

# *Chlamydia psittaci* in birds of prey, Sweden

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**Background:** *Chlamydia psittaci* is an intracellular bacterium primarily causing respiratory diseases in birds but may also be transmitted to other animals, including humans. The prevalence of the pathogen in wild birds in Sweden is largely unknown.

**Methods:** DNA was extracted from cloacae swabs and screened for *C. psittaci* by using a 23S rRNA gene PCR assay. Partial 16S rRNA and *ompA* gene fragments were sequence determined and phylogenies were analysed by the neighbour-joining method.

**Results and conclusion:** The *C. psittaci* prevalence was 1.3% in 319 Peregrine Falcons and White-tailed Sea Eagles, vulnerable top-predators in Sweden. 16S rRNA and *ompA* gene analysis showed that novel *Chlamydia* species, as well as novel *C. psittaci* strains, are to be found among wild birds.

Keywords: *Chlamydia*; Sweden; eagle; falcon; zoonosis

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Free-living birds, including birds of prey, can be a vehicle to spread infections across countries pressing the importance to keep track of the spread of infectious diseases in avian hosts, both emerging and established pathogens. *Chlamydia* mainly infects birds but can also be transmitted to humans via contaminated aerosols and cause psittacosis, a respiratory tract infection occasionally with fatal outcome. The bacteria have been studied in free-living birds to some degree but data are still limited and findings in investigated species can not be extrapolated to other species (1). Birds of prey might be especially exposed to pathogenic bacteria as they are selective hunters, often feeding on sick or crippled individuals (2). Reports of chlamydial infections in free-living raptors are rare, especially PCR-based investigations (3). The White-tailed Sea Eagle (*Haliaeetus albicilla*) and the Peregrine Falcon

(*Falco peregrinus*) are rare top-predators feeding on other birds e.g. waterfowl and gulls. The two species have small, vulnerable populations on the Scandinavian peninsula and in the Baltic Sea region after facing near extinction in the 20th century (4, 5). Here we present an investigation of the occurrence of *C. psittaci* in Swedish Peregrine Falcons and White-tailed Sea Eagles using 23S rRNA real-time PCR screening and characterization of *C. psittaci* cases by DNA sequencing of the 16S rRNA and outer membrane protein A (*ompA*) genes.

## Material and methods

Cloacal swab samples were taken from 108 Peregrine Falcon and 191 White-tailed Sea Eagle nestlings as part of national monitoring programs in the breeding seasons of 2006 and 2007 (2). Using a sterile cotton swab, a cloacal sample was taken from each nestling and

immediately put in bacterial transport medium consisting of bacterial freeze medium (Luria broth with phosphate buffered saline containing 4.4% glycerol). Samples were also collected in 2003–2006 from 20 fresh corpses of fully-grown White-tailed Sea Eagles submitted to the Swedish Museum of Natural History, Stockholm, for necropsy.

DNA was purified from the specimens using Mag-Attract DNA Mini M48 kit on a BioRobot M48 workstation (QIAGEN, Hilden, Germany). The DNA extraction process was repeated on positive samples at a separate laboratory to confirm obtained *ompA* gene sequences.

Screening was performed using a 23S rRNA gene real-time PCR specific for *C. psittaci*, *Chlamydia abortus* and *Chlamydia pecorum* (6) and was carried out on a LightCycler 2.0 (Roche Diagnostics, Basel, Switzerland). *C. psittaci* strains 6BC (ATCC nr VR-125), and the clinical isolate DC5 were used to evaluate performance of the real-time PCR detection.

The 16S rRNA gene signature sequence (298 bp) was amplified in a semi-nested PCR (7). Amplification of the 1,101 bp *C. psittaci ompA* gene fragment was performed with a previously described PCR procedure (8). To characterize the *ompA* gene sequence in the eagle nestling a semi-nested PCR amplifying a 488-bp segment was used according to Kaltenboeck et al. (9), except that the primers in the first step were 191CHOMP, and CHOMP371 and in the second step the reverse primer was replaced by CHOMP336.

After sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) the data were analyzed using DNA Baser 2.80.0 (HeracleSoftware, Lilienthal, Germany) and BioEdit 7.0.9 (Ibis Therapeutics, Carlsbad, CA). All gene sequences determined in this study were aligned with sequences published in GenBank using ClustalW (August 2010). The *ompA* based phylogenetic tree (Fig. 1) was constructed in MEGA version 4 with the neighbour-joining method using the TrN+G substitution model.

## Results and discussion

Four of the 319 raptors (1.3%) were found positive for *C. psittaci* with 23S rRNA real-time PCR screening: two falcons (1.9%) and two eagles (1%), of which one was from a dead adult and one from a nestling. The two falcon specimens, collected from well separated locations, had identical *C. psittaci* gene sequences. When compared to sequences published in GenBank, the partial 16S rRNA gene sequences (HQ616172) were identical to the type strain 6BC, except at one position in the variable region III. The *ompA* sequences (HQ616169) were novel (represented by specimen 63504, Fig. 1) and differed at seven of 1024 positions compared to 6BC. The most similar strain, differing at two positions

in *ompA*, was *C. psittaci* strain 2806/48 (EU682089), previously found in a feral pigeon in Italy (10).

The adult White-tailed Sea Eagle (specimen 60194, Fig. 1) had a 16S rRNA gene sequence (HQ616171) identical to three *C. psittaci* GenBank records, one of them being strain NJ1 (U68419). In contrast the *ompA* sequence (HQ616168) was novel and differed at 191 positions out of 1023 bp from strain NJ1 (AF269266) but only at four positions from *C. psittaci* strain 3A-CL395 (EU682087), previously isolated from a budgerigar in Germany (10). The White-tailed Sea Eagle sampled as a nestling (specimen N1091, figure) had a novel 16S rRNA gene sequence (HQ616173) differing at twelve positions from a recently discovered *Chlamydophila sp.* (GQ398031) isolated from domestic poultry in France (11). The *ompA* gene sequence (HQ616170) was most similar (71% sequence similarity) to *C. psittaci* strain VS225 (AF269259), previously found in an Orange-fronted Parakeet (*Aratinga canicularis*).

Three of four infected birds had previously unidentified strains of *C. psittaci* (Fig. 1), illustrating the lack of knowledge concerning the diversity of *C. psittaci* in free-living birds. The potential range of variation within the *Chlamydiales* is constantly being re-evaluated and several new chlamydial species have recently been identified (7, 12). This is demonstrated by the 16S rRNA and *ompA* gene sequences obtained from the White-tailed Sea Eagle nestling. The 16S rRNA gene signature sequence shared 95% sequence similarity with the *Chlamydophila* species discovered by Larocau et al. (11) and the partial *ompA* gene sequence amplified from the same specimen differed significantly from other chlamydial strains. Both the 16S rRNA and the *ompA* gene sequences indicate that this is a novel species.

*C. psittaci* prevalence in free-living birds in Sweden has only been reported in passerines where positivity rate also was low (2.9%) (13). No previous studies to identify *C. psittaci* in birds of prey have been reported from Sweden and the discovery of novel strains illustrates the shortage of data. The present study focused on top predators in the avian food chain since *C. psittaci* prevalence among the raptors should somewhat reflect the prevalence among the bird species they prey upon. Only two species of birds of prey was included in this study and it is difficult to generalize these findings to other top predators in Sweden and elsewhere. The most comprehensive review of avian host range indicates that around 15% of species in the orders *Falconiformes* and *Accipitriformes* have been found to be infected with *C. psittaci* (1). However, sampling and transport conditions, age and health state of the birds, sensitivity and specificity of detection methods are factors that may influence the test outcome and current knowledge is far from complete. In our study the DNA extraction and PCR method used had a detection capacity of

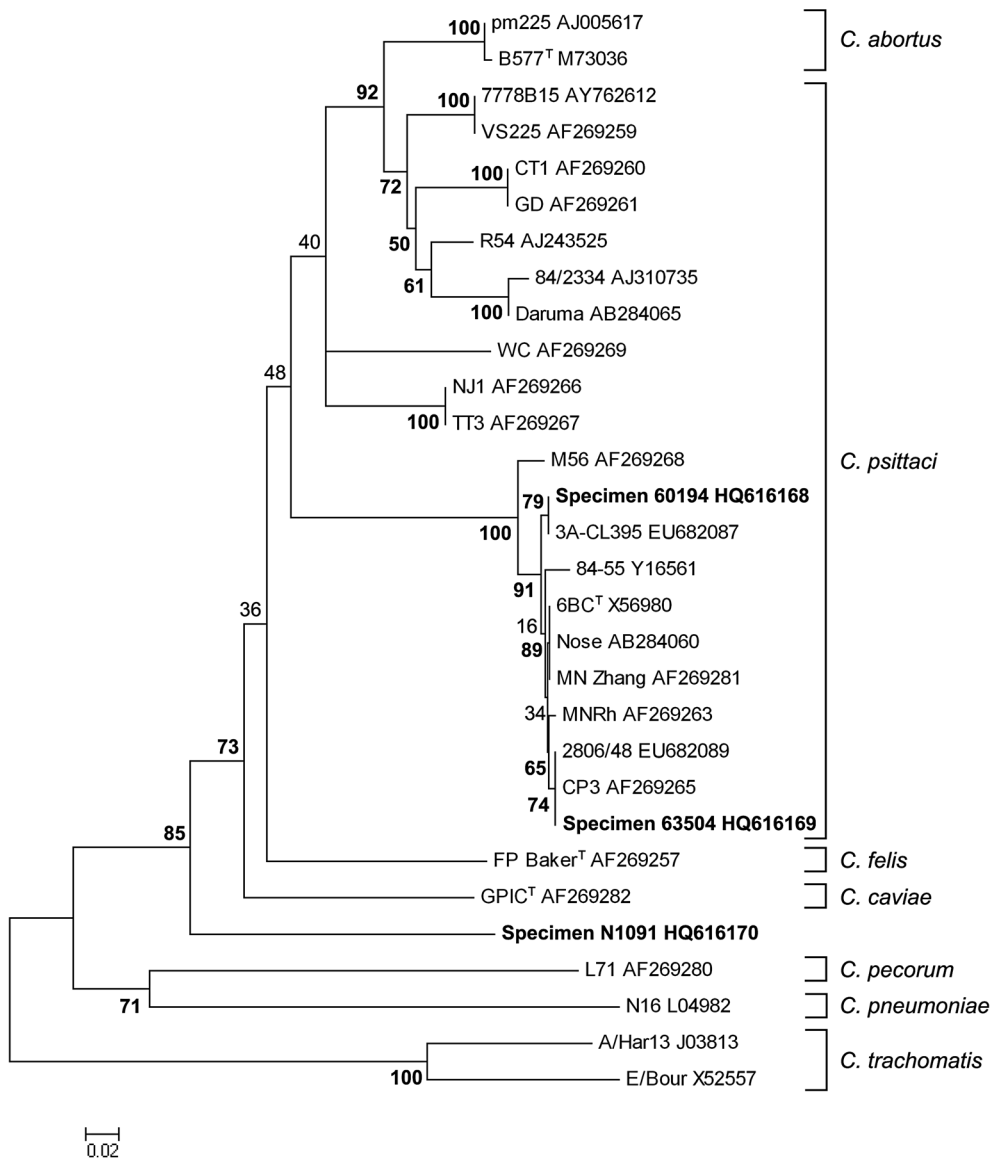


Fig. 1. Neighbour-joining phylogenetic analysis of *ompA* gene sequences from *Chlamydiaceae* strains (436–460 bp). GenBank accession numbers are provided for each strain. Specimens found in this study are in boldface. Specimen 63504 represents two Peregrine Falcons specimens that had identical *ompA* gene sequences. Specimen 60194 and N1091 are from two White-tailed Sea Eagles. T = type strain. Bootstrap was conducted with 1000 repetitions and all clades with bootstrap values over 50 are marked in boldface.

approximately 100 genome copies per swab sample, which is an adequate performance. The critical steps for detection of *C. psittaci* are the handling of specimens and the health status of the birds.

### Conclusion

Our results strengthen the view that the prevalence of *C. psittaci* among wild birds is low, but may vary depending on species, age and social behaviour. Three nestlings were infected illustrating that infection can effectively spread within the nest, either from parent to young or via prey. It would be interesting to trace the bacteria found in birds of prey from lower stages of

the food chain to get a more detailed picture of transmission routes, especially concerning the potential new species found in this study. In contrast to previous studies (14, 15) the present investigation support the notion that the risk for *C. psittaci* infections from wild birds to humans is low and is almost limited to specimens with high load of *C. psittaci* as in overtly diseased birds.

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### Conflict of interest and funding

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