

Novel *Salmonella* Variant Associated with Mortality in Two Great Spotted Woodpeckers (*Dendrocopos major*)

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ABSTRACT: Two adult Great Spotted Woodpeckers (*Dendrocopos major*) from separate sites in Great Britain were examined postmortem in 2013 and 2016. A *Salmonella* sp. was isolated from multiple tissues in both birds. Histopathology and immunohistochemistry confirmed disseminated salmonellosis. Whole-genome sequencing and biochemical analyses putatively identified both isolates as a novel variant of *Salmonella enterica* subsp. *enterica* serovar Hessarek (*S.* Hessarek). Salmonellosis has seldom been reported in Piciformes, and never before in association with *S.* Hessarek infection. These findings, therefore, add to current knowledge regarding the range of wild bird species susceptible to this *Salmonella* serovar, and our understanding of the pathogens affecting Great Spotted Woodpeckers, in particular.

Key words: Disease, *Salmonella*, surveillance, woodpecker.

Salmonellosis, resulting from infection with avian host-adapted biotypes of *Salmonella enterica* subsp. *enterica* serovar Typhimurium, is a well-known cause of mortality in passeriform species in multiple continents (Hall and Saito 2008; Lawson et al. 2010). Conversely, *Salmonella enterica* subsp. *enterica* serovar Hessarek has been isolated from only a small number of wild Passeriformes. First identified in a Common Raven (*Corvus corax*, Corvidae) found dead in Hessarek, Iran (Neel et al. 1953), *S.* Hessarek has been isolated from infrequent disease outbreaks (range: 10 to >1,000 dead birds) in Song Thrushes (*Turdus philomelos*, Turdidae; Vellarde et al. 2012) and Blackbirds (*Turdus merula*) in Europe (MacDonald et al. 1968), and Starlings (*Sturnus vulgaris*, Sturnidae) in Europe (Magistrali et al. 2008) and the

Middle East (Singer et al. 1977; Dakman et al. 2017).

Unlike in Passeriformes, salmonellosis has rarely been reported in Piciformes. Hall and Saito (2008) describe four cases in the US, although details of the species affected were not provided. We present evidence of disseminated salmonellosis in two Great Spotted Woodpeckers (GSW) *Dendrocopos major*, Picidae, in Great Britain (GB), identifying the causative agent as a novel *S.* Hessarek variant.

Disease surveillance of garden birds was conducted across GB by obtaining reports of morbidity and mortality from the public and performing postmortem examinations. Of 4,340 wild birds examined from 1992 to 2017, the majority were Passeriformes (86%: $n=3,719$), principally Fringillidae (49%: $n=2,105$), Passeridae (8%: $n=348$), and Paridae (7%: $n=311$). Piciformes constituted only 0.7% ($n=33$) of this convenience sample and comprised GSW ($n=26$) and Green Woodpeckers *Picus viridis*, Picidae ($n=7$). Trauma was the most common cause of death in Piciformes (63%), with infectious disease diagnosed in only two GSW and one Green Woodpecker (Table 1).

Due to the paucity of knowledge regarding *Salmonella* sp. infection in Piciformes, we further investigated two cases of salmonellosis diagnosed in GSWs, characterizing the strain of the bacterium involved in each. The GSWs were found dead in separate domestic gardens (approximately 15 km apart) in Surrey, England, in December 2013 (GSW1) and June 2016 (GSW2). Systematic postmortem examinations were conducted following a

TABLE 1. Piciform species examined postmortem between 1992 and 2017 as part of a wildlife health surveillance project, with a summary of the cause of death assigned, and infectious disease agents isolated. Microbiological culture was performed on the liver in all cases, in combination with small intestinal contents, lung or spleen in most cases.

Species	Cause of death	No. birds	Sites	Pathogen	No. birds
Great spotted Woodpecker (<i>Dendrocopos major</i>)	Infectious disease	2	2	<i>Salmonella</i> Hessarek variant	2
	Trauma	18	16	<i>Staphylococcus aureus</i>	1
	Predation	2	2	<i>Pasteurella multocida</i>	1
	Undetermined	3	3	Negative	
	Euthanasia	1	1	Negative	
Green Woodpecker (<i>Picus viridis</i>)	Infectious disease	1	1	<i>Yersinia pseudotuberculosis</i>	1
	Trauma	3	3	Negative	
	Predation	3	3	Negative	

standardized protocol (Lawson et al. 2010). Bacteriologic culture of the liver, small intestinal contents, and macroscopic lesions was performed by streaking samples onto *Salmonella*-selective enrichment media, incubating aerobically at 37 C, and observing after 1, 2, and 5 d. Colonial and Gram's stain morphology, and slide agglutination using poly-O antisera (Pro-Lab Diagnostics, Neston, UK), were used to confirm the isolation of *Salmonella* spp. Biochemical analysis of *Salmonella* sp. isolates was undertaken using the 20E Enterobacteriaceae biochemical test (API-BioMerieux, Marcy l'Etoile, France). Whole genome sequence data were obtained from each *Salmonella* sp. isolate, from which the multilocus sequence types (MLST) and single nucleotide polymorphism (SNP) profiles were determined using the methods of Ashton et al. (2016). The MLST, SNP, and biochemical profiles were compared to a reference human isolate of *S. Hessarek* (Short read archive reference SRR7293657).

A suite of tissues from each GSW, comprising brain, heart, kidney, liver, lung, pectoral muscle, small intestine, spleen, and others with gross lesions (where present), was fixed in 10% neutral buffered formalin and embedded in paraffin before being sectioned, stained with H&E or Gram Twort, and subjected to routine histopathologic examination. Immunohistochemistry targeting the

Salmonella common structural antigen (CSA-1) was performed using the methods of Lawson et al. (2018a).

Both GSWs were adults in poor body condition, based on reduced pectoral muscle mass and absence of fat deposits. The GSW1 was male and the GSW2 was female. Macroscopic abnormalities comprised a full thickness skin wound with tendon exposure on the right fourth digit (GSW1), circa 15 yellow-colored skin nodules (1–3 mm diameter) on the ventrum and caudo-dorsum (GSW2), and pale discoloration of liver and lung tissue (GSW2). *Salmonella* sp. was isolated from the liver, lung, and small intestinal contents of both birds. The most significant histopathologic findings were acute to subacute bacterial dermatitis (GSW1 and GSW2) and necrotizing myositis (GSW1). Gram-negative rod-shaped bacteria were observed within the skin, muscle, and lung of GSW1 and GSW2, brain and meninges of GSW1, and liver, heart, spleen, and kidney of GSW2. They were also noted intravascularly in the heart of GSW1 and GSW2, and the liver, lung, brain, and kidney of GSW2. Bacteria with *Salmonella*-specific immunoreactivity were found in all tissues examined using immunohistochemistry, colocalizing *Salmonella* with inflammatory lesions in the skin and pectoral muscle of both GSW1 and GSW2, and identifying the presence of *Salmonella* bacteria, without detect-

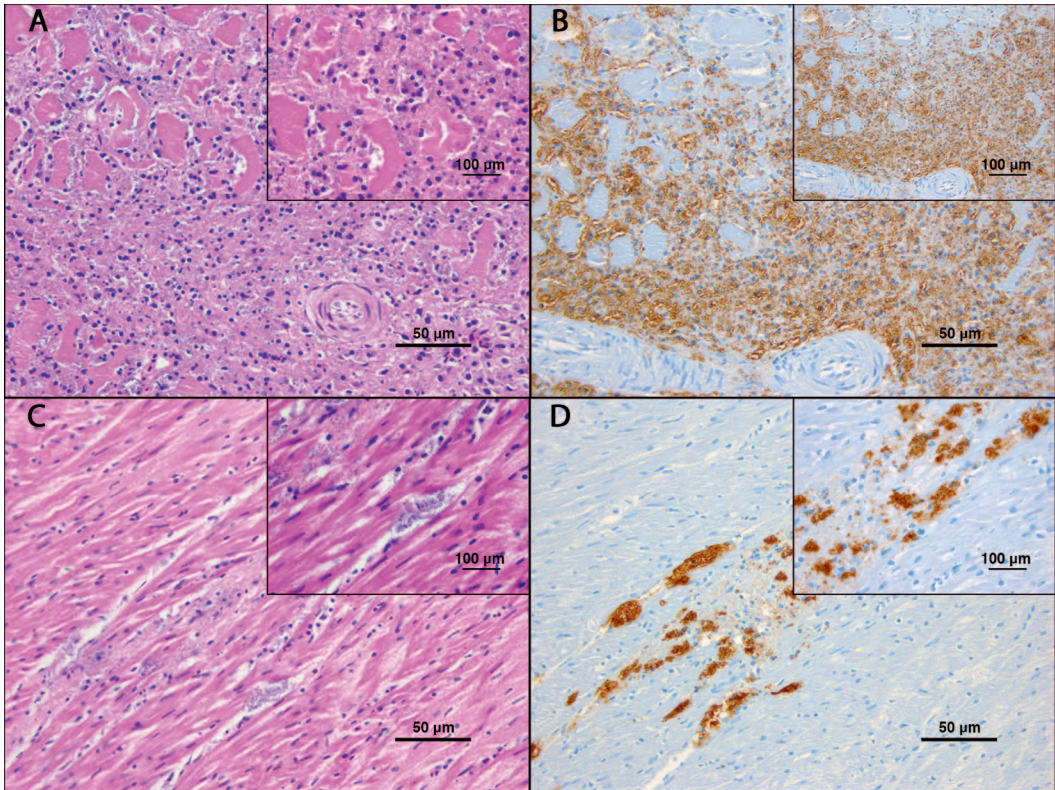


FIGURE 1. Histopathology and immunochemistry findings from the examination of two Great Spotted Woodpecker (*Dendrocopos major*) found dead in separate domestic gardens (approximately 15 km apart) in Surrey, England, in December 2013 (GSW1) and June 2016 (GSW2). (A) Necrotizing myositis with abundant intralesional bacteria in superficial pectoral muscle from GSW1. H&E. (B) Immunohistochemical reactivity for *Salmonella* common structural antigen (CSA-1) of bacteria in superficial pectoral muscle from GSW1. (C) Intravascular bacterial colonies in heart from GSW2. H&E. (D) Immunohistochemical reactivity for *Salmonella* CSA-1 in bacteria in heart tissue from woodpecker GSW2.

able inflammatory infiltrates, in the heart, gizzard, and meninges of GSW2 (Figure 1).

Biochemical analyses identified both GSW isolates as *Salmonella* sp. with the same biochemical profile, of which pronounced latent production of hydrogen disulfide was a notable feature. Serotyping demonstrated they shared the same antigenic profile (4,5,12:a:1,5), which differed from the reference *S. Hessarek* isolate (SRR7293657) (4,12,[27]:a:1,5), indicating that both GSW isolates were a 0:5 variant of *S. Hessarek*. The MLST was identical for both isolates and identified as sequence type 4896, a single locus variant of the MLST of *S. Hessarek*, sequence type 255 (SRR7293657). The SNP analyses revealed that, across the whole

genome, the isolates from GSW1 (SRR7293661) and GSW2 (SRR7293656) differed by only two SNPs, and from *S. Hessarek* (SRR7293657) by 2,690 SNPs. These results showed that both birds were infected with the same strain of *S. Hessarek* and represented the first identification of this novel *Salmonella* variant, and of *S. Hessarek*, in a member of the Piciformes.

Passeriform species have been proposed as a reservoir for *S. Hessarek* and bird-associated biotypes of *S. Typhimurium*, with occasional zoonotic spillover (Singer et al. 1977; Lawson et al. 2014). Although avian and human infection with *S. Hessarek* has been diagnosed in England, Scotland, and Wales, this serovar is uncommonly detected in GB (MacDonald

et al. 1968). Only five human isolates of *S. Hessarek* have been confirmed by Public Health England, 2004–17 inclusive, one of which was included in this study, and two of which were isolated from people with a history of international travel. This indicates that any public health risk from *S. Hessarek* is likely to be very low.

In previous avian disease incidents involving *S. Hessarek*, the most consistent features were multiple mortalities, hepatomegaly and/or splenomegaly, and detection of intravascular and intralesional bacteria where histopathology was performed (MacDonald et al. 1968; Singer et al. 1977; Velarde et al. 2012; Dakman et al. 2017). Migratory stress and high population densities have been proposed as predisposing factors for some of the events (Velarde et al. 2012; Dakman et al. 2017).

Although histopathologic findings were similar between GSW1, GSW2, and passerines that died from *S. Hessarek*-associated disease, in each of the GSW incidents there was an absence of organ enlargement and only single birds were found dead. The latter might have been the result of detection or reporting bias, or a function of species ecology because the GSW is predominantly nonmigratory and solitary.

Several contrasting features of *S. Hessarek*- and *S. Typhimurium*-associated mortality in wild birds are noteworthy. Passerine salmonellosis associated with *S. Typhimurium* infection in GB principally affects Fringillidae and Passeridae species, typically occurs in proximity to anthropogenic supplementary food sources, exhibits winter seasonality, and is characterized by granulomatous lesions in the esophagus, liver, spleen, and cecal tonsils (Lawson et al. 2018b). Greenfinch (*Chloris chloris*; Fringillidae) and House Sparrow (*Passer domesticus*; Passeridae) are the proposed reservoirs of passerine-associated biotypes of *S. Typhimurium* in GB and the species most-often documented with salmonellosis, perhaps due to their granivorous diet and flocking behavior that result in frequent opportunities for fecal-oral pathogen transmission at garden feeding stations (Lawson et al. 2018b).

In contrast, the reservoir or source of *S. Hessarek* infection in wild birds remains unknown. Mortalities have occurred in various habitats, across the year with no seasonality, and exhibited different macroscopic and histopathologic lesions. Life histories of the wild bird species documented with *S. Hessarek* infection differ from those most frequently affected by *S. Typhimurium*, and anthropogenically provisioned food has not been a common factor in recorded disease incidents.

The high similarity of the *S. Hessarek* isolates from GSW and their close geographic proximity are indicative of a single source. We considered fecal-oral transmission, typical of *Salmonella* sp. infection (Finlay 1994), as most likely, but histologic appraisal of the gastrointestinal tract was limited by autolysis in both birds. Because GSW are known to utilize garden feeding stations, these cannot be ruled out as sites for pathogen transmission. However, the GSW's predominantly insectivorous diet (Robinson 2018) reduces the probability that anthropogenically provisioned food was involved in the incidents we report. Although the skin lesions observed in both birds presented another possible route of infection, this could not be determined by histopathologic examination and is deemed unlikely given the pathogenesis of the majority of *Salmonella* sp. infections (Finlay 1994). Rather, we suspected that dermatitis and myositis were the result of hematogenous bacterial spread following initial infection. Ultimately, enhanced surveillance of GSW could aid understanding, and analysis of any further *S. Hessarek* isolates from this species (e.g., using in vivo expression technology, and whole-genome sequencing) would enable investigations into host-adaptation to the GSW (Uzzau et al. 2000).

Little is known about the disease conditions affecting Piciformes worldwide. Among those examined in GB, just one further case of infectious disease was diagnosed with yersiniosis as the cause of death (Table 1). Globally, few infectious causes of mortality have been reported in GSW; these include Usutu virus infection (Garigliany et al. 2014) in Belgium and toxoplasmosis (Jokelainen and

Vikøren 2014) in Norway. Our findings add to current understanding of the pathogens affecting GSW.

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