Prevalence of *Chlamydia psittaci* and Other *Chlamydia* Species in Wild Birds in Poland

Marta Krawiec, Tomasz Piasecki, and Alina Wieliczko

Abstract

Avian chlamydiosis is a zoonotic disease occurring in humans, poultry, and exotic birds. It has been suggested that some wild bird species play an important role as reservoirs for *Chlamydia*, especially *Chlamydia psittaci*. Whereas *C. psittaci* is the predominant chlamydial agent in birds, in the present study we have determined the prevalence of different species of *Chlamydia* among selected wild bird species in Poland using a rapid and sensitive real-time PCR method. In total, 369 free-living birds from 35 bird species and 15 orders were examined. Samples from 27 birds (7.3%) were positive for chlamydial DNA in the PCR; 22 positive samples (81.5%) belonged to *C. psittaci*, three to *Chlamydia trachomatis* (11.1%), and two (7.4%) classified only to the genus *Chlamydia*. Most of *C. psittaci*-positive samples belonged to five orders: Anseriformes, Columbiformes, Gruiformes, Phasianiformes, and Passeriformes. All *C. trachomatis* samples were obtained from Eurasian coots (Gruiformes). Two *Chlamydia*-positive samples not classified to any *Chlamydia* species were obtained from a common wood pigeon (Columbiformes) and a common buzzard (Accipitriformes). Detection of *C. psittaci* and *C. trachomatis* in free-living bird populations force to think on significance of birds as reservoir of varied *Chlamydia* species and their epidemiological importance.

Key Words: *Chlamydia psittaci*—*Chlamydia trachomatis*—Wild birds—Real-time PCR.

Introduction

CHLAMYDIOSIS IS AN IMPORTANT infectious disease of people and many animals, including birds. It is caused by the Gram-negative obligatory intracellular bacterium *Chlamydia* spp., mostly by *C. psittaci* in birds (Vanrompay 2013). The disease caused by this pathogen in birds is known as avian chlamydiosis and it can cause diseases in humans (Rehn et al. 2013). The groups of people in close contact with birds, like avian breeders, veterinarians, workers of poultry farms, etc., are particularly exposed to *Chlamydia* infections. There are few reports of cases of human-to-human psittacosis (Wallensten et al. 2014).

The *Chlamydia* spp. can cause an important disease of birds that is most commonly found in parrots (Psittaciformes) and pigeons (Columbiformes). The outcome of the illness is mostly chronic with conjunctivitis or is asymptomatic, but it might also appear as an acute disease. Depending on the chlamydial strain and the avian host, chlamydiae cause pericarditis, air sacculitis, pneumonia, lateral nasal adenitis, peritonitis, hepatitis, and splenitis.

Generalized infections result in fever, anorexia, lethargy, diarrhea, and occasionally shock and death. Chlamydiosis is a very common chronic infection of psittacine birds. Infections cause conjunctivitis, enteritis, air sacculitis, pneumonitis, and hepatosplenomegaly (Harkinezhad et al. 2009). The asymptomatic birds can play a role in the transmission of *Chlamydia* in the environment, and thereby might be significant in epidemiology of chlamydiosis. *C. psittaci* has been isolated from more than 460 avian species in the wild (Kaleta and Taday 2003); however, little is known about the prevalence of other species of *Chlamydia* among wild bird population worldwide. Some reports suggest that avian infections might be caused only by *C. psittaci* (Harkinezhad et al. 2009), but others have confirmed infections caused by other species of *Chlamydia* such as *C. pecorum* and *C. trachomatis* (Sachse et al. 2012), *C. abortus* (Herrmann et al. 2000, Pantchev et al. 2009), and *C. ibidis* (Vorimore et al. 2013).

The aim of this study was to determine the prevalence of *C. psittaci* and other *Chlamydia* spp. in various species of wild birds in Poland.

Department of Epizootiology and Clinic of Bird and Exotic Animals, The Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Wrocław, Poland.

TABLE 1. ORDERS AND SPECIES OF BIRDS EXAMINED IN THE PRESENT STUDY

Order of birds	Birds species (no.)	Total of samples
Accipitriformes	Common Buzzard (5), Sparrow Hawk (1)	6
Anatiformes	Mallard Duck (120), Velvet Scoter (30), Mute Swan (5), Long-Tailed Duck (1)	156
Apodiformes	Common Swift (9)	9
Charadriiformes	Razorbill (1), Guillemot (1), Woodcock (1)	3
Ciconiiformes	White Stork (3)	3
Columbiformes	Common Wood Pigeon (2), Rock Pigeon (2)	4
Falconiformes	Kestrel (8)	8
Gaviiformes	Red-Throated Loon (1)	1
Gruiformes	Eurasian Coot (7)	7
Passeriformes	Eurasian Siskin (7), Eurasian Coot (7), Eurasian Tree Sparrow (5), Hooded Crow (2), Great Tit (6), Bochemian Waxwing (3), Rook (6), Greenfinch (4), Black Red Start (3), Blue Tit (2), House Sparrow (1), Blackbird (1), Barn Swallow (1), Magpie (1)	49
Pelecaniformes	Gray Heron (3)	3
Phasianiformes	Common Pheasants (7)	7
Podicipediformes	Great Crested Grebe (1)	1
Strigiformes	Tawny Owl (1), Little Owl (1)	2
Suliformes	Great Cormorants (110)	110
Total		369

Materials and Methods

The samples were collected from September, 2011, to August, 2013. This study included 369 wild birds from 15 orders and 35 different species, which are presented in Table 1. All tested birds were dead, and material, including lungs, liver, spleen as well as conjunctival swabs from both eyes, were collected during necropsy. Mallard ducks, Eurasian coots, and pheasants were obtained during the hunting season by two hunting associations in accordance with local hunting laws, special permission, and hunting programs. Samples from great cormorants were obtained during the annual population culling from the lakes of the Lower Silesia region in Poland with the consent of the Regional Directorate of Environmental Protection, Wrocław, Poland (no. WPN. 6205.67.2012.MK.1). Samples from velvet scoters were collected from birds that were found dead in fishing nets on the Baltic coast between February and April of 2013. All small garden birds were found dead and brought to our University by ornithologists, veterinarians, or private individuals. The remaining tested wild bird species were delivered after death from wildlife rescue centers, where they had died before treatment procedures were started.

DNA was extracted and purified with a Syngen Tissue DNA Mini Kit, according to the manufacturer's manual (Syngen Biotech, Poland). The DNAs of reference strains were included as positive controls and were as follows: *C. suis* VR-1474 (American Type Culture Collection [ATCC], USA), *C. trachomatis* and *C. abortus* (NRVI,

Puławy, Poland), *Chlamydophila felis* strain 905 (Merial, France), and *Chlamydophila psittaci* (field isolate obtained from birds in our laboratory described previously by Piasecki et al. (2012)). *Mycoplasma* spp. ATCC 2391 (LGC Standards, Poland) and *Staphylococcus epidermidis* ATCC 35984 (PAN, Wrocław, Poland) were included as negative controls.

All DNA samples were tested by using the quantitative PCR (qPCR) procedure described by Everett et al. (1999). Products obtained with the PCR were sequenced (Genomed, Poland) and identified using BLAST (blast.ncbi.nlm.nih.gov). This test targeted the 23S ribosomal RNA gene. The oligonucleotides were synthesized by Sigma-Aldrich (Germany). Primer and probe sequences used during the study are provided in Table 2. Each 20- μ L reaction mixture for real-time PCR with the TQF/TQR primers and probe, as described by Everett et al. (1999), contained 10 μ L of KAPA PROBE FAST Bio-Rad iCycler 2x qPCR Master Mix (KapaBiosystems, USA), 10 μ M of each primer, 10 μ M of the probe, 4.2 μ L of Milli-Q water, and 5 μ L of genomic DNA. The cycling conditions were 40 cycles of 0:15 min at 95°C and 1:00 min at 60.5°C, with an initial denaturation for 3:00 min at 94°C. Real-time PCR was performed using Bio-Rad iQ5 (Bio-Rad, Poland).

Results

Chlamydia-positive results were obtained from 27 out of 369 birds, comprising 7.3% of all of examined samples. *Chlamydia*-positive birds belonged to 13 species of birds (from eight orders) including: Hooded Crow, Common Wood

TABLE 2. PRIMER AND PROBE SEQUENCES USED DURING THE STUDY BY EVERETT ET AL. 1999

Test	Primer	Sequences of oligonucleotides
Real- time PCR	TQF TQR Probe	5'-GAAAAGAACCCTTGTTAAGGGAG-3' 5'-CTTAACCTCCCTGGCTCATCATG-3' FAM-CAAAAGGCACGCCGTCAAC-TAMRA

Pigeon and Rock Pigeon, Eurasian Coot, Pheasant, Waxwing, Eurasian Tree Sparrow, Common Buzzard, Great Tit, Eurasian Siskin, Common Swift, Great Cormorant, and Mallard Ducks (Table 3).

Twenty-two positive samples (81.5%) belonged to *C. psittaci*, three (11.1%) to *C. trachomatis*, and two (7.4%) were identified just as Chlamydiae but were not classified to any *Chlamydia* species. All positive results came from DNA obtained from tissues (lungs, liver, spleen). All spleens and livers of *Chlamydia*-positive birds showed a positive

result in the real-time PCR, but only nine lungs were *Chlamydia*-positive in the same test. No positive results were seen in the swabs.

The PCR products were sequenced. Sequencing confirmed 99–100% genetic similarity to the *C. psittaci* (gi|485099180|ref|NR 102574.1|:508-637 *C. psittaci* 6BC strain 6BC 23S ribosomal RNA complete sequence [2]) and *C. trachomatis* (gi|527130011|gb|CP002677.1|:882052-882179 *C. trachomatis* RC-F(s)/342 complete genome) gene fragment sequences obtained from GenBank, respectively. Two products were

TABLE 3. CHLAMYDIAL DNA DETECTED IN FREE-LIVING BIRDS

Order of birds	Bird species/total no. of samples	No. (%) of positive samples	No. (%) of <i>Chlamydia</i> spp.-positive birds		
			<i>C. psittaci</i>	<i>C. trachomatis</i>	None classified <i>Chlamydia</i>
Accipitriformes	<i>Buteo buteo</i> Common Buzzard <i>n</i> = 5	1 (20.0)	0 (0.0)	0 (0.0)	1 (100.0)
Anseriformes	<i>Anas platyrhynchos</i> Mallard Duck <i>n</i> = 120	5 (4.16)	5 (100.0)	0 (0.0)	0 (0.0)
Apodiformes	<i>Apus apus</i> Common Swift <i>n</i> = 9	1 (11.11)	1 (100.0)	0 (0.0)	0 (0.0)
Columbiformes	<i>Columba palumbus</i> Common Wood Pigeon <i>n</i> = 2	1 (50.0)	0 (0.0)	0 (0.0)	1 (100.0)
Columbiformes	<i>Columba livia</i> Rock Pigeon <i>n</i> = 2	1 (50.0)	1 (100.0)	0 (0.0)	0 (0.0)
Gruiformes	<i>Fulica atra</i> Eurasian Coot <i>n</i> = 7	3 (42.86)	0 (0.0)	3 (100.0)	0 (0.0)
Passeriformes	<i>Spinus spinus</i> Eurasian Siskin <i>n</i> = 7	1 (14.28)	1 (100.0)	0 (0.0)	0 (0.0)
Passeriformes	<i>Corvus cornix</i> Hooded Crow <i>n</i> = 1	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)
Passeriformes	<i>Parus major</i> Great Tit <i>n</i> = 6	1 (16.67)	1 (100.0)	0 (0.0)	0 (0.0)
Passeriformes	<i>Bombycilla cedrorum</i> Waxwing <i>n</i> = 3	1 (33.33)	1 (100.0)	0 (0.0)	0 (0.0)
Passeriformes	<i>Passer montanus</i> Eurasian Tree Sparrow <i>n</i> = 5	1 (25.0)	1 (100.0)	0 (0.0)	0 (0.0)
Phasianiformes	<i>Phasianus colchicus</i> Pheasant <i>n</i> = 7	3 (42.86)	3 (100.0)	0 (0.0)	0 (0.0)
Suliformes	<i>Phalacrocorax carbo</i> Great Cormorant <i>n</i> = 110	7 (6.36)	7 (100.0)	0 (0.0)	0 (0.0)
Others	Others (22 species) <i>n</i> = 85	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	<i>n</i> = 369	27 (7.30)	22 (81.48)^a	3 (11.10)^a	2 (7.40)^a

^aThe rate of respective *Chlamydia* species number calculated from the number of all *Chlamydia*-positive (27) birds.

identified only as *Chlamydia* spp., but sequencing did not confirm a high degree of similarity of these two products to any *Chlamydia* species.

Discussion

The detection rate (7.3%) of Chlamydiae in all birds examined in the present study is in compliance with the rates reported by other authors, which showed high variability, ranging from 1% to 83% (Blomquist et al. 2012, Madani and Peighambari 2013, Ribeiro et al. 2013). The *Chlamydia*-positive detection rate in our study was also variable among different orders, as in results obtained by Kaleta and Taday (2003), who reported that many positive wild birds came from orders such as Lariformes (28%), Alciformes (26%), Sphenisciformes (25%), and Anseriformes (21%). Although all birds seem to be a host of *C. psittaci*, there are some groups and orders that are supposed to be more naturally infected than others. Waterfowl seem to have a high carrier state. In present study, the water and wetland birds (especially Anseriformes, $n = 120$, and Suliformes, $n = 110$) were the biggest group of all examined birds. However, the percentage of *Chlamydia*-positive birds for the Suliformes and Anseriformes orders were 6.36% and 4.16%, respectively. The result obtained in the order Anseriformes was lower than described by Kaleta and Taday (2003).

On the basis of the available data, *C. psittaci* has been traditionally assumed to be the only relevant chlamydial agent in birds. Results obtained in our study show that more than only the classical *C. psittaci*, such as some atypical avian strains of Chlamydiae, might be detected in free-living birds. *C. trachomatis*, a typical human *Chlamydia* species was detected in three Eurasian coots. *C. trachomatis* was previously described in other species of birds by other authors. Sachse et al. (2012) provided evidence of the occurrence of *C. trachomatis* in urban pigeons. Some authors reported the prevalence of other chlamydial species than *C. psittaci*, such as *C. pecorum* (Sachse et al. 2012) and *C. abortus* (Herrmann et al. 2000, Pantchev et al. 2009), as well as *C. ibidis* (Vorimore et al. 2013) in birds.

Further reports on wild birds or feral pigeons as a source of *C. psittaci* for humans and other animals exist (Travnicek et al. 2002, Harkinezhad et al. 2009, Zweifel et al. 2009, Dickx et al. 2010). The detection of *C. trachomatis* in the three Eurasian coots might suggest a crossover of chlamydial infections between humans and free-living birds. The three positive Eurasian coots were caught at one pond, a location at which people and whole families spend their free time.

Very often birds might be carriers of *Chlamydia* asymptotically, but sometimes avian chlamydiosis manifests as

an initial upper respiratory tract infection or progresses to a severe pneumonia, polyserositis, and/or hepatitis (Harkinezhad et al. 2009). Most of the birds examined by us did not show any pathological changes during the necropsy related to a chlamydial infection. Only one Eurasian Siskin had nodular lesions in the liver and spleen induced by a concurrent salmonellosis. This *Salmonella* infection case has been described by Krawiec et al. (2014). These findings confirm the report of Harkinezhad et al. (2009) regarding the severity of clinical signs, which depend on the host species immunity and which may be triggered by a stress factor, such as another accompanying infection.

Conclusions

The present study shows the prevalence of *Chlamydia* spp. in a population of wild birds. Mainly *C. psittaci* but also *C. trachomatis* were detected in various bird orders. The *Chlamydia*-positive birds belonged to Accipitriformes, Anatiformes, Apodiformes, Columbiformes, Gruiformes, Passeriformes, Phasianiformes, and Suliformes. Different infection rates of *Chlamydia* in wild birds as well as the detection of other species than *C. psittaci* confirm that free-living birds might be a reservoir of this zoonosis. It also shows that our knowledge on chlamydial organisms in birds is still fragmentary.

Acknowledgments

This research project was financed by the National Centre for Research and Development, project number no. 12 0126 10. The authors are grateful to Paweł Lis, PhD, for help with the biotechnological analyses and to Mieczysław Łyskawa (M.Sc., Eng.) and Piotr Zajiczek for their help with collecting the samples.

Author Disclosure Statement

No competing financial interests exist.

Address correspondence to:

Alina Wieliczko

Department of Epizootiology and Clinic of Bird
and Exotic Animals

The Faculty of Veterinary Medicine

Wrocław University of Environmental and Life Sciences

Pl. Grunwaldzki 45

50-366 Wrocław

Poland

E-mail: alina.wieliczko@up.wroc.pl