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Acute necrotising pneumonitis associated with *Suttonella ornithocola* infection in tits (Paridae)

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ABSTRACT

Suttonella ornithocola, first isolated from the lungs of British tit species in 1996, was found to be a novel bacterium belonging to the family *Cardiobacteriaceae*. Comprehensive surveillance of garden bird mortality across Great Britain between April 2005 and April 2009 involved post mortem and microbiological examination of 82 tits (Paridae; multiple species) and six long-tailed tits (Aegithalidae; *Aegithalos caudatus*). *S. ornithocola* was isolated from six birds submitted from six incidents of morbidity and mortality involving Paridae and Aegithalidae species with a wide geographical distribution. The mortality incidents occurred sporadically at low incidence throughout the study period, which suggested that the infection is endemic in native bird populations, with a seasonal peak during early spring. Histopathological examination showed multiple foci of acute pulmonary necrosis associated with Gram-negative cocco-bacillary bacteria. These findings supported the hypothesis that *S. ornithocola* is a primary pathogen of tits in Great Britain.

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Introduction

A variety of infectious diseases has been reported to cause morbidity and mortality of garden birds in Great Britain and continental Europe. The pathogens can be (1) bacterial, such as *Salmonella* Typhimurium (Pennycott et al., 1998; Refsum et al., 2003) and *Escherichia coli* serotype O86 (Foster et al., 1998), (2) viral, for example, avian pox (Weli et al., 2004) and (3) protozoal (e.g., *Trichomonas gallinae*; Lawson et al., 2006). Finches (greenfinch *Carduelis chloris*, chaffinch *Fringilla coelebs* and siskin *Carduelis spinus*) and house sparrows *Passer domesticus* are the species in which mortality incidents have most frequently been documented in Great Britain (Pennycott et al., 1998, 2006), whilst reports of infectious disease in tit species (Paridae and Aegithalidae) are comparatively rare (Kirkwood et al., 2006).

Members of the Paridae, and to a lesser extent the Aegithalidae, are common birds in garden habitats in Great Britain, particularly those with feeding stations (Toms, 2003), and their breeding populations are widely distributed across the country (Risely et al., 2008). Kirkwood et al. (2006) reported investigations into the morbidity and mortality of members of the Paridae and Aegithalidae at 11 disparate gardens in the spring of 1996; the species involved comprised the blue tit (*Cyanistes caeruleus*), coal tit (*Periparus ater*), great tit (*Parus major*) and long-tailed tit (*Aegithalos caudatus*). A total of 34 dead birds were reported, ranging from 1 to 10 per incident. Post mortem examinations revealed pulmonary congestion, but no other significant abnormalities. The bacterium, *Suttonella ornithocola*, was isolated from the lungs of affected birds (Kirkwood et al., 2006) and was identified as a novel bacterium belonging to the family *Cardiobacteriaceae* (Foster et al., 2005). Kirkwood et al. (2006) postulated that *S. ornithocola* infection might have caused the deaths of these birds, but could reach no firm conclusion as to the association between *S. ornithocola* and disease or mortality.

In this article, we describe six further mortality incidents affecting Paridae and Aegithalidae species that occurred between April 2005 and April 2009, from which *S. ornithocola* was isolated from lung tissue.

Material and methods

A national surveillance programme (the Garden Bird Health initiative (GBHi)) was launched in April 2005 to investigate causes of mortality in garden birds across Great Britain. The GBHi utilised a combination of two independent and complementary reporting schemes: (1) the opportunistic reports of garden bird morbidity and mortality solicited from the general public, and (2) systematic surveillance via the

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British Trust for Ornithology's (BTO) Garden Bird Watch (GBW)[1] volunteer network. Birds found dead in gardens were selected for post mortem investigation based on fresh carcass availability and were submitted by post. Details of the date found, geographical location and clinical signs observed were recorded.

Post mortem examinations were performed using a standardised protocol by a regional network of disease investigation laboratories comprising: the Institute of Zoology (London, England); the Department of Veterinary Pathology, University of Liverpool (Wirral, England); the Wildlife Veterinary Investigation Centre (Cornwall, England), and the Scottish Agricultural College (Ayrshire, Scotland). The age, sex and bodyweight (BW) were recorded for each bird examined. Birds were classed as juveniles until the post-juvenile body moult was complete. First year birds beyond their post-juvenile moult and adult birds were not differentiated; all were classified as adult for the purpose of this study. Sex was assigned based on gonadal inspection and/or plumage characteristics. The state of carcass preservation was described in each case as mild, moderate or advanced state of autolysis. Systematic external and internal examinations of body systems were performed and any gross lesions described. A subjective measure of body condition (emaciated, thin, moderate or good) was made based on visual inspection of the pectoral muscle and fat deposits.

Lung, liver and/or contents from the mid small intestinal loop were sampled aseptically from the majority of carcasses and examined for the presence of pathogenic bacteria. Briefly, lung and liver were plated directly onto each of the following media: Colombia blood agar supplemented with 5% horse or sheep blood (CBA; QCM laboratories or E and O laboratories); incubated under aerobic conditions, and Chocolate blood agar (CCBA; QCM laboratories); incubated under 5–10% CO_2 conditions.

Small-intestinal contents were plated directly onto each of the following media: (1) xylose–lysine deoxycholate (XLD) agar (QCM laboratories), or MacConkey agar without salt (E and O laboratories) and Brilliant green agar (E and O laboratories), incubated under aerobic conditions; (2) CBA, incubated under aerobic conditions; (3) Campylobacter blood free selective medium (modified CCDA-Preston; QCM laboratories), incubated under microaerophilic conditions; and (4) immersed into selenite Salmonella-selective enrichment broth (QCM laboratories or E and O laboratories) under aerobic conditions for 24 h followed by subculture onto XLD agar aerobically.

At the Institute of Zoology, liver also was plated onto CBA and incubated anaerobically. All culture media were incubated at 37 °C, with the exception of selenite broth at the Scottish Agricultural College, which was incubated at 42 °C. Heart blood was examined using the same protocol as the liver.

Suttonella ornithocola isolates were identified by their morphology (Gram-negative rod to coccobacillus, 0.5–0.6 × 0.6–1.3 μm), preference for aerobic and capnophilic (or carboxyphilic) culture conditions, oxidase positive reaction and biochemical test results (API 20NE, BioMérieux), as described by Kirkwood et al. (2006). 16S rRNA genes were amplified by PCR using universal primers pA and sequenced to confirm the isolate identity as *S. ornithocola* (Foster et al., 2005).

Where the state of carcass preservation permitted, samples from a range of organs (including any lesions found) were fixed in neutral-buffered 10% formalin and were processed for histopathological examination using routine methods. Tissue sections were stained using haematoxylin and eosin or Gram-Twort stains.

Results

A total of 82 members of the family Paridae (30 blue tit *Cyanistes caeruleus*, 11 coal tit *Periparus ater*, 40 great tit *Parus major*, six marsh tit *Parus palustris*) and six members of the family Aegithalidae (all long-tailed tits *Aegithalos caudatus*) were examined post mortem between April 2005 and April 2009. The age distribution comprised 69 adults, 14 juveniles, and five birds for which the age category was undetermined.

Bacteriology was performed on at least one tissue from each bird carcass submitted for post mortem examination, except for one bird where the level of decomposition precluded meaningful examination. Bacterial culture of the lung was performed on 61% (54/88) of the birds examined, which included 80% (20/25) of those diagnosed as having died of infectious disease, 63% (17/27) of those diagnosed as having died due to trauma other than predation, 56% (15/27) of those for which the cause of death was undetermined, 29% (2/7) of those diagnosed as having died from predation, and 50% (1/2) of those diagnosed as having died from 'other' causes.

S. ornithocola was isolated from the lungs of six tits considered to have died as a result of infectious disease (Table 1); no evidence of the bacterium was found in the tits which died from other causes. Liver was cultured from 72% (63/88) of birds examined; small-intestinal contents were cultured from 88% (77/88) of birds examined and heart blood was cultured from two birds. *S. ornithocola* was isolated from the liver of three birds and from the heart blood of a single bird, all of which were also positive for the bacterium on lung culture (Table 2).

All *S. ornithocola* isolates had morphological and culture characteristics typical of those reported previously for this bacterium (Foster et al., 2005; Kirkwood et al., 2006). For each isolate, 16S rRNA sequence data was identical to that of the type strain (Genbank Accession Number AJ717394).

Birds were submitted from mortality incidents throughout Great Britain and *S. ornithocola* infection was found in England, Scotland and Wales (Fig. 1). Details of *S. ornithocola*-positive mortality incidents are presented in Table 1. Several individuals of multiple species were seen to be affected in 5/6 incidents. Mortality or morbidity of blue tits was reported in each incident. Where sick birds were observed, they exhibited non-specific signs of malaise, such as lethargy and fluffed-up plumage (Table 1). No evidence of contemporaneous morbidity or mortality was observed in other garden bird species at affected sites. All incidents occurred in the spring, between the end of January and the end of April, and all were observed around garden feeding stations.

A summary of the details of the confirmed cases of *S. ornithocola* infection is presented in Table 2. All cases were in fully-fledged birds that had hatched prior to the calendar year of death. Typically, poor facial plumage condition was observed with peri-oral matting of feathers and there usually was urate staining around the vent. In each case, the upper alimentary tract was empty and the gizzard contained only dark-stained grit, indicating that the bird had not recently fed. Pulmonary congestion was noted in four cases, whilst the respiratory tract appeared grossly normal in the remaining two birds. No other significant abnormalities were noted. Microscopic examinations of wet smear preparations of small-intestinal contents were performed in 4/6 cases and all were negative for metazoan or protozoan parasites.

Histopathological examination was performed on the lung from 4/6 birds from which *S. ornithocola* was cultured and from multiple tissues from two of these birds. A list of the tissues examined is presented in Table 2. Multiple foci of acute pulmonary necrosis associated with clusters of Gram-negative rods were present in three birds (Fig. 2). A mixture of Gram-positive and Gram-negative rods was present in some of the air spaces and surrounding tissue in the fourth bird (Incident 2), but there was no evidence of tissue necrosis. The lungs of two birds (Incidents 2 and 5) were markedly congested and a small number of parabronchi in these birds contained acellular eosinophilic material (oedema fluid). Large mononuclear cells with foamy cytoplasm (macrophages) and lymphocytes were present within, and lining, air spaces in three birds (Incidents 2, 5 and 6). It is possible that some of the foamy cells lining the air spaces were reactive type II pneumocytes, but no additional investigations were conducted to investigate this further. No abnormalities were detected in the other organs examined.

Of the 82 tits (Paridae) and six long-tailed tits examined post mortem between April 2005 and April 2009, infectious disease was determined to be the sole cause of death, or an important contributory factor to the cause of death, in 25 (28%) birds. *S. ornithocola* was considered to be the primary pathogen in six (7%) birds, avian pox in eight (9%), trichomonosis in five (6%) and miscellaneous pathogens in the remaining six birds. Of the non-infectious causes of death, seven (8%) tits died of predation, 27 (31%) of other trauma and two (2%) of other causes (gizzard impaction).

The cause of death was undetermined for 27 birds as the more autolysed the carcass, the less chance there was of reaching a diagnosis. The cause of death was undetermined for 4/15 (27%) cases in

[1] See: http://www.bto.org/gbw.

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