Ornis Fennica 89:34–43. 2012

Bacterial prevalence in the Dunnock (*Prunella modularis*) in sub-alpine habitats of the Western Carpathians, Slovak Republic

Jana Kisková, Zuzana Hrehová, Marián Janiga, Martin Lukáň, Martina Haas & Zuzana Čuvalová

J. Kisková, Z. Hrehová, M. Janiga, M. Lukáň & M. Haas, Institute of High Mountain Biology, University of Žilina, Tatranská Javorina 7, 059 56 Slovak Republic. Correspondent author's e-mail jakiskova@gmail.com
Čuvalová, Z., State Veterinary and Food Institute Dolný Kubín, Janoškova 1611/58, 026 01 Dolný Kubín, Slovak Republic. E-mail cuvalova@svpudk.sk

Received 3 November 2010, accepted 25 July 2011

Birds may be important vectors of bacterial infections. The prevalence of some bacterial species in the digestive tract of the Dunnock (*Prunella modularis*), the numerically dominant avian species of sub-alpine habitats of the Western Carpathians, Slovak Republic, was studied. Species of bacteria were detected in pharyngeal and cloacal swabs obtained from 97 individuals using PCR assay. Seven *Yersinia* species were found in the samples, with the highest prevalence of *Y. enterocolitica* (34.0% of individuals). Three other genera of the family Enterobacteriaceae were detected in the samples: *Serratia* spp. (26.8%), *Erwinia* spp. (15.5%) and *Pantoea* spp. (45.4%). *Erwinia* spp. showed a significantly higher prevalence in the pharynx than in the cloaca. Three non-Enterobacteriaceae species were detected using species-specific primers: *Pseudomonas fluorescens* (39.2%), *Pseudomonas fragi* (28.9%) and *Stenotrophomonas maltophilia* (13.4%). Generally, bacterial contamination of birds was higher in April than in May–July. *Pseudomonas fluorescens* showed statistically significant differences between months and between adults and juveniles, while the occurrence of other types of bacteria was marginally lower in juveniles than in adults. The bacterial incidence and richness were similar between males and females.

1. Introduction

Bacterial micro-organisms may play an important role in the life of their hosts. Some gastro-intestinal micro-oganisms, for example, affect the correct functioning of the intestine, the utilization of nutrients, protect against pathogenic organisms and stimulate the immune response of the organism (Kyle & Kyle 1993). There are bacteria that survive in the host but do not affect its physical condition or behavior. On the other hand, some bacterial groups may negatively influence individual fitness and reproductive success and therefore represent an important factor in natural and sexual selection (Zuk 1991). The Enterobacteriaceae are a large family of Gram-negative, facultative anaerobic

bacteria some of which can be a normal part of the intestinal flora in humans and animals, while others are commonly found in water or soil. Some species parasitize on a variety of different organisms causing various infectious diseases (Ritchie *et al.* 1994, Saif *et al.* 2003).

Due to their great mobility, wild birds may play a significant role as sources and effective spreaders of bacterial species through fecal contamination of pastures and surface waters (Hamasaki *et al.* 1989, Kaneuchi *et al.* 1989, Niskanen *et al.* 2003). The genera *Shigella* and *Edwardsiella* do not normally occur in birds, the genera *Enterobacter*, *Hafnia*, *Serratia* and *Proteus* have a low avian pathogenicity, and genera such as *Escherichia*, *Salmonella* and *Yersinia* can cause serious illnesses, especially in poultry and other domestic birds (e.g., parrots, zebra finches, pigeons), but also in wild birds (Ritchie *et al.* 1994, Saif *et al.* 2003)

Only a few studies on bacteria in wild birds have previously been done in the sub-alpine and alpine vegetation zones. Janiga *et al.* (2006) identified the composition of microflora in the digestive tract of the Alpine Accentor (*Prunella collaris*), while Novotný *et al.* (2007) focused specifically on yersiniosis in this species. These authors described a high incidence of *Yersinia enterocolitica* (73%) and other *Yersinia* species (51%), and suggested that the genus *Yersinia* may play an important role in the ecology of Alpine Accentor. *Yersinia* is a heterogeneous group of Enterobacteriaceae, capable of persisting and growing with high prevalence in cold water and soil (Chernyanskii 1981, Kato *et al*. 1985, Gill & Reichel 1989, Fukushima *et al*. 1990). Pathogenic strains of *Yersinia* species may cause infectious gastroenteritis of their host (Nikolova *et al.* 2001).

The present study represents research done on *Yersinia* in the lower sub-alpine vegetation zone in central Europe. The Dunnock (*Prunella modularis*) was selected as the focal species as it is a numerically dominant bird species in dwarf-pine mountainous ecosystems at 950–1,700 m a.s.l. (Cramp 1988). The present study aimed at identifying bacteria within the genus *Yersinia* in the Dunnock, using methods originally developed for the Alpine Accentor by Novotný *et al.* (2007). Therefore, CIN agar was chosen. It is widely used as a specific cultivation medium for *Yersinia* species (Schiemann 1979) and is highly selective against the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Salmonella typhimurium*, *Shigella sonnei* and *Streptococcus faecalis* (Schiemann 1979, 1982). However, other genera such as *Citrobacter*, *Serratia*, *Enterobacter*, *Pantoea*, *Erwinia* or *Pseudomonas* may grow on it (Schiemann 1979, Neubauer 2000). Hence, in addition to *Yersinia* species, other types of bacteria were identified. Moreover, as the bacterial prevalence may be influenced by age and sex of the host species, and exhibit seasonal dynamics (Lombardo 1998, Lombardo & Thorpe 2000, Lucas & Heeb 2005, Janiga *et al.* 2006), the impact of these factors in affecting bacterial contamination was examined.

2. Material and methods

2.1. Sample collection

From April 2007 to July 2009, 97 free-living Dunnocks were captured with mist nets in the mountains of the Slovakian part of the Western Carpathians (from 1,000 to 1,750 m a.s.l.): the West, High, Low and Belianske Tatras, Great and Small Fatra, Babia hora, and Choč. Captured birds were aged as adults or juveniles (age ≤ 2 months). Adults were sexed by the presence of a cloacal protuberance in males (Nakamura 1990). Pharyngeal and cloacal swabs were taken for each bird with sterile swab shafts and they were placed in the pre-packaged Amies charcoal transport medium (Copan Italia S.p.A., Brescia Italy). Swabs were taken to the laboratory within 48 hours after sampling. After sampling, the birds were released.

2.2. Isolation and bacterial identification, and data analysis

In the laboratory, each swab was placed in tryptic soy broth (Agrarian society s r.o., Division of culture media, Rosina, Slovak Republic) and incubated at 26–28°C for 48 hours. Enriched broth was inoculated onto CIN agar by loop (DispoLab s r.o., Žilina, Slovak Republic) and cultivated for 48 hours at 26–28°C (recommended cultivation temperature for *Yersinia* spp.) (Devenish & Schieman

Table 1. Nucleotide sequence of the primers for amplifying the species-specific region of selected genes.

Bacteria	Examined gene	Primers	PCR product	Reference
Yersinia spp.	16S rRNA	Y1: 5'-AATACCGCATAACGTCTT-3' Y2: 5'-CTTCTTCTGCGAGTAACGTC-3'	330 bp	Neubauer *et al.* 2000
	ail gene	A1: 5'-TTAATGTGTACGCTGGGAGTG-3' A2: 5'-GGAGTATTCATATGAAGCGTC-3'	425 bp	Wannet *et al.* 2001
Pseudomonas fluorescens	16S rRNA	16SPSEfluF: 5'-TGCATTCAAAACTGACTG-3' 16SPSER: 5'-AATCACACCGTGGTAACCG-3'	850 bp	Scapellini *et al.* 2004
Pseudomonas fragi	carA	FraF: 5'-CGT CAG CAC CGA AAA AGC C-3' FraR: 5'-TGATGRCCSAGGCAGATRCC -3'	370 bp	Ercolini *et al.* 2007
Stenotrophomonas maltophilia	23S rRNA	SM1: 5'-CAGCCTGCGAAAAGTA-3' SM4: 5'-TTAAGCTTGCCACGAACAG-3'	531 bp	Whitby *et al.* 2000

1981, Head *et al.* 1982, Neubauer *et al.* 2000, Hussein *et al.* 2001, Nikolova *et al.* 2001).

In bacterial DNA extraction, one inoculation loop of mixed bacterial growth taken from CIN agar was suspended in 200 µl of lysis buffer (TE buffer + 1% Triton X-100, pH = 8). The suspension was incubated at 95°C for 10 min and then centrifuged at 12,000 xg for 5 min. Isolated mixed bacterial DNA was diluted to a concentration of 20 ng/µl in TE buffer (10mM TRIS-Cl, 1 mM EDTA, pH = 8) and stored at –20°C.

Yersinia was identified using the PCR method developed by Neubauer *et al.* (2000). The authors designed primers Y1 and Y2 for amplification of the specific region of the 16S rRNA gene of the genus *Yersinia*. Primers A1 and A2 were used to amplify a 430 bp fragment of the *ail* gene, found exclusively in pathogenic *Yersinia* strains (Wannet *et al.* 2001). DNA samples were amplified in a total volume of 25 µl which included 40–50 ng of the mixed bacterial DNA, 1x PCR buffer, 0.5 µM of each primers, 200 µM dNTPs, 1.5mM $MgCl_2$, recombinant 0.5U Taq DNA Polymerase (Invitrogen). Amplification was performed in a Techne thermal cycler. PCRs were conducted using the following conditions: initial denaturation at 94°C for 5 min, followed by 36 subsequent cycles: 94°C for 45 s, 58°C for 45 s, 72°C for 1 min, and the final extension at 72°C for 7 min. The PCR products were visualized on a 2% agarose gel stained with ethidium bromide. For the identification of *Yersinia* species, a reference strain (CCM 5671) of *Y. enterocolitica* ssp. *enterocolitica*, serovar 0:3, biovar 4 was obtained from the Czech collection of microorganisms, Masaryk University, Brno.

The PCR products were independently sequenced in both directions on a Genome Lab GeXP Single genetic analyzer (Beckman Coulter Inc.) and assembled with the sequencer software. The obtained sequences were analyzed by BLAST (GenBank) for the species identification. Our isolates were affiliated to strain cluster (species) on the basis of similarity threshold of 98.5% with 16S rRNA gene sequences in the GeneBank database, according to present day taxonomic practice (Stackebrant & Ebers 2006, Cousin *et al.* 2008).

The presence of *Y. pseudotuberculosis* in samples was verified also by another PCR assay using an AmpliSens *Yersinia pseudotuberculosis* kit (a special PCR-diagnostic kit for *Y. pseudotuberculosis* developed by the Central Research Institute of Epidemiology of Federal State Institution of Science, Moscow, Russia). Serotypes of the isolated *Yersinia* species were not identified.

In some samples, the presence of *Yersinia* was not confirmed using the method described above. Thus, these *Yersinia*-negative samples may have represented species of bacteria other than *Yersinia*: other types of bacteria species could also grow on CIN agar. Therefore, samples of bacterial cultures negative for Yersinia were frozen in Skim Milk Powder (Oxoid) and sent for analysis to the State Veterinary and Food Institute in Dolný Kubín, Slovakia. Here, bacterial cultures were inoculated onto blood agar, Endo agar and CIN agar and cultivated (24–48 hours) at two different temperatures, viz. 25°C and 37°C. Pure bacterial isolates were identified by colony morphology and examined by conventional biochemical tests (catalase and oxidase tests) and API 20E, API

Table 2. Species and prevalence of pharyngeal and cloacal bacteria in Dunnocks in the Western Carpathians, Slovakia.

Bacterial genus/species	16S rRNA gene	Identity (%)	Prevalence (n^a = 97) Total N^b (%)	Pharynx *N* (%)	Cloacae *N* (%)
***Yersinia* spp.**			46 (47.4)	28 (28.9)	27 (27.8)
Y. enterocolitica	FE 8073	100	33 (34.0)	20 (20.6)	17 (17.5)
	PO/Y/1-3	100			
	ARCTIC-P11	98.5			
Y. kristensenii	ATCC 33638	98.5	14 (14.4)	10 (10.3)	6 (6.2)
	991	99.5			
Y. molareti	H279-36/86	99.5	4 (3.1)	2 (2.1)	2 (2.1)
Y. intermedia	253	98.5	2 (2.1)	2 (2.1)	0 (0)
Y. aleksici	Y 388	98.5	2 (2.1)	2 (2.1)	0 (0)
Y. bercovieri	H632-36/85	98.5	2 (2.1)	2 (2.1)	1 (1.03)
Y. fredericksenii	WS 52/02	99.5	2 (2.1)	0 (0)	2 (2.06)
***Serratia* spp.**			26 (26.8)	20 (20.6)	10 (10.3)
No determined isolates	F2	100	1 (1)	(1)	1 (1)
	D1	99	1 (1)	1 (1)	0 (0)
	A7	99	1 (1)	1 (1)	0 (0)
	01WB03.2-5	99	1 (1)	0 (0)	1 (1)
S. liquefaciens	ATCC29573	99	14 (14.4)	9 (9.3)	5 (5.2)
S. proteamaculans	wg-2	100	13 (13.4)	9 (9.3)	5 (5.2)
S. fonticola	LR 15	100	11.(11.3)	11 (11.3)	2 (2.1)
S. plymouthica	ZW-22	100	7 (7.2)	4 (4.1)	3 (3.1)
S. grimesii	DSQ3	100	4 (4.1)	3 (3.1)	1 (1)
***Erwinia* spp.**			15 (15.5)	14 (14.4)	1 (1)*
No determined isolates	01WB03.2-26	100	5 (5.2)	4 (4.1)	1 (1)
	CYEB-26	100	5 (5.2)	4 (4.1)	0 (0)
E. persicina	BK 19	99	5 (5.2)	5 (5.2)	0 (0)
E. billingiae	Eb661	99.5	2 (2.1)	2 (2.1)	0 (0)
	EZ2-11-1	98.5			
E. rhapontici	BE 2-1-27	98.5	2 (2.1)	2 (2.1)	0 (0)
	N3SM14	98.5			
E. pyrifoliae	Ejp556	98.5	2 (2.1)	2 (2.1)	0 (0)
	Ep1/96	99			
E. amylovora	CFBP1430	100	2 (2.1)	2 (2.1)	0 (0)
	ATCC 49946	100			
Other					
Pantoea dispersa	G28A	99.5	44 (45.4)	35 (36.1)	25 (25.8)
	CIP 102701	98.5			
Pseudomonas fluorescens	ND^c	–	38 (39.2)	25 (25.8)	22 (22.7)
Pseudomonas fragi	ND	–	28 (28.9)	19 (19.6)	13 (13.4)
Stenotrophomonas maltophilia	ND	–	13 (13.4)	7 (7.2)	7 (7.2)

n^a = number of examined birds; N^b = number of positive individuals (pharyngeal and/or cloacal culture positive), relative value in brackets; ND^c – no determined strains; and * = significant difference in the prevalence between pharynx and cloaca (χ^2 $P \leq 0.05$).

20NE (bioMérieux SA, Marcy-l'Etoile, France) and ENTEROtest 24 systems (PLIVA-Lachema Diagnostica s.r.o., Brno, Czech Republic). Because other types of bacteria species were identified in these samples, swabs from all birds were subsequently examined for presence of these non-*Yersinia* species by PCR assay using original purified mixed DNA and species-specific primers (Table 1). A chi-square test (STATISTICA version 8) was used to compare differences in the bac-

Table 3. The prevalence of bacterial genera or species in the pharynx and cloacae of Dunnock, listed by host age and sex. Sample size (number of examined birds) for adults 80, juveniles 17, males 35 and females 45. The numerical values show the number (and percent) of positive individuals; *P* columns show results of chi-square comparisons between adults and juveniles, or between males and females (if significant, probability is given).

Bacterial genus/species	Adults	Juveniles	*P*	Males	Females	*P*
Yersinia spp.	41 (51.3)	5 (29.4)	–	16 (45.7)	25 (55.6)	–
Serratia spp.	22 (27.5)	3 (17.6)	–	8 (22.9)	14 (31.1)	–
Erwinia spp.	14 (17.5)	1 (5.9)	–	5 (14.3)	9 (20)	–
Pantoea dispersa	39 (48.8)	5 (29.4)	–	17 (48.6)	22 (48.9)	–
Pseudomonas fluorescens	35 (43.8)	3 (17.7)	0.04	15 (42.9)	20 (44.4)	–
Pseudomonas fragi	26 (32.5)	2 (11.8))	–	10 (28.6)	16 (35.6)	–
Stenotrophomonas maltophilia	10 (12.5)	3 (17.7)	–	5 (14.3)	5 (11.1)	–

terial prevalence depending on sex, age and season.

3. Results

A summary of the bacteria identified in swabs from 97 Dunnocks is presented in Table 2. Four genera of the family Enterobacteriaceae: *Yersinia*, *Serratia*, *Erwinia* and *Pantoea* were identified using PCR methods according to Neubauer *et al.* (2000) and a comparison of PCR-products to the nucleotide sequences were done in BLAST (GenBank). *Yersinia* showed the highest prevalence of these bacteria (47.4%). Seven *Yersinia* species were detected in the samples with the highest prevalence of *Y. enterocolitica* (34.0%). *Yersinia pseudotuberculosis* was not detected in any of pharyngeal or cloacal swabs, even when using the special AmpliSens *Yersinia pseudotuberculosis* kit. The presence of the *ail* gene associated with the pathogenic strains of *Yersinia* was not detected in any of the examined samples.

Apart from members of the family Enterobacteriaceae, three other species were found using species-specific primers in the PCR: *Pseudomonas fluorescens*, *Pseudomonas fragi* and *Stenotrophomonas maltophilia*. Only *Erwinia* showed a significantly higher incidence in the pharynx (14.4%) than in the cloaca (1%). Such differences were not observed in the other bacterial groups.

All species of bacteria tended to occur with a higher frequency in adult individuals than in juveniles, except *Stenotrophomonas maltophilia*. However, a statistically significant difference was found only in *Pseudomonas fluorescens* (Table 3).

The distribution (richness) of bacterial species was not significantly linked with sex or age (Table 4). Adult females had a marginally greater variety of bacteria than had males or juveniles, particularly in terms of *Yersinia* species. Only *Y. enterocolitica* and Y. *kristensenii* were detected in males and juveniles, while these and the other *Yersinia* species were found in adult females. Most females were contaminated with either *Y. enterocolitica* or *Y. kristensenii*, but one female was contaminated with four, one with five and one with six different *Yersinia* species.

Species of the genus *Serratia* occurred with a similar distribution in both sexes: one to four species per bird. In juveniles, only three types of *Serratia* (two to three species per bird) were de-

Table 4. The richness of bacterial species in Dunnocks according to host age and sex, expressed as arithmetic mean ± SD of bacterial species per bird. All chi-square comparisons between adults and juveniles, or between males and females were statistically non-significant.

Variable	Total	Adults	Juveniles	Males	Females
Mean ± SD	4.21 ± 2.52	4.18 ± 2.48	4.50 ± 3.08	3.92 ± 2.30	4.36 ± 2.62
Sample size	63	57	6	24	33

Table 5. The prevalence of bacterial genera in pharynx and cloacae in Dunnocks according to the month of sampling. Sample sizes were 10 for April, 32 for May, 25 for June and 30 for July. The numerical values show the number (and percent) of positive individuals; the *P* column shows results of chi-square comparisons between adults and juveniles, or between males and females (if significant, probability is given).

Bacterial genus/species	April	May	June	July	*P*
Yersinia spp.	8 (80)	15 (46.9)	10 (40)	13 (43.3)	–
Serratia spp.	4 (40)	8 (25)	6 (24)	7 (23.3)	–
Erwinia spp.	2 (20)	6 (18.8)	3 (12)	4 (13.3)	–
Pantoea dispersa	8 (80)	17 (53.1)	7 (28)	10 (33.3)	–
Pseudomonas fluorescens	8 (80)	14 (43.8)	7 (28)	7 (23.3)	0.01
Pseudomonas fragi	4 (40)	11 (34.4)	7 (28)	5 (16.7)	–
Stenotrophomonas maltophilia	3 (30)	3 (9.4)	2 (8)	2 (6.7)	–

tected. The distribution of *Erwinia* species varied between one and two species per individual. All five species were detected in females, three in males (*E. rhapontici*, *E. persicina and E. billingiae)* and only one strain in juveniles (no determined species 01WB03.3-26).

The prevalence of all bacterial groups peaked in April (between 20% and 80%). In the subsequent months, the incidence decreased in most bacteria to 20%–30%, but only *Pseudomonas fluorescens* showed a statistically significant difference among the four months of collection (Table 5).

4. Discussion

We detected several bacterial genera/species and seven species of *Yersinia* in the digestive tracts of Dunnocks. Among these seven, *Yersinia enterocolitica* showed the highest prevalence, which corresponds with Novotný *et al.* (2007) in the congeneric Alpine Accentor living in alpine zone of high mountains. Other *Yersinia* species were not determined in their study. The rich bacterial fauna, reported here for the Dunnock, differs somewhat from results of other studies. Janiga *et al.* (2006) reported that *Y. enterocolitica* heavily dominated the bacterial flora in the Alpine Accentor, with *Yersinia intermedia* detected in only one accentor individual. Moreover, contrary to the present study, Janiga *et al.* (2006) did not report *Erwinia*, *Pseudomonas fragi* or *Stenotrophomonas maltophilia*. These differences could be caused by different use of habitats between these species, but also by the low number of examined individuals of Alpine Accentors, and the use of different methods in identifying bacteria.

Avian bacterial contaminations follow seasonal dynamics in which social behavior, food, physiological state, sex or some environmental conditions may play a role in shaping these dynamics (Lombardo 1998, Faustino *et al.* 2004, Lucas & Heeb 2005). Indeed, bacterial transmission in birds takes place through infected food, self-preening (Lamberski *et al.* 2003, Zampiga *et al.* 2004), allo-preening (Adkins-Regan & Robinson 1993, Pozis-Francois *et al.* 2004) and copulation (Sheldon 1993, Lombardo 1998, Kulkarni & Heeb 2007). The highest prevalence of all bacterial species in the present study was detected in April. In the present study area, this is also the beginning of the breeding season of Dunnocks – the birds begin to mate. Avian copulation involves cloacal contact, which has been documented to host rich bacterial communities (Lombardo 1998, Lombardo & Thorpe 2000, Lucas & Heeb 2005). Hence, the risks of bacterial transmission during copulation are expected to be particularly high. Dunnocks have variable mating systems, from monogamy over polyandry to polygynandry (Davies 1985), and copulation with multiple partners further increases the risk of bacterial contamination and spread.

Certain types of bacteria are adapted to cold environments, such as *Yersinia* species (Chernyanskii 1981, Kato *et al*. 1985, Gill & Reichel 1989, Fukushima *et al*. 1990), *Pantoea dispersa* (Selvakumar *et al.* 2008) and *Pseudomonas* species (Meyer *et al.* 2004). Therefore, it is not surprising to find these bacteria in the climatic conditions of the present study, with high prevalence in April

(late winter and early spring). Similarly, Janiga e*t al.* (2006) detected the highest prevalence of *Y. enterocolitica* in the Alpine Accentor in April.

Interestingly, only *Pseudomonas fluorescens* showed a statistically significant variation in prevalence depending on the month of collection. The alpine environment is characterized by marked environmental changes which influence the spatial distribution of carbon in soil, and the great metabolic flexibility allows *Pseudomonas* to utilize carbon from different sources and thus inhabit variable environments (Palleroni *et al.* 1972, Lipson *et al.* 2000). Lipson *et al.* (2000) described an increase of microbial biomass during fall and winter, followed by a rapid decline after snow-melt in the spring. A similar shift in metabolic activity by *Pseudomonas fluorescens* might thus explain the significant decrease in the prevalence from April to May–July, when the the snow begins to melt in the present study area. This theory receives support from other studies: Mancinelli *et al*. (1984) identified this bacterium as being the most prevalent genus cultured from the alpine tundra soil, Meyer *et al*. (2004) described the isolation and characterization of 17 cold-tolerant strains of fluorescent *Pseudomonas* from high-alpine soil in Colorado, and Janiga *et al.* (2006) detected *Pseudomonas* only in cold months in the Alpine Accentor. A significantly lower prevalence of *Pseudomonas fluorescens* in juveniles than in adults also suggests a lower incidence of this bacterium in the sub-alpine environment in the warmer months.

Diet may influence the composition of bacterial flora in birds (Brittingham *et al.* 1988). All of the bacteria reported in the present study are ubiquitous in nature and have been isolated from water, soil and plants (Chernyanskii 1981, Gavini *et al.* 1989, Berg *et al.* 1999, Meyer *et al.* 2004). Therefore, they can be transferred to the bird through food (e.g., contaminated water or insects) and colonize its digestive tract. *Erwinia* species are exclusive parasites of plants and do not grow or survive in animals (Beer 1979, Huang *et al.* 2002, Gonzáles 2007). This fact could be the cause of their significantly higher prevalence in the pharynx compared to the cloaca. Thus, *Erwinia* species can infect the oral cavity, specifically the pharynx, but it does not survive in the intestine.

A larger spectrum of some bacterial species was found in females than in males, although significant differences in the prevalence of bacteria between sexes were not found. There is a relationship between bacterial transmission and sex in birds, leading to an asymmetry in bacterial occurrence between sexes, which can be explained by the fact that males transfer sperm to females and, given the brevity of copulation, males have a lower chance of bacterial infection from the cloacal contact (Sheldon 1993, Lombardo & Thorpe 2000, Westneat & Rambo 2000, Kulkarni *et al.* 2007). Females, however, have prolonged exposure to the ejaculate (potentially contaminated by bacteria) once it enters the reproductive tract, and so the possibility of infection during the breeding season is higher. Brittingham *et al.* (1988) reported differences in occurrence of some bacterial species between granivorous (e.g., passerines) and omnivorous (e.g., woodpeckers) bird species, but did not find differences between males and females in any of the investigated species. They speculated that the prevalence of bacteria may be influenced more by a bird's food than by their social organization. The genus *Yersinia* showed no significant differences in distribution between males and females in the Dunnock (present study) or in the Alpine Accentor (Novotný *et al.* 2007). Based on these observations, sex may not be the decisive factor in the distribution of bacteria among individuals, and both sexes are equally at risk of bacterial contamination from the environment, at least for the two dunnock species and in the studied environment.

There was a tendency towards a lower incidence of bacterial contamination in juvenile than in adult Dunnocks, as earlier reported for the Alpine Accentor for the same genera of bacteria (*Yersinia*, *Serratia* and *Pantoea*; Janiga *et al.* 2006). Similarly, Novotný *et al.* (2007) reported a lower prevalence of *Yersinia* in juvenile than in adult Alpine Accentors. The lower occurrence in juveniles suggests that bacterial contamination in birds increases with age. Young birds may thus be particularly frequently colonized by bacteria during their first breeding season.

Two findings, reported here, are of methodological importance. Firstly, the use of just one PCR array allows several bacterial species or genera to be detected. The genetic stability of the 16S rRNA gene sequence allows it to be used for molecular identification of many bacteria species (Neubauer 2000). However, because the target se-

quences of both primers, Y1 and Y2, are presented in some *Yersinia*, *Serratia*, *Erwinia* and *Pantoea* species, these bacteria may also give rise to a PCR product of roughly the same size (Neubauer 2000). Secondly, CIN agar is a recommended specific medium for *Yersinia* cultivation (Schiemann 1979), but as the present results demonstrate, some other bacterial genera or species may also grow on this medium, such as some groups of Enterobacteriaceae, *Pseudomonas* species or *Stenotrophomonas malthophilia*.

To conclude, the genus *Yersinia* occurs with a high frequency in Dunnocks inhabiting the sub-alpine zone of the Slovak Western Carpathians. Sex and age did not influence the bacterial prevalence in Dunnocks, although their effect has been reported for other bird species (see above). The highest prevalence of bacteria was detected in early spring (April), which is the mating season of Dunnocks. We suggest contact during mating increases the risk of a bacterial contamination in bird individuals. This mechanism is ecologically important, as some strains of *Yersinia enterocolitica* can be pathogenic (see above), although no pathogenic strains were detected in the present samples. The other bacterial species determined here are relatively harmless and common environmental bacteria. Hence, these can be identified as representing normal transient components of microflora in Dunnocks.

Rautiaisen elimistön bakteerit subalpiinisessa ympäristössä Länsi-Karpaateilla, Slovakiassa

Linnut voivat olla merkittäviä bakteeri-infektioiden välittäjiä. Tässä tutkimuksessa selvitettiin joidenkin bakteerilajien esiintyvyyttä rautiaisen (*Prunella modularis*) ruuansulatuselimistössä. Rautiainen on runsain lintulaji Länsi-Karpaateilla Slovakiassa. Bakteerinäytteitä otettiin kurkusta ja peräaukosta kaikkiaan 97 yksilöltä ja käsiteltiin PCR-tekniikalla. Näytteistä löytyi seitsemän *Yersinia*-lajia, joista *Y. enterocolitica* oli yleisin (34,0 % rautiaisyksilöistä). Näytteistä löytyi myös kolmen muun Enterobacteriaceae-heimon edustajia: *Serratia*- (26,8 %), *Erwinia*- (15,5 %) ja *Pantoea*-suvun lajeja (45,4 %). *Erwinia*-bakteereita löytyi merkitsevästi yleisemmin kurkusta kuin peräaukosta.

Kolme muuta, Enterobacteriaceae-heimoon kuulumatonta, lajia olivat *Pseudomonas fluorescens* (39,2 %), *Pseudomonas fragi* (28,9 %) ja *Stenotrophomonas maltophilia* (13,4 %). Yleisesti ottaen bakteeriprevalenssi oli korkeampi huhtikuin touko–heinäkuussa, mutta vain *Pseudomonas fluorescens*-lajilla oli merkitseviä eroja kuukausien ja rautiaisikäluokkien välillä. Bakteerien esiintyvyys ja lajirikkaus olivat samanlaisia sukupuolten välillä.

References

Adkins-Regan, E. & Robinson, T.M. 1993: Sex differences in aggressive behaviour in Zebra finches (*Poephila guttata*). — Journal of Comparative Psychology 107: 223–229.

Beer, S.V. 1979: Fireblight inoculum: sources and dissemination. — Bulletin OEPP/EPPO Bulletin 9: 13–25.

Berg, G., Roskot, N. & Smalla, K. 1999: Genotypic and phenotypic relationships between clinical and environmental isolates of *Stenotrophomonas maltophilia*. — Journal of Clinical Microbiology 37: 3594–600.

Brittingham, M.C., Temple, S.A. & Duncan, R.M. 1988: A survey of the prevalence of selected bacteria in wild birds. — Journal of Wildlife Diseases 24: 299–307.

Cousin, S., Brambilla, E., Yang, J. & Stackebrandt, E. 2008: Culturable aerobic bacteria from the upstream region of a karstwater rivulet. — International Microbiology 11: 91–100.

Cramp, S. 1988: The birds of the Western Palearctic. Vol. 5. — Oxford University Press. Oxford.

Davies, N.B. 1985: Cooperation and conflicts among dunnocks, *Prunella modularis*, in a variable mating system. — Animal Behaviour 33: 628–648.

Devenish, J.A. & Schieman, D.A. 1981: An abbreviated scheme for identification of *Yersinia enterocolitica* isolated from food enrichments on CIN agar. — Canadian Journal of Microbiology 27: 937–941.

Ercolini, D., Russo, F., Blaiotta, G., Pepe, O., Mauriello, G. & Villani, F. 2007: Simultaneous detection of *Pseudomonas fragi*, *P. lundensis* and *P. putida* from meat by use of a multiplex PCR assay targeting the *carA* gene. — Applied and Environmental Microbiology 73: 2354–2359.

Faustino, C.R., Jennelle, C.S., Connolly, V., Davis, A.K., Swarthout, E.C., Dhondt, A.A. & Cooch, E.G. 2004: *Mycoplasma gallisepticum* infection dynamics in a house finch population: seasonal variation in survival, encounter and transmission rate. — Journal of Animal Ecology 73: 651–669.

Fukushima, H., Gomyoda, M. & Kaneko, S. 1990: Mice and moles inhabiting mountainous areas of Shimane peninsula as sources of infection with *Yersinia*

pseudotuberculosis. — Journal of Clinincal Microbiology 28: 2448–2455.

Gavini, F,. Mergaert, J., Beji, A., Mielcarek, Ch., Izard, D., Kersters, K. & De Ley, J. 1989: Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. — International Journal of Systematic Bacteriology 39: 337–345.

Gill, C.O. & Reichel, M.P. 1989: Growth of the cold-tolerant pathogens *Yersinia enterocolitica*, *Aeromonas hydrophila* and *Listeria monocytogenes* on high-pH beef packaged under vacuum or carbon dioxide. — Food Microbiology 6: 223–230.

Gonzáles, A.J. 2007: *Erwinia persicina* causing chlorosis and necrotic spots in leaves and tendrils of *Pisum sativum* in Southeastern Spain. — Disease Notes 91: 460.

Hamasaki, S.I., Hayashidani, H., Kaneko, K.I., Ogawa, M. & Shigeta, Y. 1989: A survey for *Yersinia pseudotuberculosis* in migratory birds in coastal Japan. — Journal of Wildlife Diseases 25: 401–403.

Head, C.B., Whitty, D.A. & Ratnam, S. 1982: Comparative study of selective media for recovery of *Yersinia enterocolitica*. — Journal of Clinical Microbiology 16: 615–621.

Huang, H.C., Erickson, R.S., Yanke, L.J., Mundel, H.H. & Hsieh T.F. 2002: First report of pink seed of common bean caused by *Erwinia rhapontici*. — Plant Disease 86: 921

Hussein, H.M., Fenwick, S.G. & Lumsden, J.S. 2001: A rapid and sensitive method for the detection of *Yersinia enterocolitica* strains from clinical samples. — Letters of Applied Microbiology 33: 445–449.

Chernyanskii, V.F. 1981: The simultaneous isolation of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* in the arctic regions. — Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii 6: 104–105.

Janiga, M., Sedlárová, A., Rigg, R. & Novotná, M. 2006: Patterns of prevalence among bacterial communities of alpine accentors (*Prunella collaris*) in the Tatra Mountains. — Journal of Ornithology 148: 135–143.

Kaneuchi, C.H., Shibata, M., Kawasaki, T., Kariu, T., Kanzaki, M. & Maruyama, T. 1989: Occurrence of *Yersinia spp*. in migratory birds, ducks, seagulls and swallows in Japan. — Japanese Journal of Veterinary Research 51: 805–808.

Kato, Y., Ito, K., Kubokura, Y., Maruyama, T., Kaneko, K.I. & Ogawa M. 1985: Occurrence of *Yersinia enterocolitica* in wild-living birds and Japanese serows. — Applied and Environmental Microbiology 49: 198–200.

Kulkarni, S. & Heeb, P. 2007: Social and sexual behaviours aid transmission of bacteria in birds. — Behavioural Processes 74: 88–92.

Kyle, P.D. & Kyle, G.Z. 1993: An evaluation of the role of microbial flora in the saliva transfer technique for handrearing chimney swifts (*Chaetura pelagica*). — Wildlife Rehabilitation 8: 65–71.

Lamberski, N., Hull, A.C., Fish, A.M., Beckmen, K. & Morishita, T.Y. 2003: A survey of the choanal and cloacal aerobic bacterial flora in free-living and captive red-tailed hawks (*Buteo jamaicensis*) and Cooper's hawks (*Accipiter cooperii*). — Journal of Avian Medicine and Surgery 17: 131–135.

Lipson, D.A., Schmidt, S.K. & Monson, R.K. 2000: Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass. — Soil Biology and Biochemistry 32: 441–448.

Lombardo, M.P. & Thorpe, P.A. 2000: Microbes in tree swallow semen. — Journal of Wildlife Diseases 36: 460–468.

Lombardo, M.P. 1998: On the evolution of sexually transmitted diseases in birds. — Journal of Avian Biology 29: 314–321.

Lucas, F.S. & Heeb, P. 2005: Environmental factors shape cloacal bacterial communities in great and blue tit nestlings. — Journal of Avian Biology 36: 510–516.

Mancinelli, R.L. 1984: Population-dynamics of alpine tundra soil bacteria, Niwot Ridge, Colorado Front Range, USA. — Arctic and Alpine Research 16: 185–192.

Meyer, A.F., Lipson, D.A., Martin, A.P., Schadt, W. & Schmidt S.K. 2004: Molecular and metabolic characterization of cold-tolerant alpine soil *Pseudomonas* sensu stricto. — Applied and Environmental Microbiology 70: 483–489.

Nakamura, M. 1990: Cloacal protuberance and copulation behavior of the alpine accentor (*Prunella collaris*). — Auk 107: 284–295.

Neubauer, H., Hensel, A., Aleksic, S. & Meyer, H. 2000: Identification of *Yersinia enterocolitica* within the genus *Yersinia*. — Systematic and Applied Microbiology 23: 1–5.

Nikolova, S., Tzvetkov, Y., Najdenski, H. & Vesselinova, A. 2001: Isolation of pathogenic *Yersiniae* from wild animals in Bulgaria. — Journal of Veterinary Medicine 48: 203–209.

Niskanen, T., Waldenström, J., Fredriksson, A.M., Olsen, B. & Korkeala, H. 2003: *virF*-Positive *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* found in migratory birds in Sweden. — Applied and Environmental Microbiology 69: 4670–4675.

Novotný, M., Fečková, M., Janiga, M., Lukáň, M., Novotná, M. & Kovalčíková, Z. 2007: High incidence of *Yersinia enterocolitica* (Enterobacteriaceae) in alpine accentors *Prunella collaris* of the Tatra Mountains. — Acta Ornithologica 42: 137–143.

Palleroni, N.J. & Doudoroff M. 1972: Some properties and taxonomic subdivisions of genus *Pseudomonas*. — Annual Review of Phytopathology 10: 73–100.

Pozis-Francois, O., Zahavi, A. & Zahavi, A. 2004: Social play in Arabian babblers. — Behaviour 141: 425–450.

Ritchie, B.W., Harrison G.J. & Harrison L.R. 1994: Avian medicine: principles and application. — Lake Worth, FL Wingers Publishing Inc., Florida.

Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M.,

McDougald, L.R. & Swayne, D.E. 2003: Diseases of poultry. — Wiley-Blackwell Publishing, Iowa State Press.

Scarpellini, M., Franzetti, L. & Galli, A. 2004: Development of PCR assay to identify *Pseudomonas fluorescens* and its biotype. — FEMS Microbiological Letters 236: 257–260.

Selvakumar, G., Kundu S., Joshi, P., Nazim, S., Gupta, A.D., Mishra, P.K. & Gupta H.S. 2008: Characterization of a cold-tolerant plant growth-promoting bacterium *Pantoea dispersa* 1A isolated from a sub-alpine soil in the North Western Indian Himalayas. — World Journal of Microbiology and Biotechnology 24:955–960.

Sheldon, B.C. 1993: Sexually-transmitted disease in birds – occurrence and evolutionary significance. — Philosophical Transactions of the Royal Society of London Ser. B 339: 491–497.

Schiemann, D.A. 1979: Synthesis of a selective agar medium for *Yersinia enterocolitica*. — Journal of Microbiology 25: 1298.

Schiemann, D.A. 1982: Development of a two-step enrichment procedure for recovery of *Yersinia enterocolitica* from food. — Applied and Environmental Microbiology 43: 14.

Stackebrant, E., & Ebers, J. 2006: Taxonomic parameters revisited: tarnished gold standards. — Microbiology Today 33: 152–155.

Wannet, W.J.B., Reessink, M., Brunings, H.A. & Maas, H.M.E. 2001: Detection of pathogenic *Yersinia enterocolitica* by rapid and sensitive duplex PCR Assay. — Journal of Clinical Microbiology 39: 4483–4486.

Westneat, D.F. & Rambo, T.B. 2000: Copulation exposes female red-winged blackbirds to bacteria in male semen. — Journal of Avian Biology 31: 1–7.

Whitby, P.W., Carter, K.B., Burns, J.L., Royall, J.A., Lipuma, J.J. & Stull, T. L. 2000: Identification and detection of *Stenotrophomonas maltophilia* by rRNA-directed PCR. — Journal of Clinical Microbiology 38: 4305–4309.

Zampiga, E., Hoi, H. & Pilastro, A. 2004: Preening, plumage reflectance and female choice in budgerigars. — Ethology Ecology and Evolution 16: 339–349.

Zuk, M. 1991: The role of parasites in sexual selection: current evidence and future directions. — Advances in the Study of Behavior 21: 39–68.