***Graphical Abstract**

***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Highlights

- Griffon vultures and pig farms that provide the pig carcasses at supplementary feeding stations share several zoonotic *Salmonella* strains

- A high proportion of griffon vultures were infected with monophasic *Salmonella* Typhimurium 1,4,[5],12:i:-

- Pig carcasses-to-vulture transmission and cross-infection with *Salmonella* spp. occurs at SFS.

Supplementary feeding stations for conservation of vultures could be an important source of monophasic *Salmonella* Typhimurium 1,4,[5],12:i:-

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ABSTRACT

Vultures are nature's most successful scavengers, feeding on the carcasses of dead animals present in the field. Availability of domestic carrion has been unstable due to rapidly changing agro-grazing economies and increasing sanitary regulations that may require burial or burning of livestock carcasses. Thus, several griffon vulture (*Gyps fulvus*) recoveries are based on European legislation that guarantees the animals' welfare, avoids intense persecution of the vultures and allows the feeding of threatened wildlife in supplementary feeding stations (SFS). However, in recent years, many studies have speculated on the likelihood that avian scavengers may be infected by feeding on pig carcasses at SFS from intensive livestock. In this context, the present study evaluated whether free-living griffon vultures and pig farms share zoonotic *Salmonella* strains to test the hypothesis that vulture are infected during consumption of carcasses provided at SFS. Here, the occurrence, serotypes and genomic DNA fingerprinting (phage typing and pulsed-field gel electrophoresis) of isolated strains were carried out in griffon vultures and pig farms authorised to provided carcasses at SFS in Castellón province (eastern Spain). The bacteriological analyses revealed that 21.1% of vultures and 14.5% for pig farms samples tested were *Salmonella*-positive. Monophasic *S.* Typhimurium 1,4,[5],12:i:- was the most frequently isolated serovar. Comparison of *Salmonella* strains isolated from vultures and pig farms revealed that monophasic *S.* Typhimurium 1,4,[5],12:i:-, *S.* Derby and *S.* Rissen strains were highly genetically homogeneous (similar DNA fingerprint). In conclusion, the current study indicates that free-living griffon vultures and pig farms that provide the carcasses at SFS share several zoonotic *Salmonella* strains. On this basis, and although transmission could be bidirectional, our result seems to corroborate the pig carcasses-to-vulture transmission and cross-infection at SFS. As an immediate *Salmonella*

control strategy in wild avian scavengers, we suggest the implementation of a programme to guarantee that solely pig carcasses from *Salmonella*-free farms arrive at SFS.

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

1. Introduction

Wild birds have repeatedly been highlighted as a source in the dissemination of *Salmonella* spp. (Tizard, 2004; Hilbert et al., 2012; Krawiec et al., 2015) and links have been documented between *Salmonella* contamination of modern pig production and wild birds (Andrés et al., 2013; Andrés-Barranco et al., 2014). Furthermore, the number of wildlife species acting as reservoirs, amplifiers and disseminators is unknown (Molina-López et al., 2011). Hence, the role of wildlife as a *Salmonella* reservoir is of increasing interest (Hilbert et al., 2012). *Salmonella* stands out as one of the most most common causes of human bacterial food poisoning (EFSA, 2017).

During the past century, the availability of domestic carrion has been unstable due to rapidly changing agro-grazing economies and increasing sanitary regulations that may require burial or burning of livestock carcasses. The conservation and reintroduction of avian scavengers would therefore not have been possible without European Regional legislation to ensure their welfare and avoid their intense persecution, as well as the European ruling that permitted the feeding of threatened wildlife in SFS (Camiña-Cardenal et al., 2004; Margalida et al., 2011; Cortés-Avizanda et al., 2016). In the late 1960s, conservationists created “vulture restaurants” or SFS as a way to increase the availability of food resources (Bijleveld, 1974; Gilbert et al., 2007; Donázar et al., 2009; Fielding et al., 2014). At community level, SFS has been widely accepted as an effective management tool among conservationists and managers (Cortés-Avizanda et al., 2016). Encouraging fallen stock to be left in situ is ecologically harmonious, inexpensive and an efficient management method for the conservation of scavengers (Donázar et al., 2009).

Salmonella has been isolated in vultures in several studies, but an especially remarkable finding is the unexpected abundance of *Salmonella* ser. Typhimurium, one of the most common *Salmonella* serovars in foodborne illness outbreaks related with pork consumption (Millán et al., 2004; Molina-López et al., 2011:2015; Marin et al., 2014; Jurado-Tarifa et al., 2016; Blanco, 2018). Besides, to date it remains unknown whether *Salmonella* can cause clinical illness in avian scavengers (Blanco, 2018), which could have potential implications for conservation. Notably, *S.* Typhimurium, including monophasic variants (1,4,[5],12:i- and 1,4,12:i-), represented 21.8% of all reported serovars of confirmed human cases in 2016 in the EU (EFSA, 2017). In particular, *S.* Typhimurium accounted for 63.6% of the isolates reported in pig samples (EFSA, 2017). After the prion crisis, pig carcasses have been the scavengers’ main foodstuff provided at SFS (Blanco et al., 2016; Green et al., 2016; Blanco, 2018). Thus, a recent study carried out by Blanco (2018) in Segovia province (central Spain) supports the role of pig carcasses as a primary source of *Salmonella*, and the risk of scavenger infection in SFS, based on the concordance of serotypes and resistance patterns in an obligate scavenger partially relying on pig carcasses. Our driving hypothesis was that pig farms could be one source of vulture transmission and a cross-infection route of *Salmonella* at SFS. In this context, the present study evaluated whether free-living griffon vultures and pig farms share zoonotic *Salmonella* strains to test the hypothesis that vultures could be infected during consumption of pig carcasses provided at SFS.

2. Material and methods

2.1. Animals

This study was conducted within the conservation project for endangered species in the Valencia Region. The study population is located at the Cinctorres observatory (Castellón province, eastern Spain). In 2008, 236 breeding pairs were found in this area (93% of the breeding pairs in the Community of Valencia) (GVA, 2008). All the experimental procedures used in this study were performed in accordance with Directive 2010/63/EU EEC on animal experiments. The Department of Infrastructure, Planning and Environment of the Valencian Regional Government (Generalitat Valenciana) granted the ethical and animal welfare permission to take samples.

2.2. Sample collection

The vultures were live-captured in two sessions in September and in October 2016, during the observatory’s normal ringing schedule as part of the reserve's monitoring programme. A total of 104 free-living griffon vultures were captured using a remotely activated purposebuilt cage (for more details, see Marin et al., 2014). The age of the animals was determined according to the plumage characteristics and the colour of the bill and eye, classified as juvenile (less than 2 years), sub-adult (from 2 to 5 years) and adult (more than 5 years). Base on our previous results where there were no age-related differences in relation to the presence of *Salmonella* (Marin et al., 2014), data from all individuals was pooled. From each vulture, one cloacal sample was obtained using sterile cotton swabs (Cary Blair sterile transport swabs, Deltalab, Barcelona, Spain). The cotton swab was inserted 1 to 2 cm into the cloaca to collect a suitable sample. At each sampling day, samples of the facilities in close contact with pig carcasses during supplementary feeding (warehouse of cadavers where farmers legally dispose of dead animals, trucks that transport the carcasses from the warehouse to the SFS and pig carcasses at SFS) were collected. A total of 20 sterile cotton swabs were taken from 20 pig carcasses deposited in the SFS (10 samples per day). In addition, before the animals were loaded and delivered to the SFS point, 30 sterile cotton swabs samples were taken directly from the surface of the 2 trucks (floor and wall) that transport the carcasses from the warehouse of cadavers to the SFS (15 samples per truck and day). Moreover, 20 sterile cotton swabs were taken from the warehouse of cadavers (container walls) where farmers disposed of dead livestock (2 samples per container and day). Finally, during the week after the intensive sampling described above, a total of 11 pig farms that provide the carcasses were sampled to determine the potential transfer of *Salmonella* isolates from pig carcasses to vultures. Five pens (four in the corners and one in the middle of the barn) were chosen in each farm. Briefly, 500 gr of faeces were collected in pools from different points of the pens in sterile containers and transported under refrigeration to the laboratory. All samples were analysed within 24 h of collection. The experimental design of this study is shown in Fig. 1.

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 PulseNet (www.pulsenetinternational.org). Genomic DNA was digested with the restriction enzyme XbaI (Roche Applied Science, Indianapolis, IN) and the restriction fragments were separated by electrophoresis in a CHEF-DR III System (Bio-Rad, Hercules, CA, USA). Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA) was used to compare the PFGE patterns by cluster analysis using Dice coefficient and unweighted pair group method with arithmetic averages (UPGMA dendrogram type).

2.5. Statistical analysis

We tested whether occurrence of *Salmonella spp.* was related to sampling point. To do so, we fitted a generalised linear model (GLM) where occurrence of *Salmonella spp.* was the response variable and the sampling point (pig faeces, warehouse of cadavers, trucks that transport the carcasses from the warehouse to the SFS, carcasses in SFS and vultures), session (1 and 2) and their interaction were fixed effects. For this analysis, the error was designated as having a binomial distribution and the probit link function was used. Binomial data for each sample were assigned a 1 if *Salmonella spp.* was isolated or a 0 if not. The sampling point x session interaction effect was included in the analysis, but this was discarded because it was not significant. In addition, we tested whether occurrence of *Salmonella spp.* was related to ages of vultures, using a GLM as previously. To do so, we fitted GLM where occurrence of *Salmonella spp.* was the response variable, and age of vultures (juveniles, sub-adult and adult) was the fixed effect. As estimators of the relative quality of the model, Akaike information criterion (AIC) and Bayesian information criterion (BIC) were considered. A P value <0.05 was considered to indicate a statistically significant difference. Analyses were carried out using a commercially available software program (SPSS 21.0 software package; SPSS Inc., Chicago, IL, 2002).

3. Results

3.1. *Salmonella* occurrence

Salmonella spp. was detected in all of the sampling points. The proportion of *Salmonella*-positive samples were in decreasing order: 82.8% for trucks that transport the carcasses from the warehouse to the SFS (100% of the trucks), 40.0% for pig carcasses disposed of in SFS, 32.3% for warehouse of cadavers (100% of the containers), 21.1% for vultures and 14.5% for pig faeces at farm. The sampling point clearly has a significant effect on occurrence of *Salmonella spp.* (Table 1), as revealed by the model analyses (deviance of 88.05% with AIC and BIC values of 43.546 and 64.043 respectively). No significant differences in occurrence of *Salmonella spp.* were found between ages of vultures (Table 2). P values for this difference did not achieve significance (Bonferroni test, P=0.617).

Serovar identification was obtained for 69 pooled samples (95.8%), with 3 isolates remaining undetermined. All belonged to one of two subspecies: enterica (93.9%) and salamae (6.1%). A total of 8 serotypes were identified (pooling all sampling point positive samples, Table 3). The most predominant serotype was monophasic *S.* Typhimurium 4,12:i:- detected in 49.3% of positive samples (pooling all sampling point positive) followed by *S.* Panama (23.2%), *S.* London (13.0%), *S.* 4,12:b[-] (5.8%), *S.* Derby (2.9%), *S.* Rissen 6,7: f,g: [-](2.9%), *S.* Typhimurium 4,12:i: 1,2 (1.4%) and *S.* Kedougou (1.4%).

3.2. *Genetic characterisation of Salmonella isolates*

All isolates were first screened by ERIC-PCR and subsets of 46 were selected according to their different profiles and origin for further analysis by PFGE. Thus, a total of 16 isolates from vultures, 5 from pooled faecal samples from the farms, 7 from the warehouse of cadavers, 12 from the trucks that transport the carcasses from the warehouse to the SFS and 6 from carcasses deposited in SFS were examined by PFGE.

Monophasic *S.* Typhimurium 1,4,[5],12:i:- isolates were identical (>90% genetic homology) and belonged to samples from vultures and pig faeces from farms and carcasses disposed of in SFS (Fig. 2). In addition, *S.* Derby isolates were identical (>90% genetic homology) and belonged to samples from vultures and pig faeces from farms (Fig. 2). *Salmonella* Rissen isolates were identical (>85% genetic homology) and belonged to samples from vultures and pig faeces from farms (Fig. 2). Finally, *Salmonella* Kedougou isolates were identical (>90% genetic homology) and belonged to samples from vultures and carcasses disposed of in SFS (Fig. 2).

4. Discussion

This study demonstrated that free-living vulture strains (some monophasic *S.* Typhimurium 1,4,[5],12:i:-, *S.* Derby and *S.* Rissen) displayed genomic DNA fingerprinting patterns similar to those observed in *Salmonella* strains from pig farms, suggesting that pig farms would introduce *Salmonella* infection into vultures at SFS. This is further supported by the hypothesis proposed by several authors (Millán et al., 2004; Marin et al., 2014; Vela et al., 2015; Blanco et al., 2016; Blanco, 2018). Besides, *Salmonella* serovars isolated in vultures in the current study have frequently been recorded in vultures in Spain (Marin et al., 2014; Blanco, 2018), and are also often seen in modern pig production (EFSA, 2017). Interestingly, Blanco (2018) not only found similar *Salmonella* serotypes between faeces of vultures feeding on pig carcasses and the pig carcasses, but also identified similar antimicrobial multiresistant patterns between these serotypes. However, no studies have based the results on a molecular identification of DNA polymorphisms to differentiate strains and accurately trace their diffusion. Today, the PFGE system is considered the gold standard for use in epidemiological studies of *Salmonella* (Zou et al., 2013). Thus, monophasic *S.* Typhimurium 1,4,[5],12:i:-, *S.* Derby and *S.* Rissen strains isolated in free-living vultures and pig farms authorised to provide carcasses at SFS were highly genetically homogeneous (similar DNA fingerprint). This supports the idea that cross-infection and contamination occurs between pig farms and free-living vultures. Additionally, monophasic *S.* Typhimurium and *S.* Derby are included in the top five most commonly reported serovars in human salmonellosis cases acquired in EU during 2016 (EFSA, 2017). This highlights the role of SFS in the potentiation of griffon vultures as reservoirs, amplifiers and disseminators of *Salmonella*, but also for conservation and reintroduction of avian scavengers, as it remains unknown today whether *Salmonella* can cause clinical illness in this species (Blanco, 2018). Indeed, several studies have reported on the role of pig farms in *Salmonella* transmission among wild birds (Andrés et al., 2013; Andrés-Barranco et al., 2014). Furthermore, different pathways whereby wildlife can be involved in human salmonellosis have been documented (Hilbert et al., 2012).

Salmonella occurrence in the current study doubled that of previous studies, where the *Salmonella*-positive rate was lower than 10% in captive scavengers (Millán et al., 2004; Molina-López et al., 2011:2015; Jurado-Tarifa et al., 2016), but was reduced compared to a recent study carried out in central Spain on free-living scavengers, where 61.0% of griffon vultures were *Salmonella*-positive (Blanco, 2018). Strikingly, our previous study carried out in the same observatory and with a similarly large number of samples showed a high level of the bacterium in comparison with the current study (Marin et al., 2014). In this context, *Salmonella* determination is challenging due to intermittent day-to-day shedding and within-day shedding by particular individuals, which could explain the slight differences in occurrence among experiments (Tizard, 2004; Daoust and Prescott, 2007). Nevertheless, cloacal swab is the preferable method to determine the identity of each individual host and prevent cross-contamination by vectors, as well as environmental factors. In spite of this particular point, some *Salmonella* serovars, such as *S.* Typhimurium, monophasic *S.* Typhimurium 1,4,[5],12:i:- and *S.* Derby, have frequently been recorded in vultures throughout different regions of Spain (Millán et al., 2004; Molina-López et al., 2011:2015; Marin et al., 2014; Jurado-Tarifa et al., 2016; Blanco, 2018). In this scenario, one might suggest that our results do not seem to be specific to our area of study. Nevertheless, further research is required to assess the contribution of pig production as a primary source of *Salmonella* in scavenger infection in SFS compared with zoonotic agents in other geographical areas. In fact, this situation should not be considered exclusive to swine production, as poultry and beef production have recently been implicated in large outbreaks of multi-drug-resistant *Salmonella* both in Europe and North America (Mindlin et al., 2013; Laufer et al., 2015; CDC, 2016).

In Spain, carcasses generally disposed of in SFS often come from intensive livestock farming with pigs (Camiña and Montelío, 2006; Blanco, 2018), mainly because Spain is the second largest swine producer in the EU and fourth worldwide (Marquer et al., 2014). In particular, 7 out of 11 farms analysed in the current study were *Salmonella*-positive, where monophasic *S.* Typhimurium 1,4,[5],12:i:- was isolated in 5 of them. Currently, monophasic variants of *S.* Typhimurium (1,4,[5],12:i- and 1,4,12:i-) have emerged as a public health threat, as it is the third most frequently isolated serovar from human cases of salmonellosis in Europe, representing 8.3% of confirmed human cases in 2015 (Andres and Davies, 2015). Monophasic *S.* Typhimurium constitutes a high proportion of the multi-drug-resistant *Salmonella* isolates and its occurrence in pigs has been increasing since 2010 (Andres and Davies, 2015). The worldwide spread of monophasic *S.* Typhimurium 1,4,[5],12:i:- in swine populations is likely related to the selective advantage offered by multi-drug-resistant profiles associated with stable genetic elements, also carrying virulence features. These bacterial lineages are well adapted to the porcine host and are prevalent in human infections as a result of contaminated pig meat (EMA, 2017). In Spain, monophasic *S.* Typhimurium serovar accounted for 31.3% of the isolates from pigs in 2015 (Andres and Davies, 2015). Matching with this, a more recent study found that several serotypes isolated from egyptian and griffon vultures faeces at an SFS presented a resistance pattern simultaneously resistant to aminopenicillins, aminoglycosides and tetracyclines, including *S.* Typhimurium 4,12:i:1,2 and the monophasic *S.* Typhimurium 4,12:i:- serotypes (Blanco, 2018). This observation is in line with other studies, which have associated pig carcasses with avian scavenger contamination with veterinary pharmaceuticals and the creation of new resistances and the amplification of these acquired pathogens (Blanco et al., 2016; 2017a; Blanco, 2018). In addition, different studies have highlighted the potential impact of pig carcasses disposed in the SFSs on development of fungal and parasitic infections in wild avian scavengers (Blanco et al., 2017b; 2017c; Pitarch et al., 2017), although conceptually food security and food safety can potentially be better assured in the SFSs (Margalida et al., 2014). To circumvent this problem, in France conservationists, vets and stakeholders promoted the development of individual SFS, with the principle that each farmer directly recycles their carcasses at their own SFS, avoiding carcass displacement and limiting potential dissemination of pathogens, and furthermore providing carcasses spread more spatially for vultures, in a more natural way (Dupont et al., 2012). In this context, it is worth noting that the *Salmonella* status of the facilities in close contact with griffon vultures during supplementary feeding in this study clearly demonstrated that both the trucks that transport the carcasses from the warehouse to the SFS and the warehouse of cadavers could be an important source of cross-contamination (Dorr et al., 2009). As a measure for practical implementation, if each farm directly recycles its carcasses at its own SFS, authorities should be taking into account sanitary assurances that these farms are pathogen-free. In Spain, some Regional Governments have restricted the supply of feeding stations with swine carcasses (Blanco et al., 2018). The repercussions of this change on avian scavenger populations should be evaluated.

In conclusion, the current study indicates that free-living griffon vultures and pig farms that provide the carcasses at SFS share several zoonotic *Salmonella* strains based upon their DNA fingerprint, including monophasic *S.* Typhimurium and *S.* Derby. Taken together with previous studies and although transmission and cross-infection could be bidirectional, our result seems to corroborate the pig carcasses-to-vulture transmission and cross-infection at SFS. However, the current study contains some important biases and limitations. Our results were located at only one SFS. In addition, bidirectional transmission of *Salmonella* has not been evaluated. Under this scenario, there is an urgent need to avoid infection risk and prevent the spread of *Salmonella*, but also to find new strategies to keep the feeding stations as a useful tool for scavenger conservation and assess the potential role of these wild fauna in *Salmonella* epidemiology. Nowadays, initiatives promoting low-intensity farming practices and the use of carcasses from free-ranging ruminants left in the countryside for scavenger consumption are being proposed (Blanco, 2018). As an immediate *Salmonella* control strategy in wild avian scavengers, we suggest the implementation of a programme to ensure that only pig carcasses from *Salmonella*-free farms arrive at SFS. Moreover, we emphasise the need for continuous local surveillance programmes to identify the potential risk to wildlife and the environment.

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Figure legends

Fig 1. Experimental scheme design to evaluate whether free-living griffon vultures captured during the observatory's normal ringing programme and pig samples from farms authorised to provided carcasses share zoonotic *Salmonella* strains. Thus, the occurrence, serotypes and genomic DNA fingerprinting (phage typing and PFGE) of *Salmonella* spp. strains isolated at different sampling points: (A) pig faeces on farms; (B) warehouse of cadavers where farmers legally dispose of dead animals; (C) trucks that transport the carcasses from the warehouse to the supplementary feeding station; and (D) pig carcasses disposed in the supplementary feeding station and griffon vultures in Cinctorres observatory located in Castellón province (eastern Spain).

Fig 2. Dendrogram showing the XbaI profiles of *Salmonella* spp. strains identified from free-living vultures, pig faeces samples from farms authorised to provided carcasses at SFS and several discrete samples obtained from facilities in close contact with pig carcasses during supplementary feeding (warehouse of cadavers where farmers legally dispose of dead animals, trucks that transport the carcasses from the warehouse to the SFS and pig carcasses at SFS) in Cinctorres observatory, located in Castellón province (eastern Spain).

Table 1
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Table 1. Generalised linear model showing the relation between *Salmonella spp.* occurrence, sampling points and session when samples were taken. The estimate of the parameters (including the sign), the standard error of the parameters and the p-value are shown.

Parameter	Estimate	Standard error	p-value
Intercept	-1.029	0.208	<0.001
Sampling point (Truck that transported carcasses)	2.005	0.250	<0.001
Sampling point (Vultures)	0.253	0.345	0.311
Sampling point (Warehouse of cadavers)	0.604	0.352	0.055
Sampling point (Carcasses in SFS)	0.802	0.313	0.023
Sampling point (Pig faeces)	Ref.		
Session (1)	-0.52	0.185	0.778
Session (2)	Ref.		

Table 2. Occurrence of *Salmonella* spp. in free-living griffon vultures *(Gyps fulvus)* from different ages captured during the observatory's normal ringing programme. The age of the animals was determined according to the plumage characteristics and the colour of the bill and eye (juvenile, less than 2 years; sub-adult, between 2 to 5 years; adult, more than 5 years).

Age	**n**	***Salmonella*** **(%)**
Juveniles	9	33.3
Sub-adult	17	23.5
Adult	78	19.2
All	104	21.2

n: number of birds analysed

Table 3
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Table 3. Salmonella serovars isolated from free-living griffon vultures *(Gyps fulvus*), pig faeces samples from farms authorised to provided carcasses in the supplementary feeding station (SFS) and several samples points obtained from the facilities in close contact with pig carcasses during supplementary feeding (warehouse, trucks that transport the carcasses from the warehouse to the SFS and pig carcasses at SFS) in Cinctorres observatory located in Castellón province (Eastern Spain).

Subspecies	**Serovar**	**Vultures n (%)**	**Pig farms n (%)**	**Warehouse of cadavers[#] n (%)**	**Trucks* n (%)**	**Carcasses in SFS n (%)**
enterica	Typhimurium monophasic 1,4,[5],12:i:-	18 (82.6)	6 (75.0)	2 (30.0)	-	5 (75.0)
enterica	Typhimurium 4,12:i: 1,2	1 (4.3)	-	-	-	-
enterica	Rissen 6,7: f,g: [-]	1 (4.3)	1 (12.5)	-	-	-
enterica	Kedougou	1 (4.3)	-	-	-	-
enterica	Derby	1 (4.3)	1 (12.5)	-	-	-
enterica	Panama	-	-	4 (40.0)	11 (55.0)	1 (12.5)
enterica	London	-	-	-	9 (45.0)	-
salamae	4,12:b[-]	-	-	3 (30.0)	-	1 (12.5)

n: number of isolated. [#]Warehouse of cadavers where farmers legally dispose of dead animals. *Trucks that transport the carcasses from the warehouse to the supplementary feeding station.

Figure 1
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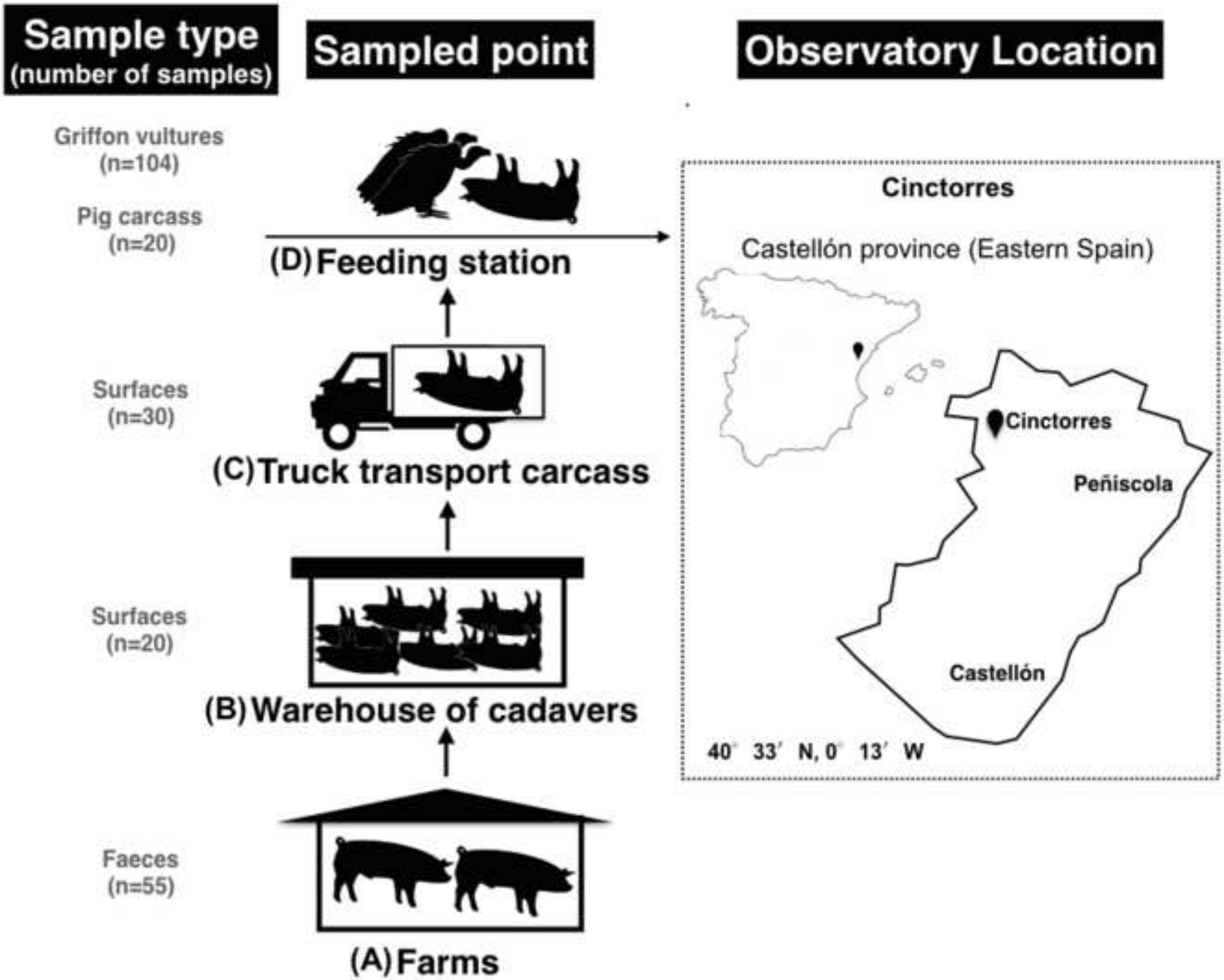

Figure 2

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