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Genomic analysis of *Salmonella enterica* serovar Typhimurium from wild passerines in England and Wales

Running title

Genome sequencing of passerine *Salmonella* Typhimurium

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Abstract

Passerine salmonellosis is a well-recognised disease of birds in the order Passeriformes, including common songbirds such as finches and sparrows, caused by infection with *Salmonella enterica* serovar Typhimurium. Previous research has suggested that some subtypes of *S.* Typhimurium – definitive phage types (DT) 40, 56 variant, and 160 – are host-adapted to passerines, and that these birds may represent a reservoir of infection for humans and other animals. Here, we have used whole genome sequences of 11 isolates from British passerines, five isolates of similar DTs from humans and a domestic cat, and previously published *S.* Typhimurium genomes including similar DTs from other hosts to investigate the phylogenetic relatedness of passerine salmonellae in comparison with other *S.* Typhimurium, and investigate possible genetic features of the distinct disease pathogenesis of *S.* Typhimurium in passerines. Our results demonstrate that the 11 passerine isolates and 13 other isolates, including those from non-passerine hosts, were genetically closely related, with a median pairwise single nucleotide polymorphism (SNP) difference of 130 SNPs. These 24 isolates did not carry antimicrobial resistance genetic determinants or the *S.* Typhimurium virulence plasmid. Although our study does not provide evidence of *Salmonella* transmission from passerines to other hosts, our results are consistent with the hypothesis that wild birds represent a potential reservoir of these *Salmonella* subtypes, and thus, sensible personal hygiene precautions should be taken when feeding or handling garden birds.

Importance

Passerine salmonellosis, caused by certain definitive phage types (DTs) of *Salmonella* Typhimurium, has been documented as a cause of wild passerine mortality since the 1950s in many countries, often in the vicinity of garden bird feeding stations. To gain

47 better insight into its epidemiology and host-pathogen interactions, we genome-sequenced a
48 collection of eleven isolates from wild passerine salmonellosis in England and Wales.
49 Phylogenetic analysis showed these passerine isolates to be closely related to each other and
50 to form a clade distinct from other strains of *S.* Typhimurium, which included a multidrug
51 resistant isolate from invasive non-typhoidal *Salmonella* disease which shares the same phage
52 type as several of the passerine isolates. Closely related to wild passerine isolates and within
53 the same clade were four *S.* Typhimurium isolates from humans as well as isolates from
54 horses, poultry, cattle, an unspecified wild bird, and a domestic cat and dog with similar DTs
55 and/or multi-locus sequence types. This suggests the potential for cross-species transmission
56 and the genome sequences provide a valuable resource to investigate passerine salmonellosis
57 further.

58 **Introduction**

59 Passerine salmonellosis is a well-described disease caused by *Salmonella enterica* subspecies
60 *enterica* serovar Typhimurium (*S*. Typhimurium) which has been reported in Europe, North
61 America, Asia and Australasia, with the earliest reports in the 1950s (2, 11-13, 16, 18, 33, 45,
62 50). Whilst the disease can occur year-round, passerine salmonellosis is highly seasonal in
63 many countries; incidents are typically observed during the cold winter months, frequently in
64 the vicinity of supplementary feeding stations for wild birds within domestic gardens (13,
65 33). Gregarious and granivorous species in the finch (Fringillidae) and sparrow (Passeridae)
66 families are primarily affected; in Great Britain, these include the greenfinch (*Chloris*
67 *chloris*) and house sparrow (*Passer domesticus*) (33, 45). Affected birds exhibit non-specific
68 signs of malaise, including lethargy and fluffed-up plumage, and therefore attract the
69 attention of members of the public. Macroscopic lesions most commonly include focal to
70 multifocal necrosis of the upper alimentary tract, liver and spleen, sometimes in combination
71 with hepatomegaly and splenomegaly (11, 16, 33).

72

73 Biotyping of passerine-derived *S*. Typhimurium isolates from Great Britain in recent decades
74 has confirmed the majority (≥90%) to be definitive phage types (DT) 40, 56 variant (56v) and
75 160 (33, 45): limited data indicate that DT56(v) isolates belong to multi-locus sequence type
76 (ST)568 and DT40 isolates to ST19 (21), which is one of the most common *S*. Typhimurium
77 sequence types (1). Pulsed-field gel electrophoresis has identified high levels of genetic
78 similarity amongst *S*. Typhimurium isolates from British passerines both within and between
79 *Salmonella* DTs (34). Whilst these *S*. Typhimurium DTs account for a small proportion of
80 *Salmonella* isolated from other species, infection has been found in livestock (17, 46),
81 humans (2, 14, 32, 44, 57) and companion animals (e.g., cat) (48), and therefore appear not

wholly restricted in their host range. Little is known regarding the mechanisms of disease pathogenesis and only limited characterisation of passerine-derived *S.* Typhimurium isolates has been performed using PCR virulotyping. This has demonstrated the absence of both the fimbriae-related associated virulence gene, *pefA*, and the SPI-1 *sopE* gene (20), the latter having been associated with enteritis and epidemics in human isolates. Based on epidemiological and microbiological investigations, wild passerines are proposed to be the primary source of infection with these *S.* Typhimurium DTs for humans, livestock and companion animals, through a range of potential exposure routes including direct contact with sick and dead wild birds, indirect contact with wild bird faeces in outdoor environments and activities related to garden bird feeding, and predation of diseased birds (17, 32, 48).

Whilst whole-genome sequencing (WGS) is increasingly being applied to human bacterial pathogens, and is offering profound insight into their biology (10, 27), few studies have utilised this approach for the study of bacterial infections in wildlife (5). Limited WGS data from passerine-derived *S.* Typhimurium isolates are available, and such information would offer considerable insight into the epidemiology and disease pathogenesis of these strains. Therefore, in this study, we used WGS to characterise eleven *S.* Typhimurium isolates from British passerines belonging to DT40 (four isolates), DT56(v) (five isolates), along with two isolates belonging to phage types DT81 and DT87(v). We include a further five DT40 and DT56(v) isolates from humans and a domestic cat, along with *S.* Typhimurium genomes from diverse geographical, temporal, and host backgrounds, to evaluate whether or not the salmonellae from passerines had a distinct phylogenetic signature, which has been suggested previously but not confirmed (32). We also determine the genetic content of the passerine isolates, including virulence factors and prophages, to identify if there are unique genetic features that may explain the distinct pathogenesis of the infection in passerines.

Materials and Methods

Isolate selection

A sample of eleven *S.* Typhimurium isolates derived from passerines with confirmed salmonellosis were selected for WGS from an available archive (Table 1). This culture collection was obtained through pathological investigations of wild birds found dead across Great Britain since the early 1990s that have been conducted at the Institute of Zoology (32, 33). Isolates were selected that had already been fully biotyped (serotype and phage type (3)) and for which pulsed-field gel electrophoresis (PFGE) groupings, using either the PulseNet Rapid *Escherichia coli* method with slight modifications (34), the PulseNet USA *Salmonella* method (32), or both, were available from previous studies. Selection focused on the two most common phage types known to cause passerine salmonellosis in Great Britain, *S.* Typhimurium DT40 and DT56(v). Two isolates of both these definitive phage types were selected from both 2001 and 2006, providing representation of a 5-year interval. Isolates were chosen from salmonellosis cases with a wide geographical distribution across England and Wales. In addition, to capture isolate diversity, three *S.* Typhimurium isolates derived from passerine salmonellosis cases with variant biotyping or PFGE grouping results were included in the study: these comprised a DT87(v) and DT81 isolate, and a DT56(v) isolate that had a distinct PFGE profile and was in a separate PFGE group, designated PFGE group 8 with the PulseNet *E. coli* protocol (34), and group 9 for the *Salmonella* protocol (32), and which did not cluster with the majority of DT56(v) isolates with either protocol. Isolates were selected from cases in the species most commonly affected by salmonellosis: greenfinch (n=6), house sparrow (n=4) and a single goldfinch (*Carduelis carduelis*), and with typical seasonality, December to February inclusive, for the disease. No DT160 isolates were available in the archive.

131

132 Five *S.* Typhimurium isolates submitted to and genome sequenced by Public Health England
133 (PHE) in 2014 that matched the passerine isolates (DT40 or DT56(v)/ST568), were also
134 included in the analysis. These comprised two DT56(v)/ST568 isolates from humans, one
135 DT56(v)/ST568 isolate from a domestic cat, one DT40/ST19 isolate from a human and one
136 DT40/ST568 isolate from a human (Table 1). To place the passerine, human and feline
137 isolates in phylogenetic context, additional *S.* Typhimurium genomes were included in the
138 analysis (Supplementary Table 1). These included seven genomes with their associated
139 plasmids: LT2 (40), SL1344 (29), DT104 (38), A130 (41), SO4698-09 (47), D23580 (26),
140 and DT2 (25) (hereafter called 'reference' genomes); the A130 (26) isolate is a DT56(v)
141 multiple drug resistant isolate from human non-typhoidal *Salmonella*-associated invasive
142 disease in Malawi. In addition, a 'context' collection of genomes was included, comprising
143 42 *S.* Typhimurium genomes from a broad temporal, host and geographical range described
144 in Okoro et al (41), and nine genomes from Petrovska et al (47), which were either ST568
145 (five genomes), or of the same definitive phage types as those associated with passerines
146 (DT40: two genomes, DT160: two genomes).

147

148 Antimicrobial susceptibility testing

149 The 11 passerine strains were raised from the -80^{o}C archive and grown at 37^{o}C on blood agar
150 plates with 5% horse blood (Oxoid, Basingstoke, UK) or in Luria-Bertani (LB) broth (Sigma-
151 Aldrich Company Ltd., Gillingham, UK). Antimicrobial susceptibility testing was performed
152 with Vitek 2 Compact using the Standard *Enterobacteriaceae* Card AST-N206 (bioMérieux,
153 Basingstoke, UK).

154

155 Whole genome sequencing

156 Genomic DNA was extracted from overnight cultures of the 11 passerine strains using the
157 MasterPure™ Complete DNA and RNA Purification Kit (Cambio Ltd, Cambridge, UK).
158 Illumina library preparation was carried out as described (49) and sequencing performed
159 using the HiSeq 2000 technology following the manufacturer's standard protocols (Illumina
160 Inc., Little Chesterford, UK), generating 100bp paired end reads. The five isolates from PHE
161 were sequenced as described in (4); short read data can be found at the PHE Pathogens
162 BioProject PRJNA248792 at NCBI.

163

164 Sequence analysis

165 Draft *de novo* assemblies of each isolate were constructed using Velvet (63), then scaffolded
166 using SSPACE (6) and GapFiller (7), as described in (43). For the passerine and PHE
167 genomes, *in silico* PCR virulotyping was performed for the virulence-associated genes
168 examined in Hughes et al. (20) and the non-redundant genes examined in Skyberg et al. (54),
169 along with a number of fimbriae-related genes (Supplementary Table 2), by searching for the
170 forward and reverse primer sequences in the draft assemblies; results were confirmed by
171 mapping sequence reads to the genes of interest using BWA-MEM (35). These results were
172 compared to those of the reference Typhimurium genomes. Prokka (53) was used to annotate
173 the draft genomes, and a pan-genome was constructed using Roary as described in (42), using
174 a blastp percentage identity threshold of 95%, distinguishing between core genes - defined as
175 found in at least 95% of isolates - and the accessory genome. The accession numbers of
176 annotated assemblies of the 11 passerine, four human and one feline isolates are listed in
177 Supplementary Table 3. A phylogenetic tree was reconstructed using the concatenated core
178 gene alignment, aligned with MAFFT (24) within Roary (42), using RAxML (55) with a

gamma correction for among site rate variation. To assess the presence or absence of the *S.* Typhimurium virulence plasmid in the passerine and PHE isolates, the reads were mapped against the LT2 chromosome and virulence plasmid (pSLT) using SMALT (61), and coverage over the plasmid was visually inspected.

The presence of acquired antimicrobial resistance (AMR) genes was assessed using the ResFinder-2.1 Server (http://cge.cbs.dtu.dk/services/ResFinder-2.1/) (62). The multi-locus sequence type (MLST) was extracted from the assemblies using the Centre for Genomic Epidemiology server, (www.cbs.dtu.dk/services/MLST) (31); MLST of the five PHE isolates were determined by a modified version of SRST (22). The draft *de novo* assemblies of the passerine, PHE and reference Typhimurium genomes were searched for prophage sequences, using the PHAST server (64).

Accession numbers

Accession numbers for the short reads of the 11 passerine isolates are ERS217356 – ERS217366. The accession numbers for the five isolates from Public Health England are SRR1968278, SRR1969075, SRR1967749, SRR1969317 and SRR1965151. These accessions, and those for the annotated assemblies for the passerine and PHE isolates, are found in Supplementary Table 3.

Results

Whole genome analysis and phylogeny

Comparative whole genome analysis of the 74 isolates included in this study showed that the core genome consisted of 3,890 genes, encompassing 11,724 variable polymorphic sites.

Based on these variable sites, we constructed a core gene phylogenetic tree (Figure 1) demonstrating that the ST568 isolates clustered together, whereas the ST19 isolates were found in multiple clades of the phylogenetic tree. Three of the four PHE human isolates as well as the feline isolate clustered with the 11 passerine isolates, hereafter called 'Clade A'; the human isolate (H142780372) from south east England in 2014 was phylogenetically closer to sample DT177, isolated from a human in the UK, and is in the same clade as the UK bovine SO4698-09 reference monophasic *S.* Typhimurium genome. Also clustering within Clade A were the other ST568s from the context genomes, along with two DT40/ST19 and one DT160/ST19 isolates (Supplementary Table 1), which included one human, one canine, one bovine, three equine, one chicken, and two other bird isolates, one of which is from a passerine and the other an unspecified wild bird (without further information). Between these 24 isolates of Clade A, there was a median pairwise distance of 130 SNPs (range 18 – 406) between isolates in the 3,890 genes included in the core gene alignment. Between isolates within Clade A and those outside Clade A, there was a median pairwise distance of 766 SNPs (range 306 – 1603) in the core genes.

In addition to the 3,890 core genes identified, there were 829 genes found in 15 - <95% of isolates, and 4,575 genes that were found in fewer than 15% of isolates. An analysis of Clade A identified that there were 1,306 genes that were uniquely found in a Clade A isolate, but the majority of these genes (1,303) were found in four or fewer of the 24 isolates. There were no genes that were both unique to Clade A and found in each of the 24 isolates, at the cut-offs examined.

In silico PCR typing, prophage identification, presence/absence of pSLT

225 Most of the various virulence and fimbriae-related genes, with some exceptions, were found
226 in the 23 passerine, PHE, and reference genome isolates. The genes found in all isolates were
227 *prgH*, *sopB*, *invA*, *spiC*, *sifA*, *misL*, *pipD*, *sitC*, *orfL*, *iroN*, *lpfC*, *msgA*, *orgA*, *pagC*, *sipB*,
228 *spaN* (all isolates with one change in the *spaN* primer sequences), *spiA* and *tolC*. No isolate
229 was found to carry *cdtB*. The exceptions, where genes were variably found in the isolates, are
230 listed in Table 2. The majority of genes were found with no changes in the primer sequences,
231 with a few exceptions ('costs') as marked in Table 2. The number of intact, incomplete, and
232 questionable prophages, as well as the identity of the intact prophages, are reported in
233 Supplementary Table 4. For all isolates in Clade A, there was no mapping coverage over the
234 entire virulence plasmid, pSLT, of the *S.* Typhimurium LT2 reference genome, indicating
235 that they do not carry the virulence plasmid commonly found in Typhimurium isolates and
236 present in 42 out of 50 non-Clade A isolates in this study.

237

238 Antimicrobial resistance

239 All 11 passerine isolates sequenced here were susceptible *in vitro* to all of the antimicrobials
240 tested; ampicillin, amoxicillin/clavulanic acid, amikacin, aztreonam, ceftazidime, cefalotin,
241 ciprofloxacin, cefotaxime, cefuroxime, cefuroxime axetil, ertapenem, cefepime, cefoxitin,
242 gentamicin, meropenem, tigecycline, tobramycin, trimethoprim and piperacillin/tazobactam.
243 Analysis of acquired resistance genes found that all possessed *aac(6')-Iaa* (NC_003197);
244 although able to confer resistance to certain aminoglycosides (37, 52), it has been shown to
245 be a cryptic resistance gene which is not expressed (37, 51). No SNPs in *gyrA*, *gyrB*, *parC* or
246 *parE,* known to confer resistance to quinolones, were identified in these isolates. Thus, the
247 phenotypic susceptibility profile of the isolates is in congruence with the absence of AMR

248 determinants in the genomes. No antimicrobial resistance determinants were found in the
249 other Clade A genomes.

250

251 **Discussion**

252 Salmonellosis is a well-known cause of mortality in some wild passerine species, and
253 represents a potential zoonotic reservoir. Specific DTs of *S.* Typhimurium are believed to be
254 host-adapted to garden birds, and their isolation from humans has been taken as indicative of
255 transmission from garden birds (32). WGS currently provides the highest resolution available
256 to investigate the relatedness and gene content of bacteria, and to our knowledge, this study
257 represents the first comparison of multiple genome sequences of *S.* Typhimurium from
258 passerines. We have also included four human and one feline isolates with the same phage
259 types as the passerine isolates, as well as 58 *S.* Typhimurium obtained from multiple different
260 host species, multiple countries, and over a 72-year period, to compare and contrast the
261 bacteria from the different host species to investigate further if wild birds are a plausible
262 reservoir of infection.

263

264 All of the 11 passerine isolates clustered together, with three of the four PHE human isolates,
265 the PHE feline isolate, and with six ST568, two DT40/ST19 and one DT160/ST19 context
266 isolates from previously published Typhimurium studies (Figure 1). The passerine isolates
267 included the two commonest DTs found in garden birds, DT56(v) and DT40, but also isolates
268 representing less common DTs. The DT81 passerine isolate clustered with DT56(v) isolates,
269 as did the DT56 and DT141 isolates from the context collection. The DT87(v) isolate
270 clustered with the passerine DT40 isolates. Sample PM1422/05, selected as it was DT56(v)
271 but had a variant PFGE grouping, clustered with the other DT56(v) isolates. There was no

evidence of clustering by passerine host species or by year of isolation. The feline isolate and three of the four human isolates from PHE also clustered with the passerine isolates, adjacent to those with the same DT. The one exception was sample H142780372 from a human, which was DT40/ST19, but genetically more similar to the *S.* Typhimurium reference genomes than to the other isolates with phage type DT40. One DT160/ST19 context isolate, a common DT found in passerines but isolated from a horse in the UK in 1998, clustered with the DT40/ST19 isolates in Clade A; the second DT160 isolate in the context collection, which was ST2866, was outside of Clade A. There was relatively low genetic variability in the core genomes of the isolates in Clade A, which included isolates over an 18-year period and from different hosts, with a median pairwise difference of 130 SNPs. In contrast, there were 784 SNPs different between the A130 and D23580 isolates, which are both ST313 from Malawi, and sampled seven years apart (26). Here, neither ST nor DT were predictive of inclusion in Clade A, as ST19, a common *S.* Typhimurium ST (1), was found in multiple clades of the tree, as were DT56(v), DT40 and DT160 (Figure 1). Even though non-ST19 isolates clustered more closely based on ST than by DT, the STs represented in this collection are all single-locus variants of ST19, and thus offer minimally informative data to distinguish isolates. Therefore, the core genome SNPs provided the greatest information about the relatedness of isolates.

Antimicrobial resistance in non-typhoidal *Salmonella* is common, and in some places it has been increasing in recent years (9). In a report examining antimicrobial sales and AMR in UK food-producing animals, the prevalence of *S.* Typhimurium resistant to at least one antimicrobial ranged between 65.6 – 88.6% in the years 2004 – 2013 (59). Whilst a growing body of research has found evidence of AMR in *Salmonella* sp. isolates derived from free-living wildlife including birds (8, 23), this study, as with others on *S.* Typhimurium derived

297 from British passerines (20, 32), found no phenotypic evidence of AMR. This was supported
298 by an absence of acquired resistance genes or known SNPs conferring resistance in the
299 passerine isolates. This was also true for the Clade A isolates from the context collection
300 from non-passerine hosts. Only limited incidents of AMR in salmonellae from passerines
301 have been reported previously all outside of the UK, involving Corvidae (36) and Thraupidae
302 (39) species, and a single isolate from a Fringillidae species with phenotypic resistance to
303 sulphamethoxazole (19). This is in contrast to the A130 isolate from a human in Malawi (26),
304 which although also DT56(v), is resistant to ampicillin, kanamycin, trimethoprim and
305 sulphonamides, and is phylogenetically distinct from the DT56(v) cluster in Clade A. This is
306 unsurprising, as all of the Clade A DT56(v) isolates in this study are ST568, whereas A130 is
307 ST313, part of the epidemic of multi-drug resistant *S.* Typhimurium ST313 that is a major
308 cause of invasive salmonellosis in humans in sub-Saharan Africa (26). Whilst four of the
309 passerine isolates and two of the context isolates were DT40/ST19, there was one human
310 isolate (H142780372) that was also DT40/ST19, but was not part of Clade A. These results
311 further highlight the advantage of utilising the higher resolution of WGS over PFGE and
312 phage typing in understanding the patterns of disease in *Salmonella*.

313

314 The results of the *in silico* PCR virulotyping were broadly similar to those observed by
315 Hughes et al. (20). None of the isolates in Clade A had either the SPI-1 *sopE* gene or the
316 virulence-plasmid located *pefA* and *spvB* genes, the latter two being expected as these isolates
317 did not carry pSLT. The DT40/ST19 human isolate H142780372, which was not in Clade A,
318 did contain a gene similar to *sopE*, which had 37 SNPs compared to the reference *sopE*
319 nucleotide sequence but 99% amino acid identity. All 11 passerine isolates contained *prgH*,
320 *sopB*, *invA*, *spiC*, *sifA*, *misL*, *pipD*, *sitC* and *orfL*, which are all found within *Salmonella*
321 Pathogenicity Islands, and also *iroN*, a siderophore. This is in agreement with the passerine-

322 derived *S.* Typhimurium examined previously (20). Also positive for these genes, but lacking
323 *sopE* and *pefA*, were the three human and one feline isolates in Clade A. The seven reference
324 Typhimurium isolates contained all examined genes from Hughes et al (20), with the
325 exception of *sopE*, which was found only in SL1344 and SO4698-09, and *pefA*, which was
326 not found in SO4698-09. For the non-redundant genes examined using the Skyberg et al.
327 primers (54), *lpfC*, *msgA*, *orgA*, *pagC*, *sipB*, *spaN*, *spiA* and *tolC* were found in all isolates,
328 whereas pSLT-associated *spvB* was only found in six of the reference Typhimurium
329 sequences (excluding SO4698-09), and *cdtB*, a cytolethal distending toxin found in *S.* Typhi,
330 was not found in any isolate. These results are in contrast to Krawiec et al. (28), who found a
331 more variable presence of virulence genes in the *Salmonella* isolates from wild birds they
332 examined.

333

334 The virulence plasmid, pSLT, was absent in all Clade A isolates, as well as the ST19 isolate
335 SARA3 and the seven isolates in the clade containing the monophasic Typhimurium
336 reference genome SO4698-09. An early estimate was that 88% of *S.* Typhimurium carry the
337 virulence plasmid (15), although there are notable exceptions where it is less common, such
338 as in the European monophasic Typhimurium epidemic strains (47). There was some
339 mapping over part of the plasmid for the isolate XT1456/06, which, when compared to the
340 reference genome SL1344, was identified as similar to colicin plasmid pCol1B9 (29). This
341 plasmid is associated with horizontal gene transfer via conjugation to *E. coli* during infection
342 in mice (56). At least part of the shufflon region encoding the variable pilus tip antigen in the
343 XT1456/06 plasmid was rearranged compared to the plasmid in SL1344, which is thought to
344 be related to sex pilus binding specificity (56).

345

346 The PHAST analysis (Supplementary Table 4) indicated that the 15 passerine and PHE Clade
347 A isolates had intact Gifsy-1 (similar to that in SO4698-09) and ST64B prophages, in
348 common with several of the reference genomes. However, long-read sequencing is necessary
349 to identify the exact composition and orientation of the prophages in these isolates. Whilst
350 there are no individual genes present uniquely in every Clade A isolate, it is also possible that
351 pseudogenes or SNPs may be related to adaptation to specific hosts or a systemic rather than
352 gastrointestinal infection lifestyle, as has been identified previously (26, 30, 60). The loss of
353 diverse metabolic pathways that allow persistence in the gastrointestinal tract of the chicken
354 during experimental infection is a feature common to the galliform-adapted serovar *S.*
355 Gallinarum (30), *S.* Typhimurium DT2 associated with feral pigeons (25) and *S.*
356 Typhimurium African ST313 isolates (26); this shared signature appears to be an early stage
357 in host adaption. In addition, passerine salmonellosis has a global distribution and the
358 comparison of WGS data of passerine-derived *S.* Typhimurium isolates from continental
359 Europe, Asia, Australasia and North America would be worthwhile to investigate the genetic
360 relationships between international isolates.

361

362 This analysis has demonstrated the genomic similarity of the 11 *S.* Typhimurium obtained
363 from passerines in this study. It has also identified that 13 other isolates, from humans,
364 companion animals (cat and dog), horses, cattle, chicken, a finch and another unspecified
365 wild bird and all from the UK, were also genetically related to the passerine isolates. What
366 this has shown is that, in addition to forming a separate phylogenetic cluster, the isolates
367 appear also to be defined by the lack of a virulence plasmid and antimicrobial resistance
368 determinants. Previously, it has been stated that wild bird populations could act as a reservoir
369 of human infections with some *S.* Typhimurium subtypes (32). Multiple studies have shown
370 infection in domestic cats with passerine-associated *S.* Typhimurium subtypes, with exposure

371 believed to occur when they predate diseased wild birds: indeed, the condition in cats is
372 colloquially known as "songbird fever" (58). The genomic analyses presented here are
373 consistent with wild birds acting as a potential reservoir of these particular *Salmonella*
374 subtypes, but the data do not represent true transmission events, as the passerine isolates were
375 obtained from 2001 – 2006, whereas only two of the remaining 13 Clade A isolates were
376 obtained during this period. This study provides the basis to pursue an active collection of
377 contemporaneous isolates from humans and passerines to identify more conclusively the
378 sources and sinks of these particular DTs. Whilst it is important from a public health
379 perspective to recognise that this reservoir exists, the risk should be kept in context: a
380 previous study (32) found that passerine-associated *S.* Typhimurium phage types (DTs 40,
381 56(v) and 160) accounted for only 1.6% of *S.* Typhimurium isolates and 0.2% of all
382 *Salmonella* isolates recovered from humans in England and Wales over the period 2000-
383 2010. Nevertheless, awareness of this potential health risk should be raised and the public
384 who feed garden birds encouraged to take sensible personal hygiene precautions when
385 handling or feeding wild birds. The genome sequences investigated here demonstrate the
386 relatedness between *Salmonella* strains infecting wild passerines, and some of those found in
387 other hosts including humans. Furthermore, they provide an important resource to investigate
388 further the epidemiology, disease pathogenesis and putative host-adaption of these
389 salmonellae.

390

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393 incidents and submitted dead birds for post-mortem examination. The pathological
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620 Figure legends

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622 Figure 1. Maximum-likelihood mid-point rooted phylogeny based on 3,890 core genes of
623 *Salmonella* Typhimurium from passerines and other host species, with *S.* Typhimurium
624 reference and context genomes; black blocks represent data not known. Scale bar represents
625 the number of substitutions per site in the core gene alignment.

626 Table 1. Identity and source of new *Salmonella* Typhimurium genomes investigated in this study.

Strain name	Region	Host species	Sample type	Date of isolation	DT	PFGE *E. coli* protocol	PFGE *Salmonella* protocol	MLST	Reference for information/ genomes
PM1402/06	Cheshire, UK	Greenfinch	Post mortem liver	Nov-06	40	6	1	19	(34); this study
XT1456/06	Gwent, UK	Goldfinch	Post mortem liver	Dec-06	81	5		568	(34); this study
PM108/01	Powys, UK	Greenfinch	Post mortem spleen	Feb-01	56v	5	5	568	(34); this study
PM1422/05	Glamorgan, UK	Greenfinch	Post mortem liver	Dec-05	56v	8	9	568	(34); this study
PM65/01	Lancashire, UK	House sparrow	Post mortem kidney	Jan-01	40	6	1	19	(34); this study
PM132/06	Leicestershire, UK	Greenfinch	Post mortem liver	Feb-06	56v	5	5	568	(34); this study
XT062/01	Cheshire, UK	Greenfinch	Post mortem liver	Jan-01	87v	5		19	(34); this study
PM1377/06	Kent, UK	House sparrow	Post mortem small intestine	Nov-06	56v	5	5	568	(34); this study
PM100/01	Shropshire, UK	Greenfinch	Post mortem spleen	Feb-01	40	6	1	19	(34); this study
PM54/01	Nottinghamshire, UK	House sparrow	Post mortem crop	Jan-01	56v	5	5	568	(34); this study
PM1356/06	Devon, UK	House sparrow	Post mortem liver	Nov-06	40	6	1	19	(34); this study
H144540642	West Midlands, UK	Human	Faeces	05/11/2014	56v			568	Public Health England
H143320447	West Midlands, UK	Human	Faeces	12/08/2014	56v			568	Public Health England
H143540876	Sussex, Surrey and Kent, UK	Domestic cat		27/08/2014	56v			568	Public Health England
H142780372	Sussex, Surrey and Kent, UK	Human	Faeces	04/07/2014	40			19	Public Health England
H143120429	West Midlands, UK	Human	Faeces	29/07/2014	40			568	Public Health England

627 Table 2. Results showing differences between the passerine and PHE isolates in Clade A and
628 the reference *S.* Typhimurium genomes of the *in silico* PCR virulotyping analysis and
629 confirmatory mapping for the Hughes et al (20) and Skyberg et al (54) primers and the
630 fimbriae-associated primers; 'cost' refers to a mismatch in the primer sites.

Isolate	***sopE***	***pefA***	***fimA***	***msgA***	***spvB***
PM1402/06	0	0	1	1	0
XT1456/06	0	0	1	1	0
PM108/01	0	0	1	1	0
PM1422/05	0	0	1	1	0
PM65/01	0	0	1	1	0
PM132/06	0	0	1	1	0
XT062/01	0	0	1	1	0
PM1377/06	0	0	1	1	0
PM100/01	0	0	1	1	0
PM54/01	0	0	1	1	0
PM1356/06	0	0	1	1	0
H142780372	1*	0	1	1	0
H143120429	0	0	1	1	0
H143320447	0	0	1	1	0
H143540876	0	0	1	1	0
H144540642	0	0	1	1	0
SO4698-09	1	0	1	1	0
A130	0	1	1	1	1
DT104	0	1	1	1	1
SL1344	1	1	1	1	1
D23580	0	1	1^	1	1
DT2	0	1	1	1^	1
LT2	0	1	1	1	1

631 * cost of 2

632 ^ cost of 1