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Salmonella Amager, *Campylobacter jejuni,* and Urease-positive Thermophilic *Campylobacter* Found in Free-flying Peregrine Falcons (*Falco peregrinus*) in Sweden

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ABSTRACT: Rare species with small population sizes are vulnerable to perturbations such as disease, inbreeding, or random events. The threat arising from microbial pathogens could be large and other species could act as reservoirs for pathogens. We report finding three enteric bacterial species, *Salmonella* Amager, *Campylobacter jejuni*, and urease-positive thermophilic *Campylobacter*, in nestling free-flying peregrine falcons (*Falco peregrinus*) in Sweden in 2000. *Campylobacter jejuni* isolates exhibited marked genetic similarities to an isolate from a human, providing a possible association between a human-associated strain of this bacterium and peregrine falcons.

Key words: *Campylobacter jejuni*, epidemiology, peregrine falcon, *Salmonella* Amager, wild birds.

Historically, peregrine falcons (*Falco peregrinus*) were moderately common in Sweden, with an estimated population of 900–1,400 breeding pairs (Lindberg et al., 1988). The species, being a top predator that feeds on other bird species, was extremely vulnerable to substances that bioaccumulate (i.e., mercury and organic pesticides) and that were introduced into agricultural practices in the 1940s (Lindberg, 1983). Exposure to these substances affected survival and reproduction, and in the mid 1970s, fewer than 20 breeding pairs remained in the country (Lindberg et al., 1988). A recovery program with captive breeding and release of immature birds, together with a national ban on the use of harmful chemicals, have successfully increased the wild population to almost 100 pairs. Despite this remarkable recovery, the peregrine falcon still has a vulnerable conservation status in Sweden (Gärdenfors, 2000), and small changes in fecundity and mortality rates could reduce the population again.

Infectious diseases form a potential threat to the small Swedish population. There are reports of mortality of captive falcons from *Salmonella* Typhimurium infection (Sykes et al., 1981; Wernery et al., 1998) and epizootics of *Salmonella* infection in other birds (Hurvell et al., 1974; Tauni and Österlund, 2000), but data on occurrence of pathogens in free-flying peregrine falcons are still limited (Keymer, 1972; Kirkpatrick and Trexler-Myrén, 1986). In this study, we investigated occurrence of *Salmonella* and *Campylobacter* in the Swedish peregrine falcon population and identified the isolates to species and strain level with phenotypic and genetic characterization techniques.

Fecal swabs were collected from 69 peregrine falcon nestlings during banding in a national monitoring program in 2000. Of these, 62 samples were obtained from falcons in 31 broods (mean, two nestlings per brood) from southern (n=21 individuals from 11 broods) and northern (n=41 individuals from 20 broods) breeding areas in Sweden. Seven additional samples were taken from captive nestlings hatched at a breeding station at Nordens Ark, Bohuslän, Sweden. The nestlings that were sampled during this investigation constituted 44% of the total number of peregrine falcons hatched in Sweden during that year.

Fecal samples were collected by sterile swabs inserted into the cloaca of the birds and were stored in transport medium with

charcoal (Transwab, BioDisc, Solna, Sweden) at refrigerator temperature until analyzed. At each nest, remains of prey found in the nests were identified to species but were not analyzed for the occurrence of bacteria.

All samples were cultured by previously described procedures for detection of *Campylobacter* (Broman et al., 2002) and according to a routine method used by accredited Swedish clinical bacteriology laboratories to detect *Salmonella* from stools (Palmgren, 2002). The latter method is optimized for detection of *Salmonella* serovars that are pathogenic to humans. To isolate *Salmonella,* each sample was enriched in selenite cystine enrichment broth (Oxoid AB, Stockholm, Sweden) at 37 C for 18–24 hr before the broth was plated onto selective xylose-lysine-desoxycholate agar and incubated at 37 C for 20–24 hr. Putative *Salmonella* isolates were further examined by carbohydrate fermentation tests and by analyzing sensitivity to bacteriophages. The isolates were characterized according to the Kauffmann-White serotyping scheme (Kauffmann, 1972) to serovar level. To isolate thermophilic *Campylobacter* species, samples were plated onto selective medium (42.5 g/l Columbia Agar Base [Becton Dickinson, Cockeysville, Maryland, USA]; 5% citrated horse blood, 10 mg/l vancomycin, 2,500 IE/l polymyxin B, 5 mg/l trimethoprim) and incubated at 42 C in a microaerophilic atmosphere (85% N_2, 10% CO_2, 5% O_2) for 72 hr, at which time the plates were examined for bacterial growth. Presumptive *Campylobacter* spp. were identified by limited phenotypic characterization (cell morphology; oxidase, catalase, and hippurate hydrolysis test; urease production; susceptibility to nalidixic acid).

We used two methods for a detailed genetic identification of *Campylobacter* isolates. First, we used a polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method that produces species-specific fragment patterns of all thermophilic *Campylobacter* species (Fermér and Engvall, 1999). As a complement and validation of the first method, a multiplex PCR method, with primer pairs specific for *Campylobacter jejuni* and *Campylobacter coli* (Vandamme et al., 1991), also was used. In both methods, boiled lysates were used as templates for the PCR reactions.

Two *Salmonella* isolates were obtained from two siblings in a brood sampled in northern Sweden. These isolates were identified as *Salmonella* Amager.

In six (9%) of the samples, thermophilic *Campylobacter* spp. were isolated. Of these, two were characterized as *C. jejuni* by phenotypic and genotypic methods. These isolates were further investigated by macrorestriction profile (MRP) with pulsed-field gel electrophoresis and the use of *Sma*I and *Kpn*I for the digestions according to a previously described protocol (Broman et al., 2002), with the exceptions that a CHEF apparatus model DR III (Bio-Rad Laboratories, Sundbyberg, Sweden) was used for electrophoresis and that gels were digitally captured by GelDoc 2000 (Bio-Rad) and optimized with QuantityOne (v.4 software, Bio-Rad). Macro restriction profiles were compared with a database of *C. jejuni* profiles obtained from black-headed gulls (*Larus ridibundus*) (n=80), chickens (n=50), and humans (n=50) in the Malmö region of southern Sweden with GelCompare software (Applied Maths, Kortrijk, Belgium). They were also visually compared with an additional 172 profiles originating from a variety of wild bird species and with 49 human isolates obtained in Kalmar county, southern Sweden. The *Sma*I and *Kpn*I MRP of the falcon isolates were identical to each other and to the profile of a *C. jejuni* isolated from a young man from southern Sweden in the summer of 1999 (Fig. 1).

The remaining four *Campylobacter* isolates from two nests were characterized as hippurate hydrolysis–negative thermophilic *Campylobacter* spp. The PCR-RFLP method (Fermér and Engvall, 1999) re-

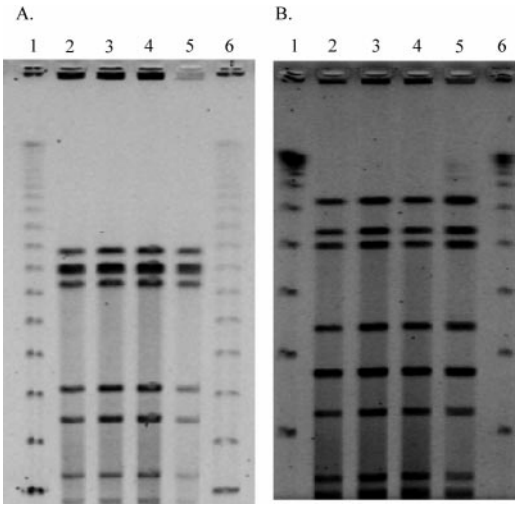

FIGURE 1. Macrorestriction profiles (MRPs) of *Campylobacter jejuni* isolates of peregrine falcon and human origin. (A) *Sma*I MRPs. (B) *Kpn*I MRPs. Lanes 2 and 3: peregrine isolates. Lane 4: isolate obtained from dropping in nest. Lane 5: human isolate. Lanes 1 and 6: molecular size marker; lambda ladder.

vealed that all four isolates produced an identical digestion pattern. This pattern strongly resembled that produced by typical *Campylobacter lari,* but the isolates in this study produced a slightly longer smallest fragment (ca. 10–20 bp longer). Positive reactions in the urease test and sensitivity to nalidixic acid strongly indicated that these four isolates belonged to the urease-positive thermophilic campylobacter group (most often denoted UPTC).

Campylobacter jejuni is commonly found in many bird species (e.g., Broman et al., 2002; Waldenström et al., 2002). However, finding that the isolates, after digestion with the enzymes (*Sma*I and *Kpn*I), were genetically indistinguishable from a human clinical isolate is noteworthy. *Campylobacter jejuni* is a heterogeneous organism, exhibiting a multitude of genotypes. We have hundreds of MRP from wild birds and from human isolates in our data bank, and only occasionally do we find complete matches between isolates from the two hosts (Broman et al., 2002). The findings reported here, therefore, indicate that a bacterial pathogen that causes disease in humans is found in peregrine falcons. This pathogen could be from a source common to both species. Considering the diet of peregrine falcons, with a large proportion of gulls, pigeons, and corvids, one likely pathway could be consumption of a prey species that also occurs in urban areas.

Campylobacter bacteria belonging to the UPTC group, by some authors considered a biovar of *C. lari* (Vandamme et al., 1991), have been isolated from gulls (Kaneko et al., 1999), shellfish (Wilson and Moore, 1996), river water (Bolton et al., 1987), seawater (Bolton et al., 1985), and a few humans (Mégraud et al., 1988). Even though there seems to be an aquatic link with many of these isolates, it is unlikely that UPTCs are environmental organisms. Their optimal growth temperature of 42 C indicates that they might only multiply well in warm-blooded hosts, most likely birds, because they typically have higher body temperatures than mammals.

We do not know whether the bacteria found in these birds are pathogenic to their host. The UPTCs are generally considered to be of low pathogenicity in humans, but no work regarding pathogenicity has been performed in wild birds. *Campylobacter jejuni,* which is a pathogen in humans, is often regarded as nonpathogenic in birds, but even though older birds seem to be unaffected by colonization, there are reports of enteric disease and mortality in young broiler chickens because of *C. jejuni* infection. To our knowledge, only one published investigation has dealt with this question in a wild bird species. Twenty-seven trapped herring gulls (*Larus argentatus*) that were naturally colonized by *C. jejuni* were caged and observed for signs of disease. All birds appeared healthy, and the infection was eliminated in all but one individual at resampling 4 wk later (Glünder et al., 1992). It is important to note, though, that in a strongly heterogeneous organism like *C. jejuni,* virulence properties can differ between isolates. Also, it is not unlikely that

host species differ in their susceptibility to infection and in severity and type of clinical manifestations.

In the case of the *Salmonella* Amager isolates, no direct link could be established between the falcons and other sources. We noted, however, remains of several black-headed gulls, a scavenging bird species often associated with human activities, at the infected nest, and in a previous year, remains of spaghetti have been found at the same nest.

The occurrence of salmonellosis in Sweden is extremely low. In 2000, fewer than 4,850 human cases were reported in a population of nearly 9 million. Of these, only 691 human cases were considered domestically acquired. Prevalence of *Salmonella* infections in domestic animals reported in 2000 was less than 0.1% (Swedish National Veterinary Institute, 2001). Except for the two falcon isolates in this study, no *Salmonella* Amager was reported in Sweden in the year 2000, and only one or two isolates of *Salmonella* Amager had been reported each year during the last 10 yr. This bacterium has been described in a few outbreaks in humans in Russia and India (Beliakov et al., 1969; Oak et al., 1984), and all known human cases of *Salmonella* Amager infection in Sweden were associated with travel to southern Europe or Africa. No reservoirs for this bacterium are known from Sweden (Swedish Institute for Infectious Disease Control, R. Wollin, pers. comm.).

All infected peregrine nestlings in this study fledged, but there is no other information on their further development and survival. Thus, we have no information about whether the birds in which *Salmonella* or *Campylobacter* were detected were detrimentally affected by their infections. Nevertheless, finding *Salmonella* Amager and *C. jejuni* in peregrine falcons is noteworthy and shows that this rare falcon species is exposed to enteropathogens. Further work is needed to establish the source of these infections.

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LITERATURE CITED

BELIAKOV, V. D., I. A. SHIFRIN, R. I. BEISLEKHEM, G. I. NEDBAI, AND P. N. IAKUNIN. 1969. On food poisoning caused by *Salmonella amager*. Gigiena i Sanitariia 34: 46–48. [In Russian.]

BOLTON, F. J., A. V. HOLT, AND D. N. HUTCHINSON. 1985. Urease-positive thermophilic campylobacters. Lancet 8439: 1217–1218.

———, D. COATES, D. N. HUTCHINSON, AND A. F. GODFREE. 1987. A study of thermophilic campylobacters in a river system. Journal of Applied Bacteriology 62: 167–176.

BROMAN, T., H. PALMGREN, S. BERGSTRÖM, M. SELLIN, J. WALDENSTRÖM, M.-L. DANIELSSON-THAM, AND B. OLSEN. 2002. *Campylobacter jejuni* in black-headed gulls (*Larus ridibundus*): Prevalence, genotypes, and influence on *C. jejuni* epidemiology. Journal of Clinical Microbiology 40: 4594–4602.

FERMÉR, C., AND E. O. ENGVALL. 1999. Specific PCR identification and differentiation of the thermophilic campylobacters, *Campylobacter jejuni, C. coli, C. lari,* and *C. upsaliensis.* Journal of Clinical Microbiology 37: 3370–3373.

GLÜNDER, G., U. NEUMANN, AND S. BRAUNE. 1992. Occurrence of *Campylobacter* spp. in young gulls, duration of *Campylobacter* infection and reinfection by contact. Journal of Veterinary Medicine B 39: 119–122.

GÄRDENFORS, U. 2000. Rödlistade arter i Sverige—The 2000 red list of Swedish species. Artdatabanken, Statens Lantbruksuniversitet (SLU), Uppsala, Sweden.

HURVELL, B., K. BORG, A. GUNNARSSON, AND, J. JEVRING. 1974. Studies on *Salmonella typhimurium* infections in passerine birds in Sweden. International Congress on Game Biology 11: 493–497.

KANEKO, A., M. MATSUDA, M. MIYAJIMA, J. E. MOORE, AND P. G. MURPHY. 1999. Urease-positive thermophilic strains of *Campylobacter* isolated from seagulls (*Larus* spp.). Letters in Applied Microbiolology 29: 7–9.

KAUFFMANN, F. 1972. Serological diagnosis of *Sal-*

monella-species. Kauffmann-White-schema. Scandinavian University Books, Denmark Munksgaard, Copenhagen, Denmark, 125 pp.

KEYMER, I. F. 1972. Diseases of birds of prey. The Veterinary Record 90: 579–594.

KIRKPATRICK, C. E., AND V. P. TREXLER-MYRÉN. 1986. A survey of free-living falconiform birds for *Salmonella.* Journal of the American Veterinary Medical Association 189: 997–998.

LINDBERG, P. 1983. Relations between the diet of Fennoscandian peregrines *Falco peregrinus* and organochlorines and mercury in their eggs and feathers, with a comparison to the gyrfalcon *Falco rusticolus.* PhD Thesis, Department of Zoology, University of Göteborg, Sweden, pp. 272.

———, P. J. SCHEI, AND M. WIKMAN. 1988. The peregrine falcon in Fennoscandia. *In* Peregrine falcon populations: Their management and recovery, T. J. Cade, J. H. Enderson, C. G. Thelander, and C. M. White (eds.). The Peregrine Fund, Boise, Idaho, pp. 159–172.

MÉGRAUD, F., D. CHEVRIER, N. DESPLACES, A. SEDALLIAN, AND J. L. GUESDON. 1988. Urease-positive thermophilic campylobacter (*Campylobacter laridis* variant) isolated from an appendix and from human fees. 1988. Journal of Clinical Microbiology 26: 1050–1051.

OAK, S. D., S. N. SAXENA, M. L. MAGO, P. C. JOHN, L. N. RAO BHAU, A. R. AGNIHOTRI, V. M. KENDRE, AND R. M. PATHAK. 1984. *S. amager* (3,10: y:1,2): A new *Salmonella* serotype isolated for the first time in India. Indian Journal of Pathology and Microbiology 27: 105–106.

PALMGREN, H. 2002. Importance of wild birds in the spread of *Salmonella.* PhD Thesis, Infectious Diseases, Umeå University Medical Faculty, Umeå, Sweden, 125 pp.

SWEDISH NATIONAL VETERINARY INSTITUTE. 2001. Trends and sources of zoonotic infections recorded in Sweden during 2000. Uppsala, Sweden, 24 pp.

SYKES, G. P., C. MURPHY, AND V. HARDASWICK. 1981. *Salmonella* infection in a captive peregrine falcon. Journal of the American Veterinary Medical Association 179: 1269–1271.

TAUNI, M. A., AND A. ÖSTERLUND. 2000. Outbreak of *Salmonella typhimurium* in cats and humans associated with infection in wild birds. Journal of Small Animal Practice 41: 339–341.

VANDAMME, P., B. POT, AND K. KERSTERS. 1991. Differentiation of campylobacters and campylobacter-like organisms by numerical analysis of one-dimensional electrophoretic protein patterns. Systematic and Applied Microbiology 14: 57–66.

WALDENSTRÖM, J., T. BROMAN, I. CARLSSON, D. HASSELQUIST, R. P. ACHTERBERG, J. A. WAGENAAR, AND B. OLSEN. 2002. Prevalence of *Campylobacter jejuni, Campylobacter lari,* and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. Applied and Environmental Microbiology 68: 5911–5917.

WERNERY, U., R. WERNERY, R. ZACHARIAH, AND J. KINNE. 1998. Salmonellosis in relation to chlamydiosis and pox and *Salmonella* infections in captive falcons in the United Arab Emirates. Zentralblat Veterinary Medicine B 45: 577–583.

WILSON, I. G., AND J. E. MOORE. 1996. Presence of *Salmonella* spp. and *Campylobacter* spp. in shellfish. Epidemiology and Infection 116: 147–153.

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