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Published in:
Journal of Applied Microbiology

DOI:
10.1111/j.1365-2672.2004.02232.x

2004

Journal of Applied Microbiology 2004, **96,** 834–843 doi:10.1111/j.1365-2672.2004.02232.x

Diversities and similarities in PFGE profiles of *Campylobacter jejuni* isolated from migrating birds and humans

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2003/763: received 28 August 2003, revised 11 December 2003 and accepted 13 December 2003

ABSTRACT

T. BROMAN, J. WALDENSTRÖM, D. DAHLGREN, I. CARLSSON, I. ELIASSON AND B. OLSEN. 2004.

Aims: To genetically sub-type *Campylobacter jejuni* strains isolated from migratory birds, and to compare these with clinical strains collected in the same area and corresponding time period, with the aim to increase our knowledge on sub-types occurring among wild birds and their possible impact on human disease.

Methods and Results: We sub-typed *C. jejuni* strains from migrating birds ($n = 89$) and humans ($n = 47$), using macrorestriction profiling by pulsed-field gel electrophoresis. Isolates from migrant birds often exhibited sub-types with higher levels of similarity to isolates from birds of the same species or feeding guild, than to isolates from other groups of birds. Likewise, could the vast majority of sub-types found among the migrant bird isolates not be identified among sub-types from human cases. Only two bird strains, one from a starling (*Sturnus vulgaris*) and one from a blackbird (*Turdus merula*), had sub-types that were similar to some of the human strain sub-types.

Conclusions: Isolates from one bird species, or feeding guild, often exhibited high similarities, indicating a common transmission source for individuals, or an association between certain sub-types of *C. jejuni* and certain ecological guilds or phylogenetic groups of birds. Sub-types occurring among wild birds were in general distinctively different from those observed in patients. The two bird isolates that were similar to human strains were isolated from bird species that often live in close associations with human settlements.

Significance and Impact of Study: Wild birds have often been mentioned as a potential route for transmission of *C. jejuni* to humans. Our study demonstrates that strains isolated from birds most often are different from clinical strains, but that some strain similarities occur, notably in birds strongly associated with human activities.

Keywords: *Campylobacter jejuni*, epidemiology, migration, PFGE, wild birds.

INTRODUCTION

During the past decade, *Campylobacter jejuni* has been recognized as a major health problem, with increasing incidence of bacterial enteritis (Friedman *et al.* 2000). Certain risk factors have been identified, including consumption of undercooked poultry meat or cross-contamination from raw poultry to other food products (Harris *et al.* 1986; Studahl and Andersson 2000), drinking contaminated water or milk (Hopkins *et al.* 1984), travelling abroad or contact with puppies (Neal and Slack 1997). Recent case-control studies have shown, however, that far from all cases could be explained by known risk factors for infection (Neal and Slack 1997; Rodrigues *et al.* 2001). It is therefore good reason to believe that less well-known sources may be of equal or even greater importance in the epidemiology of *C. jejuni* (Neal and Slack 1997). The microorganism is known to occur naturally in both domesticated and wild mammals and birds, and there seems to be a strong association between *C. jejuni* and birds (Luechtefeld *et al.*

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1980; Kapperud and Rosef 1983; Waldenström *et al.* 2002). Consequently, wild birds are often suggested as potential reservoirs for *C. jejuni* in nature, and as possible sources for human infections. Several investigations have been performed to clarify the occurrence of *Campylobacter* spp. among wild birds (Kapperud and Rosef 1983; Pacha *et al.* 1988; Lévesque *et al.* 2000; Waldenström *et al.* 2002). However, few have used genotyping methods to investigate whether bacterial isolates found among birds are comparable with those isolated from humans with clinical disease. The only major study published so far focused on a single bird species, the black-headed gull (*Larus ridibundus*) (Broman *et al.* 2002). In that investigation, only a few gull strains had genotypic profiles identical to those of human strains, and the majority of the sub-typed gull strains were of a sub-type not found in any of the clinical strains. In a recently reported investigation there existed marked differences in the prevalence of *C. jejuni* between different bird taxa, and the prevalence was significantly related to the host's feeding guild (main feeding habits), thus establishing a possible link between foraging and *C. jejuni* colonization (Waldenström *et al.* 2002). An important question is whether the observed difference in sub-types of *C. jejuni* from black-headed gulls and humans is a general phenomenon, or if some bird species may be more important than others as sources for *C. jejuni* infection in man.

In the present investigation, all hippurate positive *Campylobacter* spp. isolates collected from a total of 1794 migratory birds (Waldenström *et al.* 2002) were compared, using macrorestriction profiling (MRP) by pulsed-field gel electrophoresis (PFGE), to investigate the distribution of *C. jejuni* sub-types among groups of birds. The bird isolates were also compared with isolates from human clinical cases collected during the same time period and in the same geographical area, to investigate the possible impact of wild birds on human *C. jejuni* infections.

MATERIALS AND METHODS

Sample origin and cultivation

All bird isolates used in this study originated from an investigation of the prevalence of *Campylobacter* spp. in different taxa and guilds of migratory birds at Ottenby Bird Observatory (56°12′N, 16°24′E), SE Sweden, during the year 2000 (Waldenström *et al.* 2002). A total of 1794 migrating birds were sampled in the spring between 14 March and 15 June, and in the autumn between 1 July and 17 November. Details of trapping methods, bird identification, sampling procedure and primary isolation of bacteria have been presented elsewhere (Waldenström *et al.* 2002). All isolates with *Campylobacter* growth characteristics were subjected to hippurate hydrolysis test (Hwang and Ederer 1975), and isolates with positive reactions ($n = 89$) were regarded as putative *C. jejuni*. The phenotypic species identification of all hippurate hydrolysis positive isolates was later confirmed by genotypic testing of purified chromosomal DNA (Puregene DNA Isolation Kit; Gentra Systems, Minneapolis, MN, USA) by a *C. jejuni*- and *Campylobacter coli*-specific multiplex PCR (Vandamme *et al.* 1997). Isolates with negative reactions for both primer pairs were subjected to a previously described combined PCR and restriction fragment analysis method (Fermér and Olsson-Engvall 1999 and Engvall 1999). Further, isolates with positive reactions in the hippurate hydrolysis test, negative reactions for both *C. jejuni* and *C. coli* in the multiplex PCR, but with *C. coli*-specific digestion patterns after digestion of 23S rRNA PCR products were retrieved from freezing, and sub-cultured repeatedly with extreme care taken to pick a single colony for the subsequent sub-culture. Hippurate hydrolysis test, multiplex PCR and restriction fragment analysis of 23S r*DNA* PCR products were thereafter repeated.

For comparison, all domestically acquired human clinical isolates of hippurate hydrolysis positive campylobacters ($n = 47$), collected during the same study period as the bird isolates and within the same geographical area (Kalmar County) as Ottenby Bird Observatory, were included in the study. In order to avoid a false high homology between human isolates, only one isolate was included from each patient. Human faecal samples were cultured as previously described for avian faecal samples (Waldenström *et al.* 2002).

Typing with PFGE and computer analysis

All *C. jejuni* isolates were subjected to MRP and PFGE, using *Sma*I restriction enzyme, according to a previously described protocol (Broman *et al.* 2002), with the exception that a CHEF apparatus model DR III (Bio-Rad Laboratories, Sundbyberg, Sweden) was used for the electrophoresis, and gels were digitally captured by GelDoc 2000 (Bio-Rad Laboratories) and optimized with QuantityOne version 4 software (Bio-Rad Laboratories). When two fragments of similar size were suspected of being superimposed on each other after PFGE of *Sma*I-digests (*i.e.* broad or strong bands on the gel), a second electrophoresis was performed to identify double bands. Ramping parameters further separating fragments between approx. 200 and 350 kb were 18·3–30·8 s for 33·5 h, and for fragments between approx. 120 and 250 kb, 6·8–21·8 s for 31·4 h. With the two latter programmes, electrophoresis was performed at 14°C and Tris–borate–EDTA (TBE)-buffer was replaced after approx. 24 h. The digital gel photographs of *Sma*I digests were analysed with the Molecular Analyst Software Fingerprinting Plus software (version 1.6, Bio-Rad Laboratories) in order to produce a UPGMA dendrogram. Strains

that were non-digestable by *Sma*I were excluded from further analysis. Fragments of all sizes were included, and a band position tolerance of 1·0% and an increase tolerance of 2·0% were used for the analysis. To further sub-divide isolates with similar *Sma*I restriction patterns, all isolates with MRPs clustering at a 90% or higher similarity level, were digested with *Kpn*I and the resulting products subsequently separated by PFGE as previously described (Broman *et al.* 2002). A number of cases were observed where isolates from different *Sma*I similarity groups had a considerable proportion of fragments in common. Therefore, all *Kpn*I digests were investigated together, rather than separately, and a dendrogram was produced for these digests. The approach was the same as for the *Sma*I digest, with the exception that fragments shorter than 48·5 kbp were excluded from analysis of *Kpn*I digests, and that an increase tolerance of 1·4% was used.

Bird isolates were linked to the feeding guild of the respective host in order to investigate if certain *C. jejuni* MRP types could be associated with certain feeding habits (Waldenström *et al.* 2002). Isolates originating from guilds that were represented by less than five isolates were excluded from this analysis.

RESULTS

Species identification

Multiplex PCR confirmed the putative species identification for 80 of the 89 hippurate hydrolysis positive isolates as *C. jejuni*. Nine isolates that phenotypically had been characterized as *C. jejuni* were repeatedly negative in the multiplex PCR, both for *C. jejuni* and *C. coli*. Four of these produced typical *C. jejuni*-specific digestion patterns after *Alu*I treatment of 23S r*DNA* PCR products. The remaining five multiplex-PCR negative isolates produced *C. coli*-specific patterns after *Alu*I digestion. These were isolates Dunlin 6, 20 and 27, and Broad-billed Sandpiper 2 and 3. Renewed testing of repeated single colony sub-cultures gave the same results as before sub-culturing, excluding the possibility that the conflicting results were caused by mixed infections of *C. jejuni* and *C. coli*.

MRP by PFGE and computer analysis

Three *C. jejuni* bird isolates were indigestible by *Sma*I and therefore excluded from the analysis (Table 1). The five isolates for which species identification was uncertain produced variants of *Sma*I MRPs that are commonly found among *C. jejuni* strains. We therefore treated them as putative *C. jejuni* isolates, and included them in the computer analysis. Four of these five isolates clustered at 100% similarity to isolates where species identification was unambiguous (Fig. 1), and were referred to *Kpn*I analysis (see below). In the *Kpn*I dendrogram three of the four grouped together, but produced profiles that in no sense could be regarded as atypical when compared to profiles from isolates that had been clearly identified as *C. jejuni*. The fifth isolate with uncertain species identification had, in this dataset, a unique *Sma*I profile (Fig. 1) and was not referred to *Kpn*I analysis.

In the *Sma*I dendrogram (Fig. 1) isolates from several of the host species frequently clustered with other isolates originating from the same species. This was particularly true for isolates from dunlins (*Calidris alpina*), song thrushes (*Turdus philomelos*), starlings (*Sturnus vulgaris*) and humans. Linking of the bird isolates to the feeding guild of their respective host showed that 32 of the 37 isolates originating from shoreline-foraging invertebrate feeders (Guild H) clustered, together with two isolates from another guild, above a similarity level of 68% in the *Sma*I dendrogram (Fig. 1). The 32 isolates originating from ground-foraging invertebrate feeders (Guild B) were more scattered throughout the dendrogram, but these isolates commonly occurred together in groups. The only other guild represented by more than five isolates was raptors (Guild A) with nine isolates. No considerable similarity was observed for *Sma*I profiles of isolates originating from birds within this guild.

Throughout the *Sma*I dendrogram, groups of isolates could be identified with profiles that clustered at or above the 90% level of similarity. A total of 92 isolates in the dataset were included in such groups, hereafter referred to as similarity groups (denoted from a to z in Fig. 1). The number of isolates per similarity group ranged from two to 10. All isolates from the 26 *Sma*I similarity groups were subjected to *Kpn*I digestion and computer analysis.

A large number of isolates in the dataset produced *Sma*I profiles consisting of only five fragments (Fig. 1). Isolates producing variants of this five-fragment pattern were by computer analysis referred to the similarity groups a through j, and n. The *Kpn*I further subdivided most of these groups, and isolates from different *Sma*I groups frequently occurred intermingled with isolates from other *Sma*I groups after computer analysis of *Kpn*I patterns (Fig. 2). Even so, were all isolates from *Sma*I similarity groups a through f referred to one sub-cluster in the *Kpn*I dendrogram, and thus separated from all but one of the isolates from *Sma*I similarity groups g through j and n. The only isolate from the latter *Sma*I similarity groups, that after *Kpn*I analysis was referred to the sub-cluster containing isolates from *Sma*I similarity groups a through f, was an isolate from a little stint (Little Stint 2). Consequently, did all isolates from the shoreline invertebrate feeding guild that produced a five-fragment-*Sma*I pattern and had been included in one of the *Sma*I similarity groups, cluster together in the *Kpn*I dendrogram. All but one of the

Table 1. Origin of *C.jejuni* isolates from birds, dates of isolation and feeding guilds

Isolate†	Date of isolation	Feeding guild‡	Isolate†	Date of isolation	Feeding guild‡
Sparrowhawk 1	21 Sep	A	Ruff 1	3 Aug	H
Jackdaw 1	6 May	L	Wood Sandpiper 1*	21 Jul	H
Jackdaw 2	17 May	L	Long-eared Owl 1	4 Nov	A
Jackdaw 3	6 May	L	Long-eared Owl 2	12 Oct	A
Jackdaw 4*	10 Jun	L	Long-eared Owl 3	17 Oct	A
Dunnock 1*	22 Sep	C	Long-eared Owl 4	9 Nov	A
Yellowhammer 1	19 Oct	C	Long-eared Owl 5	17 Apr	A
White Wagtail 1	4 Sep	D	Long-eared Owl 6	3 Nov	A
White Wagtail 2	15 Sep	D	Long-eared Owl 7	21 Apr	A
Dunlin 1	2 Aug	H	Long-eared Owl 8	13 Oct	A
Dunlin 2	19 Jul	H	Starling 1	22 Jul	B
Dunlin 3	26 Jul	H	Starling 2	14 Jun	B
Dunlin 4	24 Jul	H	Starling 3	22 Jul	B
Dunlin 5	3 Aug	H	Starling 4	22 Jul	B
Dunlin 6	3 Aug	H	Starling 5	22 Jul	B
Dunlin 7	4 Aug	H	Starling 6	23 Jul	B
Dunlin 8	23 Jul	H	Starling 7	10 Aug	B
Dunlin 9	3 Aug	H	Starling 8	3 Nov	B
Dunlin 10	19 Jul	H	Starling 9	3 Nov	B
Dunlin 11	3 Aug	H	Starling 10	22 Jul	B
Dunlin 12	24 Jul	H	Reed Warbler 1	7 Oct	G
Dunlin 13	9 Aug	H	Winter Wren 1	7 Apr	G
Dunlin 14	19 Jul	H	Redwing 1	11 Oct	B
Dunlin 15	24 Jul	H	Redwing 2	3 Nov	B
Dunlin 16	2 Aug	H	Redwing 3	29 Oct	B
Dunlin 17	23 Jul	H	Redwing 4	27 Sep	B
Dunlin 18	26 Jul	H	Redwing 5	29 Oct	B
Dunlin 19	3 Aug	H	Blackbird 1	5 Apr	B
Dunlin 20	28 Jul	H	Blackbird 2	26 Mar	B
Dunlin 21	4 Aug	H	Blackbird 3	20 Oct	B
Dunlin 22	25 Jul	H	Blackbird 4	5 Nov	B
Dunlin 23	22 Jul	H	Blackbird 5	27 Mar	B
Dunlin 24	2 Aug	H	Blackbird 6	4 Nov	B
Dunlin 25	3 Aug	H	Blackbird 7	7 Oct	B
Dunlin 26	4 Aug	H	Song Thrush 1	4 Apr	B
Dunlin 27	20 Jul	H	Song Thrush 2	7 Oct	B
Dunlin 28	24 Jul	H	Song Thrush 3	29 Sep	B
Dunlin 29	25 Jul	H	Song Thrush 4	6 Oct	B
Curlew Sandpiper 1	21 Jul	H	Song Thrush 5	6 Oct	B
Little Stint 1	29 Jul	H	Song Thrush 6	20 Sep	B
Little Stint 2	28 Jul	H	Song Thrush 7	7 Oct	B
Broad-billed Sandpiper 1	21 Jul	H	Fieldfare 1	8 Sep	B
Broad-billed Sandpiper 2	21 Jul	H	Fieldfare 2	15 Nov	B
Broad-billed Sandpiper 3	21 Jul	H	Mistle Thrush 1	27 Mar	B
Broad-billed Sandpiper 4	24 Jul	H			

†Sparrowhawk (*Accipter nisus*), Jackdaw (*Corvus monedula*), Dunnock (*Prunella modularis*), Yellowhammer (*Emberiza citrinella*), White Wagtail (*Motacilla alba*), Dunlin (*Calidris alpina*), Curlew Sandpiper (*Calidris ferruguinea*), Little Stint (*Calidris minuta*), Broad-billed Sandpiper (*Limicola falcinellus*), Ruff (*Philomachus pugnax*), Wood Sandpiper (*Tringa glareola*), Long-eared Owl (*Asio otus*), Starling (*Sturnus vulgaris*), Reed Warbler (*Acrocephalus scirpaceus*), Winter Wren (*Troglodytes troglodytes*), Redwing (*Turdus iliacus*), Blackbird (*Turdus merula*), Song Thrush (*Turdus philomelos*), Fieldfare (*Turdus pilaris*), Mistle Thrush (*Turdus viscivorus*)

‡Guilds; A = Raptors; B = Ground-foraging Invertebrate Feeders; C = Ground-foraging Granivores; D = Ground-foraging Insectivores; G = Reed and Herbaceous Plant-Foraging insectivores; H = Shoreline-Foraging Invertebrate Feeders (Shorebirds); L = Opportunistic Feeders.

*Isolate indigestible by *Sma*I.

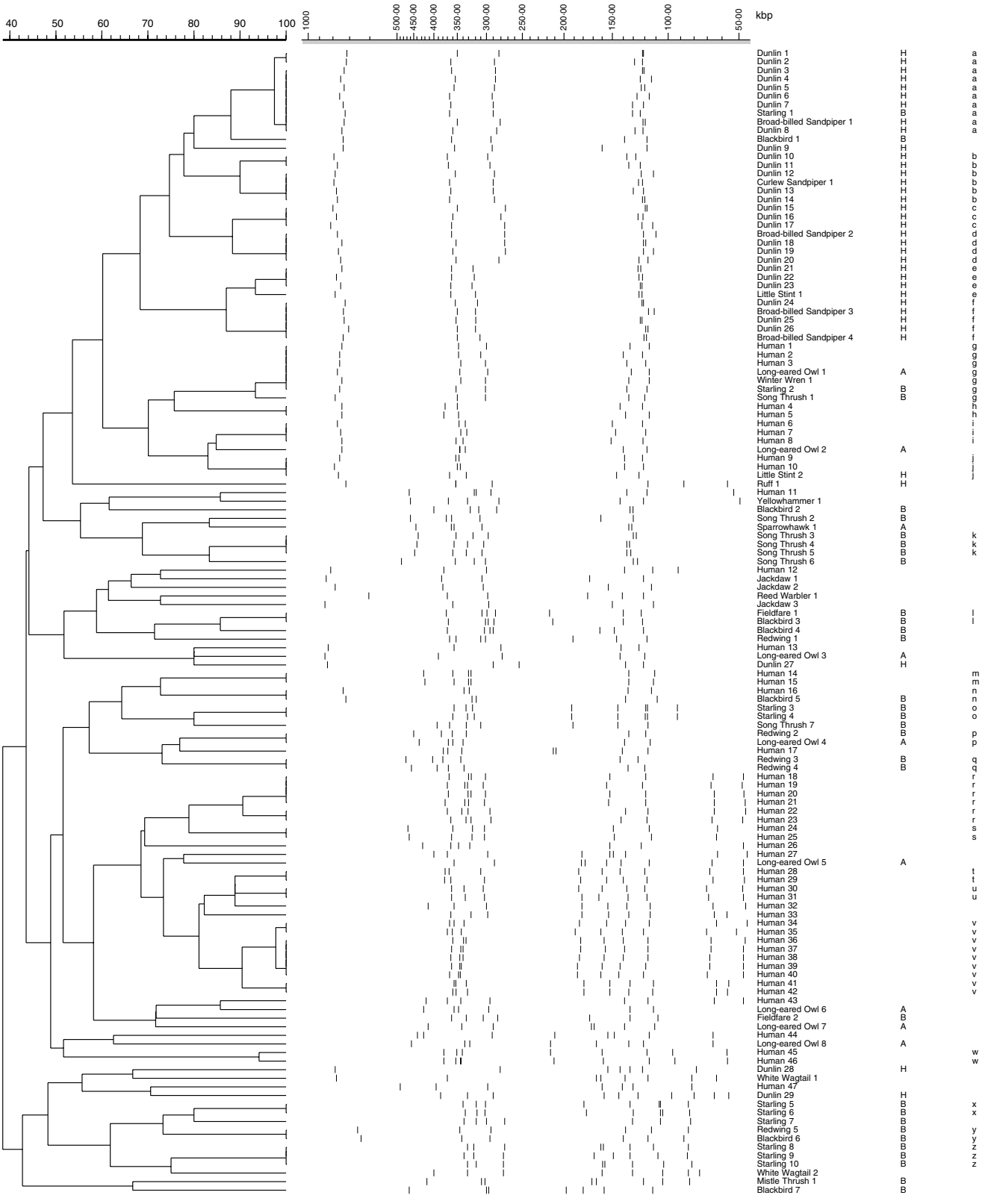


Fig. 1 Dendrogram of *Sma*1 MRPs of *C. jejuni* from migratory birds and humans. Feeding guild of bird host species, and similarity group belonging for isolates clustering above 90% similarity is also shown (see text for details)

isolates with similar *Sma*I patterns that originated from other bird species or from humans were referred to other sub-clusters.

Nine similarity groups included only human isolates, while six consisted of isolates from one given bird species. Isolates in eight similarity groups were of mixed bird origin, while three similarity groups (g, j and n) contained isolates from both humans and birds. *Kpn*I digestion and analysis further subdivided the mixed bird/human *Sma*I similarity groups g and j (Fig. 2). Only one of the bird isolates from *Sma*I group g (Starling 2) produced a *Kpn*I MRP that was identical to the *Kpn*I pattern of a human isolate (Human 1). The other two bird isolates, and one of those from patients, had unique *Kpn*I profiles. The remaining two human isolates had profiles that were mutually identical. Similarity group j was subdivided after *Kpn*I analysis, so that the above-mentioned isolate from a little stint (Little Stint 2) produced a MRP that was highly different from the patterns of the two human isolates. The latter two, however, had identical profiles, and in addition also clustered at an 89% similarity level to two of the human isolates from *Sma*I group g. The two isolates from the third of the mixed bird/human *Sma*I similarity groups (group n; isolates Human 16 and Blackbird 5) had, also after *Kpn*I digestion, similar patterns and clustered at a level of 96%. An additional clinical isolate (Human 7) from *Sma*I group i, was shown to have a *Kpn*I pattern that was identical to the one produced by the human isolate of group n. Visual examination revealed that, even if they by the computer analysis had been referred to different sub-clusters of the *Sma*I dendrogram, the isolates in groups n and i had similar *Sma*I profiles.

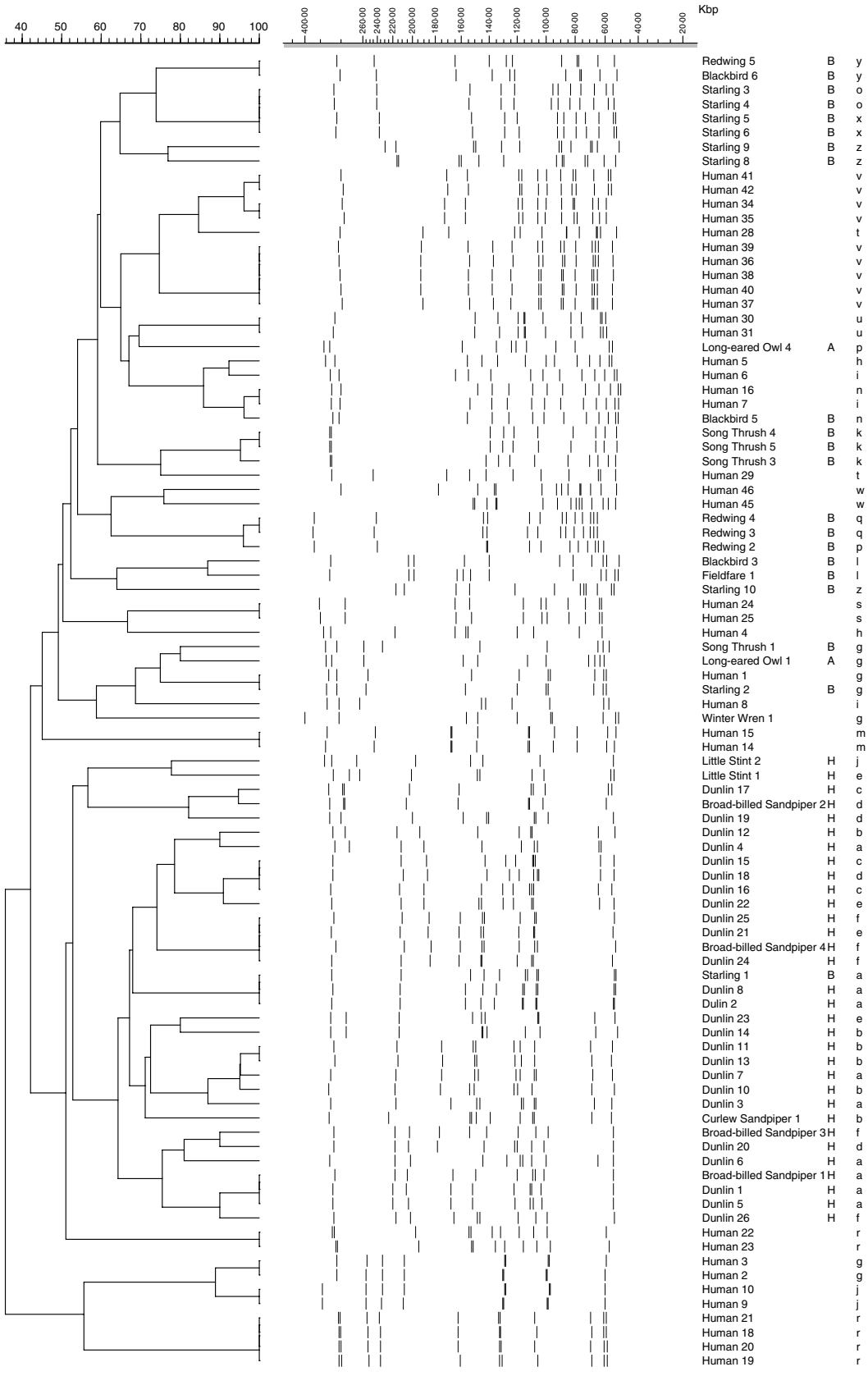
DISCUSSION

In a previous study where we presented a survey of thermotolerant *Campylobacter* spp. among migrating birds in Sweden, we showed that the bacteria were unequally distributed among the investigated bird species (Waldenström *et al.* 2002). Colonization by thermotolerant *Campylobacter* spp., and also by *C. jejuni per se*, was more frequently found among birds belonging to certain species and feeding guilds. In the present investigation, we sub-typed, using MRP by PFGE, all *C. jejuni* isolates ($n = 89$) that were obtained from the 1794 sampled migratory birds. We compared isolates from different groups of birds to investigate if certain sub-types tended to be more prevalent among certain bird host species, or among groups of species that have similar feeding habits. In order to investigate if any influence could be observed on human disease from *C. jejuni* strains occurring among migrating birds, we also included all clinical, domestically acquired, hippurate hydrolysis positive *Campylobacter* isolates ($n = 47$) that were obtained in the region during the corresponding time period.

The population structure of *C. jejuni* has long been poorly understood, but results from recent investigations based on multilocus sequence typing (MLST) showed that *C. jejuni* is a highly diverse organism, with a weakly clonal population structure (Dingle *et al.* 2002). The genetic instability of *C. jejuni* can be expected to have consequences for all methods used for sub-typing of the organism. Acquisition of foreign DNA, and random recombination of large DNA segments, may well cause alterations detectable using MRP by PFGE (Wassenaar *et al.* 2000). This has previously been indicated by investigations where profile variability has been observed in *C. jejuni* isolated from broiler chicken meat and live chicken (Wassenaar *et al.* 1998; Hänninen *et al.* 1999). Even so, has MRP by PFGE been of considerable use for the identification of infection sources and transmission routes during *C. jejuni* outbreaks (Olsen *et al.* 2001; Hänninen *et al.* 2003), and can be considered useful for the investigation of isolates from sources with considerable epidemiological links.

The finding that the majority of isolates from shoreline-foraging invertebrate feeders (Guild H) were referred to a single sub-cluster, separated from most isolates with similar restriction patterns that originated from other host species, should be regarded with caution as a similarity comparison based on only five genetic characters inherently will be relatively indecisive. It is notable therefore, that when isolates of the different *Sma*I similarity groups (isolates clustering ≥90% similarity) were referred to *Kpn*I digestion and subsequent computer analysis, all isolates from shoreline-foraging invertebrate feeders still clustered together (Fig. 2), and thus were separated from all but one of the isolates with five-fragment *Sma*I profiles from other hosts. Also isolates from the second largest guild in the dataset, the ground-foraging invertebrate feeders (Guild B), often clustered together in groups after both *Sma*I and *Kpn*I digestions.

The observed grouping of isolates from hosts of different guilds and species could have various explanations. It could, for example, be a reflection of a common source of infection. Such a source could, depending on the migratory behaviour of a given species, either exist at a common breeding or wintering site, a common roosting locality during migration, or locally in the area surrounding Ottenby. Another possible explanation could be that different sub-types of *C. jejuni*, in a more general sense, may be associated with different biotopes. This hypothesis is supported by the findings of the above mentioned MLST investigation, where it was shown that some clonal complexes only could be found among isolates from certain sources, as e.g. sand samples (Dingle *et al.* 2002). This finding in turn, may indicate that certain lineages of *C. jejuni* can be particularly resistant to environmental stresses, for e.g. saline, and that they therefore more commonly are transmitted to certain groups of hosts, like


40 50 60 70 80 90 100
400·00 280·00 240·00 220·00 200·00 180·00 160·00 140·00 120·00 100·00 80·00 60·00 40·00 20·00
Kbp
Redwing 5 B y
Blackbird 6 B y
Starling 3 B o
Starling 4 B o
Starling 5 B x
Starling 6 B x
Starling 9 B z
Starling 8 B z
Human 41 v
Human 42 v
Human 34 v
Human 35 v
Human 28 t
Human 39 v
Human 36 v
Human 38 v
Human 40 v
Human 37 v
Human 30 u
Human 31 u
Long-eared Owl 4 A p
Human 5 h
Human 6 i
Human 16 n
Human 7 i
Blackbird 5 B n
Song Thrush 4 B k
Song Thrush 5 B k
Song Thrush 3 B k
Human 29 t
Human 46 w
Human 45 w
Redwing 4 B q
Redwing 3 B q
Redwing 2 B p
Blackbird 3 B l
Fieldfare 1 B l
Starling 10 B z
Human 24 s
Human 25 s
Human 4 h
Song Thrush 1 B g
Long-eared Owl 1 A g
Human 1 g
Starling 2 B g
Human 8 i
Winter Wren 1 g
Human 15 m
Human 14 m
Little Stint 2 H j
Little Stint 1 H e
Dunlin 17 H c
Broad-billed Sandpiper 2 H d
Dunlin 19 H d
Dunlin 12 H b
Dunlin 4 H a
Dunlin 15 H c
Dunlin 18 H d
Dunlin 16 H c
Dunlin 22 H e
Dunlin 25 H f
Dunlin 21 H e
Broad-billed Sandpiper 4 H f
Dunlin 24 H f
Starling 1 B a
Dunlin 8 H a
Dulin 2 H a
Dunlin 23 H e
Dunlin 14 H b
Dunlin 11 H b
Dunlin 13 H b
Dunlin 7 H a
Dunlin 10 H b
Dunlin 3 H a
Curlew Sandpiper 1 H b
Broad-billed Sandpiper 3 H f
Dunlin 20 H d
Dunlin 6 H a
Broad-billed Sandpiper 1 H a
Dunlin 1 H a
Dunlin 5 H a
Dunlin 26 H f
Human 22 r
Human 23 r
Human 3 g
Human 2 g
Human 10 j
Human 9 j
Human 21 r
Human 18 r
Human 20 r
Human 19 r

◄

Fig. 2 Dendrogram of *Kpn*1 MRPs of *C. jejuni* from migratory birds and humans. Feeding guild of bird host species, and *Sma*I similarity group belonging for isolates clustering above 90% similarity in the *Sma*I dendrogram is also shown (see text for details)

shoreline feeding birds. Finally, the trend that strains from birds of given guilds largely had similar restriction patterns could indicate that there may exist some level of association between certain lineages of *C. jejuni* and certain host species or guilds of birds. During spring migration, most birds stop only briefly at Ottenby before they continue towards their respective breeding areas. Consequently, the *C. jejuni* strains isolated from spring migrants could reflect acquisition of the microorganisms either at the wintering areas or during migration. The situation is different during autumn migration, when certain bird species stay and feed in the Ottenby area for a period of days or even weeks before they continue southwards. Such lingering behaviour is especially apparent in several species of shoreline feeders, and for that reason an influence of the local environment on *C. jejuni* colonization cannot be completely disregarded for these species. Consequently, *C. jejuni* strains isolated among autumn migrating individuals may originate either from the breeding area, from stopover sites on the south-bound migrations, or for some species from the local environment in the Ottenby region. The shoreline-foraging invertebrate feeders that gather at Ottenby during autumn migration arrive from various parts of northern Fennoscandia and the Russian Tundra. It is therefore unlikely that the relative similarity observed among *C. jejuni* sub-types from these birds could be a reflection of infections acquired at a common breeding site or roosting place. Because many shorebirds remain in the Ottenby region for some time during the autumn migration, the finding could be the result of local transmission of certain sub-types through a shared feeding habitat. One circumstance that speaks for a local transmission of *C. jejuni* is the finding that a starling, caught while feeding on the shore, was colonized by a *C. jejuni* strain (Starling 1) that shared both *Sma*I and *Kpn*I patterns with strains isolated from dunlins. For most of the year starlings and shoreline-foraging invertebrate feeders do not occur together, but during autumn migration starlings commonly forage along shores together with birds of the shoreline-foraging invertebrate feeder guild. These two groups of birds could, for that reason, be exposed to each other's *C. jejuni* strains during this particular time of the year. There exist, however, other findings that speak against local transmission and that may instead support the hypothesis of association between different sub-types of *C. jejuni* and certain species or guilds of hosts. Six other starling strains in the dataset also originated from individuals that were sampled at the shore (Starlings 3, 4, 5, 6, 7, and 10). None of these was colonized by a *C. jejuni* of a sub-type that resembled those found among the true shorebirds. Instead, several of the starling isolates had sub-types that were identical or highly similar among them. For example, had isolates Starling 3, 4, 5, and 6 in between themselves identical *Kpn*I profiles. Visual examination showed that also their respective *Sma*I profiles were highly similar, even though computer analysis had referred them to two separate sub-clusters of the *Sma*I dendrogram. During autumn migration, starlings from various parts of northern Fennoscandia congregate and migrate together in growing flocks. We therefore cannot exclude the possibility that these four individuals, sampled during two subsequent days, had arrived from one common breeding area, or had all roosted at one common locality during the flight to Ottenby. It is notable, however, that isolate Starling 7, obtained 18 days later, produced a *Sma*I profile that differed from those of the above four isolates only through what could be the effect of one genetic event, that is the loss of one *Sma*I restriction site. *Kpn*I digestion showed that the isolate Starling 7 had a *Kpn*I profile that was indistinguishable from those of the other four (result not shown). It should also be remembered that among isolates from shorebirds, not one was found that had a sub-type resembling those found among isolates from these shoreline feeding starlings, even though these birds occurred together on the seaweed banks. In this context, yet one other group of isolates could be mentioned; Redwing 4, 3, and 2 had highly similar profiles both after *Sma*I and *Kpn*I digestions. These three were collected on 27 September, 29 October, and 3 November respectively, and a common origin for their respective hosts is unlikely. Thus, in several cases the genotypic similarities observed among isolates originating from a given host species or feeding guild cannot be explained by a common origin of the hosts or by influence of the local environment at Ottenby. This may indicate that certain sub-populations of *C. jejuni*, to some extent, are associated with certain bird hosts. These findings are consistent with those of a previous study, where it was found that variants of one sub-type dominated among *C. jejuni* strains originating from black-headed gulls that were sampled over a considerable time-period (Broman *et al.* 2002).

Among the 89 *C. jejuni* isolates from wild birds we only found two that produced both *Sma*I and *Kpn*I MRPs that were identical or very similar (≥90% similarity) to isolates from humans (Fig. 1 and Fig. 2). The first of these originated from a starling (*Sturnus vulgaris*), and the second from a blackbird (*Turdus merula*). These two bird species often occur close to human settlements. The starling is typically associated with open grasslands, pastures, or lawns, and is commonly found in both agricultural and urban areas. The blackbird often nests in gardens. In contrast to these, the majority of the other bird species that

were included in the investigation rarely come in close contact with human settlements or human activities during the time of the year that preceded the samplings. For some species though, transmission of bacteria from birds to humans could be plausible during the actual sampling period. This is particularly so for several shoreline-feeding species, where the autumn migration to a large extent coincides with the main summer holiday period (July–August). As many feeding areas are located in close proximity to beaches and other recreational areas, it is not unlikely that people may become exposed to *C. jejuni* excreted by shoreline-feeding birds. Still, as we did not find more than remote restriction profile similarities between shorebird and human strains when digestion patterns of both enzymes were analysed, we did not find any indications of humans sharing *C. jejuni*-types with shoreline-feeding bird species. Based on these results, it appears that the species of shoreline-foraging invertebrate feeding birds investigated in this study had little impact on human *C. jejuni* infections in the region. It should be noted that isolates undigestable by *Sma*I were excluded from further analysis in this investigation. This strategy will not have affected the comparison between human and bird isolates, as no such isolates were found among the clinical cases in the present dataset. Such isolates do, however, occasionally occur also among humans and may therefore deserve further consideration.

The results of this investigation serve as a reminder that wild birds constitute a heterogeneous group of different species. It is possible that the ecology of *C. jejuni* sub-types that frequently colonize one given group of birds may differ substantially from the ecology of *C. jejuni* types colonizing another group of birds. We hypothesize that certain sub-populations of *C. jejuni* may largely be associated with certain bird species, and that not all necessarily will be equally important for human infections. For natural reasons we have no direct epidemiological data that link the patients that were infected with *C. jejuni* of the same sub-types as those colonizing the above discussed starling and blackbird to the corresponding bird hosts, apart from a coincidence in time and space. Therefore, we do not know whether the respective isolates were of the same strains. Still it is notable that the only two bird isolates that strongly resembled human isolates were obtained from bird species that are strongly associated with human settlements. The results of the genotypic comparison between isolates from birds and humans may therefore have been different, had the investigation focused on bird species that live closer to human activities.

ACKNOWLEDGEMENTS

Many thanks goes to Paul D. Haemig for valuable comments on the manuscript. This work was supported financially by the Health Research Council of Southeast Sweden (2001–02), the Center for Environmental Research, the Uddenberg-Nordingska Foundation, and the Medical Faculty of Umeå University. This is contribution no. 191 from Ottenby Bird Observatory.

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