1 **Genomic analysis of *Salmonella enterica* serovar Typhimurium from wild passerines in**

2 **England and Wales**

3

4 **Running title**

5 Genome sequencing of passerine *Salmonella* Typhimurium

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23 **Abstract**

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

42 **Importance**

43 Passerine salmonellosis, caused by certain definitive phage types (DTs) of *Salmonella*
44 Typhimurium, has been documented as a cause of wild passerine mortality since the
45 1950s in many countries, often in the vicinity of garden bird feeding stations**.** To gain
46 better insight into its epidemiology and host-pathogen interactions, we genome-sequenced a

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

56 **Introduction**

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

70

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

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

90

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

105 **Materials and Methods**

106 Isolate selection

107 A sample of eleven *S.* Typhimurium isolates derived from passerines with confirmed
108 salmonellosis were selected for WGS from an available archive (Table 1). This culture
109 collection was obtained through pathological investigations of wild birds found dead across
110 Great Britain since the early 1990s that have been conducted at the Institute of Zoology (32,
111 33). Isolates were selected that had already been fully biotyped (serotype and phage type (3))
112 and for which pulsed-field gel electrophoresis (PFGE) groupings, using either the PulseNet
113 Rapid *Escherichia coli* method with slight modifications (34), the PulseNet USA *Salmonella*
114 method (32), or both, were available from previous studies. Selection focused on the two most
115 common phage types known to cause passerine salmonellosis in Great Britain, *S*. Typhimurium
116 DT40 and DT56(v). Two isolates of both these definitive phage types were selected from both
117 2001 and 2006, providing representation of a 5-year interval. Isolates were chosen from
118 salmonellosis cases with a wide geographical distribution across England and Wales. In
119 addition, to capture isolate diversity, three *S.* Typhimurium isolates derived from passerine
120 salmonellosis cases with variant biotyping or PFGE grouping results were included in the
121 study: these comprised a DT87(v) and DT81 isolate, and a DT56(v) isolate that had a distinct
122 PFGE profile and was in a separate PFGE group, designated PFGE group 8 with the PulseNet
123 *E. coli* protocol (34), and group 9 for the *Salmonella* protocol (32), and which did not cluster
124 with the majority of DT56(v) isolates with either protocol. Isolates were selected from cases in
125 the species most commonly affected by salmonellosis: greenfinch (*n*=6), house sparrow (*n*=4)
126 and a single goldfinch (*Carduelis carduelis*), and with typical seasonality, December to
127 February inclusive, for the disease. No DT160 isolates were available in the archive.

128

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

144

145 Antimicrobial susceptibility testing

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

151

152 Whole genome sequencing

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

160

161 Sequence analysis

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

177 the passerine and PHE isolates, the reads were mapped against the LT2 chromosome and
178 virulence plasmid (pSLT) using SMALT (61), and coverage over the plasmid was visually
179 inspected.

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

187

188 Accession numbers

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

194

195 **Results**

196 Whole genome analysis and phylogeny

197 Comparative whole genome analysis of the 74 isolates included in this study showed that the
198 core genome consisted of 3,890 genes, encompassing 11,724 variable polymorphic sites. Based
199 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

Most of the various virulence and fimbriae-related genes, with some exceptions, were found in the 23 passerine, PHE, and reference genome isolates. The genes found in all isolates were

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

234

235 Antimicrobial resistance

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

247

248 **Discussion**

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

260

261 All of the 11 passerine isolates clustered together, with three of the four PHE human isolates,
262 the PHE feline isolate, and with six ST568, two DT40/ST19 and one DT160/ST19 context
263 isolates from previously published Typhimurium studies (Figure 1). The passerine isolates
264 included the two commonest DTs found in garden birds, DT56(v) and DT40, but also isolates
265 representing less common DTs. The DT81 passerine isolate clustered with DT56(v) isolates,
266 as did the DT56 and DT141 isolates from the context collection. The DT87(v) isolate clustered
267 with the passerine DT40 isolates. Sample PM1422/05, selected as it was DT56(v) but had a
268 variant PFGE grouping, clustered with the other DT56(v) isolates. There was no evidence of
269 clustering by passerine host species or by year of isolation. The feline isolate and three of the
270 four human isolates from PHE also clustered with the passerine isolates, adjacent to those with
271 the same DT. The one exception was sample H142780372 from a human, which was

272 DT40/ST19, but genetically more similar to the *S*. Typhimurium reference genomes than to the
273 other isolates with phage type DT40. One DT160/ST19 context isolate, a common DT found
274 in passerines but isolated from a horse in the UK in 1998, clustered with the DT40/ST19
275 isolates in Clade A; the second DT160 isolate in the context collection, which was ST2866,
276 was outside of Clade A. There was relatively low genetic variability in the core genomes of the
277 isolates in Clade A, which included isolates over an 18-year period and from different hosts,
278 with a median pairwise difference of 130 SNPs. In contrast, there were 784 SNPs different
279 between the A130 and D23580 isolates, which are both ST313 from Malawi, and sampled
280 seven years apart (26). Here, neither ST nor DT were predictive of inclusion in Clade A, as
281 ST19, a common *S*. Typhimurium ST (1), was found in multiple clades of the tree, as were
282 DT56(v), DT40 and DT160 (Figure 1). Even though non-ST19 isolates clustered more closely
283 based on ST than by DT, the STs represented in this collection are all single-locus variants of
284 ST19, and thus offer minimally informative data to distinguish isolates. Therefore, the core
285 genome SNPs provided the greatest information about the relatedness of isolates.

286

287 Antimicrobial resistance in non-typhoidal *Salmonella* is common, and in some places it has
288 been increasing in recent years (9). In a report examining antimicrobial sales and AMR in UK
289 food-producing animals, the prevalence of *S.* Typhimurium resistant to at least one
290 antimicrobial ranged between 65.6 – 88.6% in the years 2004 – 2013 (59). Whilst a growing
291 body of research has found evidence of AMR in *Salmonella* sp. isolates derived from free-
292 living wildlife including birds (8, 23), this study, as with others on *S*. Typhimurium derived
293 from British passerines (20, 32), found no phenotypic evidence of AMR. This was supported
294 by an absence of acquired resistance genes or known SNPs conferring resistance in the
295 passerine isolates. This was also true for the Clade A isolates from the context collection from
296 non-passerine hosts. Only limited incidents of AMR in salmonellae from passerines have been

297 reported previously all outside of the UK, involving Corvidae (36) and Thraupidae (39) species,
298 and a single isolate from a Fringillidae species with phenotypic resistance to
299 sulphamethoxazole (19). This is in contrast to the A130 isolate from a human in Malawi (26),
300 which although also DT56(v), is resistant to ampicillin, kanamycin, trimethoprim and
301 sulphonamides, and is phylogenetically distinct from the DT56(v) cluster in Clade A. This is
302 unsurprising, as all of the Clade A DT56(v) isolates in this study are ST568, whereas A130 is
303 ST313, part of the epidemic of multi-drug resistant *S.* Typhimurium ST313 that is a major
304 cause of invasive salmonellosis in humans in sub-Saharan Africa (26). Whilst four of the
305 passerine isolates and two of the context isolates were DT40/ST19, there was one human isolate
306 (H142780372) that was also DT40/ST19, but was not part of Clade A. These results further
307 highlight the advantage of utilising the higher resolution of WGS over PFGE and phage typing
308 in understanding the patterns of disease in *Salmonella*.

309

310 The results of the *in silico* PCR virulotyping were broadly similar to those observed by Hughes
311 et al. (20). None of the isolates in Clade A had either the SPI-1 *sopE* gene or the virulence-
312 plasmid located *pefA* and *spvB* genes, the latter two being expected as these isolates did not
313 carry pSLT. The DT40/ST19 human isolate H142780372, which was not in Clade A, did
314 contain a gene similar to *sopE*, which had 37 SNPs compared to the reference *sopE* nucleotide
315 sequence but 99% amino acid identity. All 11 passerine isolates contained *prgH*, *sopB*, *invA*,
316 *spiC*, *sifA*, *misL*, *pipD*, *sitC* and *orfL*, which are all found within *Salmonella* Pathogenicity
317 Islands, and also *iroN*, a siderophore. This is in agreement with the passerine-derived *S.*
318 Typhimurium examined previously (20). Also positive for these genes, but lacking *sopE* and
319 *pefA*, were the three human and one feline isolates in Clade A. The seven reference
320 Typhimurium isolates contained all examined genes from Hughes et al (20), with the exception
321 of *sopE*, which was found only in SL1344 and SO4698-09, and *pefA*, which was not found in

322 SO4698-09. For the non-redundant genes examined using the Skyberg et al. primers (54), *lpfC*,
323 *msgA*, *orgA*, *pagC*, *sipB*, *spaN*, *spiA* and *tolC* were found in all isolates, whereas pSLT-
324 associated *spvB* was only found in six of the reference Typhimurium sequences (excluding
325 SO4698-09), and *cdtB*, a cytolethal distending toxin found in *S.* Typhi, was not found in any
326 isolate. These results are in contrast to Krawiec et al. (28), who found a more variable presence
327 of virulence genes in the *Salmonella* isolates from wild birds they examined.

328

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

340

341 The PHAST analysis (Supplementary Table 4) indicated that the 15 passerine and PHE Clade
342 A isolates had intact Gifsy-1 (similar to that in SO4698-09) and ST64B prophages, in common
343 with several of the reference genomes. However, long-read sequencing is necessary to identify
344 the exact composition and orientation of the prophages in these isolates. Whilst there are no
345 individual genes present uniquely in every Clade A isolate, it is also possible that pseudogenes

346 or SNPs may be related to adaptation to specific hosts or a systemic rather than gastrointestinal
347 infection lifestyle, as has been identified previously (26, 30, 60). The loss of diverse metabolic
348 pathways that allow persistence in the gastrointestinal tract of the chicken during experimental
349 infection is a feature common to the galliform-adapted serovar *S*. Gallinarum (30), *S*.
350 Typhimurium DT2 associated with feral pigeons (25) and *S*. Typhimurium African ST313
351 isolates (26); this shared signature appears to be an early stage in host adaption. In addition,
352 passerine salmonellosis has a global distribution and the comparison of WGS data of passerine-
353 derived *S*. Typhimurium isolates from continental Europe, Asia, Australasia and North
354 America would be worthwhile to investigate the genetic relationships between international
355 isolates.

356

357 This analysis has demonstrated the genomic similarity of the 11 *S*. Typhimurium obtained from
358 passerines in this study. It has also identified that 13 other isolates, from humans, companion
359 animals (cat and dog), horses, cattle, chicken, a finch and another unspecified wild bird and all
360 from the UK, were also genetically related to the passerine isolates. What this has shown is
361 that, in addition to forming a separate phylogenetic cluster, the isolates appear also to be
362 defined by the lack of a virulence plasmid and antimicrobial resistance determinants.
363 Previously, it has been stated that wild bird populations could act as a reservoir of human
364 infections with some *S*. Typhimurium subtypes (32). Multiple studies have shown infection in
365 domestic cats with passerine-associated *S*. Typhimurium subtypes, with exposure believed to
366 occur when they predate diseased wild birds: indeed, the condition in cats is colloquially known
367 as "songbird fever" (58). The genomic analyses presented here are consistent with wild birds
368 acting as a potential reservoir of these particular *Salmonella* subtypes, but the data do not
369 represent true transmission events, as the passerine isolates were obtained from 2001 – 2006,
370 whereas only two of the remaining 13 Clade A isolates were obtained during this period. This

371 study provides the basis to pursue an active collection of contemporaneous isolates from
372 humans and passerines to identify more conclusively the sources and sinks of these particular
373 DTs. Whilst it is important from a public health perspective to recognise that this reservoir
374 exists, the risk should be kept in context: a previous study (32) found that passerine-associated
375 *S*. Typhimurium phage types (DTs 40, 56(v) and 160) accounted for only 1.6% of *S*.
376 Typhimurium isolates and 0.2% of all *Salmonella* isolates recovered from humans in England
377 and Wales over the period 2000-2010. Nevertheless, awareness of this potential health risk
378 should be raised and the public who feed garden birds encouraged to take sensible personal
379 hygiene precautions when handling or feeding wild birds. The genome sequences investigated
380 here demonstrate the relatedness between *Salmonella* strains infecting wild passerines, and
381 some of those found in other hosts including humans. Furthermore, they provide an important
382 resource to investigate further the epidemiology, disease pathogenesis and putative host-
383 adaption of these salmonellae.

384

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612 Figure legends

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

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

Table 2. Results showing differences between the passerine and PHE isolates in Clade A and the reference *S.* Typhimurium genomes of the *in silico* PCR virulotyping analysis and confirmatory mapping for the Hughes et al (20) and Skyberg et al (54) primers and the 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

* cost of 2

^ cost of 1