Chlamydia psittaci in Swedish Wetland Birds: A Risk to Zoonotic Infection?

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Research Note—

Chlamydia psittaci in Swedish Wetland Birds: A Risk to Zoonotic Infection?

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SUMMARY. *Chlamydia psittaci* in birds may be transmitted to humans and cause respiratory infections, sometimes as severe disease. Our study investigated the *C. psittaci* prevalence in migratory birds in Sweden by real-time PCR. Fecal specimens or cloacal swabs were collected from 497 birds from 22 different species, mainly mallards (*Anas platyrhynchos*), at two bird observatories in Sweden. DNA from *C. psittaci* was found in six (1.2%) birds from three different species. Five of the positive specimens were infected with four novel strains of *C. psittaci*, based on sequencing of partial 16S rRNA gene and *ompA* gene, and the sixth was indentified as a recently described *Chlamydiaceae*-like bacterium. Considering exposure to humans it is concluded that the risk of zoonotic infection is low.

RESUMEN. *Nota de Investigación*—Presencia de *Chlamydia psittaci* en aves de los humedales de Suecia: ¿Un riesgo de zoonosis? La *Chlamydia psittaci* en las aves puede ser transmitida a los seres humanos y puede causar infecciones respiratorias, y a veces enfermedad grave. Este estudio investigó la prevalencia de *C. psittaci* en las aves migratorias en Suecia mediante PCR en tiempo real. Las muestras fecales o hisopos cloacales se obtuvieron de 497 aves de 22 especies diferentes, sobre todo patos reales (*Anas platyrhynchos*), en dos observatorios de aves en Suecia. El ADN de *C. psittaci* se encontró en seis (1.2%) de las aves a partir de tres especies diferentes. Cinco de las muestras positivas estaban infectadas con cuatro cepas nuevas de *C. psittaci*, basados en la secuenciación parcial del gene de ARN ribosomal 16S y del gene *ompA*, mientras que el sexto fue identificado como una bacteria del tipo *Chlamydiaceae* recientemente descrita. Considerando la exposición a los seres humanos, se concluye que el riesgo de infección zoonótica es bajo.

Key words: *Chlamydia psittaci*, chlamydiosis, mallard, *Anas platyrhynchos*, *ompA*, 16S RNA gene

Abbreviations: dNTP = deoxyribonucleotide triphosphate; dTTP = deoxythymidine triphosphate; dUTP = deoxyuridine triphosphate

The intracellular pathogen *Chlamydia psittaci* primarily infects the respiratory, pharyngeal, and cloacal epithelia in birds, but can also be transmitted to humans. The disease has been reported since the late 1800s and became recognized worldwide in the late 1920s, when an outbreak of pneumonia in North America and Europe was found to originate from Amazon parrots imported from Argentina (5). Originally, human infection was thought to originate solely from psittacine birds, and was therefore named psittacosis. Nowadays *Chlamydia* bacteria are known to infect a large number of bird species worldwide (7).

Migratory wild bird species can potentially function as long-range dispersers of microorganisms transmissible to humans. Recently, a number of reports have been published concerning the occurrence of *C. psittaci* in free-living birds (11,16,20), but knowledge is still limited. Migrating long distances is an energy-demanding activity, and prolonged, physical strain may lead to immunosuppression, which in turn increases the risk of activating latent infections (13,17).

The order Anseriformes includes many species of ducks and other game birds. *Chlamydia psittaci* has previously been isolated from these types of birds, both free living and domestic (8,11,18). *Chlamydia psittaci* infections have also been found in several species of waterfowl and other wetland birds (3,12). Previous reports show that free-living birds can spread chlamydial infection to domestic fowl, for example via common standing water (5).

Human chlamydiosis is regarded as a rare, but potentially severe disease. The bacteria can cause respiratory symptoms, including pneumonia, but patients can also be asymptomatic (19). Because the clinical picture varies from mild infection to fatal disease, it is difficult to distinguish chlamydiosis from other respiratory diseases, and laboratory diagnosis is required. *Chlamydia psittaci* usually spread through inhalation of contaminated aerosols, contact with secretion from eyes or exhalation, or contact with feces. Shedding of the bacteria through feces occurs intermittently and can continue for several months in both symptomatic and asymptomatic carriers (4,15). Humans in close contact with birds in their profession or leisure time, such as poultry farmers, veterinarians, bird owners, and hunters of game birds, are considered to be at greatest risk of being infected (6,17). In Sweden around 10 cases per year of human chlamydiosis have been reported the last decade according to the Swedish Institute for Infectious Disease Control.

This study used a real-time PCR amplifying a part of the 23S rRNA gene to investigate the occurrence of *C. psittaci* in cloacal specimens from migratory waterfowl in Sweden. Positive specimens were genotyped with the use of PCR amplification and DNA sequencing of the 16S rRNA gene and *ompA* gene. The study mainly focused on mallards although several other species of birds were also tested.

MATERIALS AND METHODS

Setting. Wild birds (n = 497) were caught during autumn migration in 2006 and 2007 at Ottenby Bird Observatory, on Öland, a Swedish

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Table 1. Specimens screened for the presence of *Chlamydophila psittaci* according to species.

Bird species	Birds	Positive
Barnacle goose (*Branta leucopsis*)	2[A (2)]	
Black-headed gull (*Chroicocephalus ridibundus*)	3[A (3)]	
Black tern (*Chlidonias niger*)	1	
Buff-breasted sandpiper (*Tryngites subruficollis*)	1[A (1)]	
Caspian gull (*Larus argentatus cachinnans*)	2[A (2)]	
Common eider (*Somateria mollissima*)	4[A (3)]	
Common tern (*Sterna hirundo*)	56[A (34)]	1
Dunlin (*Calidris alpina*)	8[A (8)]	
Eurasian teal (*Anas crecca*)	8[A (3)]	
Eurasian wigeon (*Anas penelope*)	5[A (1)]	
Great black-backed gull (*Larus marinus*)	2[A (2)]	
Great cormorant (*Phalacrocorax carbo*)	3	
Grey heron (*Ardea cinerea*)	1	
Greylag goose (*Anser anser*)	1	
Herring gull (*Larus argentatus*)	10[A (3)]	1
Jack snipe (*Lymnocryptes minimus*)	1[A (1)]	
Little tern (*Sternula albifrons*)	15[A (10)]	
Lure duck (mallard, *Anas platyrhynchos*)	14[A (14)]	1
Mallard (*Anas platyrhynchos*)	349[A (160)]	3
Northern pintail (*Anas acuta*)	1	
Ruddy turnstone (*Arenaria interpres*)	3[A (2)]	
Tawny owl (*Strix aluco*)	3[A (3)]	
Wood sandpiper (*Tringa glareola*)	4[A (4)]	
Total	497	6[B]

[A]Species where samples were not pooled. Number of specimens not pooled in parentheses.

[B]Positive: PCR product obtained from the 23S RNA gene. All positive specimens were from Ottenby; *C. psittaci* was not detected in any specimens from Hornborgasjön.

island in the Baltic Sea, as previously described (9). The specimens originated from 22 different bird species, although the majority came from migratory mallards (Table 1). Cloacal swabs were taken from each bird and the same bird was often sampled at different dates, giving a total of 549 specimens from 387 birds from Ottenby. With the use of similar methods, an additional 203 samples were collected from 110 birds trapped at Lake Hornborgasjön in south central Sweden from May to the end of August in 2007. Out of 752 specimens, 506 (67%) were pooled in groups of three or four birds of the same, or similar, species.

Nucleic acid extraction. DNA was extracted with the MagAttract Viral RNA M48 extraction kit ($n = 192$) in combination with the M48 Biorobot (both supplied by Qiagen, Hilden, Germany) or by using the NucliSens easyMAG standard reagents on the NucliSens easyMAG instrument (BioMérieux, Marcy l'Etoile, France).

PCR screening and amplification. A real-time PCR detecting the 23S rRNA gene of *C. psittaci, Chlamydia pneumoniae*, and *Chlamydia pecorum* was performed on a LightCycler 2.0 Real-Time Thermal Cycler (Roche Diagnostics, Basel, Switzerland), and the primers CHL23SUP and CHL23SDN and the probes CHL23LCR and CP23FLU as previously described (2). The reaction mix contained 5 µl of extracted DNA in a total volume of 20 µl. This included 1 µM of each primer and 1 µM of each probe, FastStartHybProbe from Roche containing FastStart Taq DNA Polymerase, reaction buffer dNTP mix (with dUTP instead of dTTP), and 10 mM $MgCl_2$, and additional 4.5 mM $MgCl_2$. The *C. psittaci* strains 6BC and DC5 were used to evaluate the real-time PCR detection In each PCR run a positive control with 6BC was included as well as a negative control of water.

The 16S rRNA gene signature sequence (298 bp) was amplified in a seminested PCR using primers 16SIGF and ChlamR and HotStarTaq DNA polymerase (Qiagen, Hilden, Germany) as described in our previous study (1). In the second step 16SIGR was used as reversed primer. Amplification of the partial *rnpB* gene (325 bp) was performed as previously described (1) with the use of the primers BH3 and BH2 designed on basis of *C. psittaci* sequences. Amplification of the 1101-bp *C. pscittaci ompA* gene fragment was performed with the previously described PCR procedure (6). Amplification of the partial *ompA* gene (422 bp) from the herring gull specimen was performed as a seminested PCR (1). The PCR was carried out with the use of Expand High Fidelity DNA polymerase (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions, except using 10 plus 30 cycles.

DNA sequencing. Sequencing PCR products using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), and subsequent ethanol purification, was carried out according to the manufacturer's instructions. Additional sequencing primers for the partial 16S rRNA gene included the primer 16SIGR and for the *ompA* gene additional sequencing primers were used, as previously described (6).

When sequencing the *ompA* gene from the herring gull specimen additional sequencing primers were used as previously described (1). Sequencing was carried out on all DNA strands using an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) and 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 compared to sequences published in GenBank (August 2010) using the Basic Local Alignment Search Tool (BLAST). Relevant sequences were downloaded and alignments were produced using ClustalW, included in BioEdit 7.0.9.

RESULTS AND DISCUSSION

Screening of 752 cloacal specimens from 497 birds resulted in 6 birds positive for *C. psittaci* with 23S RNA PCR: 3 wild (specimens 50376, 50338, and 1192) and 1 domestic mallard (specimen 55435), 1 herring gull (specimen 67754), and 1 common tern (specimen 21; Table 1). However, continued characterization showed that the herring gull specimen was not a *C. psittaci* strain (see below). The remaining five cases were identified as novel *C. psittaci* strains based on the partial *ompA* gene, although they showed similarities to known strains (Table 2). *Chlamydia psittaci* has mainly been studied in feral pigeons and domesticated birds where studies have shown a prevalence ranging between 19% and 96% (10). The occurrence of these bacteria among wild birds is less examined, even though it is known that over 430 bird species have been shown to have the infection (7). In our study *C. psittaci* were found in only 1% of the specimens, suggesting a low prevalence in Swedish migratory aquatic birds. This prevalence is slightly lower than expected in comparison to previously reported prevalence in aquatic and other migratory birds (6,13,14,16). As mentioned, *C. psittaci* is shed intermittently and may have been present in latent form among some birds when the specimens were collected, resulting in false negatives. The samples we examined were cloacal or fecal swabs and the result may have been different if samples had been taken in, for example, the pharynx. Even so, the relatively low prevalence of *C. psittaci* in the specimens indicates that the spread of the infection to humans via waterfowl and shorebirds is not a large threat. However, investigations of birds are needed for an increased understanding of the spread of the disease.

The duck trap holds between 8 and 12 domestic mallards (lure ducks), which are used to attract birds to the trap. They were initially without *C. psittaci* infection, but one sample from these birds was positive for *C. psittaci* at a later sampling occasion. Three wild mallards were *C. psittaci*–positive and had identical bacterial 16S rRNA gene sequences. The last mallard found positive was also

Table 2. Nucleotide differences between partial *ompA* gene sequences (839–872 bp) found in this study, and those representing other *Chlamydiaceae* spp.[A]

No.	Species [sequences]	Number of nucleotide differences[B]															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*C. psittaci* M56 [AF269268]	ID															
2	*C. psittaci* [L04980]	58	ID														
3	*C. pneumoniae* [L04982]	286	284	ID													
4	*C. abortus* B577 [M73036]	188	200	252	ID												
5	*C. psittaci* WC [AF269269]	170	166	259	157	ID											
6	*C. psittaci* VS225 AF269259	177	172	249	72	136	ID										
7	*C. psittaci* isolate 1V [EF028916]	169	171	266	99	146	100	ID									
8	*C. psittaci* Mat116 [AB284058]	50	28	279	195	174	174	168	ID								
9	*C. psittaci* 6BC [X56980]	53	6	285	199	164	172	170	26	ID							
10	*C. psittaci* strain 84-55 [Y16561]	61	15	288	203	168	176	173	33	9	ID						
11	*C. psittaci* NJ1 [AF269266]	152	148	248	134	123	101	125	155	146	150	ID					
12	*C. psittaci*, sample 21 [JQ679391] (this study)	179	173	251	72	138	**2**	102	175	173	177	102	ID				
13	*C. psittaci,* sample 1192 [JQ679392] (this study)	164	168	260	101	142	99	**50**	166	166	169	121	101	ID			
14	*C. psittaci,* sample 55435 [JQ679394] (this study)	74	79	280	207	184	189	187	**73**	75	83	172	191	179	ID		
15	*C. psittaci,* sample 50338 [JQ679393] (this study)	75	80	280	207	185	189	188	**73**	76	84	171	191	180	**1**	ID	
16	*C. psittaci,* sample 50376 (this study)	75	80	280	207	185	189	188	**73**	76	84	171	191	180	**1**	**0**	ID

[A]Differences in size of the gene sequences required shortening of all sequences, however, not affecting the overall outcome.
[B]The number at the top of each column corresponds to the species number as defined for each row.

sampled 4 days earlier, but tested negative for *C. psittaci* in that specimen. The mallards may initially have been asymptomatic carriers with no shedding, but when experiencing stress from being captured or migrating, the bacteria would start shedding and therefore not be detected the first time. As they were collected at the same place in a short period of time it is likely that the birds infected each other. The lure duck did not share a pen with the wild birds but shared the same water. This indicates that the bacteria can be highly infectious and spread from free-living birds and may be a threat to domestic birds via direct contact or a shared water source.

A limitation in this study was pooling of specimens. One of the mallards found positive for *C. psittaci* was part of a pool of four mallard specimens. Likewise, the positive common tern was from a pool of four specimens from common terns. Because of the overall low prevalence of *C. psittaci* the pools were assumed to consist of one positive specimen and three negative specimens. This was confirmed for the pool of mallards when original specimens from the four birds were screened and sequenced for the *ompA* gene. This was not possible for the pool of common terns, most likely due to poor quality of specimens thawed several times.

Genotyping was applied to characterize the 23S RNA–positive cases. A partial region of the *ompA* gene was amplified and sequenced together with a signature sequence of the 16S rRNA gene from the *C. psittaci*–positive specimens. The five cases identified as *C. psittaci* showed close relatedness to *C. psittaci* in both the partial 16S rRNA gene sequence and the partial *ompA* gene sequence. The partial 16S rRNA gene sequences found in specimens 50376, 50338, and 55435 are identical and appear to be from a novel strain, differing with four positions from *C. psittaci* strain prk/Daruma (D85710), previously isolated from a king parakeet (*Alisterus* sp.) in Japan. The partial *ompA* gene sequence was found to be the same in two of the specimens and differed least from *C. psittaci* strain Mat116, (AB284058) previously isolated from a chestnut-fronted macaw (*Ara severa*) in Japan; the third differed from the other two in one position of 1028 bp (Table 2).

The fourth mallard, specimen 1192, and the common tern, specimen 21, were infected with unidentified strains of *C. psittaci*, which differed from the other isolated strains in *ompA* gene sequences. The partial 16S rRNA gene sequence found in specimen 1192 was found to be identical to *C. psittaci* strain prk/Daruma (D85710) and *C. abortus* strain Ov/B577 (D85709). The partial *ompA* gene sequence found in the 1192 specimen differed least from *C. psittaci* isolate 1V (EF028916) previously isolated from a hooded crow in Russia (Table 2). The partial 16S rRNA gene sequence found in the common tern specimen was identical to *Chlamydia* sp. Rostinovo-70 (DQ663788) and differed in one point mutation from numerous *C. psittaci* strains. The partial *ompA* gene sequence isolated from the common tern was most simliar to *C. psittaci* strain VS225 (AF269259; Table 2) previously isolated from an orange-fronted parakeet (*Aratinga canicularis*) in Texas, USA.

Genotyping of the herring gull specimen was shown to contain a recently discovered *Chlamydiaceae*-like bacterium (1). This was confirmed in partial sequencing of three gene sequences, 16S, *ompA* and *rnpB*. The signature 16S rRNA sequence was identical to that of sequences found in specimens from the Bering Sea. The *ompA* gene sequence JQ679395 differed in 30 positions from previously found *ompA* sequences. The *rnpB* gene sequence found in the herring gull specimen differed in two point mutations from the sequence in glaucous-winged gulls (*Larus glaucescens*). The presence of the novel *Chlamydiaceae*-like bacterium in the herring gull specimen is interesting, as this bacterium has earlier only been identified in specimens from the Bering Sea. To test for laboratory contamination DNA extraction was performed at two different laboratories (Uppsala, Sweden and Kalmar, Sweden) by different methods to exclude false results from the herring gull specimen. The sequence differences in the *ompA* and the *rnpB* genes compared to the recently found sequences from the Bering Sea was not unexpected, given the vast geographical differences between the findings. This gives rise to many questions concerning the spread and nature of this bacterium.

In summary, 1% of 497 birds were found to be infected with *C. psittaci* by sequencing, and in addition one case of a novel *Chlamydiaceae*-like bacterium was detected. It was concluded that the risk of zoonotic infection is low.

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