Chlamydiaceae in wild, feral and domestic pigeons in Switzerland and insight into population dynamics by Chlamydia psittaci multilocus sequence typing

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Abstract: Feral pigeons, common wood pigeons and Eurasian collared doves are the most common representatives of the Columbidae family in Switzerland and are mostly present in highly populated, urban areas. Pigeons may carry various members of the obligate intracellular Chlamydiaceae family, particularly Chlamydia (C.) psittaci, a known zoonotic agent, and C. avium. The objective of the study was to identify the infection rates of common free-roaming pigeons for different Chlamydia species with the overall aim to assess the risk pigeons pose to public health. In this study, 431 pigeons (323 feral pigeons, 34 domestic pigeons, 39 Eurasian collared doves, 35 common wood pigeons) from several geographic locations in Switzerland were investigated for the presence of Chlamydiaceae. Samples consisted of pooled choanal-cloacal swabs (n = 174), liver samples (n = 52), and paired swab and liver samples from 205 pigeons (n = 410). All 636 samples were screened using a Chlamydiaceae family-specific 23S rRNA real-time PCR (qPCR). Subsequent species identification was performed by DNA-microarray assay, sequencing of a 16S rRNA gene fragment and a C. psittaci specific qPCR. In total, 73 of the 431 pigeons tested positive for Chlamydiaceae, of which 68 were positive for C. psittaci, four were C. avium-positive and one pigeon was co-infected with C. avium and C. psittaci. The highest infection rates were detected in feral (64/323) and domestic pigeons (5/34). Common wood pigeons (2/35) and Eurasian collared doves (2/39) revealed lower infection rates. Additionally, multilocus sequence typing of twelve selected C. psittaci-positive samples revealed closely related sequence types (ST) between and within different Swiss cities. Furthermore, liver and corresponding swab samples from the same bird were colonized by the same ST. Considering the high infection rates of C. psittaci in domestic and feral pigeons, close or frequent contact to these birds poses a human health risk.

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Abstract

Feral pigeons, common wood pigeons and Eurasian collared doves are the most common representatives of the Columbidae family in Switzerland and are mostly present in highly populated, urban areas. Pigeons may carry various members of the obligate intracellular Chlamydiaceae family, particularly Chlamydia (C. psittaci), a known zoonotic agent, and C. avium. The objective of the study was to identify the infection rates of common free-roaming pigeons for different Chlamydia species with the overall aim to assess the risk pigeons pose to public health. In this study, 431 pigeons (323 feral pigeons, 34 domestic pigeons, 39 Eurasian collared doves, 35 common wood pigeons) from several geographic locations in Switzerland were investigated for the presence of Chlamydiaceae. Samples consisted of pooled choanal-cloacal swabs (n = 174), liver samples (n = 52), and paired swab and liver samples from 205 pigeons (n = 410). All 636 samples were screened using a Chlamydiaceae family-specific 23S rRNA real-time PCR (qPCR). Subsequent species identification was performed by DNA-microarray assay, sequencing of a 16S rRNA gene fragment and a C. psittaci specific qPCR. In total, 73 of the 431 pigeons tested positive for Chlamydiaceae, of which 68 were positive for C. psittaci, four were C. avium-positive and one pigeon was co-infected with C. avium and C. psittaci. The highest infection rates were detected in feral (64/323) and domestic pigeons (5/34). Common wood pigeons (2/35) and Eurasian collared doves (2/39) revealed lower infection rates. Additionally, multilocus sequence typing of twelve selected C. psittaci-positive samples revealed closely related sequence types (ST) between and within different Swiss cities. Furthermore, liver and corresponding swab samples from the same bird were colonized by the same ST. Considering the high infection rates of C. psittaci in domestic and feral pigeons, close or frequent contact to these birds poses a human health risk.
Introduction

Members of the *Chlamydiaceae* family are gram negative, obligate intracellular bacteria with a biphasic developmental cycle. The single genus *Chlamydia* (*C.*) consists of thirteen species and three *Candidatus* species [1–3]. The most well-known chlamydial species harboured by birds is *C. psittaci*, which has been reported in at least 467 bird species belonging to 30 different orders [4]. This pathogen causes asymptomatic to severe systemic infections in several bird species, depending on susceptibility of the host species, immune status, infectious dose and virulence of the strain involved [5]. Transmission can occur by inhalation of contaminated dust, feather particles and respiratory tract secretions [6]. In birds, bacterial shedding can be intermittently activated by stressful events such as breeding, migration or other illnesses, without presentation of clinical symptoms [5].

*C. psittaci* is a zoonotic agent causing ornithosis, an influenza-like illness in humans, potentially leading to atypical pneumonia with sometimes fatal outcome [7]. Humans contract disease during close contact with infected birds by inhalation of respiratory secretions or dust from dried feces [6].

Based on the outer membrane protein A (*ompA*), *C. psittaci* is divided into nine genotypes and several subtypes, which are more or less associated with different hosts. Seven of these genotypes are generally found in avian hosts (*A–F* and *E/B*) [8–11]. Genotypes *A* and *F* are primarily found in psittacine birds, *B* in pigeons, *C* in ducks and geese, and *D* in turkeys. Genotype *E* infects a broad range of birds including pigeons [11], while *E/B* has been described in ducks [9]. Human infections are most frequently associated with genotype *A*, causing more severe infections than other genotypes [12–15].

*C. avium*, another chlamydial species infecting birds, was first described in 2014 [16] and has so far been reported in feral pigeons from Italy, France, Germany and the Netherlands, in a parrot from Germany and in a mallard from Poland [16–18]. To date, it is still unclear whether it causes disease in birds, if it has zoonotic potential or how it is transmitted.

Pigeons may become infected with several chlamydial species, including *C. psittaci* (the most common *Chlamydia* species identified in pigeons), *C. avium*, *C. abortus*, *C. pecorum* and *C. trachomatis* [19]. In Swiss feral pigeons, *C. psittaci* is the only species of the family *Chlamydiaceae* identified to date [20–22] and in general, research in avian *Chlamydia* seems to focus on *C. psittaci* and feral pigeons. Worldwide, several studies on *C. psittaci* in feral pigeons have been conducted, revealing a seroprevalence of up to 95.6%, while chlamydial DNA could be detected in up to 50% of the tested pigeons [23]. Especially in cities, where feral pigeons find easy access to food sources, they can build large populations of more than 300–400 pigeons per km², leading to more stressed and diseased birds and thus to an increased risk for pathogen transmission to humans [5, 24]. Additionally, the close contact to feral pigeons through feeding, or even briefly passing areas with a high pigeon density, may increase the likelihood for zoonotic transmission of *C. psittaci* [23]. Whether any of the other *Chlamydiaceae* harboured by pigeons, apart from *C. psittaci*, may cause psittacosis-like disease in humans is not known.

The Swiss feral pigeon population has been stable or, in some areas, slightly declining in the last 20 years [25], due to different population management programs, e.g. culling schemes or reduction of food availability. In 2001, Lucerne introduced a population management program [26, 27] primarily focusing on banning public feeding and building two pigeon lofts to attract the birds. Droppings accumulating in the lofts are disposed resulting in a decreased fecal load in the city decreasing the risk of disease transmission to humans. In 2012, a different population management program was introduced in Berne consisting of catching all available pigeons, followed by euthanasia of clinically unhealthy birds and endoscopic sterilization of males. In addition, all caught pigeons were ringed and placed in one of five pigeon lofts. Both
of these city loft programs were successful: the pigeon population decreased from around 7'000 individuals in 2001 to 2'500 in 2015 in Lucerne and from around 10'000 in 2011 to currently 1'500 birds in Berne [27, 28]. In Zurich, the feral pigeon population is managed primarily by culling. In 2019, an estimated 16'000 feral pigeons lived in the city of Zurich [29].

Switzerland is home to five different species of free-roaming pigeons. In detail, the four wild pigeon species common wood pigeon (*Columba palumbus*), stock dove (*Columba eonas*), Eurasian collared dove (*Streptopelia decaocto*), and European turtle dove (*Streptopelia turtur*), and feral pigeons (*Columba livia domestica*). In areas inhabited by humans, feral pigeons, common wood pigeons and Eurasian collared doves are well documented with a tendency towards increasing populations for the last two species [25]. These three pigeon species are potential hosts for *Chlamydia psittaci* [4] and possibly other *Chlamydia* species. However, there is no data available about the presence of *C. psittaci* and other *Chlamydia* species in Swiss wild pigeons. The present study aimed at collecting baseline data on the presence of *Chlamydiaeae* in three different free roaming Swiss pigeon species (feral and domestic pigeons, common wood pigeons and Eurasian collared dove), with insights into the population genetics of *C. psittaci* by using typing schemes such as ompA genotyping and multilocus sequence typing (MLST) [30, 31].

### Materials and methods

#### Samples

A total of 636 samples from 431 pigeons belonging to the three species i) *Columbia livia domestica*, i.e. domestic (homing pigeons, fancy pigeons, flying/sporting pigeons) and feral pigeons (“city pigeons”), ii) *Streptopelia decaocto* (Eurasian collared dove) and iii) *Columba palumbus* (common wood pigeon) from different geographical areas in Switzerland between May 2014 and October 2018 were analyzed. Individual samples consisted of combined choanal/cloacal swabs (c/c-swabs; n = 174) and liver samples (n = 52). Additionally, paired samples of c/c-swab and liver (n = 107), and cloacal swab (c-swab) and liver (n = 98) were available (Table 1). Samples derived from the diagnostic service of the National Reference Centre for Poultry and Rabbit Diseases (NRGK) and originated from birds found at various locations admitted to the rehabilitation center of the Swiss Ornithological Institute based in the Canton of Lucerne (n = 58) and from feral pigeons inhabiting three of the five pigeon lofts in Berne (loft A, **Lucerne, Kriens, Horw, Emmen, Emmenbruecke, Rothenburg**

### Table 1. Number of pigeons according to type of pigeon, sample material and place of origin.

|                     | Single samples | Paired samples | Place of origin | Total no. of pigeons |
|---------------------|----------------|----------------|-----------------|----------------------|
|                     | C/c-swab | Liver | C/c-swab + liver | C-swab + liver | Berne | Greater Lucerne area* | Greater Zurich area | Various** *** |          |
| Feral pigeon        | 142      | 47    | 36               | 98             | 123  | 23                      | 142               | 35                 | 323        |
| Domestic pigeon     | 17       | 2     | 15               | 0              | 0    | 8                       | 0                 | 26                 | 34         |
| Eurasian collared  dove | 12    | 1     | 26               | 0              | 0    | 2                       | 3                 | 34                 | 39         |
| Common wood pigeon  | 3        | 2     | 30               | 0              | 0    | 13                      | 1                 | 21                 | 35         |
| Total               | 174      | 52    | 107              | 98             | 123  | 46                      | 146               | 116                | 431        |

*C/c-swab = combined choanal/cloacal swab; **C-swab = cloacal swab
**Lucerne, Kriens, Horw, Emmen, Emmenbruecke, Rothenburg
***More rural places as compared to the city areas within the cantons Lucerne, Obwalden, Nidwalden, Schwyz, Zug, Aargau, Solothurn, Zurich, Schaffhausen, Basel-Land, Thurgau and St. Gallen

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n = 25; loft B, n = 49; loft C, n = 23). All loft birds were tested on the same day excluding repeated sampling of individuals. Additional samples from feral pigeons culled by the game warden in the context of the local population control program in the city of Zurich and surrounding areas (greater Zurich area) (n = 142) completed the sample set (Table 1). The majority of the rehabilitation center pigeons were found in rural regions, like small villages or farmland (Table 1). Upon collection and until DNA extraction, the swabs and liver samples were stored at -20˚C, samples from Zurich were stored at -80˚C. A complete list of samples is provided in S1 Table.

DNA extraction

DNA was extracted using a commercial kit (Genomic DNA from tissue, NucleoSpin® Tissue from Macherey-Nagel, Düren, Germany) according to the manufacturer’s instructions. For each extraction lot, a negative extraction control was prepared by using “Buffer T1” instead of the sample. Extracts were stored at -20˚C until further analysis was performed. Extracted DNA was measured on a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) to determine DNA quantity and quality (260/280 value).

Chlamydiaceae screening of extracted DNA

All extracted DNA samples (n = 636) were investigated according to the decision tree as depicted in Fig 1. All primers and probes used in this study are listed in Table 2.

Quantitative and conventional PCRs

All quantitative PCRs (qPCR; Fig 1, Table 2) were run on an Applied Biosystems® 7500 Real-Time PCR System (Thermo Fisher Scientific). As internal amplification control, eGFP was added to the reaction mix [33].

Products from all conventional PCRs (Fig 1, Table 2) were purified using the QIAquick® PCR Purification Kit (Qiagen) according to the manufacturer’s instructions.

Purified amplicons were Sanger sequenced by Microsynth (Balgach, Switzerland) [38]. The obtained sequences were assembled and analyzed using the CLC Main Workbench 8 software and compared against the NCBI database using the BLASTn tool (NCBI, https://blast.ncbi.nlm.nih.gov/) or the MLST database (http://pubmlst.org/chlamydiales/).

Chlamydiaceae qPCR

The samples were analyzed with the 23S rRNA-based Chlamydiaceae family-specific qPCR [32] as modified by Blumer et al. [37]. The cycle conditions were 95˚C for 20 s, followed by 45 cycles of 95˚C for 3 s, and 60˚C for 30 s. All samples were tested in duplicate and the cycle threshold was set at 0.1 in each run. A sevenfold dilution series of _C. abortus_ DNA with a known number of DNA copies was included in each run as a standard curve allowing the software of the Applied Biosystems® 7500 Real-Time PCR System to calculate the number of copies in positive samples of that run. Molecular grade water was used as a negative control. Samples were interpreted as positive if the mean cycle threshold (Ct value) was < 38. Samples with higher Ct values or inhibited amplification were re-tested in duplicate. Samples repeatedly showing a Ct value > 38 were considered as positive. The _Chlamydiaceae_ copy number per μl was determined directly by the PCR instrument using the standard curve, calculating the percentage of _Chlamydia_-DNA out of total DNA (_Chlamydia-%)._
DNA microarray assay

The sample DNA, including internal control DNA (Intype IC-DNA, Qiagen Labor, Leipzig, Germany), was amplified and biotin labelled using the method described by Borel et al. [34]. The cycle conditions were 96˚C for 10 min, followed by 39 cycles of 94˚C for 30 s, 50˚C for 30 s and 72˚C for 30 s and a last step of 72˚C for 4 min. The labelled DNA was hybridized using the Hybridization Kit 245200100 (Alere Technologies GmbH; now Abbott, Chicago, Illinois, USA) and analyzed using the ArrayStrip™ system (ChlamType-23S AS-4 Kit, Alere Technologies GmbH, Jena, Germany), as established by Borel et al [34]. With the current kit, eleven Chlamydia species and nine Chlamydia-like organisms can be identified.
16S ribosomal RNA (rRNA) PCR

The conventional 16S rRNA PCR was performed as described by Pospischil et al. [35], using the primers 16S IGF (short) and 16S IGR (short) to amplify a sequence of 298 base pairs (bp) (Table 2).

Per sample, a 50 μl reaction mix was prepared, containing 1 μl (< 150 ng/μl) sample template, 1x PCR buffer with MgCl₂ (Roche Diagnostics GmbH), 0.5 mM MgCl₂ Stock Solution

Table 2. Primers and probes and their final concentration (nM) in the PCR reagent mix for different quantitative PCR (qPCR) and conventional PCR (PCR) tests used in this study.

| Method                  | Target          | Primer & Probe | Sequence (5’-3’) | Final conc. of primers and Probes in the PCR reagent mix | Amplicon size (base pairs) | Annealing temperature (˚C) | references               |
|------------------------|-----------------|----------------|------------------|----------------------------------------------------------|-----------------------------|-----------------------------|---------------------------|
| **Chlamydiaceae**      |                 |                |                  |                                                          |                             |                             |                           |
| 23S rRNA qPCR          | 23S rRNA        | Ch23S-F        | CTGAAACCAGTAGTTACCTAAGCGGT | 500 nM                                                   | 111                          | 60                          | Ehrlich et al. [32]       |
|                        |                 | Ch23S-R        | ACCCTCCCGGTCTTAACTTAACTCC |                                                           |                             |                             |                           |
|                        |                 | Ch23S-p        | FAM-CTCTCAGTGAAAGGCAGCGCG-TAMRA | 200 nM                                   |                             |                             |                           |
|                        |                 | eGFP           | GACCACTACCCAAGAGAAACAC | 200 nM                                                   | 177                          |                             | Hoffmann et al. [33]      |
|                        |                 | eGFP-10-R      | CTTGTCAGCTCCTGCTATGC |                                                           |                             |                             |                           |
|                        |                 | eGFP-HEX       | HEX-AGCACCACAGCTCCCTGAGCA-BHQ1 | 276                                                  |                             |                             |                           |
| DNA microarray         | 23S rRNA        | U23F-1         | ATGGAAAGCCGGAGGAAAGAA | 500 nM                                                   | 171                          | 50                          | Ehrlich et al., Borel et al. [32, 34] |
| assay PCR              |                 | 23R-22         | biotin-CCYACTAGATGGTACCTTC | 50 nM                                                  | 276                          |                             | Hoffmann et al. [33]      |
|                        |                 | eGFP           | CAGCCAACACGTCTATATCATG |                                                           |                             |                             |                           |
|                        |                 | eGFP-11-F      | CAGCCAACACGTCTATATCATG | 200 nM                                                   | 276                          |                             |                           |
|                        |                 | eGFP-10-Bio    | Bio-CTTGTACAGCTCCCTGCTATGC | 400 nM                                   |                             |                             |                           |
| 16S rRNA PCR           | 16S rRNA        | 16S IGF (short)| GATGAGGCATGCAAAGTGAAC | 300 nM                                                   | 278                          | 58                          | Pospischil et al. [36]    |
|                        |                 | 16S IGR (short)| CACGTTGTCGCGCATCTTC | 300 nM                                                   | 278                          | 58                          | Pospischil et al. [36]    |
| C. psittaci ompA       | ompA            | CppOMP1-F      | CCACTAGTTGGAAGGTTCTCA | 900 nM                                                   | 76                           | 60                          | Pantchev et al. [36]      |
| qPCR                   |                 | CppOMP1-R      | CTGGCGGGATCTAATGGG |                                                           |                             |                             |                           |
|                        |                 | CppOMP1-S      | FAM-CCYACTAGATGGTGAC-TAMRA | 200 nM                                   |                             |                             |                           |
|                        |                 | eGFP           | GACCACTACCCAAGAGAAACAC | 900 nM                                                   | 132                          |                             |                           |
|                        |                 | eGFP-2-R       | GAACCTCAGCAAGCAGCATG |                                                           |                             |                             |                           |
|                        |                 | eGFP-Hex       | HEX-AGCACCACAGCTCCCTGAGCA-BHQ1 | 200 nM                                   |                             |                             |                           |
| MLST PCR               | enoA            | YPenoA3        | CCTATGATGAAATCTCATATGG | 200 nM                                                   | 450–500                      | 53                          | Pannekoek et al. [31]     |
|                        |                 | YPenoA4        | CCCAACCCTAATATCTTTTCTTC | 200 nM                                                   | 450–500                      | 53                          | Pannekoek et al. [31]     |
|                        | fumC            | YPfumC1        | GGCTTCTCGAGGTAGTATGC | 500–600                                                   | 53                           |                             |                           |
|                        |                 | YPfumC2        | CGAAATGATGACTACCTACTCA | 500–600                                                   | 53                           |                             |                           |
|                        | gatA            | YPgatA3        | GCCCTAGTAGTAAAGAATGCGG | 500–600                                                   | 53                           |                             |                           |
|                        |                 | YPgatA4        | CCCCTCTGATGGAACCTAAGGC | 500–600                                                   | 53                           |                             |                           |
|                        | gidA            | YPgida1        | GCTTATGAGAGCTGTCCTGGC | 500–600                                                   | 53                           |                             |                           |
|                        |                 | YPgida2        | CGCTGTTTCTTCTACCAAGG | 500–600                                                   | 53                           |                             |                           |
|                        | hemN            | YPhemN1        | GGATCCATTTTGCGAGGAGGG | 500–630                                                   | 53                           |                             |                           |
|                        |                 | YPhemN2        | CCTGGAGAGTTTCTACTACAG | 500–630                                                   | 53                           |                             |                           |
|                        | hflX             | YPfhlX3        | GAGATTTTTCGTAAGGAGGG | 500–510                                                   | 53                           |                             |                           |
|                        |                 | YPfhlX4        | GTAAAAACCTCTCTAGTAAAGGC | 500–510                                                   | 53                           |                             |                           |
|                        | oppA            | YPoppA3        | ATCGGCAAAGATTACCAATGGG | 500–610                                                   | 53                           |                             |                           |
|                        |                 | YPoppA4        | GGGCGAGTTTAGTGAACCTCGC | 500–610                                                   | 53                           |                             |                           |
| ompA PCR               | ompA            | ompA F (CTU)   | ATGAATTTTCAATTCATACGG | 200 nM                                                   | 1200                         | 49                          | Sachse et al. [30]        |
|                        |                 | ompA rev       | TCCCTGAAATCTGAAATGGAC | 200 nM                                                   | 1200                         | 49                          | Sachse et al. [30]        |

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C. psittaci quantitative PCR

The C. psittaci-specific qPCR was performed according to the protocol as described by Pantchev et al. [36]. The reaction mix contained 4 μl (＜150 ng/μl) sample template, 1 μl eGFP template, 1x TaqMan Universal PCR MasterMix, 900 nM of the primers CppsOMP1-F and CppsOMP1-R, 200 nM probe CppsOMP1-S, 900 nM of the primers eGFP-1-F and eGFP-2-R and 200 nM probe eGFP-HEX [33] in a final volume of 25 μl.

C. psittaci typing

Twelve C. psittaci strains were selected for further characterization by performing C. psittaci-specific multilocus sequence typing (MLST) as described by Pannekoek et al. [31] and genotyping based on the major outer membrane protein (ompA) gene [30]. Selection aimed at creating a sample subset that represents the diversity of the main sample set. It was based on (I) geographical location (city, pigeon loft), (II) pigeon species and (III) positivity in a set of paired samples. Samples finally included were: swab samples of five feral pigeons from the lofts A, B, and C in Berne (1, 2, and 2 pigeons, respectively), two feral pigeons from Zurich, and two feral pigeon from the greater Lucerne area; a matching liver sample to one of the feral pigeons from the greater Lucerne area; a swab sample of a domestic pigeon from the greater Lucerne area, and one swab sample from an Eurasian collared dove from Inwil. To make sure that MLST analysis will be successful, only samples with a Chlamydia-% above 0.002 were selected. Thus, a sample from a common wood pigeon could not be included.

Multilocus sequence typing

The conventional PCRs targeting seven housekeeping genes were performed as previously described [31]. For each sample, a 50 μl reaction mix was used, containing 1x AmpliTaq Gold™ 360 Master Mix (Thermo Fisher Scientific), 200 nM of each primer (Table 2) and 3 μl sample template with a DNA concentration of 25 ng/μl. Cycling conditions were 95°C for 10 min, followed by 35 cycles of 95°C for 30 s, 53°C (enOA, fumC, gidA, hemN, hflX) or 60°C (gatA, oppA) for 30 s, 72°C for 60 s and a final step at 72°C for 7 min. If amplification resulted in weak bands, a modified cycling protocol with 40 cycles of 95°C for 60 s, 53°C/60°C for 60 s, 72°C for 90 s was used. Subsequently, the PCR products were analyzed as mentioned above. The alignments of the concatenated sequences (3098 bp) were generated using MAFFT as implemented in Geneious 11.0.5 [39]. The mid-point rooted Bayesian tree was constructed using the concatenated MLST 3098 bp MAFFT alignment with the MrBayes program (as implemented in Geneious). The tree parameters included: GTR +G nucleotide model, with 4 MCMC chains with million generations, sampled every 1000 generations and with the first 100 000 trees discarded as burn-in. The additional strains used for Bayesian analysis as shown in Fig 2 are listed in S2 Table. The MLST sequences generated in this study are deposited in PubMLST/Chlamydiales (https://pubmlst.org/chlamydiales/).

OmpA genotyping

Each reaction mix with a final volume of 50 μl contained 1x AmpliTaq Gold 360 master mix (Thermo Fisher Scientific), 200 nM of the primers CTU and ompA rev [30] and 3 μl sample
template with a DNA concentration of 25 ng/μl. Cycling conditions were 10 min at 95˚C, followed by 35 cycles of 95˚C for 30 s, 49˚C for 30 s, 72˚C for 60 s and a final elongation at 72˚C for 7 min. If amplification resulted in weak bands, a modified cycling protocol with 40 cycles of 95˚C for 60 s, 49˚C for 60 s, 72˚C for 90 s was used. Alignments of the 1050 bp sequences and Bayesian analysis was performed as described above. The C. psittaci strains used for Bayesian analysis as shown in Fig 3 are listed in S2 Table. The sequences generated in this study are available in Genbank under accession numbers MK805041—MK805052.

**Ethics statement**

Samples for this study included swab and organ samples of pigeons taken during necropsy and choanal/cloacal swab samples of live animals collected by a certified veterinarian for diagnostic use. Therefore, no approval of the ethics committee was sought.

**Results**

*Chlamydiaceae* PCR revealed an average infection rate of 16.9% in Swiss pigeons

A total of 70 swab (18.5%) and 22 liver samples (8.6%) of a total of 73 pigeons (16.9%) were positive for *Chlamydiaceae* (Table 3).
In feral pigeons, *Chlamydiaceae* infection rates differed according to geographical origin. While pigeons from the greater Zurich area showed an infection rate of 27.5% (39/142), pigeons from the greater Lucerne area and Berne showed lower overall infection rates of 15.5% (19/123) and 17.4% (4/23), respectively. The results from the pigeons of the three lofts within Berne, however, differed greatly with 4/23 (17.4%) *Chlamydiaceae*-positive pigeons in Loft C, but only 1/25 (4.0%) and 2/49 (4.1%) in Loft A and B. The latter infection rates were similar to the overall infection rate determined for feral pigeons from the more rural places of different cantons (Lucerne, Obwalden, Nidwalden, Schwyz, Zug, Aargau, Solothurn, Zurich, Schaffhausen, Basel-Land, Thurgau, St. Gallen), where only 2/35 (5.7%) individuals were positive for

![Bayesian phylogenetic tree of ompA gene sequences from 40 Chlamydia psittaci strains from avian and mammalian hosts.](https://doi.org/10.1371/journal.pone.0226088.g003)

**Fig 3.** Bayesian phylogenetic tree of *ompA* gene sequences from 40 *Chlamydia psittaci* strains from avian and mammalian hosts. The host is color-labeled as depicted in the legend. M56 taxa was used as an outgroup. Samples from this study are marked in bold letters. The posterior probability values are displayed on the tree nodes.

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### Table 3. Number of samples (positive/total) by pigeon species tested for *Chlamydiaceae*.

| Species               | Swabs only | Paired samples: | Liver only | Total no. of pigeons |
|-----------------------|------------|----------------|------------|----------------------|
|                       | swabs      | liver          |            |                      |
| Feral pigeons         | 20/142     | 42/129         | 18/129     | 64/323               |
| Domestic pigeons      | 4/17       | 1/15           | 0/15       | 5/34                 |
| Common wood pigeon    | 0/3        | 1/30           | 1/30       | 2/35                 |
| Eurasian collared dove| 0/12       | 2/26           | 2/26       | 2/39                 |
| Total                 | 24/174     | 46/205         | 21/205     | 73/431               |

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**Chlamydiaceae.** Similar infection rates again were detected in the Swiss wild pigeon species Eurasian collared dove (2/39; 5.1%) and common wood pigeon (2/35; 5.7%).

Different results were also detected for the different sample materials. A comparison of the results for the paired swab and liver samples (410 samples of 205 pigeons) revealed a total of 46/205 (22.4%) swab samples and 21/205 (10.2%) liver samples to be positive for *Chlamydiaceae*. These samples originated from 19 pigeons positive in both samples (swab and liver), 27 pigeons positive in the swab sample only and two pigeons positive in the liver sample only. In average, the chlamydial load (*Chlamydia-%*) in swab samples was 10^4 times higher than in the matching liver sample with only one pigeon showing a higher *Chlamydia-%* in the liver sample compared to the associated swab sample (S3 Table).

**C. psittaci** is the predominant chlamydial species in Swiss pigeons

All 92 *Chlamydiaceae* positive samples originating from 73 pigeons were further investigated by the Arraymate microarray assay. Of these, 53 samples (57.6%) of 37 individuals could be identified as *C. psittaci*, five samples (5.4%) of 4 individuals as *C. avium* and one (1.1%) as a mixed infection of *C. psittaci* and *C. avium*. The remaining 33 samples (35.9%) could not be further identified by microarray assay and were subjected to the conventional 16S rRNA PCR, as were the five *C. avium*-positive samples and the mixed-infected sample for confirmation reasons. Sequencing of PCR products was successful for 13 samples. Of those, the five samples from two domestic and two feral pigeons previously identified with a single infection with *C. avium* by microarray assay, were confirmed to be positive for *C. avium*, whereas the other eight samples from feral pigeons, including the one previously identified with a mixed infection of *C. avium* and *C. psittaci*, were identified as *C. psittaci*.

The 25 yet unidentified samples originating from 22 feral pigeons, two domestic pigeons, one common wood pigeon and one Eurasian collared dove, were further investigated with the *C. psittaci ompA* qPCR. All 25 samples were positive for *C. psittaci* (Table 4), including the samples with Ct values > 38 in the *Chlamydiaceae* qPCR.

**Different STs identified in different pigeon populations belong to OmpA genotype B and E**

MLST analysis of twelve selected samples revealed that pigeons within one city were infected with *C. psittaci* strains from the same or closely related sequence types (ST). Between cities, however, the identified STs were distinct (Fig 2, S4 Table). Additionally, three new STs were detected due to a single nucleotide polymorphism (SNP) in the house keeping gene *hflX* (ST212, feral pigeons from Zurich), a SNP in the house keeping gene *gatA* (ST216, Eurasian collared dove from Inwil) and due to a novel combination of house keeping gene alleles (ST213, Feral pigeon from Lucerne). Additionally, the analysis of the paired swab (377_T) and liver sample (377_Li) of one pigeon showed the same ST (ST27) in both samples (S4 Table).

| Species               | *C. psittaci* | *C. avium* | *C. psittaci + C. avium* |
|-----------------------|---------------|------------|-------------------------|
| Feral pigeons         | 61/323        | 2/323      | 1/323                   |
| Domestic pigeons      | 3/34          | 2/34       | 0/34                    |
| Common wood pigeon    | 2/35          | 0/35       | 0/35                    |
| Eurasian collared dove| 2/39          | 0/39       | 0/39                    |
| Total                 | 68/431 (15.8%)| 4/431 (0.9%)| 1/431 (0.2%)            |

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OmpA typing of the twelve strains revealed, that 9/12 strains shared 100% sequence identity with the reference *C. psittaci* strain CP3 and thus belong to *ompA* genotype B. The strains identified as ST26 (194_T) and ST216 (451_T) both shared 99.9% sequence identity with the *C. psittaci* CP3 *ompA* sequence, showing a synonymous (A → G) single nucleotide polymorphism (SNP) on position 748 and a non-synonymous (A → G) SNP on position 466, and were denoted genotype B"and B", respectively. Additionally, both strains showed 99.7% and 99.9% sequence identity with the novel genotype B’ detected in a horse from Queensland, Australia (strain Qld/H/Pl, Accession no. MG587894.1) (S4 Table). The strain identified as ST213 (394_T) belongs to genotype E, sharing 100% sequence identity with the previously described reference strain MN. Phylogenetic analysis of the *ompA* gene is shown in Fig 3.

Discussion

Different infection rates of feral pigeons in different geographical areas

The percentage of *Chlamydiaceae*-positive feral pigeons originating from Berne was notably higher in loft C (32.7%) as compared to lofts A and B (4.0% and 4.1%) although pigeons of all three lofts were kept under equal conditions. It can also be expected that the *C. psittaci* strains infecting the pigeons of the different lofts express similar virulence, since samples taken from five feral pigeons originating from these three different lofts were all typed as ST55 by MLST and *ompA* genotype B, the most common genotype found in pigeons [23, 40–42]. A possible explanation for the differences in infection rate might be the geographical location of the lofts with loft C being situated in the outskirts of Berne in close proximity to a forest and lofts A and B being located in an urban environment. However, distances to forest areas in Switzerland are relatively short: Switzerland is a small country with a total area of 41,285 km² and one third covered by forests. Apart from bodies of water (>4%), and mountains above tree line, there is no area without any smaller or larger wood nearby.

While a study on feral pigeons from Lucerne from 2007 detected *Chlamydiaceae* DNA in 2/60 (3.3%) individuals, the present study, tested 4/23 (17.4%) of feral pigeons from the greater Lucerne area positive for *Chlamydiaceae*. Since the pigeons from the current study had been admitted to a wildlife hospital, sampled animals predominantly suffered from trauma or disease, which could have induced or increased chlamydial shedding due to stress [5].

In contrast to a previous study on feral pigeons from Zurich from 2008 [21], where 10/24 (42.7%) pigeons tested positive for *Chlamydiaceae*, the infection rate of 39/142 (27.4%) detected in the present study was much lower. It is unclear whether the amount of infected birds decreased over time or whether the difference is biased due to different sample sizes.

Since the infection rate seemed to be higher in the city of Zurich as compared to other Swiss cities (see above), which implemented different population management programs, the type of program might influence urban *Chlamydiaceae* epidemiology. The two population management programs in Berne and Lucerne were more effective than culling to achieve a smaller and healthier pigeon population. It was shown that culling, as performed in Zurich, only results in a temporary population size decrease; most likely due to the high compensatory potential of pigeons [43]. Additionally, the continuous extraction of animals by culling might result in an increased contact rate between individual pigeons due to the frequent restructuring of the population [44], thus leading to a potential increase of disease transmission events within the population.

The infection rate of *Chlamydiaceae*-positive feral pigeons (2/35, 5.7%) originating from rather rural geographical locations is comparable with the infection rate detected in wild pigeon species in the present study. This might be explained by the lower population density...
of feral pigeons in small villages and on farmland as compared to urban areas and which is comparable to that of common wood pigeons throughout Switzerland [25].

The infection rate of \textit{C. psittaci} in feral pigeons varies widely between different European countries or cities, e.g. from 2.4\% in Utrecht, the Netherlands [18], to 50\% in Vinica, Republic of North Macedonia [23], and may even vary from year to year [19]. The infection rates seen in the greater Lucerne area and Berne are comparable to those of other cities in Europe [19, 23, 45]. Zurich on the other hand, shows one of the highest infection rates throughout Europe [23].

\textbf{Infection rates of domestic pigeons, Eurasian collared doves and common wood pigeons}

Literature on \textit{Chlamydiaceae} infection rates for free-roaming pigeons other than feral pigeons is scarce. In the present study, 5/34 (14.7\%) domestic pigeons were positive for \textit{Chlamydiaceae} which is comparable to infection rates reported in domestic pigeons elsewhere in Europe such as Slovenia with a infection rate of 16\% [46] and Germany with 12.8–42.6\% [47].

The infection rate of 2/39 (5.1\%) Eurasian collared doves being positive for \textit{C. psittaci} seems relatively low when compared to the results of a recent study on urban collared dove populations from Italy, where 46/76 (61\%) were positive for \textit{C. psittaci} [48]. However, comparison of results is difficult since birds from the present study originated from different locations, most-likely belonging to different colonies, while the Italian study analysed individuals from one population.

The analysis of common wood pigeons identified 2/35 (5.7\%) individuals to be positive for \textit{C. psittaci}; a similar infection rate (1/25, 4.0\%) as detected by Sharples et al. [49] by \textit{ompB} PCR in common wood pigeons in the UK.

\textbf{Molecular identification of \textit{C. psittaci} strains and infection rate with \textit{C. avium}}

As expected, the present study revealed that \textit{C. psittaci} is the predominant \textit{Chlamydia} species identified in the three species of free-roaming Swiss pigeons with 68/73 \textit{Chlamydiaceae}-positive pigeons positive for \textit{C. psittaci}, 4/73 positive for \textit{C. avium}, and 1/73 positive for both \textit{Chlamydia} species. Confirmation by 16S rRNA PCR of \textit{C. psittaci}, but not \textit{C. avium} in the sample initially identified as mixed-infected might be explained by a higher copy number of \textit{C. psittaci} in the sample, which was then predominantly amplified by the 16S rRNA PCR.

\textit{C. psittaci} is the most common \textit{Chlamydia} species identified in pigeons [19] and for Swiss feral pigeons the only \textit{Chlamydia} species described so far [20–22].

\textit{C. avium} was first described in 2014 [16]. So far, \textit{C. avium} has been found in pigeons from France, Germany, Italy, and the Netherlands [16, 18]. In Germany, co-infections of \textit{C. avium} and \textit{C. psittaci} were detected in two young pigeons, both showing respiratory symptoms [16]. Szymańska-Czerwińska and Niemczuk [50] suggested that co-infection with \textit{C. psittaci} and \textit{C. avium} may cause clinical symptoms in birds. However, the pigeon tested positive for both of these \textit{Chlamydia} species in the present study was euthanized due to an open metacarpal fracture. Apart from that, no clinical symptoms were observed. Although \textit{C. avium} has been found mainly in pigeons, more recent studies reported \textit{C. avium} infections in a parrot in Germany and a mallard duck in Poland [16, 17]. The zoonotic potential of \textit{C. avium} is still unknown.

MLST analysis of selected \textit{C. psittaci} strains revealed the same or closely related STs within a population, but distinct STs in different geographic locations, supporting the hypothesis that the exchange between different pigeon populations is limited [43, 44]. Therefore, it can be assumed that direct pathogen transmission between different populations is occurring only
infrequently. Additionally, the same ST (ST27), ompA genotype B was detected in swab and liver of the same pigeon. A finding that was expected, indicating that infections are caused by a single genotype also being able to induce a systemic infection. As expected, most *C. psittaci*-positive samples (9/12) that were further investigated by ompA sequencing were identical to the strain CP3 and thus belonged to genotype B, the genotype responsible for the majority of the *C. psittaci* infections in pigeons and thought to be endemic in the European pigeon population [23]. Two pigeons were infected by a strain showing 99.9% identity to strain CP3, but had a synonymous (sample 194_T) and a non-synonymous (sample 451_T) SNP compared to strain CP3 and were denoted ompA genotype B' and B#. Both strains additionally showed high identity (99.9% and 99.7%) with the strain Qld/H/Pl (Accession no. MG587894.1) isolated from the placenta of a horse abortion in Queensland, Australia. It may therefore be speculated that certain *C. psittaci* strains carried by birds have the potential to infect horses leading to abortion. One feral pigeon (394_T) was infected by a *C. psittaci* strain belonging to genotype E, which is frequently found in pigeons worldwide and has been detected in several other avian species from Europe and Asia, [23, 48, 51–54]. This genotype has been associated with several outbreaks in ducks and turkeys as well as fatal cases of chlamydiosis in ratites [6].

**Public health concerns**

The detection of *C. psittaci*, a zoonotic agent, in all investigated pigeon species proves that not only feral and domestic pigeons may represent a potential public health hazard, but also wild pigeon species such as the common wood pigeon or Eurasian collared dove. Due to intermittent shedding, even clinically healthy pigeons or pigeons with a negative result in a PCR analysis might still be a source of infection [55, 56]. OmpA analysis of selected *C. psittaci* strains detected genotype B and E, both considered to be less pathogenic to humans than the closely related genotype A, predominantly found in psittacine birds [12, 14, 57, 58]. However, all genotypes of *C. psittaci* are considered zoonotic. A major risk factor identified for human *C. psittaci* infection was unprotected daily contact to domestic pigeons (i.e. contact to feather dust and fecal matter) [59].

**Conclusion**

Of the four pigeon types investigated, feral pigeons showed the highest rate of infection with *Chlamydiaceae* (19.8%), followed by domestic pigeons (14.7%). While the identification of *Chlamydiaceae* in wild pigeon species from Switzerland was described for the first time, the infection rate was much lower (5.1% for Eurasian collared doves, 5.7% for common wood pigeons). *Chlamydia psittaci* was the most common *Chlamydia* species detected and the presence of *C. avium* in Switzerland was reported for the first time.

**Supporting information**

S1 Table. Comprehensive list of samples collected, analyses performed and according results. (n.a.—not available, n.d.—not done, n.i.—not identified). (XLSX)

S2 Table. *Chlamydia psittaci* strains shown in Figs 2 and 3. These strains are either *Chlamydia psittaci* reference strains or were isolated from horse abortions, humans showing severe respiratory symptoms and from birds originating from Europe and Australia. Additionally, strains isolated from other mammals from Germany are listed. (XLSX)
S3 Table. Percentage of Chlamydia DNA out of total DNA (Chlamydia-%) from paired swab and liver samples.

(XLSX)

S4 Table. Results for MLST analysis and ompA genotyping for the 12 selected C. psittaci samples.

(XLSX)

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References
1. Vorimore F, Hsia R-C, Huot-Creasy H, Bastian S, Deruyter L, Passet A, et al. Isolation of a New Chlamydia species from the Feral Sacred Ibis (Threskiornis aethiopicus): Chlamydia ibidis. PLoS ONE. 2013; 8:e74823. https://doi.org/10.1371/journal.pone.0074823 PMID: 24073223
2. Taylor-Brown A, Rüegg S, Polkinghorne A, Borel N. Characterisation of Chlamydia pneumoniae and other novel chlamydial infections in captive snakes. Vet Microbiol. 2015; 178:88–93. https://doi.org/10.1016/j.vetmic.2015.04.021 PMID: 25944652
3. Taylor-Brown A, Bachmann NL, Borel N, Polkinghorne A. Culture-independent genomic characterisation of Candidatus Chlamydia sanzinia, a novel uncultivated bacterium infecting snakes. BMC Genomics. 2016; 17:710. https://doi.org/10.1186/s12864-016-2055-x PMID: 27595750
4. Kaleta EF, Taday EMA. Avian host range of Chlamydophila spp. based on isolation, antigen detection and serology. Avian Pathol. 2003; 32:435–61. https://doi.org/10.1080/03079450310001593613 PMID: 14522700
5. Shewen PE. Chlamydial infection in animals: a review. Can Vet J. 1980; 21:2–11. PMID: 6988075
6. Harkinezhad T, Geens T, Vanrompay D. Chlamydophila psittaci infections in birds: a review with emphasis on zoonotic consequences. Vet Microbiol. 2009; 135:68–77. https://doi.org/10.1016/j.vetmic.2008.09.046 PMID: 19054633

7. Vanrompay D, Harkinezhad T, van de Walle M, Beeckman D, van Droogenbroeck C, Verminnen K, et al. Chlamydophila psittaci transmission from pet birds to humans. Emerging Infect Dis. 2007; 13:1108–10. https://doi.org/10.3201/eid1307.070074 PMID: 18214194

8. Vanrompay D, Butaye P, Sayada C, Ducatelle R, Haesebrouck F. Characterization of avian Chlamydia psittaci strains using omp1 restriction mapping and serovar-specific monoclonal antibodies. Research in Microbiology. 1997; 148:327–33. https://doi.org/10.1016/S0923-2508(97)81588-4 PMID: 9765811

9. Geens T, Desplanques A, van Loock M, Börner BM, Kaleta EF, Magnino S, et al. Sequencing of the Chlamydia psittaci ompA gene reveals a new genotype, E/B, and the need for a rapid discriminatory genotyping method. J Clin Microbiol. 2005; 43:2456–61. https://doi.org/10.1128/JCM.43.5.2456-2461.2005 PMID: 15872282

10. Andersen AA. Serotyping of Chlamydia psittaci isolates using serovar-specific monoclonal antibodies with the microimmunofluorescence test. J Clin Microbiol. 1991; 29:707–11. PMID: 1890172

11. Andersen AA. Two new serovars of Chlamydia psittaci from North American birds. J Vet Diagn Invest. 1997; 9:159–64. https://doi.org/10.1177/10406387970900209 PMID: 9211235

12. Rehn M, Ringberg H, Runehagen A, Herrmann B, Olsen B, Petersson AC, et al. Unusual increase of psittacosis in southern Sweden linked to wild bird exposure, January to April 2013. Euro Surveill. 2013; 18:20478.

13. Read TD, Joseph SJ, Didelot X, Liang B, Patel L, Dean D. Comparative analysis of Chlamydia psittaci genomes reveals the recent emergence of a pathogenic lineage with a broad host range. MBio 2013. https://doi.org/10.1128/mBio.00604-12 PMID: 23532978

14. Wallensten A, Fredlund H, Runehagen A. Multiple human-to-human transmission from a severe case of psittacosis, Sweden, January-February 2013. Euro Surveill. 2014; 19.

15. Cadario ME, Frutos MC, Arias MB, Origlia JA, Zelaya V, Madariaga MJ, et al. Epidemiological and molecular characteristics of Chlamydia psittaci from 8 human cases of psittacosis and 4 related birds in Argentina. Rev Argent Microbiol. 2017; 49:323–7. https://doi.org/10.1016/j.ram.2017.04.001 PMID: 28734713

16. Sachse K, Laroucau K, Riege K, Wehner S, Dilcher M, Creasy HH, et al. Evidence for the existence of two new members of the family Chlamydiaceae and proposal of Chlamydia avium sp. nov. and Chlamydia gallinacea sp. nov. Syst Appl Microbiol. 2014; 37:79–88. https://doi.org/10.1016/j.syapm.2013.12.004 PMID: 24461712

17. Szymańska-Czerwińska M, Mitura A, Niemczuk K, Zaręba K, Jodełko A, Pluta A, et al. Dissemination and genetic diversity of chlamydial agents in Polish wildfowl: Isolation and molecular characterisation of avian Chlamydia abortus strains. PLoS ONE. 2017; 12:e0174599. https://doi.org/10.1371/journal.pone.0174599 PMID: 28350846

18. Burt SA, Röring RE, Heijne M. Chlamydia psittaci and C. avium in feral pigeon (Columba livia domestica) droppings in two cities in the Netherlands. Vet Q. 2018; 38:63–6. https://doi.org/10.1080/01652176.2018.1482028 PMID: 29806552

19. Sachse K, Kuehlewind S, Ruettger A, Schubert E, Rohde G. More than classical Chlamydia psittaci in urban pigeons. Vet Microbiol. 2012; 157:476–80. https://doi.org/10.1016/j.vetmic.2012.01.002 PMID: 22296995

20. Haag-Wackernagel D. Human diseases caused by feral pigeons. In: Feare CJ, Cowan DP (eds) Advances in vertebrated pest management. p. 31–58.

21. Zweifel D, Hoop R, Sachse K, Pospischil A, Borel N. Prevalence of Chlamydiophila psittaci in wild birds-potential risk for domestic poultry, pet birds, and public health? Eur. J. Wildl. Res. 2009; 55:575–81.

22. Geigenfeind I, Vanrompay D, Haag-Wackernagel D. Prevalence of Chlamydia psittaci in the feral pigeon population of Basel, Switzerland. J Med Microbiol. 2012; 61:261–5. https://doi.org/10.1099/jmm.0.034025-0 PMID: 21921110

23. Magnino S, Haag-Wackernagel D, Geigenfeind I, Helmecke S, Dovc A, Pruanker-Radovcic E, et al. Chlamydial infections in feral pigeons in Europe: Review of data and focus on public health implications. Vet Microbiol. 2009; 135:54–67. https://doi.org/10.1016/j.vetmic.2008.09.045 PMID: 18977610

24. Dinetti M, Gallo-Orsi U. Colombi e storni in città: Manuale pratico di gestione. Milano: Il verde; 1998.

25. Knaus P. Schweizer Brutvogelatlas 2013–2016; 2018.

26. Haag-Wackernagel D. Tauben in der Stadt. 2016. https://www.unibas.ch/dam/jcr:b3108793-ee4d-4949-9f6f-2766e899517d/Broschuere_Tauben_in_der_Stadt_2016.pdf.

27. Keller M. Projekt "Stadttauben Luzern" 2001–2006. 2007. Accessible at www.stadtluzern.ch/de.
28. Stadt Luzern U. Zahlen und Fakten: Die Tauben von Luzern. 2015. https://www.stadtluzung.ch/_docn/1140174/Stadtauben_Facts_2015.pdf.

29. Stadt Zürich. Tauben. 2019. https://www.stadt-zuerich.ch/td/de/index/gsz/natuerliche_vielzahl/tiere/Voegel/tauben.html.

30. Sachse K, Laroucau K, Hotzel H, Schubert E, Ehrlich R, Slickers P. Genotyping of *Chlamydia psittaci* using a new DNA microarray assay based on sequence analysis of ompA genes. BMC Microbiol. 2008; 8:63. https://doi.org/10.1186/1471-2180-8-63 PMID: 18419800

31. Pannekoek Y, Dickx V, Beeckman DSA, Jolley KA, Keijzers WC, Vreys E, et al. Multi locus sequence typing of *Chlamydia* reveals an association between *Chlamydia psittaci* genotypes and host species. PLoS ONE. 2010; 5:e14179. https://doi.org/10.1371/journal.pone.0014179 PMID: 21152037

32. Ehrlich R, Slickers P, Goellner S, Hotzel H, Sachse K. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol Cell Probes. 2006; 20:60–3. https://doi.org/10.1016/j.mcp.2005.09.003 PMID: 16330186

33. Hoffmann B, Depner K, Schirmieher M, Beer M. A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestviruses. J Virol Methods. 2006; 136:200–9. https://doi.org/10.1016/j.jvirem.2006.05.020 PMID: 16806503

34. Borel N, Kempf E, Hotzel H, Schubert E, Torgerson P, Slickers P, et al. Direct identification of chlamydiae from clinical samples using a DNA microarray assay: a validation study. Mol Cell Probes. 2008; 22:55–64. https://doi.org/10.1016/j.mcp.2007.06.003 PMID: 17714911

35. Pospischil A, Kaiser C, Hofmann-Lehmann R, Lutz H, Hilbe M, Vaughan L, Borel N. Evidence for *Chlamydia* in wild mammals of the Serengeti. J Wildl Dis. 2012; 48:1074–8. https://doi.org/10.7589/2011-10-298 PMID: 23060512

36. Pantchev A, Sting R, Bauerfeind R, Tyczka J, Sachse K. New real-time PCR tests for species-specific detection of *Chlamydia psittaci* and *Chlamydia abortus* from tissue samples. Vet J. 2009; 181:145–50. https://doi.org/10.1016/j.tvjl.2008.02.025 PMID: 18413292

37. Blumer S, Greub G, Waldvogel A, Hässig M, Thoma R, Tschuor A, et al. *Wadidia, Parachlamydia* and *Chlamydiaceae* in bovine abortion. Vet Microbiol. 2011; 152:385–93. https://doi.org/10.1016/j.vetmic.2011.05.024 PMID: 21658867

38. Staub E, Marti H, Biondi R, Levi A, Donati M, Leonard CA, et al. Novel *Chlamydia* species isolated from snakes are temperature-sensitive and exhibit decreased susceptibility to azithromycin. Sci Rep. 2018; 8:136. https://doi.org/10.1038/s41598-018-23897-z

39. Dolz G, Solórzano-Morales Á, Angelova L, Tien C, Fonseca L, Bonilla MC. Chlamydia psittaci genotype B in a pigeon (*Columba livia*) inhabiting a public place in San José, Costa Rica. Open Vet J. 2013; 3:135–9. PMID: 26623327

40. Sariya L, Prompiram P, Tangsudjai S, Poltep K, Chamsai T, Mongkolphan C, et al. Detection and characterization of *Chlamydia psittaci* in asymptomatic feral pigeons (*Columba livia domestica*) in central Thailand. Asian Pac J Trop Med. 2015; 8:94–7. https://doi.org/10.1093/bioinformatics/bts199 PMID: 22543367

41. Wang X, Zhang N-Z, Ma C-F, Zhan X-X, Zhao Q, Ni H-B. Epidemiological Investigation and Genotype of Chlamydia Exposure in Pigeons in Three Provinces in Northern China. Vector Borne Zoonotic Dis. 2018; 18:181–4. https://doi.org/10.1089/vbz.2017.2214 PMID: 29350589

42. Giunchi D, V. Y, Emilio N, Vanni L, Soldatini C. Feral Pigeons: Problems, Dynamics and Control Methods. In: Soloneski S, editor. Integrated Pest Management and Pest Control—Current and Future Tactics. InTech; 2012. https://doi.org/10.5772/31536

43. Soł D, Senar JC. Urban pigeon populations: stability, home range, and the effect of removing individuals. Can. J. 1995; 73:1154–60.

44. Čechová L, HaláMOVÁ M, Kalinová Z, Čislíková L, HaláN M, Valenčaková A. Detection of *Chlamydia psittaci* in feral pigeons (*Columba livia domestica*) in Slovakia and their characterisation. Ann Agric Environ Med. 2016; 23:75–8. https://doi.org/10.5604/12321966.1196856 PMID: 27007521

45. Dovc a, Jereb G, Krapež U, Gregurč-Gračner G, Pintarić Š, Slavec B, et al. Occurrence of Bacterial and Viral Pathogens in Common and Noninvasive Diagnostic Sampling from Parrots and Racing Pigeons in Slovenia. Avian Dis. 2016; 60:487–92. https://doi.org/10.1637/11373-011116-Reg PMID: 27309292

46. Teske L, Ryll M, Rubbenstroth D, Häne I, Hartmann M, Kreienbrock L, Rautenschlein S. Epidemiological investigations on the possible risk of distribution of zoonotic bacteria through apparently healthy pigeons (*Columba livia domestica*). J Wildl Dis. 2016; 52:465–73. https://doi.org/10.7589/2015-04-006
48. Donati M, Laroucau K, Delogu M, Vorimore F, Aaziz R, Cremonini E, et al. Chlamydia psittaci in Eurasian Collared Doves (Streptopelis decaocto) in Italy. J Wildl Dis. 2015; 51:214–7. https://doi.org/10.7589/vjr.2014-01-010 PMID: 26741324

49. Sharples E, Baines SJ. Prevalence of Chlamydia psittaci-positive cloacal PCR tests in wild avian casualties in the UK. Vet Rec. 2009; 164:16–7. https://doi.org/10.1136/vr.164.1.16 PMID: 19122179

50. Szymańska-Czerwińska M, Niemczuk K. Avian Chlamydiosis Zoonotic Disease. Vector Borne Zoonotic Dis. 2016; 16:1–3. https://doi.org/10.1089/vbz.2015.1839 PMID: 26741325

51. Dickx V, Kalmar ID, Tavernier P, Vanrompay D. Prevalence and genotype distribution of Chlamydia psittaci in feral Canada geese (Branta canadensis) in Belgium. Vector Borne Zoonotic Dis. 2013; 13:382–4. https://doi.org/10.1089/vbz.2012.1131 PMID: 23654298

52. van Loock M, Geens T, de Smit L, Nauwynck H, van Empel P, Naylor C, et al. Key role of Chlamydia psittaci on Belgian turkey farms in association with other respiratory pathogens. Vet Microbiol. 2005; 107:91–101. https://doi.org/10.1016/j.vetmic.2005.01.009 PMID: 15795081

53. Jeong J, An I, Oem J-K, Wang S-J, Kim Y, Shin J-H, et al. Molecular prevalence and genotyping of Chlamydia spp. in wild birds from South Korea. J Vet Med Sci. 2017; 79:1204–9. https://doi.org/10.1292/jvms.16-0516 PMID: 28579580

54. Beckmann KM, Borel N, Pocknell AM, Dagleish MP, Sachse K, John SK, et al. Chlamydiosis in British Garden Birds (2005–2011): retrospective diagnosis and Chlamydia psittaci genotype determination. Ecohealth. 2014; 11:544–63. https://doi.org/10.1007/s10393-014-0951-x PMID: 24947738

55. Takahashi T, Takashima I, Hashimoto N. Shedding and transmission of Chlamydia psittaci in experimentally infected chickens. Avian Dis. 1998; 32:650–6. PMID: 3060085

56. West A. A Brief Review of Chlamydiophila psittaci in Birds and Humans. J Exot Pet Med. 2011; 20:18–20.

57. Branley J, Bachmann NL, Jelocnik M, Myers GSA, Polkinghorne A. Australian human and parrot Chlamydia psittaci strains cluster within the highly virulent 6BC clade of this important zoonotic pathogen. Sci Rep. 2016; 6:30019. https://doi.org/10.1038/srep30019 PMID: 27488134

58. de Boeck C, Dehollolinge C, Dumont A, Spierenburg M, Heijne M, Gysens I, et al. Managing a cluster outbreak of psittacosis in Belgium linked to a pet shop visit in The Netherlands. Epidemiol Infect. 2016; 144:1710–6. https://doi.org/10.1017/S0950268815003106 PMID: 26669637

59. Harkinezhad T, Verminnen K, de Buyzere M, Rietzschel E, Bekkaert S, Vanrompay D. Prevalence of Chlamydiophila psittaci infections in a human population in contact with domestic and companion birds. J Med Microbiol. 2009; 58:1207–12. https://doi.org/10.1099/jmm.0.011379-0 PMID: 19528151