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Detection of *Chlamydiaceae* in Swiss wild birds sampled at a bird rehabilitation centre

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ABSTRACT

Background Annually, 800–1500 wild birds are admitted to the rehabilitation centre of the Swiss Ornithological Institute, Sempach, Lucerne, Switzerland. The workers of the centre come in close contact with the avian patients and might therefore be exposed to zoonotic agents shed by these birds, such as *Chlamydia psittaci*.

Methods In the present study, 91 choanal, 91 cloacal and 267 faecal swabs from 339 wild birds of 42 species were investigated using a stepwise diagnostic approach.

Results *Chlamydiaceae* were detected in 0.9 per cent (0.3–2.6 per cent) of birds (n=3), all of them members of the Columbidae family. The *Chlamydiaceae* species of two of these birds (one Eurasian collared dove, one fancy pigeon) were identified as *C psittaci* types B and E by PCR and outer membrane protein A genotyping.

Conclusion The findings of the current study suggest that zoonotic transmission of *Chlamydiaceae* is very unlikely for songbird and waterfowl species tested herein, while pigeons might pose a risk to workers at rehabilitation centres.

INTRODUCTION

The rehabilitation centre of the Swiss Ornithological Institute is located in Sempach, Lucerne, Switzerland. In the last 10 years, between 800 and 1500 birds were admitted for treatment annually. The workers of the rehabilitation centre come in close contact with the birds and their faeces during the time of treatment. Thus, the workers might be exposed to zoonotic agents shed by these birds, such as Chlamydia psittaci. Several Chlamydiaceae species are harboured by wild birds, for example, C abortus, C avium, C pecorum and Candidatus C ibidis.[1–7] C psittaci, the causative agent of psittacosis/ornithosis in human and avian chlamydiosis, has been detected in more than 460 avian species.[8] The clinical signs in C psittaci-infected birds are variable, depending on age, species and immune status of the host, and the pathogenicity of the causative strain.[9] Clinical signs include respiratory, enteric and ocular signs, but asymptomatic infections are common.[10] In Switzerland, 46 cases in birds have been reported to the Federal Food Safety and Veterinary Office from 2010 to 2019.[11] Transmission from birds to humans occurs through inhalation of feather dust or aerosols from urine, dried faeces, and respiratory and eye secretions.[12] Most infected humans remain asymptomatic or have mild symptoms, but in some cases C psittaci causes severe pneumonia.

Studies investigating the occurrence of *Chlamydiaceae* in European wild birds reported infection rates ranging from 2.8 per cent to 14.8 per cent determined using PCR or immunoassay.[5 13–18] In Switzerland, two PCR-based studies focusing on pigeons reported infection rates of 8.4 per cent and 16.9 per cent,[19 20] and another PCR-based study reported infection rates of 14.3 per cent in pigeons, 0.4 per cent in songbirds and 4.3 per cent in waterfowl.[21] The highest infection rates of *Chlamydiaceae* were observed in Columbiformes and Psittaciformes, where *C psittaci* is the most frequently detected species.[22 23] Passeriformes were only occasionally diagnosed with chlamydiosis.[24 25]

The current study aims to investigate whether admitted wild birds pose a risk of zoonotic chlamydial infection to workers at a Swiss wild bird rehabilitation centre.

MATERIALS AND METHODS

Samples

Samples were obtained from the wild bird rehabilitation centre of the Swiss Ornithological Institute in Sempach, Lucerne, Switzerland, between April and October 2018 (online supplemental table 1). Around two-thirds of the birds were admitted during the peak season from May to mid-July, the majority of which were juveniles. Submitted avian patients received care depending on their condition, for example, feeding, cleaning, veterinary care, treatment or surgery if necessary. The staff of the rehabilitation centre

(six persons, among one veterinarian) come into close contact with the birds while handling them, although manual handling is reduced to the necessary minimum. Juvenile birds are kept in small boxes with household paper or foam pads as substrate. Fledged birds are transferred to cages with foam pads or an outdoor aviary. The boxes and foam pads are cleaned every one to two hours using an industrial dishwasher; cages are cleaned with water and soap—depending on the amount and quality of faeces—daily or twice daily. Disinfection of the cages (Meliseptol rapid, B Braun) is carried out before each reassignment. The outdoor aviaries are cleaned when necessary using a high-pressure cleaner at 60°C while wearing a protective mask and goggles.

This study included 339 wild birds representing nine orders and 42 species, as shown in table 1.

The age of 316 birds was known, of which 163 were nestlings, 126 juveniles and 27 adults. Dry choanal (n=91) and cloacal swabs (n=91) (FLOQSwabs, COPAN Flock Technologies) were obtained from deceased birds only. Dry faecal swabs (n=267) were taken from living birds after defecation. Samples were stored at −80°C until further processing.

DNA extraction

A commercial kit (Genomic DNA from tissue, NucleoSpin Tissue from Macherey-Nagel) was used to extract the DNA of choanal and cloacal swabs. DNA of the faecal samples was extracted with the Macherey-Nagel NucleoSpin stool kit according to the manufacturer's instructions. Extracted DNA was stored at −20°C until further use.

Real-time qPCR assays for detection of *Chlamydiaceae*

First, all samples were tested in duplicates with a 23S rRNA-based *Chlamydiaceae* family-specific quantitative PCR (qPCR) (111 bp) modified to include an internal positive amplification control (IPC; enhanced green fluorescent protein (eGFP))[26–28] on an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific). The cycle conditions were 95°C for 20 seconds, followed by 45 cycles of 95°C for three seconds and 60°C for 30 seconds. For every sample, a 25 µl reaction mix was prepared, including 12.5 µl TaqMan Universal PCR MasterMix, 500 nM of the primers 'CH23S-F' (5'-CTGAAACCAGTAGCTTATAAGCGGT-3') and 'CH23S-R' (5'-ACCTCGCCGTTTAACTTAACTCC-3'), 200 nM of the probe 'CH23S-P' (5' FAM-CTCATCATGCAAAAGGCACGCCG-TAMRA 3'), and 200 nM each of the primers 'eGFP-1-F' (5'-GACCACTACCAGCAGAACAC-3') and 'eGFP-10-R' (5'-CTTGTACAGCTCGTCCATGC-3'), and the probe 'eGFP-HEX' (5'-HEX-AGCACCCAGTCCGCCCTGAGCA-BHQ1-3'). A sevenfold dilution series of *C abortus* DNA with a known number of DNA copies was included in each run as a positive control and standard curve. Molecular grade water was included as a negative control in each run. Samples were interpreted as positive if the mean cycle threshold (Ct value) was less than 38. Samples with higher Ct values or inhibited amplification were retested in duplicate. Samples repeatedly showing a Ct value greater than 38 were considered positive. Samples with inhibited amplification were retested undiluted and tenfold diluted, both in duplicates.

Secondly, in samples positive for *Chlamydiaceae*, a *C psittaci*-specific qPCR (76 bp) was performed as previously described, modified to include an IPC.[29 30] The reaction mix contained 4 µl (<150 ng/µl) sample template, 1 µl eGFP template, 1 x TaqMan Universal PCR MasterMix, 900 nM of the primers 'CppsOMP1-F' (5'-CACTATGTGGGAAGGTGCTTCA-3') and 'CppsOMP1-R' (5'-CTGCGCGGATGCTAATGG-3'), 200 nM probe 'CppsOMP1-S' (5'-FAM-CGCTACTTGGTGTGAC-TAMRA-3'), 900 nM of the primers 'eGFP-1-F' (5'-GACCACTACCAGCAGAACAC-3') and 'eGFP-2-R' (5'-GAACTCCAGCAGGACCATG-3'), and 200 nM probe 'eGFP-HEX' (5'-HEX-AGCACCCAGTCCGCCCTGAGCA-BHQ1-3') in a final volume of 25 µl.

Outer membrane protein A genotyping PCR

Samples that were positive for *C psittaci* in qPCR were subjected to an outer membrane protein A (*ompA*) genotyping PCR. Per sample, a reaction mix with a final volume of 50 µl containing 25 µl REDTaq ReadyMix (Merck KGaA), 200 nM of the primers 'ompA F (CTU)' (5'-ATGAAAAAACTCTTGAAATCGG-3') and 'ompA rev' (5'-TCCTTAGAATCTGAATTGAGC-3'), and 3 µl sample template with a DNA concentration of 25 ng/µl was prepared.[31] Cycling conditions were 10 minutes at 95°C, followed by 35 cycles of 95°C for 30 seconds, 49°C for 30 seconds, 72°C for 60 seconds and a final elongation at 72°C for seven minutes. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions. Purified amplicons were Sanger-sequenced by Microsynth. The obtained sequences were assembled and analysed using the Geneious Prime software version 2019.2.3 and compared against the National Center for Biotechnology Information database using the BLASTn tool (https://blast.ncbi.nlm.nih.gov/).

All primers and probes used in this study were obtained from Microsynth.

RESULTS

Chlamydiaceae were detected in 0.9 per cent (95 per cent confidence interval: 0.3–2.6 per cent) of the birds (n=3) of three different species, namely in one of five (20 per cent, 3.6–62.5 per cent) fancy pigeons (*Columba livia domestica*), one of five (20 per cent, 3.6–62.5 per cent) common wood pigeons (*Columba palumbus*), and one of four (25 per cent, 4.6–69.9 per cent) Eurasian collared doves (*Streptopelia decaocto*), as shown in table 1. Five individual samples were positive for *Chlamydiaceae*: both choanal and cloacal swabs from the Eurasian collared dove and fancy pigeon, and a faecal swab from the common wood pigeon. Both choanal and cloacal swabs

Table 1 Species, number of sample types and number of individual birds per total number of birds tested for *Chlamydiaceae* using real-time PCR in this study

Order	Family	Species name (Latin)	Species name (English)	Number of available and *Chlamydiaceae**-positive swab samples per anatomical site			*Chlamydiaceae*-positive birds (%, 95% CI)	Mean Ct value, *Chlamydiaceae* quantitative PCR	Chlamydia psittaci-positive birds (%)	Accession number of *ompA* study sequence
				Faeces	Choana	Cloaca				
Anseriformes	Anatidae	*Anas platyrhynchos*	Mallard	0/26	0/4	0/4	0/29 (0, 0–11.7)		n.d.	
		Cygnus olor	Mute swan	0/0	0/1	0/1	0/1 (0, 0–79.4)		n.d.	
		Mergus merganser	Common merganser	0/9	0/0	0/0	0/9 (0, 0–29.9)		n.d.	
Apodiformes	Apodidae	*Apus apus*	Common swift	0/15	0/7	0/7	0/20 (0, 0–16.1)		n.d.	
		Tachymarptis melba	Alpine swift	0/3	0/0	0/0	0/3 (0, 0–56.2)		n.d.	
Charadriiformes	Laridae	*Larus michahellis*	Yellow-legged gull	0/3	0/1	0/1	0/4 (0, 0–49.0)		n.d.	
Ciconiiformes	Ciconiidae	*Ciconia ciconia*	White stork	0/3	0/2	0/2	0/5 (0, 0–43.5)		n.d.	
Columbiformes	Columbidae	*Columba livia domestica*	Fancy pigeon	0/4	1/1	1/1	1/5 (20, 3.6–62.5)	Choana: 28.0 Cloaca: 29.3	1/1 (100)	MT450277
		Columba livia domestica	Feral pigeon	0/5	0/2	0/2	0/6 (0, 0–39.0)		n.d.	
		Columba palumbus	Common wood pigeon	1/4	0/1	0/1	1/5 (20, 3.6–62.5)	Faeces: 37.9	0/1 (0)	
		Streptopelia decaocto	Eurasian collared dove	0/2	1/2	1/2	1/4 (25, 4.6–69.9)	Choana: 36.0 Cloaca: 16.9	1/1 (100)	MT450278
Gruiformes	Rallidae	*Crex crex*	Corn crake	0/1	0/0	0/0	0/1 (0, 0–79.4)		n.d.	
		Fulica atra	Eurasian coot	0/2	0/1	0/1	0/3 (0, 0–56.2)		n.d.	
Passeriformes	Acrocephalidae	*Acrocephalus scirpaceus*	Eurasian reed warbler	0/0	0/1	0/1	0/1 (0, 0–79.4)		n.d.	
	Emberizidae	*Emberiza citrinella*	Yellowhammer	0/1	0/1	0/1	0/1 (0, 0–79.4)		n.d.	
	Fringillidae	*Carduelis carduelis*	Goldfinch	0/8	0/1	0/1	0/8 (0, 0–32.4)		n.d.	
		Chloris chloris	European greenfinch	0/4	0/4	0/4	0/7 (0, 0–35.4)		n.d.	
		Coccothraustes coccothraustes	Hawfinch	0/1	0/1	0/1	0/2 (0, 0–65.8)		n.d.	
		Fringilla coelebs	Common chaffinch	0/4	0/1	0/1	0/4 (0, 0–49.0)		n.d.	
		Serinus serinus	European serin	0/1	0/0	0/0	0/1 (0, 0–79.4)		n.d.	
	Hirundinidae	*Delichon urbicum*	Common house martin	0/2	0/0	0/0	0/2 (0, 0–65.8)		n.d.	
		Hirundo rustica	Barn swallow	0/4	0/2	0/2	0/6 (0, 0–39.0)		n.d.	
	Motacillidae	*Motacilla alba*	White wagtail	0/3	0/0	0/0	0/3 (0, 0–56.2)		n.d.	
	Muscicapidae	*Erithacus rubecula*	European robin	0/0	0/1	0/1	0/1 (0, 0–79.4)		n.d.	
		Ficedula hypoleuca	European pied flycatcher	0/1	0/0	0/0	0/1 (0, 0–79.4)		n.d.	
		Muscicapa striata	Spotted flycatcher	0/3	0/0	0/0	0/3 (0, 0–56.2)		n.d.	
		Phoenicurus ochruros	Black redstart	0/6	0/2	0/2	0/8 (0, 0–32.4)		n.d.	
	Paridae	*Cyanistes caeruleus*	Eurasian blue tit	0/18	0/7	0/7	0/25 (0, 0–13.3)		n.d.	
		Parus major	Great tit	0/15	0/3	0/3	0/18 (0, 0–17.6)		n.d.	
	Passeridae	*Passer domesticus*	House sparrow	0/40	0/11	0/11	0/48 (0, 0–7.4)		n.d.	
		Passer montanus	Eurasian tree sparrow	0/5	0/1	0/1	0/6 (0, 0–39.0)		n.d.	

Continued

Table 1 Continued

Order	Family	Species name (Latin)	Species name (English)	Number of available and *Chlamydiaceae**-positive swab samples per anatomical site			*Chlamydiaceae*-positive birds (%, 95% CI)	Mean Ct value, *Chlamydiaceae* quantitative PCR	Chlamydia psittaci-positive birds (%)	Accession number of *ompA* study sequence
	Sittidae	*Sitta europaea*	Eurasian nuthatch	0/0	0/1	0/1	0/1 (0, 0–79.4)		n.d.	
	Sturnidae	*Sturnus vulgaris*	Common starling	0/4	0/2	0/2	0/6 (0, 0–39.0)		n.d.	
	Sylviidae	*Sylvia atricapilla*	Eurasian blackcap	0/7	0/1	0/1	0/7 (0, 0–35.4)		n.d.	
		Sylvia borin	Garden warbler	0/1	0/0	0/0	0/1 (0, 0–79.4)		n.d.	
	Turdidae	*Turdus merula*	Blackbird	0/50	0/22	0/21	0/67 (0, 0–5.4)		n.d.	
		Turdus philomelos	Song thrush	0/3	0/3	0/3	0/4 (0, 0–49.0)		n.d.	
		Turdus pilaris	Fieldfare	0/2	0/1	0/1	0/3 (0, 0–56.2)		n.d.	
		Turdus viscivorus	Mistle thrush	0/1	0/0	0/0	0/1 (0, 0–79.4)		n.d.	
Pelecaniformes	Ardeidae	*Ardea cinerea*	Grey heron	0/0	0/2	0/2	0/2 (0, 0–65.8)		n.d.	
Piciformes	Picidae	*Dendrocopos major*	Great spotted woodpecker	0/3	0/1	0/1	0/3 (0, 0–56.2)		n.d.	
		Picus viridis	European green woodpecker	0/3	0/0	0/1	0/4 (0, 0–49.0)		n.d.	
Total				1/267	2/91	2/91	3/339 (0.9, 0.3–2.6)		2/3 (66.7)	

*All faecal swabs were obtained from living birds after defecation. Both choanal and cloacal swabs were available from 89 deceased birds. From one blackbird only a choanal swab was available. From one Eurasian blue tit a faecal and a choanal swab were available. Only a cloacal swab was obtained from one Eurasian blue tit and one European green woodpecker. All three swab types were obtained from 20 birds that died or were euthanased during treatment due to trauma or disease.
CI, confidence interval; Ct, cycle threshold; n.d., not determined; *ompA*, outer membrane protein A.

from the Eurasian collared dove and fancy pigeon were positive for *C psittaci* by species-specific qPCR. *OmpA* genotyping classified the organism detected in the cloacal sample of the Eurasian collared dove as *C psittaci* B. *C psittaci* detected in the choanal swab of the fancy pigeon belonged to the *ompA* genotype E.

C psittaci was not detected in the *Chlamydiaceae*-positive faecal swab of the common wood pigeon, and *ompA* genotyping was not successful due to low copy numbers (table 1). Thus, it was not possible to specify the detected *Chlamydiaceae* in this sample.

DISCUSSION

The workers at the rehabilitation centre belong to the population at risk for zoonotic diseases transmitted by birds. Among all bird orders, Columbiformes and psittacine birds show the highest *Chlamydia* prevalence, ranging between 3.4 per cent and 50 per cent.[22 23] In this study, three of 20 (15 per cent, 5.2–36.2 per cent) of the Columbiformes were positive for *Chlamydiaceae*, whereof two were positive for *C psittaci*. This is in accordance with the findings of other studies performed in Switzerland.[20 21] *C psittaci* genotype B, which was found in one Eurasian collared dove, is the predominant genotype in the European pigeon population.[22 32 33] Genotype E, which was found in one fancy pigeon, infects a variety of avian species and is frequently found in pigeons worldwide.[34–37] Both genotypes are zoonotic, but human infection is mostly associated with genotype A causing a more severe course of disease.[38–40]

Chlamydiaceae were not detected in any other bird order included in this study. Partly, the low infection rate might be due to the selection of study samples. The majority of birds (n=246) were tested via faecal swabs only. Testing of faecal swabs has been shown to be a less sensitive method for detection of *Chlamydiaceae* compared with choanal swabs.[41] Furthermore, most birds were nestlings (n=163) or juveniles (n=126), which were previously shown to have lower *Chlamydiaceae* prevalence than adult birds.[18] However, these circumstances reflect the real conditions in the rehabilitation centre during the peak season. The findings in this study are in accordance with those of Zweifel and others,[21] reporting low infection rates of non-*C psittaci-Chlamydiaceae* in songbirds (0.4 per cent) and waterfowl (4.3 per cent). In this study, excretion of *Chlamydiaceae* under stressful conditions in a bird rehabilitation centre was only present in pigeons. As pigeons mostly harbour *C psittaci*, they may therefore pose a hazard to workers at rehabilitation centres. In order to minimise the risk of zoonotic chlamydial transmission, protective equipment (eg, gloves, masks) should always be used and appropriate hygiene measures (eg, washing hands) should be adhered to when handling wild birds.[42]

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Ethics approval All animal housing and sampling were conducted in strict accordance with the Swiss law of animal welfare. None of the birds was killed for this study. The birds of which choanal and cloacal swabs were taken were euthanased due to incurable trauma or disease before sampling.

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Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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