Original Contribution

Chlamydiosis in British Garden Birds (2005–2011): Retrospective Diagnosis and Chlamydia psittaci Genotype Determination

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Abstract: The significance of chlamydiosis as a cause of mortality in wild passerines (Order Passeriformes), and the role of these birds as a potential source of zoonotic Chlamydia psittaci infection, is unknown. We reviewed wild bird mortality incidents (2005–2011). Where species composition or post-mortem findings were indicative of chlamydiosis, we examined archived tissues for C. psittaci infection using PCR and ArrayTube Microarray assays. Twenty-one of 40 birds tested positive: 8 dunnocks (Prunella modularis), 7 great tits (Parus major), 3 blue tits (Cyanistes caeruleus), 2 collared doves (Streptopelia decaocto, Order Columbiformes), and 1 robin (Erithacus rubecula). Chlamydia psittaci genotype A was identified in all positive passerines and in a further three dunnocks and three robins diagnosed with chlamydiosis from a previous study. Two collared doves had genotype E. Ten of the 21 C. psittaci-positive birds identified in the current study had histological lesions consistent with chlamydiosis and co-localizing Chlamydia spp. antigens on immunohistochemistry. Our results indicate that chlamydiosis may be a more common disease of British passerines than was previously recognized. Wild passerines may be a source of C. psittaci zoonotic infection, and people should be advised to take appropriate hygiene precautions when handling bird feeders or wild birds.

Keywords: Chlamydia psittaci, chlamydiosis, collared dove Streptopelia decaocto, Order Passeriformes, passerine, wild bird

INTRODUCTION

Chlamydiosis is a disease of birds and mammals, including people, caused by infection with the Gram-negative, intracellular bacterium, Chlamydia (Chlamydophila) psit-
infiltrates, and biliary hyperplasia (Vanrompay et al. 1995). Hepatic histiocytosis, hepatic periportal inflammatory cell infiltration in acute cases; other findings can include splenic and/or hepatic, renal, and/or myocardial necrosis may be evident (Vanrompay et al. 2009). Microscopic lesions are variable: splenic, hepatic, renal, and/or myocardial necrosis may be evident in acute cases; other findings can include splenic and/or hepatic histiocytosis, hepatic periportal inflammatory cell infiltrates, and biliary hyperplasia (Vanrompay et al. 1995).

Avian chlamydiosis has been diagnosed in a variety of wild bird species in Europe, particularly columbiforms (Order Columbiformes) such as collared doves (Streptopelia decaocto), feral pigeons (Columbia livia), and wood pigeons (Columba palumbus) (Bracewell and Bevan 1986; Magnino et al. 2009). Also, chlamydiosis has been diagnosed occasionally in passerines (Order Passeriformes) (Simpson and Bevan 1989; Holzinger-Umlauf et al. 1997; Pennycott et al. 2009). The first reported occurrence of the disease in passerines in Britain was in 1988, when robins (Erithacus rubecula), dunlins (Tringa ochropus) and Paridae (tit species) were affected in a garden in south-west England (Simpson and Bevan 1989). Subsequently, Pennycott et al. (2009) reported mortality of Fringillidae (finches), Paridae, and robins in a Scottish garden in 2008, in which trichomonosis was considered the primary cause of disease and death, but in which concurrent chlamydiosis was diagnosed in some of the birds examined. Colville et al. (2012) described a further six incidents which affected Paridae and/or dunnocks and/or robins in England in 2009 (1 incident) and 2011 (5 incidents).

*Chlamydia psittaci* is currently classified into seven ompA genotypes, each of which appears to have a certain host predilection: genotype A (parrots), B (pigeons), C (ducks and geese), D (turkeys), E (pigeons, ducks and other species), F (parakeets), and E/B (ducks, turkeys and pigeons) (Vanrompay et al. 1997; Geens et al. 2005; Sachse et al. 2009). These data are derived mainly from studies of captive or farmed birds and feral pigeons: *C. psittaci* genotypes infecting wild passerines have rarely been determined (Kaleta and Taday 2003; Kalmar et al. 2013). In a recently proposed extension of the ompA typing scheme, subgroups of genotypes A (A-VS1, A-6BC and A-8455), E/B (EB-E30, EB-859 and EB-KKCP), and D (D-NJ1 and D-9N) were described, and six further avian genotypes were identified [in corvids, parrots, an oriental stork (*Ciconia boyciana*), and a brown skua (*Stercorarius antarcticus lomnergi*)] (Sachse et al. 2008).

While pigeons and doves appear to be the major wild bird reservoir of *C. psittaci* across Europe (Bracewell and Bevan 1986; Magnino et al. 2009), variable and potentially high prevalences of *C. psittaci* infection have been demonstrated in some wild passerine populations. For example, in Germany, 215 of 399 (54%) clinically healthy tits [including 30 of 43 (70%) blue tits (*Cyanistes (Parus) caeruleus*), 169 of 318 (53%) great tits (*Parus major*) and 12 of 32 (38%) marsh tits (*Poecile (Parus) palustris*)] were found to be *Chlamydia* sp. positive from cloacal and pharyngeal swabs using immunofluorescent antibody testing (Holzinger-Umlauf et al. 1997). Olsen et al. (1998) detected *C. psittaci* in 9 of 219 (3%) passerines sampled in Sweden (using PCR on fecal samples), including 2 of 29 (7%) robins and 1 of 21 (5%) great tits. Observation of sick birds was not reported; therefore, it seems likely that birds sampled in this study were apparently healthy. Others have failed to detect *C. psittaci* infection in passerines: Zweifel et al. (2009) from 527 passerines [including 211 chaffinches (*Fringilla coelebs*), 47 great tits and 12 robins] sampled in Switzerland (by PCR on cloacal swabs), and Pruken-Radovíc et al. (2005) from 53 passerines (including 15 robins) sampled in Croatia (by ELISA on cloacal swabs). The prevalence of *C. psittaci* infection in wild passerines in Britain is unknown.

*Chlamydia psittaci* infection causes a range of symptoms in human beings (in which the disease is termed “psittacosis”), ranging from asymptomatic infection or mild, flu-like illness to severe respiratory disease that, in rare cases, can be fatal (Smith et al. 2011; Rehn et al. 2013). Human cases have most often been attributed not only to direct or indirect contact with infected captive psittacine birds (Palmer 1982; Wreght and Taylor 1988; Smith et al. 2011), but also to contact with poultry (particularly ducks) (Palmer 1982; Gaede et al. 2008; Laroocau et al. 2009) and racing and feral pigeons (Haag-Wackernagel and Moch 2004; Harkinezhad et al. 2009; Magnino et al. 2009). The origins of human psittacosis cases, however, are often undetermined [e.g., Health Protection Agency (HPA), and Department for Environment, Food & Rural Affairs (Defra) 2012]. Other wild bird species have been implicated in...
some psittacosis outbreaks (Williams et al. 1998; Telfer et al. 2005; Herrmann et al. 2006; Rehn et al. 2013), including wild passerines, which were the suspected source of an outbreak that affected at least 25 people in southern Sweden in early 2013 (Rehn et al. 2013).

Wild bird carcasses tend not to be tested for C. psittaci infection routinely due to financial constraints (molecular tests are required to obtain a diagnosis) (Pennycook et al. 2009); therefore, the prevalence of chlamydioides in British passerines has been under-investigated (Colvile et al. 2012). Here, we conducted a retrospective survey of selected garden bird carcasses submitted by members of the public across England and Wales in order to investigate the significance of chlamydioides as a cause of disease in these species. We use the term “chlamydioides” to describe cases in which C. psittaci infection was detected in birds which had gross, histological, and immunohistochemical findings consistent with the disease. We conducted C. psittaci genotyping of positive cases in order to further our understanding of the epidemiology of the infection in British garden birds.

**Methods**

**Wild Bird Cases**

Reports of sick and dead wild birds were received from the general public through a national disease surveillance network established as part of the Garden Bird Health initiative (GBHi) (Robinson et al. 2010). Morbidity and mortality incidents were reported either on an ad hoc basis or through a systematic volunteer scheme (Robinson et al. 2010). A detailed description of each incident was obtained, including the species and number of birds affected, date when sick and/or dead birds were first observed, location, and clinical signs. If available, carcasses suitable for post-mortem examination (PME) were submitted.

On receipt, carcasses were either refrigerated at 4°C and examined within 48 h, or frozen at −20°C and examined at a later date. PMEs followed a standardized protocol, as described by Lawson et al. (2011). Birds were assigned to the age classes “Nestling,” “Juvenile,” (fully fledged and independent from nest) or “Adult” (any individual beyond its post-juvenile molt), and sex was determined, where possible, on the basis of plumage characteristics or gonad inspection. Carcasses were weighed, and body condition was subjectively assessed (as “Emaciated,” “Thin,” “Normal,” or “Fat”) on the basis of subcutaneous fat deposits and pectoral muscle condition. Samples (liver, small-intestinal content, and tissues with macroscopic lesions) were routinely submitted for microbiological examination using a standardized protocol (Lawson et al. 2011). A saline mount preparation of small-intestinal contents was examined microscopically for parasites. A standard range of tissues from each case was frozen at −20°C pending further testing and, where the state of carcass preservation permitted, tissue samples were fixed in neutral-buffered 10% formalin pending histological examination. Tissues were submitted for further tests (in addition to those described below) as indicated by the macroscopic findings, including culture and PCR to detect *Trichomonas* sp. infection (Robinson et al. 2010), and histopathology and PCR to detect avipoxvirus infection (Lawson et al. 2012).

Cases were selected for C. psittaci testing from an archive of 1,578 passerine and columbiform carcasses received at the Institute of Zoology from across England and Wales, 2005–2011, on the basis of either (1) having gross lesions consistent with previously reported chlamydioides incidents (hepatomegaly and/or splenomegaly and/or serositis), or (2) having been from a mortality incident in which the species assemblage of sick and dead birds was consistent with previously reported passerine chlamydioides incidents (involvement of robins and/or Paridae and/or dunnocks). In addition, tissues from six passerine carcasses in which chlamydioides had already been diagnosed (Colvile et al. 2012) were submitted for molecular C. psittaci testing.

**Molecular Detection of C. psittaci Infection**

DNA was extracted from frozen/thawed liver, or from pooled liver and spleen where both archived tissues were available, using a Biosprint 15 DNA Blood Kit (Qiagen Ltd., Manchester, M15 6SH, UK) according to the manufacturer’s instructions. The purified DNA was stored at 4°C, until the molecular analyses were performed.

All samples were examined by real-time PCR with primers specific for the 23S rRNA gene (Family Chlamydiaceae) using an ABI 7500 thermocycler (Applied Biosystems, Foster City, California, USA) following methods described by Ehricht et al. (2006) and Zweifel et al. (2009) which had a detection limit of 1 inclusion-forming unit (ifu) (Ehricht et al. 2006). A positive control (C. abortus DNA) and a negative control (reaction mix with molecular biology grade water) were included in each PCR run. Each sample was tested in duplicate. When both Ct-values were <38, a sample was considered as positive (Zweifel et al.
Samples for which one or both duplicates gave Ct-values of >38 were considered as questionably positive. If amplification was absent in both duplicates, the sample was interpreted as negative, and no further molecular tests were performed. Positive and questionably positive samples were further examined using each of the following three tests:

1. A *Chlamydia* species-specific 23S ArrayTube (AT) Microarray assay (Alere Chip Technologies GmbH, Jena, Germany) as described by Borel et al. (2008), which had a detection limit of 1 ifu (Ehricht et al. 2006).

2. A *C. psittaci* ompA real-time PCR, which had a detection limit of 2 ifu, as described by Pantchev et al. (2009). Each sample was tested in duplicate with positive (*C. psittaci* DNA) and negative (molecular grade water) controls included. A sample was considered as positive when the average Ct-value was <36 (Pantchev et al. 2009), and as questionable positive when the average Ct-value was >36.

3. A *C. psittaci* genotyping assay, as described by Sachse et al. (2008). In the case of weak signals where the ompA genotype could not be accurately identified by the software, the assignment was done manually based on the closest match; these cases were termed “weak positive.” The lowest amount of DNA required for correct typing was equivalent to 2 ifu (Sachse et al. 2009).

Samples were considered positive for *C. psittaci* if they were positive (including—for the *C. psittaci* genotyping assay—“weak positive”) on at least one of these three further tests.

**Histology and Immunohistochemistry**

In *C. psittaci*-positive cases for which formalin-fixed tissues were available, the significance of the infection was investigated using histopathological examination and immunohistochemistry.

Formalin-fixed tissues were prepared for histopathological examination using routine methods (Bancroft 2008), and 5-μm-thick sections were examined using various stains including H&E, Ziehl-Neelsen, Giemsa, Periodic Acid-Schiff, and Gram-Twort.

*Chlamydia* spp.-specific immunohistochemistry, using anti-chlamydial lipopolysaccharide antibody (mouse IgG1, clone 13/4; Santa Cruz Biotechnology Inc., California, USA), was performed on paraffin-embedded, formalin-fixed tissue sections following the methodology described by Buxton et al. (1996).

A diagnosis of chlamydiosis was made for *C. psittaci*-positive cases which had co-localization of *Chlamydia* spp.-specific immunolabeling with histological lesions consistent with the disease [such as splenic, hepatic, renal and/or myocardial necrosis, splenic and/or hepatic histiocytosis, hepatic periportal inflammatory cell infiltrates, and biliary hyperplasia (Vanrompay et al. 1995)].

**Results**

**Wild Bird Cases**

Tissues from 40 birds (from 38 mortality incidents) in the case archive fulfilled our selection criteria and were tested for *C. psittaci* infection using molecular methods. These comprised 35 passerines (from 33 mortality incidents) and 5 columbiforms (from a further 5 mortality incidents) (Table 1).

**Molecular Detection of *C. psittaci* Infection**

Tissues from 21 of the 40 selected cases tested positive for *C. psittaci* DNA: all of 8 dunnocks, 7 (of 12) great tits, 3 (of 4) blue tits, 2 (of 3) collared doves, and 1 (of 4) robins (Table 1 and Supplementary Table 1). For the positive cases, the results of each of the molecular tests are presented in Table 2. The 21 positive cases had been submitted from 20 separate mortality incidents, the details of which are presented in Table 3. Each of 4 corvids, 2 feral pigeons, 1 wren (*Troglydotes troglodytes*), 1 chaffinch, and 1 pied wagtail (*Motacilla alba*) tested were negative.

Nine *C. psittaci*-positive birds were from eight incidents of multi-species passerine mortality; eight positive birds were from incidents in which only a single bird had been observed to be sick or found dead; and four positive birds were from sites of multiple mortality where a single species had been affected [including two nestlings—a robin (Case 13) and a blue tit (Case 14)—from failed nests] (Table 3). Positive cases had either been observed with non-specific clinical signs (fluffed up plumage and/or lethargy) prior to death (11 cases), had been found dead (4 cases), had suffered trauma (including predation) (at least 6 cases), or had been euthanized for welfare reasons (2 cases). One positive blue tit (Case 21) had been submitted from an incident in which it, and other blue tits and great tits, had been observed with apparent dyspnea and ocular disease. Two positive collared doves were from separate incidents where no other sick or dead birds were observed;
there was no report of columbiform morbidity or mortality at any of the positive passerine incidents (Table 3).

Eighteen of the 21 *C. psittaci*-positive birds were adults, comprising 7 males (4 great tits, 2 dunnocks, and 1 blue tit), 6 females (4 great tits, 1 dunnock, and 1 collared dove), and 5 birds of undetermined sex (2 great tits, 2 dunnocks and 1 blue tit) (Table 3). The remaining positive birds were nestlings (see above) and a juvenile collared dove (Table 3). Positive cases had been submitted in each year of the study: 1 (of 1 tested) was from 2005, 1 (2) from 2006, 7 (12) from 2007, 1 (4) from 2008, 5 (9) from 2009, 5 (8) from 2010, and 1 (4) was from 2011. The positive birds had been found dead in all seasonal quarters of the year: 7 had been found in January–March; 7 in April–June; 2 in July–September; and 5 in October–December. Figure 1 shows the locations of positive and negative cases.

The *C. psittaci* genotype involved was determined for 17 of the 21 positive birds (Table 2). Genotype A was present in all 15 passerine cases for which the genotype was determined (7 dunnocks, 6 great tits and 2 blue tits) and was subtyped as genotype A-VS1 in 11 cases (6 dunnocks, 4 great tits and 1 blue tit) and as genotype A-6BC in 4 cases (2 great tits, 1 dunnock, and 1 blue tit). A further 3 dunnocks and 3 robins confirmed to have chlamydiosis in a previous study (Colvile et al. 2012) were also found to have been infected with genotype A-VS1. The two positive collared doves examined were infected with *C. psittaci* genotype E.

### Pathological Examination

Of the 21 *C. psittaci*-positive birds, the state of carcass preservation in six birds precluded histopathological or immunohistochemical evaluation (Supplementary Table 2). Of the 15 birds for which tissues were examined microscopically, the significance of *C. psittaci* infection was unclear in five (Supplementary Table 2), but chlamydiosis was diagnosed by histological and immunohistochemical examination in 10 (Table 4 and Fig 2): 5 dunnocks, 3 great tits, and 2 collared doves, from 9 separate mortality incidents. Of the chlamydiosis cases, body condition was “emaciated” in 6 cases, “thin” in 3 cases, and “normal” in 1 case; splenomegaly was suspected/confirmed in 7 cases, hepatomegaly was suspected/confirmed in 5 cases, and serositis was present in 4 cases (Table 4).

There was concurrent infectious disease in over half (8/15) of the *C. psittaci*-positive cases examined microscopically. Avian pox was confirmed (using PCR +/- histology +/- electron microscopy) in five *C. psittaci*-positive great tits from separate incidents, including one (Case 16) with confirmed chlamydiosis (Table 4; Supplementary Table 2). Trichomonosis was diagnosed (using PCR and histology) in one *C. psittaci*-positive dunnock (Case 3) from a mortality incident affecting predominantly finch species (Table 3). Concurrent trauma, most commonly cat predation, was either confirmed or suspected in nine *C. psittaci*-positive cases, including four cases of confirmed chlamydiosis (Table 4).

### Discussion

When garden bird carcasses from 38 mortality incidents suggestive of chlamydiosis were examined retrospectively, chlamydiosis was diagnosed in at least one bird from each of nine incidents. Ten birds, submitted from 2006–2010, were positive for the disease: eight small passerines (5...
Two collared doves. The eight passerines were from seven separate mortality incidents, which add to eight previously confirmed incidents associated with chlamydiosis in small passerines in Britain (Simpson and Bevan 1989; Pennycott et al. 2009; Colvile et al. 2012). Colvile et al. (2012) described six small passerine chlamydiosis mortality incidents in England, five of which occurred in 2011 and questioned whether there had been a recent increase in the incidence of chlamydiosis in small passerines in Britain. Here, cases of passerine chlamydiosis were identified in each year of the study, indicating that any apparent increase in incidence is most likely

### Table 2. Results of PCR, ArrayTube Microarray, and genotyping assays in C. psittaci-positive birds

| Case no. | Species       | 23S rtPCR for Chlamydiaceae | 23S ArrayTube Microarray | C. psittaci ompA rtPCR | C. psittaci genotyping assay |
|----------|---------------|-----------------------------|--------------------------|------------------------|-----------------------------|
| 1        | Blue tit      | 39.6 Ques                   | 38.6 Ques                | Genotype A-6BC         |                             |
| 2        | Dunnock       | 17.2 Pos C. psittaci        |                          |                        |                             |
| 3        | Dunnock       | 41.3 Ques                   | 38.6 Ques Weak positive  |                        |                             |
| 4        | Dunnock       | 18.8 Pos C. psittaci        | 20.4 Pos                | Genotype A-VS1         |                             |
| 5        | Great tit     | 26.3 Pos                    | 27.5 Pos                | Genotype A-VS1         |                             |
| 6        | Great tit     | 38.9 Ques C. psittaci      | 38.8 Ques Weak positive  |                        |                             |
| 7        | Great tit     | 37.1 Pos C. psittaci        | 38.2 Ques                | Genotype A-VS1         |                             |
| 8        | Great tit     | 26.0 Pos C. psittaci        | 27.0 Pos                | Genotype A-VS1         |                             |
| 9        | Dunnock       | 21.9 Pos C. psittaci        | 22.7 Pos                | Genotype A-VS1         |                             |
| 10       | Collared dove | 15.2 Pos C. psittaci        | 20.1 Pos                | Genotype E             |                             |
| 11       | Dunnock       | 19.8 Pos C. psittaci        | 23.9 Pos                | Genotype A-VS1         |                             |
| 12       | Dunnock       | 15.9 Pos C. psittaci        | 16.9 Pos                | Genotype A-VS1         |                             |
| 13       | Robin         | 40.5 Ques Neg               | 29.6 Pos Neg            |                        |                             |
| 14       | Blue tit      | 43.7 Ques C. psittaci      | 40.0 Ques Neg           |                        |                             |
| 15       | Collared dove | 13.9 Pos C. psittaci        | 17.7 Pos                | Genotype E             |                             |
| 16       | Great tit     | 26.5 Ques C. psittaci      | 30.6 Pos                | Genotype A-6BC         |                             |
| 17       | Dunnock       | 14.6 Pos C. psittaci        | 19.2 Pos                | Genotype A-6BC         |                             |
| 18       | Great tit     | 25.9 Ques C. psittaci      | 30.0 Pos                | Genotype A-6BC         |                             |
| 19       | Dunnock       | 15.9 Ques C. psittaci      | 20.9 Pos                | Genotype A-VS1         |                             |
| 20       | Great tit     | 19.0 Pos C. psittaci        | 23.4 Pos                | Genotype A-VS1         |                             |
| 21       | Blue tit      | 34.3 Pos Neg               | 39.5 Ques Genotype A-VS1 |                        |                             |
| 22       | Robin         | 16.7 Pos C. psittaci        | 18.1 Pos                | Genotype A-VS1         |                             |
| 23       | Robin         | 15.6 Pos C. psittaci        | 19.9 Pos                | Genotype A-VS1         |                             |
| 24       | Dunnock       | 19.1 Pos C. psittaci        | 23.2 Pos                | Genotype A-VS1         |                             |
| 25       | Robin         | 13.0 Pos C. psittaci        | 17.7 Pos                | Genotype A-VS1         |                             |
| 26       | Dunnock       | 23.8 Ques C. psittaci      | 27.9 Pos                | Genotype A-VS1         |                             |
| 27       | Dunnock       | 11.8 Pos C. psittaci        | 16.5 Pos                | Genotype A-VS1         |                             |

Samples were considered positive for C. psittaci if they were (1) positive or questionably positive on 23S PCR, and (2) positive (including, for the C. psittaci genotyping assay, “weak positive”) on at least one of the subsequent molecular tests.

Pos positive, Ques questionable positive, Neg negative.

Six additional chlamydiosis cases reported by Colvile et al. (2012) also submitted for molecular testing.

23S rtPCR as described by Ehricht et al. (2006) and Zweifel et al. (2009). Ct-value averaged from two duplicate samples, cut-off value 38.0.

Only one Ct-value was determined.

23S ArrayTube Microarray assay as described by Borel et al. (2008).

OmpA rtPCR as described by Pantechev et al. (2009). Ct-value averaged from at least two duplicate samples, cut-off value 36.0.

C. psittaci genotyping assay as described by Sachse et al. (2008). In the case of weak signals where the ompA genotype could not be accurately identified by the software, the assignment was done manually based on the closest match; these cases were termed “weak positive.”

dunnocks, 3 great tits) and two collared doves. The eight passerines were from seven separate mortality incidents, which add to eight previously confirmed incidents associated with chlamydiosis in small passerines in Britain (Simpson and Bevan 1989; Pennycott et al. 2009; Colvile et al. 2012). Colvile et al. (2012) described six small passerine chlamydiosis mortality incidents in England, five of which occurred in 2011 and questioned whether there had been a recent increase in the incidence of chlamydiosis in small passerines in Britain. Here, cases of passerine chlamydiosis were identified in each year of the study, indicating that any apparent increase in incidence is most likely
| Case no. | Species and signalment | Date and location | Details of mortality incident | Clinical signs (if sick birds were observed) and/or perceived cause of death (reported by members of the public) | Body condition, [bodyweight (g)] and gross findings on post-mortem examination |
|---------|------------------------|------------------|-----------------------------|--------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| 1       | Blue tit Adult         | Oct 2005 Wiltshire, England | Blue tit 1 (0)              | None reported                                                      | Normal (11.1) Suspected hepatomegaly                                           |
| 2       | Dunnock Adult male     | Jan–Feb 2006 East Sussex, England | Dunnock 2 (1) (2 individuals) | One individual was fluffed up prior to death                       | Emaciated (17.4) Suspected splenomegaly                                           |
| 3       | Dunnock Adult          | Sep 2006–Jan 2007 Staffordshire, England | Dunnock 1 (1) (1 individual) | Dunnock was fluffed up and lethargic prior to death                | Thin (17.0) Hepatomegaly, Necrotic ingluvitis                                     |
|         |                        |                  | Greenfinch 6 (some)         | Some greenfinches were fluffed up and unable to fly                |                                                                                   |
|         |                        |                  | Chaffinch 14 (some)         | None reported                                                      |                                                                                   |
|         |                        |                  | House sparrow 2 (0)         | None reported                                                      |                                                                                   |
| 4       | Dunnock Adult female   | Feb 2007 Northamptonshire, England | Dunnock 1 (0)              | Suspected window strike                                           | Emaciated (13.4) Suspected splenomegaly                                           |
| 5       | Great tit Adult female | Apr 2007 Wrexham, Wales    | Great tit 1 (0)             | None reported                                                      | Thin (14.1) Penetrating wound, rib fractures and fibrinous serositis               |
| 6       | Great tit Adult        | Sep–Oct 2007 East Sussex, England | Great tit 1 (0)             | Great tit was predated by a cat                                    | Normal (20.5) Splenomegaly, Pedunculated skin lesions on wing, Puncture wound     |
|         |                        |                  | Greenfinch 0 (1)            | Greenfinch was fluffed up and lethargic                           |                                                                                   |
| 7       | Great tit Adult        | Jul–Sep 2007 Surrey, England | Great tit 3 (3) (≥4 individuals) | Multiple individuals had skin growths, particularly on face and wing. Two of the dead great tits were euthanized | Normal (17.6) Splenomegaly, Facial skin lesions. Hemorrhage (euthanasia)           |
|         |                        |                  | Blue tit 5 (0)              |                                                                                   |                                                                                   |
| 8       | Great tit Adult male   | Jul–Oct 2007 East Sussex, England | Great tit 2 (3) (3 individuals) | Two great tits were lethargic and one other was observed to have a skin growth on wing | Normal (16.9) Splenomegaly, Suspected hepatomegaly, Fibrinous serositis, Hemorrhagic, inflamed neck lesion |
|         |                        |                  | Dunnock 1 (1) (1 individual) | Dunnock was fluffed up before death                                 |                                                                                   |
| Case no. | Species and signalment | Details of mortality incident | Clinical signs (if sick birds were observed) and/or perceived cause of death (reported by members of the public) | Body condition, [bodyweight (g)] and gross findings on post-mortem examination |
|---------|------------------------|-------------------------------|-------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| 9       | Dunnock Adult          | From the same mortality incident as Case 8 (see above) | | Emaciated (15.9) Hepatomegaly and splenomegaly |
| 10      | Collared dove Adult female | Sep 2008 Essex, England | Collared dove 1 (1) (1 individual) | Found sick following cat predation and later died | Emaciated (108.5) Serositis, air sacculitis and pericarditis. Ingluvitis. Hepatomegaly |
| 11      | Dunnock Adult male     | Nov 2008–Jan 2009 Powys, Wales | Dunnock 2 (2) (2 individuals) Robin 1 (1) (1 individual) Greenfinch 0 (1) | Dunnocks and robin were fluffed up and lethargic before death | Emaciated (15.7) Anorexia |
| 12      | Dunnock Adult male     | Feb 2009 West Sussex, England | Blue tit 1 (0) Dunnock 3 (0) | Blue tit was a possible window strike One dunnock was a possible window strike | Thin (19.2) Fractures with no associated hemorrhage |
| 13      | Robin Nestling         | Apr 2009 Surrey, England | Robin 3 (0) | All of a dutch of 3 nestlings found dead | Thin (11.0) Hepatic congestion |
| 14      | Blue tit Nestling      | May 2009 Staffordshire, England | Blue tit 6 (0) | Six of a clutch of 7 nestlings died | Thin (5.3) Suspected hepatomegaly. Anorexia |
| 15      | Collared dove Juvenile | Jun 2009 Tyne and Wear, England | Collared dove 1 (1) (1 individual) | Fledgling, seen lethargic before death | Emaciated (104) Hepatomegaly, splenomegaly and serositis |
| 16      | Great tit Adult female | Feb 2010 Wiltshire, England | Great tit 1 (1) (1 individual) | Lethargic prior to death, with skin lesion on head | Thin (15.5) Large skin lesion on head. Suspected splenomegaly |
| Case no. | Species and signalment | Date and location | Details of mortality incident | Body condition, [bodyweight (g)] and gross findings on post-mortem examination |
|---------|------------------------|------------------|------------------------------|-----------------------------------------------------------------------|
| 17      | Dunnock Adult male     | Mar 2010 Kent, England | Dunnock 1 (1) (1 individual) Blue tit 0 (1) | Thin (17.0) Wound, fracture and hemorrhage. Splenomegaly and suspected hepatomegaly. Numerous intestinal helminths |
| 18      | Great tit Adult female | Feb–Apr 2010 Surrey, England | Great tit 1 (5) (5 individuals) | Thin (13.7) Multiple skin lesions. Suspected splenomegaly. Numerous lice |
| 19      | Dunnock Adult male     | Apr 2010 Hampshire, England | Dunnock 1 (1) (1 individual) | Thin (17.0) Wounds, fractures and hemorrhage (euthanasia). Hepatomegaly and suspected splenomegaly |
| 20      | Great tit Adult female | Oct 2010 Surrey, England | Great tit 1 (0) | Normal (19.0) Multiple skin lesions. Fracture and hemorrhage. Splenomegaly |
| 21      | Blue tit Adult male    | Mar–Apr 2011 Worcestershire, England | Blue tit 6 (≥9) Great tit 0 (≥2) | Thin (8.16) Pulmonary congestion. Anorexia |
to have been due to increased diagnostic effort. Furthermore, our results show that chlamydiosis is likely to have been a commoner cause of disease in small passerines than was previously recognized.

In addition to the 10 garden birds diagnosed with chlamydiosis, a further 11 birds found dead from 2005–2011 were positive for *C. psittaci* infection. Post-mortem tissue decomposition precluded histological or immunohistochemical examination in six of these cases, while in five cases, histology was equivocal for chlamydiosis—indicating that another infectious disease may have been the primary cause of morbidity or death. Avian pox in great tits—an emerging infectious disease in Britain (Lawson et al. 2012)—was the most common concurrent infectious disease diagnosed (5 cases). In addition to chlamydiosis, a dunnock examined in the current study had trichomonosis. Concurrent chlamydiosis and trichomonosis were previously reported from a passerine mortality incident in Scotland in 2008 (Pennycott et al. 2009), and concurrent infectious disease is a common finding in other avian species affected by chlamydiosis (Vanrompay et al. 1995). At least four of the 21 *C. psittaci*-positive cases (including two cases with chlamydiosis) had evidence of cat predation. There have been rare reports of disease in cats and dogs associated with *C. psittaci* infection (Werth 1989), most commonly attributed to the animals having contact with pet parrots. The risk of pet cats or dogs acquiring the infection from wild birds is unknown but is likely to be low since there are few diagnosed cases of chlamydiosis in these companion animals.

Most (17 of 21) positive cases were selected for testing based on the presence of gross lesions typical of avian chlamydiosis (hepatomegaly, splenomegaly, and serositis), hence any *C. psittaci*-positive cases with different or no gross lesions would have been overlooked during case selection for this study. Also, only certain species were selected for diagnostic testing. It is therefore not possible to make inferences regarding the prevalence of chlamydiosis, or *C. psittaci* infection, in the general passerine population in Britain from this study. Further investigation, particularly of cases with no gross or macroscopic lesions (or clinical signs) typical of chlamydiosis, is warranted in order to explore the prevalence of *C. psittaci* infection in passerines.

Both the number and species of birds that had been observed sick or dead in each of the *C. psittaci*-positive mortality incidents we identified were highly variable. In the eight positive incidents in which there had been multi-species mortality, tits, dunnocks, robins, and finches were the most commonly affected species, as observed in previous studies (Simpson and Bevan 1989; Pennycott et al. 2009).
| Case no. | Species and signalment | Results of microbiological examination and additional tests | Histopathological findings | Immunohistochemical labeling for *Chlamydia* sp. specific antigens | Diagnoses |
|---------|------------------------|---------------------------------------------------------------|-----------------------------|---------------------------------------------------------------|-----------|
| 2       | Dunnock (Adult male)   | Liver and small intestine (SI): *Escherichia coli* 1. Spleen: no growth | Fibrinous to histiocytic hepatitis with fibrinous thrombosis of the hepatic veins. Fibrinonecrotic, focally extensive splenitis. Fibrinous pneumonia. Histiocytic, focal, mild epicarditis. Proventriculus and gizzard: histiocytic serositis. Giemsa-positive granules in Kupffer cells of the liver, with similar material in some dissociated cells (macrophages or autolyzed hepatocytes), possibly representative of Chlamydial inclusions (or conventional bacteria, such as the *E. coli* 1 isolated from the tissue). Ziehl-Neelsen (ZN) stain negative for acid-fast agents or inclusions | Intense positive immunolabeling in the heart (endo- and epicardium plus interstitial cells) and serosal surface of the trachea. Foci of positive labeling in the meninges, proventriculus and gizzard. Within the lung, spleen and liver, positive labeling in the cytoplasm of macrophage-like cells (possibly Kupffer cells in the liver) | Chlamydiosis; possible additional bacterial infection |
| 4       | Dunnock (Adult female) | Liver: mixed growth, predominantly *E. coli* 1. SI: *E. coli* 1 and *Enterococcus* sp. | Fibrinous to histiocytic hepatitis, with fibrinous thrombosis of hepatic veins. Fibrinous pneumonia. Marked atrophy of epicardial adipose tissue and pectoral muscle. Giemsa-positive, granular to linear material in Kupffer cells of the liver, possibly representative of Chlamydial inclusions (or conventional bacteria, such as the *E. coli* 1 isolated from the tissue; interpretation hindered by autolysis). ZN stain negative for acid-fast agents or inclusions | Positive immunolabeling in the heart (interstitium of the left ventricular wall, right ventricular wall cardiomyocytes, and epicardium), liver (macrophages, hepatocytes and white blood cells) and lung | Chlamydiosis; possible coli-septicemia; possible window strike |
| Case no. | Species and signalment | Results of microbiological examination and additional tests | Histopathological findings | Immunohistochemical labeling for *Chlamydia* sp. specific antigens | Diagnoses |
|----------|------------------------|---------------------------------------------------------------|-----------------------------|---------------------------------------------------------------|-----------|
| 5        | Great tit (Adult female) | Liver: confluent mixed growth, *E. coli* 1, *Moellerella wisconsinensis* & *Enterococcus* spp., Lung: mixed growth predominance *Serratia fonticola* & *M. wisconsinensis*, Coelomic cavity: few colonies *E. coli* 1, *M. wisconsinensis* & *Enterococcus* spp. | Fibrinous perihepatitis and striking cellular infiltrate of portal tracts throughout the liver parenchyma (interpretation hindered by autolysis). Multifocal, acute pulmonary edema. Moderate to marked atrophy of epicardial adipose tissue. ZN and Giemsa stain reveal no Chlamydial inclusions | Positive labeling in the heart (mainly associated with blood vessels), liver (hepatocytes and possibly Kupffer cells), kidney (interstitial tissue) and keel | Chlamydiosis; trauma (possible predation); possible additional (bacterial/viral) infection |
| 8        | Great tit (Adult male) | Lung, skin lesion and coelom: *E. coli* 1 & *Enterococcus* spp. | Vascular endothelial hypertrophy within heart and spleen, with intraleional Gram-negative organisms. Fibrinogranulomatous to mixed cellular, locally extensive, epicarditis, with intraleional Gram-negative organisms. Granulomatous to hemorrhagic, extensive dermatitis, possibly associated with an unidentified mite. Fibrinonecrotizing, focal, acute hepatitis. Fibrinonecrotizing splenitis. Mild pectoral muscle atrophy. Gram-Twort stain shows intra-endothelial organisms as Gram-negative cocccobacilli or short rods and shows similar organisms in some epicardial macrophages. ZN and Giemsa stains show no evidence of Chlamydial inclusions | Positive labeling in the heart (epicardium and heart base), spleen (white blood cells), lung (parenchyma and white blood cells), liver (cell-associated, probably macrophages), and skin (inflammatory cells) | Chlamydiosis; possible other bacterial infection |
| Case no. | Species and signalment | Results of microbiological examination and additional tests | Histopathological findings | Immunohistochemical labeling for *Chlamydia* sp. specific antigens | Diagnoses |
|----------|------------------------|---------------------------------------------------------|--------------------------|---------------------------------------------------------------|-----------|
| 9 | Dunnock (Adult) | Liver: Mixed growth. *E. coli* 1 and *Providencia stuartii*. SI and bursa of Fabricus: *E. coli* 1 | Fibrinonecrotic, marked hepatitis with multifocal probable fibrinous thrombosis and with intraleisional bacterial rods. Fibrinonecrotic splenitis. Fibrinous pneumonia with intrahistiocytic bacterial rods. Focal epicarditis. Giemsa stain shows moderate numbers of bacterial rods in blood vessels in all tissues, within pulmonary macrophages and within some of the fibrinous lesions in the liver and spleen. ZN stain negative for acid-fast agents | Positive labeling in the liver (cell-associated and extracellular labeling in sinusoids and blood vessels), spleen (subcapsule area), heart (interstitial tissue and myocardium), lung (white blood cells and pleura), and trachea (serosal surface) | Chlamydiosis; possible other bacterial infection |
| 10 | Collared dove (Adult female) | Liver, SI, crop, pericardium and lung: mixed, *E. coli* 1 and *Enterococcus* spp.. Crop: also *Candida tropicalis* & *C. albicans* 1. Crop tissue negative for *Trichomonas* sp. on culture and PCR | Severe candidiasis (crop mucosa markedly thickened, containing massive numbers of *Candida* sp. spores and pseudohyphae) and secondary bacterial infection (consistent with *E. coli* 1 infection isolated on culture). Marked, necrotic pericarditis. Liver, gizzard and small intestine: serosodometitis. Scattered cells within the spleen appear to contain large, basophilic, cytoplasmic inclusions (autolysis hinders interpretation) | Positive specific labeling in the liver, spleen, serosal surface of the small intestine and individual cells (presumed macrophages) in the lung | Chlamydiosis; cat predation; candidiasis |
| Case no. | Species and signalment | Results of microbiological examination and additional tests | Histopathological findings | Immunohistochemical labeling for *Chlamydia* sp. specific antigens | Diagnoses |
|----------|------------------------|----------------------------------------------------------|----------------------------|---------------------------------------------------------------|-----------|
| 11       | Dunnock (Adult male)   | SI content: *Campylobacter* sp., Liver and lung: no growth | Granulocytic enteritis associated with luminal and encysted trematode life stages (consistent with schistosomes but autolysis hinders interpretation). Fibrinonecrotic hepatitis. Focal epicarditis. Mild pulmonary edema (probably agonal). Sarcocystosis of the pectoral muscle. Severe atrophy of epicardial adipose tissue. ZN stain shows no acid-fast agents or inclusions. Giemsa stain faintly highlights Sarcocysts in the pectoral muscle and highlights scanty punctate material in the foci of hepatic necrosis (nuclear dust, or less likely, bacteria or Chlamydial inclusions) | Positive labeling in the lung (diffuse, in cells resembling macrophages and within blood vessels), trachea (serosal surface and intramuscular), pectoral muscle, liver (diffuse, and some associated with bile duct epithelium), proventriculus and gizzard (interstitium and mucosa), heart (cell-associated in interstitium, and myocardium), spleen, and intestines (serosal surface and mucosa) | Chlamydiosis; possible other septicaemia; parasitic enteritis; sarcocystosis (probably incidental); intestinal *Campylobacter* sp. infection (probably incidental) |
| 15       | Collared dove (Juvenile) | Liver: moderate pure growth *E. coli*. SI: confluent nearly pure *E. coli*. Crop: *Trichomonas* sp. isolated in Bushby’s medium (subclinical infection). Circovirus-specific PCR on necrotic coelomic tissue negative | Fibrinogranulomatous, extensive serositis with intralesional Gram-negative bacteria and some plant material (possible artefactual transfer, or alimentary tract rupture). Fibrinonecrotic splenitis with intralesional Gram-negative bacteria. Diffuse, marked atrophy of adipose tissue. Giemsa stain highlights bacteria in the coelomic exudate but shows no inclusions. A Periodic Acid-Schiff (PAS) preparation highlights plant matter in the exudate on the stomach and intestine but shows no fungal agents. A Gram-Twort highlights the Gram-negative bacteria as coccobacilli to short rods. ZN stain shows no acid-fast agents or inclusions | Positive labeling in the heart (predominantly cell-associated but also extracellular, often perivascular), spleen (capsule and parenchyma), crop (serosal surface), proventriculus and gizzard (serosal surfaces and intramuscular), intestine (within inflammatory cells on the serosal surface), lung (within macrophages in alveoli and interstitium), and kidney (interstitium) | Chlamydiosis; possible other Gram-negative bacterial infection; possible alimentary tract rupture; subclinical *Trichomonas* sp. infection |
| Case no. | Species and signalment | Results of microbiological examination and additional tests | Histopathological findings | Immunohistochemical labeling for *Chlamydia* sp. specific antigens | Diagnoses |
|---------|------------------------|------------------------------------------------------------|--------------------------|---------------------------------------------------------------|-----------|
| 16      | Great tit (Adult female) | Liver: Light nearly pure growth of *Serratia ficaria*. Small intestine and lung: no growth. Skin lesion: avipox PCR positive | Generalized vascular endothelial hypertrophy in most tissues, with intralesional Gram-negative, PAS-positive organisms. Acute, fibrinous pneumonia with atelectasis. Fibrinonecrotic, extensive hepatitis. Fibrinonecrotic, disseminated, acute or subacute splenitis. Proliferative, multifocally necrotizing, extensive, severe dermatitis with numerous intracytoplasmic inclusion bodies (pathognomonic for avian poxvirus infection) and with minor surface infection by bacterial cocci and mixed fungi. Apparent mild hemoparasitism (compatible with leucocytozoonosis, but other hemoprotozoa could be indistinguishable on histology). ZN and Giemsa stains show no inclusions. Gram-Twort stain shows many of the endothelial bodies as Gram-negative coccoid or short bacillary structures; PAS stain highlights most of the same structures in intense magenta | Positive immunolabeling in the liver (cell-associated, primarily perivascular) and head lesions (inflammatory cells, primarily macrophages). (Brain, trachea, heart, pectoral muscle, lung, esophagus, spleen, proventriculus and gizzard and large intestine devoid of immunolabeling) | Chlamydiosis; avian pox disease with secondary mixed infection; hemoparasitism (significance unclear) |
| Case no. | Species and signalment | Results of microbiological examination and additional tests | Histopathological findings | Immunohistochemical labeling for *Chlamydia* sp. specific antigens | Diagnoses |
|---------|------------------------|-------------------------------------------------|----------------------------|-------------------------------------------------|------------|
| 17      | Dunnock (Adult male)   | Liver, SI and peritoneum: pure isolate *E.coli* 1. | Fibrinonecrotic splenitis with intralesional, coccoid to coccobacillary, Gram-negative bacteria. Pulmonary congestion, edema and atelectasis. Generalized perivascular cellular infiltrates (interpretation hindered by autolysis). Sarcocystosis of the pectoral muscle with no evidence of myositis. ZN and Giemsa stains reveal no Chlamydial inclusions. A Gram-Twort stain shows the splenic bacteria to be Gram-negative, and apparently coccoid to coccobacillary | Positive labeling in the brain (cell-associated and possibly extracellular), trachea (muscle), lung (diffuse, cell-associated and extracellular), heart (diffuse, myocardium), crop (serosa), proventriculus (white blood cells within mucosa and lamina propria), gizzard (mucosa), liver (diffuse, cell-associated and extracellular), spleen (sub-capsular region, associated with white blood cells), large and small intestines, kidney (cell-associated), testis (cell-associated, interstitium), pectoral muscle (myofibrils) | Chlamydiosis; possible other bacterial sepsis; cat predation; heavy intestinal helminth burden; sarcocytosis (presumed incidental infection) |

* Circovirus PCR performed by Biobest Laboratories Ltd., Penicuik, Scotland, EH26 0PY, UK.
Such a species complement, however, was one of the criteria used for the selection of cases for this study and was the sole basis for the selection of cases from three incidents, so this observation will be circular. No apparent sex predisposition to *C. psittaci* infection or seasonality of infection was evident, although the relatively small sample size may provide limited inferences regarding these factors. *C. psittaci*-positive incidents were widespread geographically (Fig. 1). Two *C. psittaci*-positive cases were from Wales, where (to the authors’ knowledge) infection with *C. psittaci* in free-living passerines has not been reported previously.

The use of four very sensitive assays—one family-specific screening assay, combined with three *C. psittaci*-specific assays—ensured that the overall molecular diagnostic method was highly sensitive and specific. *C. psittaci* was characterized as genotype A in all 15 passerines in which this could be determined. The sub-genotype was determined to be A-VS1 in 11 cases and A-6BC in 4 cases. A further six passerines diagnosed with chlamydirosis in a previous study (Colvile et al. 2012) also had genotype A-VS1. Genotype A has been identified most commonly in captive psittacines (Sachse et al. 2008, 2009), but our results suggest it is also a common genotype in wild passerines in Britain. Genotype A-VS1 is the most common subtype of genotype A, with the broadest host range of all *C. psittaci* genotypes, having previously been identified in psittacines, poultry, pigeons, canaries, and pheasants (Sachse and Rütger 2014). Genotype A-6BC has been identified in a similar range of host species to A-VS1 but appears to be less prevalent (Sachse and Rütger 2014). In two collared doves with chlamydirosis (Cases 10 and 15), *C. psittaci* was characterized as genotype E. Genotype E has been identified previously in feral pigeons (Magnino et al. 2009).

Although all *C. psittaci* genotypes are potentially zoonotic (Vanrompay et al. 2007), genotype A is the most commonly identified genotype in people, including in patients with severe psittacosis (Heddem et al. 2006; Vanrompay et al. 2007; Gaede et al. 2008). *C. psittaci* genotype A was identified in all four genotyped cases in a recent outbreak of human psittacosis in southern Sweden (that affected at least 25 people), in which wild passerines were implicated as the source of infection (Rehn et al. 2013). The identification of *C. psittaci* genotype A in passerines in the current study supports wild passerines as a potential source of human infection. Case-control investigations of human psittacosis outbreaks in Australia and Sweden have identified direct or indirect contact with live or dead wild birds (Telfer et al. 2005; Rehn et al. 2013), cleaning of wild bird feeders (Rehn et al. 2013), time spent in the garden, and lawn mowing (Williams et al. 1998; Telfer et al. 2005) as risk factors for disease. It is recommended that the public take sensible hygiene precautions when handling sick or dead wild birds and garden bird feeders, and that they wet areas contaminated with bird droppings prior to cleaning to minimize aerosolization, to reduce the risk of infection with *C. psittaci* and other zoonotic pathogens (Pennycott et al. 2009; Colvile et al. 2012; Rehn et al. 2013).

Although the overall risk of *C. psittaci* transmission from wild birds to humans is likely to be low (Haag-Wackernagel and Moch 2004; Rehn et al. 2013), consider-

![Figure 2](image-url)
ing that over 12 million households provide supplementary food for garden birds in Britain (Davies et al. 2009), it is important to determine the prevalence of subclinical C. psittaci carriage in wild passerines in order to understand the risks of zoonotic transmission (Colvile et al. 2012).

**CONCLUSION**

Through this retrospective study, we almost double (from 8 to 15) the number of small passerine mortality incidents in Britain in which chlamydiosis has been diagnosed, showing that chlamydiosis may be a more common cause of disease in British passerines than was previously recognized. We diagnosed further cases of C. psittaci infection in passerines, and showed that it was unlikely to have been a primary pathogen in some birds. C. psittaci was characterized as genotype A in all the passerines (dunnocks, great tits, robins, and blue tits) from which it was determined, indicating that this is likely to be a common genotype in these species. As this genotype is known to be capable of infecting people, our results support a potential role for wild passerines in the zoonotic transmission of C. psittaci. Further research is required to determine the prevalence of C. psittaci infection in wild birds in Britain; people should be advised to take appropriate hygiene precautions when cleaning wild bird feeders or when handling sick or dead wild birds.

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