines from clades 4 (OR: 0.1, 95% CI: 0.001–0.7, $p = 0.03$) and 7 (OR: 0.2, 95% CI: 0.02–0.9, $p = 0.02$) were less likely to have *Isospora* spp. in their faeces. Insectivorous passerines were less likely to have *Isospora* spp. in their faeces than omnivorous passerines (OR adjusted for migration and species: 0.3, 95% CI: 0.1–0.9, $p = 0.03$).

Nematodes (*Trichostrongylus tenuis*, *Syngamus* spp. and ascarid ova) and cestodes (cestoda ova) were detected in faecal samples. Overall, the presence of these, non-coccidian parasites, was univariately associated with migration ($p = 0.05$) and the type of diet ($p = 0.03$). Non-coccidian parasites were more likely to be present in Passerines classified in clade 3 (OR: 5.9, 95% CI: 1.2–50, $p = 0.02$). Non-coccidian parasites are less likely to be detected in insectivorous than omnivorous passerines, which was not statistically supported by our data (OR adjusted for migration and species: 0.2, 95% CI: 0.01–0.8, $p = 0.09$).

The type of diet was univariately associated with the presence of *Syngamus* spp. ($p = 0.05$). *Syngamus* spp. were more likely to be detected in omnivorous than granivorous and insectivorous passerines. However, this also was not strongly statistically supported by our data (OR: 0.1, 95% CI: 0.01–0.8, $p = 0.09$).

Discussion

Some studies have revealed the prevalence of coccidial infection in certain species of birds in Europe (Zinke et al. 2004), whereas others have evaluated the role of foraging ecology in coccidian infections (Dolnik et al. 2010). Our study is the first study that investigated the association of intestinal parasites prevalence in European passerines with their phylogeny, migration habits and the type of diet. Our results indicated that the prevalence of intestinal parasites in passerines' faeces is 15.6%; the prevalence of intestinal parasites is not associated with migration, but is associated with the type of diet and overall phylogenetic relationship.

Although the prevalence of parasites in migratory passerines was higher than in non-migratory passerines, the difference was not found to be significant. Climate is an important determinant of transmission of parasitic infections, with adequate moisture and warm temperature essential for ova persistence and larval development in the soil or on surfaces (Brooker et al. 2006). It is expected that migratory birds have a greater chance to acquire parasite infections because by migrating they constantly dwell in a relatively warm environment throughout the year, which does not inhibit the parasite life cycle. They can be infected by parasites throughout the year, whereas non-migratory birds remaining in temperate latitudes are less likely to be infected during the cold winter period when parasites are not active. However, the effect of winter can be reduced in intensively cultivated habitats (farming) with concentrated bird or other animal population and higher micro-environmental temperatures (Tompkins and Begon 1999, Anderson 2000, Read and Taylor 2001, Hudson et al. 2006, Benchaoui 2010).

Most studies regarding the transmission of pathogens or potential pathogens are aimed at informing the human community about the risks for the disease transmission from birds to humans and/or farm animals. Seasonal bird migration has been associated with the geographic distribution of viruses (Eastern equine encephalitis virus, West Nile

virus, Influenza A virus, Newcastle disease virus), bacteria (*Anaplasma phagocytophilum*, *Borrelia burgdorferi*, *Campylobacter jejuni*, *Pasteurella multocida*, *Clostridium botulinum*, *Mycobacterium avium*), as well as protozoa and parasites (Hubálek 2004). Fewer studies, however, had the objective to recognize and follow pathogenic elements, including parasites that can be harmful only to birds. Knowing and following the prevalence and intensity of parasitic infection in any animal species may be important for future studies evaluating how global climate change may have influenced rates of parasites' prevalence (Tompkins and Begon 1999, Smallridge and Bull 2000). In the recent study by Lawson et al. (2011), trichomonosis was identified as a newly emerging significant disease in greenfinch *Carduelis chloris* population in England and continental Europe. *Trichomonas gallinae*, a protozoan parasite responsible for trichomonosis, was often isolated from pigeons, doves and birds of prey, and was not considered dangerous for garden birds (Dovc et al. 2004). However, it was recently recognized as a significant factor in an epidemic mortality and population decline of greenfinches *Carduelis chloris* and chaffinches *Fringilla coelebs* in UK/Europe (Lawson et al. 2011). Therefore, it is important that existing pathogens and potentially pathogenic organisms for specific bird population are recognized, including intestinal parasites (Thomas et al. 2005, Haque 2007, Benchaoui 2010). Our results show that Fringillidae (clade 7), which include greenfinches, together with tits (clade 4: Certhiidae, Paridae and Aegithalidae), are less likely to have parasites in their faeces than passerines classified in other clades. It is not possible to clearly define the reasons for clades 4 and 7 to be less likely positive for intestinal parasites in their faeces. Passerines from clades 4 and 7 are non-migratory birds and, based on their biology, less likely to come in close contact with concentrated source of infectious parasite particles in intensively cultivated farming environments.

Taxonomic diversification of passerines has been accompanied by extensive diversity, and has proved difficult to classify (Cramp 1988, 1992, Cramp and Perrins 1993, 1994a, b, Jancar et al. 1999, BirdLife International 2014). Molecular methods have put some more order into the taxonomy (Fregin et al. 2012, McCormack et al. 2013). However, passerines origin, phylogeny, biogeography, anatomical and morphological characteristics, migration habits and the type of diet in combination or individually can influence the presence and dissemination of intestinal parasites in passerines' faeces. These characteristics were all utilized to classify passerines in this study (Table 1).

Several parasites were detected in our bird faecal samples: *Eimeria* spp., *Isospora* spp., *Trichostrongylus tenuis*, *Syngamus* spp., cestoda ova and ascarid ova. Captive (Svobodová 1994, Quiroga et al. 2000) and wild (Svobodová 1994, McQuistion 2000) passerines can be infected with *Eimeria* spp. and *Isospora* spp., which can cause a serious clinical disease and high mortality (Giacomo et al. 1997). Less is known about the infection with *Trichostrongylus tenuis*, *Syngamus* spp., cestoda and ascarids in passerines. They can, however, significantly affect birds' health and behaviour and render them less competitive and more vulnerable in their environment, which is evident through reduced fitness to feed, court and/or breed. It can lead to either an evolutionary adaptation(s) of the host to a specific parasite, or

to a substantial decline or even an extinction of the species (Holmstad et al. 2006, Vergara et al. 2011, Martinez-Padilla et al. 2012).

Successful transmission of parasites is dependent on exposure of susceptible hosts to free-living infective stages, which are the specific parasite life cycle forms capable of infecting the host (infectious parasite particles). Parasites can be transmitted through animal (arthropod) vectors or invade feeding grounds (Anderson 2000). Some parasitic infective larvae are known to selectively ascend food plants to increase their chance to invade the bird host (Saunders et al. 2001). As evident from this study, insectivorous and omnivorous passerines are likely to be exposed to a variety of infectious parasite particles during their feeding, whereas granivorous passerines had only coccidia present in their faeces. Granivorous passerines are less likely to consume invertebrate vectors or seeds contaminated with infectious nematode/cestode parasite particles.

Omnivorous passerines are more likely to be infected with coccidia (*Eimeria* spp. and *Isospora* spp.). They are opportunistic feeders and often feed in the ecological niches, which are populated by humans or intensively cultivated by farming and are, therefore, more likely to be contaminated with greater number of infectious parasite particles (Mennerat et al. 2010). In such an environment birds can transmit parasites to mammals; transmission of *Ascaris* spp. from chickens to pigs was reported by Permin et al. (2000).

The presence of *Syngamus* spp. was associated with the type of diet. Our data also indicated that *Syngamus* spp. was more likely to be detected in omnivore passerines. *Syngamus* spp. is the gapeworm of poultry and many species of wild birds (Anderson 2000, Permin et al. 2000). Wild birds may serve as the source of *Syngamus* infections in outbreaks on poultry and game-bird farms, and vice versa (Anderson 2000). It is possible that omnivores come in closer contact with farmed bird species when seeking food, therefore, entering the concentrated source of *Syngamus* spp.

Parasites can negatively affect many species of wildlife and are the evolutionary partner in the development of the host-parasite interaction (Tompkins and Begon 1999, Read and Taylor 2001). It is important to realise that erratic changes in the environment can cause acute adaptations in microbes/parasites or in the host, which can be expressed with a variable degree of lethality for the host population (Casadevall and Pirofski 2000, Maizels and Yazdanbakhsh 2003, Hudson et al. 2006). If evolution is given time, the host-parasite relationship can co-evolve and again become harmonious enabling sustainability of both populations over time (Maizels and Yazdanbakhsh 2003, Holmstad et al. 2006, Vergara et al. 2011, Martinez-Padilla et al. 2012).

Evaluation of faeces for evidence of parasites in small birds is challenging (Dolnik 2006). A flotation method was used to concentrate faecal parasitic forms in this study. Passerine birds produced small amounts of faeces (< 1 g) and fed on abundant fruit/seed on their autumn migration, which discoloured their faeces and rendered sedimentation or a smear investigation less appropriate. Flotation method used in this study resulted in a clean sample preparation for microscopic examination with a minimal amount of distracting discoloration. Some parasite eggs, however, do not float in saline solution (Thienpont et al. 1979), and may have been missed using this method only.

The presence of parasites in passerine faeces was not strongly influenced by migration, which is intriguing because non-migratory passerines should not be exposed to infective parasite stages during the winter, whereas migratory passerines are constantly pressure by parasite infection in the warm environment.

One quarter of all European breeding passerines and near-passerine birds migrate from the European continent to sub-Saharan Africa in autumn each year, which is estimated to be over 2 billion birds (Moreau 1972, Hahn et al. 2009). Such migration can have an impact on local communities and can affect the health of birds or other animals, and humans. Perhaps most importantly in the long term, warmer global temperatures may favour parasite development and infectivity, which may significantly impact the health of several animal populations. Therefore, studies like ours are important. We need to investigate and follow the prevalence of parasites and other etiological factors that can have a negative effect on passerine bird populations. In short, only by knowing the present epidemiological situation offers the possibility to examine the influence of global warming on the prevalence of parasites in passerines.

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Supplementary material (Appendix wlb.00044 at <www.wildlifebiology.org/readers/appendix>). Appendix 1.