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*. *C. psittaci*, the causative agent of psittacosis/ornithosis in human and avian chlamydiosis, has been detected in more than 460 avian species. 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. Clinical signs include respiratory, enteric and ocular signs, but asymptomatic infections are common. In Switzerland, 46 cases in birds have been reported to the Federal Food Safety and Veterinary Office from 2010 to 2019. Transmission from birds to humans occurs through inhalation of feather dust or aerosols from urine, dried faeces, and respiratory and eye secretions. Most infected humans remain asymptomatic or have mild symptoms, but in some cases *C. psittaci* causes severe pneumonia.

**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. 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 (Melisepotol 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 defection. 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’-CTGA GGAAAATCCTTGAAATCGG-3’), and 900 nM of the primers ‘CH23S-R’ (5’-ACCTGCGGTATTAACCTTAACTCC-3’), 200 nM of the probe ‘CH23S-R’ (5’-FAM-CTCATGCAAAGG CAGCGGT-TAMRA-3’), and 200 nM each of the primers ‘eGFP-1-F’ (5’-GACCCTACCCAGCAGACAC-3’) and ‘eGFP-10-R’ (5’-CTTGTACAGCTCGTCCATGC-3’), and the probe ‘eGFP-HEX’ (5’-HEX-AGACCGAGCTCC GCCCTGAGCA-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’-CAGTATG GGGAAAGTGTCATCA-3’) and ‘CppsOMP1-R’ (5’-CTGG CGGAGATGCTAATGG-3’), 200 nM probe ‘CppsOMP1-S’ (5’-FAM-CCGTACTTGGTGTGAGC-TAMRA-3’), 900 nM of the primers ‘eGFP-1-F’ (5’-GACCGACTACGAGAAACAC-3’) and ‘eGFP-2-R’ (5’-GAATCCAGCAGGACCAGT-3’), and 200 nM probe ‘eGFP-HEX’ (5’-HEX-AGACCCAGCTCCGCCTGAGCA-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’-ATGAAAACACTCTTGAAATCGG-3’) and ‘ompA rev’ (5’-TCCTTGAACATCTTGAATGGC-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

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Table 1: Distribution of Chlamydiaceae in different wild bird species

| Bird Species          | Number of Positive Samples |
|-----------------------|----------------------------|
| Fancy pigeon          | 1                          |
| Common wood pigeon    | 0                          |
| Eurasian collared dove| 0                          |

Stalder S, et al. Vet Rec Open 2020;7:e000437. doi:10.1136/vetreco-2020-000437
| 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 |
|---------------|--------------|----------------------|------------------------|-------------------------------------------------------------------|------------------------------------------|---------------------------------------------|--------------------------------------|----------------------------------------|
| Anseriformes  | Anatidae     | Anas platyrhynchos   | Mallard                | Faeces: 0/26; Choana: 0/4; Cloaca: 0/4 | 0/29 (0, 0–11.7) | n.d.                                        | n.d.                                 | n.d.                                  | MT450277                              |
|               |              | Cygnus olor         | Mute swan              | Faeces: 0/0; Choana: 0/1; Cloaca: 0/1 | 0/1 (0, 0–79.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               |              | Mergus merganser    | Common merganser       | Faeces: 0/9; Choana: 0/0; Cloaca: 0/0 | 0/9 (0, 0–29.9) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Apodiformes   | Apodidae     | Apus apus           | Common swift           | Faeces: 0/15; Choana: 0/7; Cloaca: 0/7 | 0/20 (0, 0–16.1) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Charadriiformes| Laridae      | Larus michahellis   | Yellow-legged gull     | Faeces: 0/3; Choana: 0/1; Cloaca: 0/1 | 0/4 (0, 0–49.0) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Gruiformes    | Rallidae     | Crex crex           | Corn crake             | Faeces: 0/1; Choana: 0/0; Cloaca: 0/0 | 0/1 (0, 0–79.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               |              | Fulica atra         | Eurasian coot          | Faeces: 0/2; Choana: 0/1; Cloaca: 0/1 | 0/3 (0, 0–56.2) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Passeriformes | Acrocephalidae| Acrocephalus scirpaceus| Eurasian reed warbler | Faeces: 0/0; Choana: 0/1; Cloaca: 0/1 | 0/1 (0, 0–79.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               | Emberizidae  | Emberiza citrinella | Yellowhammer           | Faeces: 0/1; Choana: 0/1; Cloaca: 0/1 | 0/1 (0, 0–79.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Fringillidae  | Carduelis carduelis | Goldfinch          | Goldfinch              | Faeces: 0/8; Choana: 0/1; Cloaca: 0/1 | 0/8 (0, 0–32.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               | Chloris chloris | European greenfinch | European greenfinch   | Faeces: 0/4; Choana: 0/4; Cloaca: 0/4 | 0/7 (0, 0–35.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               | Coccothraustes coccothraustes | Hawfinch         | Hawfinch               | Faeces: 0/1; Choana: 0/1; Cloaca: 0/1 | 0/2 (0, 0–65.8) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Fringilla creeds | Common chiffinch | European chiffinch  | European chiffinch    | Faeces: 0/4; Choana: 0/1; Cloaca: 0/1 | 0/4 (0, 0–49.0) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Seiurus serinus| European serin | European serin      | European serin         | Faeces: 0/1; Choana: 0/0; Cloaca: 0/0 | 0/1 (0, 0–79.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Hirundinidae  | Delichon urbicum | Common house martin | Common house martin    | Faeces: 0/2; Choana: 0/0; Cloaca: 0/0 | 0/2 (0, 0–65.8) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               | Hirundo rustica | Barn swallow        | Barn swallow           | Faeces: 0/4; Choana: 0/2; Cloaca: 0/2 | 0/6 (0, 0–39.0) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Motacillidae  | Motacilla alba | White wagtail       | White wagtail          | Faeces: 0/3; Choana: 0/0; Cloaca: 0/0 | 0/3 (0, 0–56.2) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Muscicapaoidae| Erithacus rubecula | European robin     | European robin         | Faeces: 0/0; Choana: 0/1; Cloaca: 0/1 | 0/1 (0, 0–79.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               | Ficedula hypoleuca | European pied flycatcher | European pied flycatcher | Faeces: 0/1; Choana: 0/0; Cloaca: 0/0 | 0/1 (0, 0–79.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               | Muscicapa sibirica | Spotted flycatcher | Spotted flycatcher     | Faeces: 0/3; Choana: 0/0; Cloaca: 0/0 | 0/3 (0, 0–56.2) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               | Phoenicurus ochruros | Black redstart    | Black redstart        | Faeces: 0/6; Choana: 0/2; Cloaca: 0/2 | 0/8 (0, 0–32.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Paridae       | Cyanistes caeruleus | Eurasian blue tit   | Eurasian blue tit      | Faeces: 0/18; Choana: 0/7; Cloaca: 0/7 | 0/25 (0, 0–13.3) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               | Parus major | Great tit           | Great tit              | Faeces: 0/15; Choana: 0/3; Cloaca: 0/3 | 0/18 (0, 0–17.6) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
| Passeridae    | Passer domesticus | House sparrow      | House sparrow          | Faeces: 0/40; Choana: 0/11; Cloaca: 0/11 | 0/43 (0, 0–7.4) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |
|               | Passer montanus | Eurasian tree sparrow | Eurasian tree sparrow | Faeces: 0/5; Choana: 0/1; Cloaca: 0/1 | 0/6 (0, 0–39.0) | n.d.                                        | n.d.                                 | n.d.                                  |                                       |

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, 0–79.4)                          | n.d.                                        |                                      |                                          |
| Sturnidae | Sturnus vulgaris| Common starling      | 0/4                    | 0/2                                                                              | 0/6 (0, 0–39.0)                          | n.d.                                        |                                      |                                          |
| Sylviidae | Sylvia atricapilla| Eurasian blackcap   | 0/7                    | 0/1                                                                              | 0/7 (0, 0–35.4)                          | n.d.                                        |                                      |                                          |
|           | Sylvia borin | Garden warbler       | 0/1                    | 0/0                                                                              | 0/1 (0, 0–79.4)                          | n.d.                                        |                                      |                                          |
| Turdidae  | Turdus merula | Blackbird            | 0/50                   | 0/22                                                                             | 0/67 (0, 0–5.4)                          | n.d.                                        |                                      |                                          |
|           | Turdus philomelos| Song thrush         | 0/3                    | 0/3                                                                              | 0/4 (0, 0–49.0)                          | n.d.                                        |                                      |                                          |
|           | Turdus pilaris| Fieldfare            | 0/2                    | 0/1                                                                              | 0/3 (0, 0–66.2)                          | n.d.                                        |                                      |                                          |
|           | Turdus viscivorus| Mistle thrush       | 0/1                    | 0/0                                                                              | 0/1 (0, 0–79.4)                          | n.d.                                        |                                      |                                          |
| Pelecaniformes | Ardea cinerea | Grey heron            | 0/0                    | 0/2                                                                              | 0/2 (0, 0–65.8)                          | n.d.                                        |                                      |                                          |
| Piciformes | Picidae     | Dendrocopos major | Great spotted woodpecker | 0/3                                                                       | 0/3 (0, 0–56.2)                      | n.d.                                        |                                      |                                          |
|           | Picus viridis| European green woodpecker | 0/3              | 0/0                                                                              | 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 choanal 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 euthanised 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 in the European pigeon population.22 32 33 Genotype E, a new Chlamydia species isolated from a red-shouldered hawk. Syst Appl Microbiol 2019;12:15789.

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Contributors SA, BV and NB designed the study. SS and PM performed the experiments. SS, HM and NW analysed the data. SS wrote the original draft. SA, BV, NB, HM, NW and PM reviewed and edited the original draft. All authors have read and agreed to the published version of the manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

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 euthanised due to incurable trauma or disease before sampling.

Provenance and peer review Not commissioned; externally peer reviewed.

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

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