1 **Supplementary feeding stations for conservation of vultures could be an important**

2 **source of monophasic *Salmonella* Typhimurium 1,4,[5],12:i:-**

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22 **ABSTRACT**

23 Vultures are nature's most successful scavengers, feeding on the carcasses of dead animals

24 present in the field. Availability of domestic carrion has been unstable due to rapidly

25 changing agro-grazing economies and increasing sanitary regulations that may require burial

26 or burning of livestock carcasses. Thus, several griffon vulture (*Gyps fulvus*) recoveries are

27 based on European legislation that guarantees the animals' welfare, avoids intense

28 persecution of the vultures and allows the feeding of threatened wildlife in supplementary

29 feeding stations (SFS). However, in recent years, many studies have speculated on the

30 likelihood that avian scavengers may be infected by feeding on pig carcasses at SFS from

31 intensive livestock. In this context, the present study evaluated whether free-living griffon

32 vultures and pig farms share zoonotic *Salmonella* strains to test the hypothesis that vulture

33 are infected during consumption of carcasses provided at SFS. Here, the occurrence,

34 serotypes and genomic DNA fingerprinting (phage typing and pulsed-field gel

35 electrophoresis) of isolated strains were carried out in griffon vultures and pig farms

36 authorised to provided carcasses at SFS in Castellón province (eastern Spain). The

37 bacteriological analyses revealed that 21.1% of vultures and 14.5% for pig farms samples

38 tested were *Salmonella*-positive. Monophasic *S.* Typhimurium 1,4,[5],12:i:- was the most

39 frequently isolated serovar. Comparison of *Salmonella* strains isolated from vultures and pig

40 farms revealed that monophasic *S.* Typhimurium 1,4,[5],12:i:-, *S.* Derby and *S.* Rissen strains

41 were highly genetically homogeneous (similar DNA fingerprint). In conclusion, the current

42 study indicates that free-living griffon vultures and pig farms that provide the carcasses at

43 SFS share several zoonotic *Salmonella* strains. On this basis, and although transmission could

44 be bidirectional, our result seems to corroborate the pig carcasses-to-vulture transmission and

45 cross-infection at SFS. As an immediate *Salmonella* control strategy in wild avian

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46 scavengers, we suggest the implementation of a programme to guarantee that solely pig

47 carcasses from *Salmonella*-free farms arrive at SFS.

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50 **Keywords:** Avian scavengers; intensive farming; pig; environment; *Salmonella* Derby;

51 *Salmonella* Rissen.

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54 **1. Introduction**

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56 Wild birds have repeatedly been highlighted as a source in the dissemination of *Salmonella*

57 spp. (Tizard, 2004; Hilbert et al., 2012; Krawiec et al., 2015) and links have been documented

58 between *Salmonella* contamination of modern pig production and wild birds (Andrés et al.,

59 2013; Andrés-Barranco et al., 2014). Furthermore, the number of wildlife species acting as

60 reservoirs, amplifiers and disseminators is unknown (Molina-López et al., 2011). Hence, the

61 role of wildlife as a *Salmonella* reservoir is of increasing interest (Hilbert et al., 2012).

62 *Salmonella* stands out as one of the most most common causes of human bacterial food

63 poisoning (EFSA, 2017).

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65   During the past century, the availability of domestic carrion has been unstable due to rapidly

66 changing agro-grazing economies and increasing sanitary regulations that may require burial

67 or burning of livestock carcasses. The conservation and reintroduction of avian scavengers

68 would therefore not have been possible without European Regional legislation to ensure their

69　welfare and avoid their intense persecution, as well as the European ruling that permitted the

70　feeding of threatened wildlife in SFS (Camiña-Cardenal et al., 2004; Margalida et al., 2011;

71　Cortés-Avizanda et al., 2016). In the late 1960s, conservationists created "vulture

72　restaurants" or SFS as a way to increase the availability of food resources (Bijleveld, 1974;

73　Gilbert et al., 2007; Donázar et al., 2009; Fielding et al., 2014). At community level, SFS has

74　been widely accepted as an effective management tool among conservationists and managers

75　(Cortés-Avizanda et al., 2016). Encouraging fallen stock to be left in situ is ecologically

76　harmonious, inexpensive and an efficient management method for the conservation of

77　scavengers (Donázar et al., 2009).

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79　*Salmonella* has been isolated in vultures in several studies, but an especially remarkable

80　finding is the unexpected abundance of *Salmonella* ser. Typhimurium, one of the most

81　common *Salmonella* serovars in foodborne illness outbreaks related with pork consumption

82　(Millán et al., 2004; Molina-López et al., 2011:2015; Marin et al., 2014; Jurado-Tarifa et al.,

83　2016; Blanco, 2018). Besides, to date it remains unknown whether *Salmonella* can cause

84　clinical illness in avian scavengers (Blanco, 2018), which could have potential implications

85　for conservation. Notably, *S.* Typhimurium, including monophasic variants (1,4,[5],12:i- and

86　1,4,12:i-), represented 21.8% of all reported serovars of confirmed human cases in 2016 in

87　the EU (EFSA, 2017). In particular, *S.* Typhimurium accounted for 63.6% of the isolates

88　reported in pig samples (EFSA, 2017). After the prion crisis, pig carcasses have been the

89　scavengers' main foodstuff provided at SFS (Blanco et al., 2016; Green et al., 2016; Blanco,

90　2018). Thus, a recent study carried out by Blanco (2018) in Segovia province (central Spain)

91　supports the role of pig carcasses as a primary source of *Salmonella*, and the risk of scavenger

92　infection in SFS, based on the concordance of serotypes and resistance patterns in an obligate

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93   scavenger partially relying on pig carcasses. Our driving hypothesis was that pig farms could

94   be one source of vulture transmission and a cross-infection route of *Salmonella* at SFS. In

95   this context, the present study evaluated whether free-living griffon vultures and pig farms

96   share zoonotic *Salmonella* strains to test the hypothesis that vultures could be infected during

97   consumption of pig carcasses provided at SFS.

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100   **2. Material and methods**

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102   **2.1. Animals**

103   This study was conducted within the conservation project for endangered species in the

104   Valencia Region. The study population is located at the Cinctorres observatory (Castellón

105   province, eastern Spain). In 2008, 236 breeding pairs were found in this area (93% of the

106   breeding pairs in the Community of Valencia) (GVA, 2008). All the experimental procedures

107   used in this study were performed in accordance with Directive 2010/63/EU EEC on animal

108   experiments. The Department of Infrastructure, Planning and Environment of the Valencian

109   Regional Government (Generalitat Valenciana) granted the ethical and animal welfare

110   permission to take samples.

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112   **2.2. Sample collection**

113   The vultures were live-captured in two sessions in September and in October 2016, during

114   the observatory's normal ringing schedule as part of the reserve's monitoring programme. A

115   total of 104 free-living griffon vultures were captured using a remotely activated purpose-

116   built cage (for more details, see Marin et al.*,* 2014). The age of the animals was determined

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117   according to the plumage characteristics and the colour of the bill and eye, classified as

118   juvenile (less than 2 years), sub-adult (from 2 to 5 years) and adult (more than 5 years). Base

119   on our previous results where there were no age-related differences in relation to the presence

120   of *Salmonella* (Marin et al., 2014), data from all individuals was pooled. From each vulture,

121   one cloacal sample was obtained using sterile cotton swabs (Cary Blair sterile transport

122   swabs, Deltalab, Barcelona, Spain). The cotton swab was inserted 1 to 2 cm into the cloaca

123   to collect a suitable sample. At each sampling day, samples of the facilities in close contact

124   with pig carcasses during supplementary feeding (warehouse of cadavers where farmers

125   legally dispose of dead animals, trucks that transport the carcasses from the warehouse to the

126   SFS and pig carcasses at SFS) were collected. A total of 20 sterile cotton swabs were taken

127   from 20 pig carcasses deposited in the SFS (10 samples per day). In addition, before the

128   animals were loaded and delivered to the SFS point, 30 sterile cotton swabs samples were

129   taken directly from the surface of the 2 trucks (floor and wall) that transport the carcasses

130   from the warehouse of cadavers to the SFS (15 samples per truck and day). Moreover, 20

131   sterile cotton swabs were taken from the warehouse of cadavers (container walls) where

132   farmers disposed of dead livestock (2 samples per container and day). Finally, during the

133   week after the intensive sampling described above, a total of 11 pig farms that provide the

134   carcasses were sampled to determine the potential transfer of *Salmonella* isolates from pig

135   carcasses to vultures. Five pens (four in the corners and one in the middle of the barn) were

136   chosen in each farm. Briefly, 500 gr of faeces were collected in pools from different points

137   of the pens in sterile containers and transported under refrigeration to the laboratory. All

138   samples were analysed within 24 h of collection. The experimental design of this study is

139   shown in Fig. 1.

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2.3. *Salmonella* spp. isolation and identification

The procedure was based on the official method ISO 6579: 2002 recommendations (Annex D). Cotton swab samples were pre-enriched in 1:10 vol/vol Buffered Peptone Water 2.5% (BPW, Scharlau, Barcelona, Spain). Faeces samples were homogenised and 25 gr were transferred into 225 mL of BPW. All BPW enrichments were incubated at 37 ± 1 ºC for 18 ± 2 h. Next, x ul of these enrichments were inoculated onto Modified Semi-Solid Rappaport Vassiliadis agar plates (MSRV, Difco, Valencia, Spain), which were incubated at 41.5 ± 1ºC for 24–48 h. Suspicious growths on MSRV plates were selected for inoculation onto Xylose–Lysine–Deoxycholate (XLD, Liofilchem, Valencia, Spain) and ASAP (ASAP, bioMerieux, Madrid, Spain) agar plates and incubated at 37 ± 1ºC for 24–48 h. After the incubation period, 5 presumptive *Salmonella* colonies were selected and streaked onto nutrient agar plates (Scharlab, Barcelona, Spain) 37 ± 1ºC for 24 ± 3 h. *Salmonella* isolates were serotyped according to the Kauffman-White-Le Minor scheme (Grimont and Weill, 2007) and was carried out at the Laboratori Agroalimentari (Cabrils, Spain) of the Departament d'Agricultura, Ramaderia, Pesca i Alimentació.

2.4. Molecular typing of *Salmonella* strain isolates

Enterobacterial repetitive intergenic consensus (ERIC)-PCR of all *Salmonella* isolates was performed and representative isolates from the different ERIC-PCR patterns and different origin were further analysed by pulsed-field gel electrophoresis (PFGE). ERIC-PCR was performed as previously described, except that a 50ºC annealing temperature was used (Antilles et al.*,* 2015). Primer pairs used were ERIC-F (5'-AAG TAA GTG ACT GGG GTG AGC G-3') and ERIC-R (5'-ATG TAA GCT CCT GGG GAT TCA C-3') (Versalovic *et al.,* 1991). PFGE typing was performed according to the standard operating procedure of

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165   PulseNet (www.pulsenetinternational.org). Genomic DNA was digested with the restriction

166   enzyme XbaI (Roche Applied Science, Indianapolis, IN) and the restriction fragments were

167   separated by electrophoresis in a CHEF-DR III System (Bio-Rad, Hercules, CA, USA).

168   Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA) was used to compare the

169   PFGE patterns by cluster analysis using Dice coefficient and unweighted pair group method

170   with arithmetic averages (UPGMA dendrogram type).

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172   **2.5. Statistical analysis**

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174   We tested whether occurrence of *Salmonella spp.* was related to sampling point. To do so,

175   we fitted a generalised linear model (GLM) where occurrence of *Salmonella spp.* was the

176   response variable and the sampling point (pig faeces, warehouse of cadavers, trucks that

177   transport the carcasses from the warehouse to the SFS, carcasses in SFS and vultures), session

178   (1 and 2) and their interaction were fixed effects. For this analysis, the error was designated

179   as having a binomial distribution and the probit link function was used. Binomial data for

180   each sample were assigned a 1 if *Salmonella spp.* was isolated or a 0 if not. The sampling

181   point x session interaction effect was included in the analysis, but this was discarded because

182   it was not significant. In addition, we tested whether occurrence of *Salmonella spp.* was

183   related to ages of vultures, using a GLM as previously. To do so, we fitted GLM where

184   occurrence of *Salmonella spp.* was the response variable, and age of vultures (juveniles, sub-

185   adult and adult) was the fixed effect. As estimators of the relative quality of the model,

186   Akaike information criterion (AIC) and Bayesian information criterion (BIC) were

187   considered. A P value <0.05 was considered to indicate a statistically significant difference.

188   Analyses were carried out using a commercially available software program (SPSS 21.0

189   software package; SPSS Inc., Chicago, IL, 2002).

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192   **3. Results**

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194   **3.1. *Salmonella* occurrence**

195   *Salmonella spp.* was detected in all of the sampling points. The proportion of *Salmonella-*

196   positive samples were in decreasing order: 82.8% for trucks that transport the carcasses from

197   the warehouse to the SFS (100% of the trucks), 40.0% for pig carcasses disposed of in SFS,

198   32.3% for warehouse of cadavers (100% of the containers), 21.1% for vultures and 14.5%

199   for pig faeces at farm. The sampling point clearly has a significant effect on occurrence of

200   *Salmonella spp.* (Table 1), as revealed by the model analyses (deviance of 88.05% with AIC

201   and BIC values of 43.546 and 64.043 respectively). No significant differences in occurrence

202   of *Salmonella spp.* were found between ages of vultures (Table 2). P values for this difference

203   did not achieve significance (Bonferroni test, P=0.617).

204   Serovar identification was obtained for 69 pooled samples (95.8%), with 3 isolates remaining

205   undetermined. All belonged to one of two subspecies: enterica (93.9%) and salamae (6.1%).

206   A total of 8 serotypes were identified (pooling all sampling point positive samples, Table 3).

207   The most predominant serotype was monophasic *S.* Typhimurium 4,12:i:- detected in 49.3%

208   of positive samples (pooling all sampling point positive) followed by *S.* Panama (23.2%), *S.*

209   London (13.0%), *S.* 4,12:b[-] (5.8%), *S.* Derby (2.9%), *S.* Rissen 6,7: f,g: [-](2.9%), *S.*

210   Typhimurium 4,12:i: 1,2 (1.4%) and *S.* Kedougou (1.4%).

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212   **3.2. *Genetic characterisation of Salmonella isolates***

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214   All isolates were first screened by ERIC-PCR and subsets of 46 were selected according to

215   their different profiles and origin for further analysis by PFGE. Thus, a total of 16 isolates

216   from vultures, 5 from pooled faecal samples from the farms, 7 from the warehouse of

217   cadavers, 12 from the trucks that transport the carcasses from the warehouse to the SFS and

218   6 from carcasses deposited in SFS were examined by PFGE.

219   Monophasic *S.* Typhimurium 1,4,[5],12:i:- isolates were identical (>90% genetic homology)

220   and belonged to samples from vultures and pig faeces from farms and carcasses disposed of

221   in SFS (Fig. 2). In addition, *S.* Derby isolates were identical (>90% genetic homology) and

222   belonged to samples from vultures and pig faeces from farms (Fig. 2). *Salmonella* Rissen

223   isolates were identical (>85% genetic homology) and belonged to samples from vultures and

224   pig faeces from farms (Fig. 2). Finally, *Salmonella* Kedougou isolates were identical (>90%

225   genetic homology) and belonged to samples from vultures and carcasses disposed of in SFS

226   (Fig. 2).

227

228   **4. Discussion**

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230   This study demonstrated that free-living vulture strains (some monophasic *S.* Typhimurium

231   1,4,[5],12:i:-, *S.* Derby and *S.* Rissen) displayed genomic DNA fingerprinting patterns similar

232   to those observed in *Salmonella* strains from pig farms, suggesting that pig farms would

233   introduce *Salmonella* infection into vultures at SFS. This is further supported by the

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234 hypothesis proposed by several authors (Millán et al., 2004; Marin et al., 2014; Vela et al.,

235 2015; Blanco et al., 2016; Blanco, 2018). Besides, *Salmonella* serovars isolated in vultures

236 in the current study have frequently been recorded in vultures in Spain (Marin et al., 2014;

237 Blanco, 2018), and are also often seen in modern pig production (EFSA, 2017). Interestingly,

238 Blanco (2018) not only found similar *Salmonella* serotypes between faeces of vultures

239 feeding on pig carcasses and the pig carcasses, but also identified similar antimicrobial

240 multiresistant patterns between these serotypes. However, no studies have based the results

241 on a molecular identification of DNA polymorphisms to differentiate strains and accurately

242 trace their diffusion. Today, the PFGE system is considered the gold standard for use in

243 epidemiological studies of *Salmonella* (Zou et al., 2013). Thus, monophasic *S.* Typhimurium

244 1,4,[5],12:i:-, *S.* Derby and *S.* Rissen strains isolated in free-living vultures and pig farms

245 authorised to provide carcasses at SFS were highly genetically homogeneous (similar DNA

246 fingerprint). This supports the idea that cross-infection and contamination occurs between

247 pig farms and free-living vultures. Additionally, monophasic *S.* Typhimurium and *S.* Derby

248 are included in the top five most commonly reported serovars in human salmonellosis cases

249 acquired in EU during 2016 (EFSA, 2017). This highlights the role of SFS in the potentiation

250 of griffon vultures as reservoirs, amplifiers and disseminators of *Salmonella*, but also for

251 conservation and reintroduction of avian scavengers, as it remains unknown today whether

252 *Salmonella* can cause clinical illness in this species (Blanco, 2018). Indeed, several studies

253 have reported on the role of pig farms in *Salmonella* transmission among wild birds (Andrés

254 et al., 2013; Andrés-Barranco et al., 2014). Furthermore, different pathways whereby wildlife

255 can be involved in human salmonellosis have been documented (Hilbert et al., 2012).

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257 *Salmonella* occurrence in the current study doubled that of previous studies, where the

258 *Salmonella*-positive rate was lower than 10% in captive scavengers (Millán et al., 2004;

259 Molina-López et al., 2011:2015; Jurado-Tarifa et al., 2016), but was reduced compared to a

260 recent study carried out in central Spain on free-living scavengers, where 61.0% of griffon

261 vultures were *Salmonella*-positive (Blanco, 2018). Strikingly, our previous study carried out

262 in the same observatory and with a similarly large number of samples showed a high level of

263 the bacterium in comparison with the current study (Marin et al., 2014). In this context,

264 *Salmonella* determination is challenging due to intermittent day-to-day shedding and within-

265 day shedding by particular individuals, which could explain the slight differences in

266 occurrence among experiments (Tizard, 2004; Daoust and Prescott, 2007). Nevertheless,

267 cloacal swab is the preferable method to determine the identity of each individual host and

268 prevent cross-contamination by vectors, as well as environmental factors. In spite of this

269 particular point, some *Salmonella* serovars, such as *S.* Typhimurium, monophasic *S.*

270 Typhimurium 1,4,[5],12:i:- and *S.* Derby, have frequently been recorded in vultures

271 throughout different regions of Spain (Millán et al., 2004; Molina-López et al., 2011:2015;

272 Marin et al., 2014; Jurado-Tarifa et al., 2016; Blanco, 2018). In this scenario, one might

273 suggest that our results do not seem to be specific to our area of study. Nevertheless, further

274 research is required to assess the contribution of pig production as a primary source of

275 *Salmonella* in scavenger infection in SFS compared with zoonotic agents in other

276 geographical areas. In fact, this situation should not be considered exclusive to swine

277 production, as poultry and beef production have recently been implicated in large outbreaks

278 of multi-drug-resistant *Salmonella* both in Europe and North America (Mindlin et al., 2013;

279 Laufer et al., 2015; CDC, 2016).

280　　In Spain, carcasses generally disposed of in SFS often come from intensive livestock farming

281　　with pigs (Camiña and Montelío, 2006; Blanco, 2018), mainly because Spain is the second

282　　largest swine producer in the EU and fourth worldwide (Marquer et al., 2014). In particular,

283　　7 out of 11 farms analysed in the current study were *Salmonella*-positive, where monophasic

284　　*S.* Typhimurium 1,4,[5],12:i:- was isolated in 5 of them. Currently, monophasic variants of

285　　*S.* Typhimurium (1,4,[5],12:i- and 1,4,12:i-) have emerged as a public health threat, as it is

286　　the third most frequently isolated serovar from human cases of salmonellosis in Europe,

287　　representing 8.3% of confirmed human cases in 2015 (Andres and Davies, 2015).

288　　Monophasic *S.* Typhimurium constitutes a high proportion of the multi-drug-resistant

289　　*Salmonella* isolates and its occurrence in pigs has been increasing since 2010 (Andres and

290　　Davies, 2015). The worldwide spread of monophasic *S.* Typhimurium 1,4,[5],12:i:- in swine

291　　populations is likely related to the selective advantage offered by multi-drug-resistant

292　　profiles associated with stable genetic elements, also carrying virulence features. These

293　　bacterial lineages are well adapted to the porcine host and are prevalent in human infections

294　　as a result of contaminated pig meat (EMA, 2017). In Spain, monophasic *S.* Typhimurium

295　　serovar accounted for 31.3% of the isolates from pigs in 2015 (Andres and Davies, 2015).

296　　Matching with this, a more recent study found that several serotypes isolated from egyptian

297　　and griffon vultures faeces at an SFS presented a resistance pattern simultaneously resistant

298　　to aminopenicillins, aminoglycosides and tetracyclines, including *S.* Typhimurium 4,12:i:1,2

299　　and the monophasic *S.* Typhimurium 4,12:i:- serotypes (Blanco, 2018). This observation is

300　　in line with other studies, which have associated pig carcasses with avian scavenger

301　　contamination with veterinary pharmaceuticals and the creation of new resistances and the

302　　amplification of these acquired pathogens (Blanco et al., 2016; 2017a; Blanco, 2018). In

303　　addition, different studies have highlighted the potential impact of pig carcasses disposed in

304 the SFSs on development of fungal and parasitic infections in wild avian scavengers (Blanco

305 et al., 2017b; 2017c; Pitarch et al., 2017), although conceptually food security and food safety

306 can potentially be better assured in the SFSs (Margalida et al., 2014). To circumvent this

307 problem, in France conservationists, vets and stakeholders promoted the development of

308 individual SFS, with the principle that each farmer directly recycles their carcasses at their

309 own SFS, avoiding carcass displacement and limiting potential dissemination of pathogens,

310 and furthermore providing carcasses spread more spatially for vultures, in a more natural way

311 (Dupont et al., 2012). In this context, it is worth noting that the *Salmonella* status of the

312 facilities in close contact with griffon vultures during supplementary feeding in this study

313 clearly demonstrated that both the trucks that transport the carcasses from the warehouse to

314 the SFS and the warehouse of cadavers could be an important source of cross-contamination

315 (Dorr et al., 2009). As a measure for practical implementation, if each farm directly recycles

316 its carcasses at its own SFS, authorities should be taking into account sanitary assurances that

317 these farms are pathogen-free. In Spain, some Regional Governments have restricted the

318 supply of feeding stations with swine carcasses (Blanco et al., 2018). The repercussions of

319 this change on avian scavenger populations should be evaluated.

320 In conclusion, the current study indicates that free-living griffon vultures and pig farms that

321 provide the carcasses at SFS share several zoonotic *Salmonella* strains based upon their DNA

322 fingerprint, including monophasic *S.* Typhimurium and *S.* Derby. Taken together with

323 previous studies and although transmission and cross-infection could be bidirectional, our

324 result seems to corroborate the pig carcasses-to-vulture transmission and cross-infection at

325 SFS. However, the current study contains some important biases and limitations. Our results

326 were located at only one SFS. In addition, bidirectional transmission of *Salmonella* has not

327   been evaluated. Under this scenario, there is an urgent need to avoid infection risk and

328   prevent the spread of *Salmonella,* but also to find new strategies to keep the feeding stations

329   as a useful tool for scavenger conservation and assess the potential role of these wild fauna

330   in *Salmonella* epidemiology. Nowadays, initiatives promoting low-intensity farming

331   practices and the use of carcasses from free-ranging ruminants left in the countryside for

332   scavenger consumption are being proposed (Blanco, 2018). As an immediate *Salmonella*

333   control strategy in wild avian scavengers, we suggest the implementation of a programme to

334   ensure that only pig carcasses from *Salmonella*-free farms arrive at SFS. Moreover, we

335   emphasise the need for continuous local surveillance programmes to identify the potential

336   risk to wildlife and the environment.

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Figure legends

485

486 **Fig 1.** Experimental scheme design to evaluate whether free-living griffon vultures captured

487 during the observatory's normal ringing programme and pig samples from farms authorised

488 to provided carcasses share zoonotic *Salmonella* strains. Thus, the occurrence, serotypes and

489 genomic DNA fingerprinting (phage typing and PFGE) of *Salmonella* spp. strains isolated at

490 different sampling points: (A) pig faeces on farms; (B) warehouse of cadavers where farmers

491 legally dispose of dead animals; (C) trucks that transport the carcasses from the warehouse

492 to the supplementary feeding station; and (D) pig carcasses disposed in the supplementary

493 feeding station and griffon vultures in Cinctorres observatory located in Castellón province

494 (eastern Spain).

495

496 **Fig 2.** Dendrogram showing the XbaI profiles of *Salmonella* spp. strains identified from free-

497 living vultures, pig faeces samples from farms authorised to provided carcasses at SFS and

498 several discrete samples obtained from facilities in close contact with pig carcasses during

499 supplementary feeding (warehouse of cadavers where farmers legally dispose of dead

500 animals, trucks that transport the carcasses from the warehouse to the SFS and pig carcasses

501 at SFS) in Cinctorres observatory, located in Castellón province (eastern Spain).