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Eurasian griffon vultures carry widespread antimicrobial resistant *Salmonella* and *Campylobacter* of public health concern

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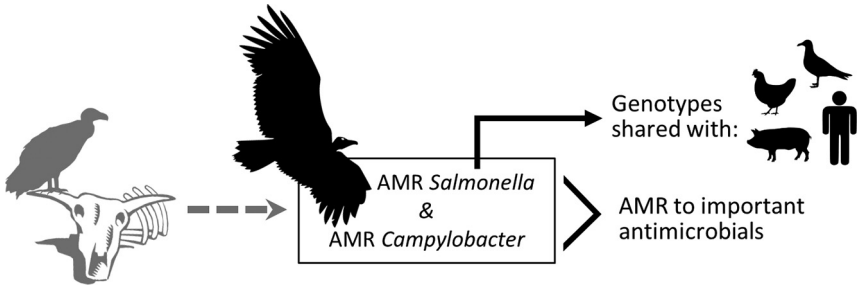
HIGHLIGHTS

- Vultures are carriers of widespread AMR zoonotic *Salmonella* and *Campylobacter*.
- Strains of monophasic *S. Typhimurium* were shared by gulls, livestock and humans.
- The majority of isolates showed resistance to at least one antimicrobial.
- AMR bacteria in vultures might originate in livestock farming.
- The high frequency of AMR to critical antimicrobials for human medicine is of concern.

GRAPHICAL ABSTRACT



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ABSTRACT

The global emergence of antimicrobial-resistant (AMR) strains of *Salmonella* and *Campylobacter* is a serious public health concern. Both bacteria are leading causes of human gastrointestinal foodborne infections and the two most reported zoonoses in the European Union. By feeding on livestock carcasses, especially from intensive farming, as well as on landfill sites, obligate avian scavengers can become infected with zoonotic pathogens and AMR strains, and can be considered large-scale sentinels of the environmental burden. In this study, we assessed the occurrence and AMR of *Salmonella* spp. and *Campylobacter* spp. in 218 Eurasian griffon vultures (*Gyps fulvus*) captured in north-eastern Spain. We isolated *Salmonella* from 8.1 % of individuals and *Campylobacter lari* from 4.7 %. Among the 10 different *Salmonella* serovars found, monophasic *S. Typhimurium* was the most frequent. Genotyping analysis revealed same strains of monophasic *S. Typhimurium* shared by gulls, livestock and humans. Isolates from both bacterial species presented AMR to important antimicrobials (tetracyclines, fluoroquinolones and β-lactams). In conclusion, this study shows that Eurasian griffon vultures in north-eastern Spain are carriers of widespread AMR zoonotic *Salmonella* and *Campylobacter*. More comprehensive analyses are still needed to understand the potential risk of spill-over from those wild birds to humans.

1. Introduction

The emergence and spread of antimicrobial-resistant (AMR) bacteria is one of the greatest threats to human medicine, veterinary medicine, and public health (Laxminarayan et al., 2013). This situation impairs our efficacy to treat a growing number of infections, increases medical costs, and could account for as many as 10 million annual deaths worldwide by

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2050 (O'Neill, 2016). AMR has multifactorial causes which is mainly due to a combination of inappropriate drug prescriptions and use, lack of appropriate drug regulations and surveillance systems in developing countries and extensive use of antimicrobials in animal production (Ayukekbong et al., 2017). In order to contain the situation, the World Health Organization (WHO) has promoted a ban on the use of critically important antimicrobials for human medicine in livestock disease control (WHO, 2017). Currently, *Salmonella* and *Campylobacter* are the leading causes of human gastrointestinal foodborne infections and the two most reported zoonoses in the European Union (EFSA and ECDC, 2021). The recent emergence of the monophasic *Salmonella Typhimurium* serovar, related with multidrug-resistance (MDR), is now a global public health emergency (Sun et al., 2020). In fact, monophasic *Salmonella Typhimurium* has become, after *Salmonella Enteritidis*, one of the most important cause of human salmonellosis in the EU (EFSA and ECDC, 2021). Overall, the global emergence of AMR strains of both *Salmonella* and *Campylobacter* is leading to an increase in mortality rates due to therapeutic failure, and is considered a serious public health concern (Eng et al., 2015; Moore et al., 2006).

While the occurrence of AMR in livestock has been broadly assessed, the role of wild animal species in the maintenance and transmission of these resistances is still poorly understood (Vittecoq et al., 2016). In general, wildlife does not naturally come into contact with antimicrobials, but can acquire AMR bacteria from human and livestock sources such as agricultural facilities, contaminated sewage and refuse dumps. Also, antimicrobials present in human residues and livestock carcasses released to the environment can promote the development of AMR bacteria in the intestinal microbiota of wildlife (Gómez-Ramírez et al., 2020; Ramey and Ahlstrom, 2020). In that sense, scavengers, due to their feeding habits and their position in the food chain, are expected to be more prone to carry *Campylobacter* and *Salmonella* and spread AMR bacteria, facilitating their dissemination (Antilles et al., 2021; Marin et al., 2014; Smith et al., 2020). Furthermore, wild bird species that can fly great distances may act as a source of bacterial dissemination across broad geographical scales (Blanco et al., 2020; Molina-Lopez et al., 2011).

Spain is home for 90 % of the European population of Eurasian griffon vultures (*Gyps fulvus*) (Del Moral and Molina, 2018). Eurasian griffon vultures are obligate avian scavengers that feed from carcasses of wild and domestic animals found by flying over extensive areas. The indiscriminate use of poison up to the 1980s contributed to a critic decline of scavenger species. To redress the situation, supplementary feeding stations using livestock carcasses have been established throughout the Iberian Peninsula to increase the availability of food resources and boost the recovery of the European populations of avian scavengers (Margalida et al., 2010). These "vulture restaurants" are also an inexpensive and efficient alternative to the industrial destruction of carcasses. As a consequence, vultures can become infected with zoonotic pathogens while feeding on livestock carcasses at these feeding stations, especially from an intensive farming origin (Blanco et al., 2020; Sevilla et al., 2020). At the same time, the disposal of sick and medicated livestock carcasses may increase the ingestion of veterinary drugs and AMR pathogens (Blanco et al., 2020). Moreover, vultures forage on organic waste in landfill sites. Such sites, where human waste is disposed, are considered a source of zoonotic pathogens and are associated with the presence of pharmaceutical products such as antimicrobials (Matejczyk et al., 2011; Musson and Townsend, 2009). As so, feeding on such sites may favour the transmission of pathogens and antimicrobial drugs to scavengers (Plaza et al., 2019). Therefore, obligate avian scavengers, such as vultures, can be potential large-scale sentinels of the environmental burden of zoonotic pathogens and AMR strains (Blanco and Bautista, 2020).

In this study, we aimed to assess the prevalence and genetic diversity of *Salmonella* spp. and *Campylobacter* spp., and their AMR strains in a population of Eurasian griffon vultures from north-eastern Spain. As such population feed on supplementary feeding stations and in a landfill, they might be more prone to be carriers of zoonotic agents. Our working hypothesis is that vultures are sentinels of the environmental burden of AMR zoonotic pathogens. This hypothesis predicts that vultures will be carriers of widespread AMR *Salmonella* and *Campylobacter* that are present in other hosts such as livestock, humans, and other wildlife species in areas with anthropogenic pressure. By comparing the genotypes of the recovered strains with strains from other regions and hosts, we further aimed to gain insight into the potential anthropogenic origin of those strains, the span of those AMR zoonotic pathogens, and the role of avian scavengers in their epidemiology.

2. Material and methods

2.1. Study area and sampling

Between June 2019 and February 2020, we collected cloacal swabs from 218 Eurasian griffon vultures in Osona (province of Barcelona, Catalonia, NE-Spain; 42°4′N, 2°12′E). This capture site is situated in the vicinities of a landfill site where vultures feed regularly on organic waste. This population of vultures also feed in supplementary feeding stations around the area. Twelve animals were captured and sampled twice and two were sampled thrice, obtaining a total of 234 samples. Vultures were captured during monthly ringing activities, using a portable trap with fixed side panels (5.2 × 5.2 m) and self-locking swinging doors. Two cloacal swabs were obtained from each individual, which were placed in Amies transport medium containing charcoal (Deltalab, Rubí, Spain) and were maintained at 4 °C and processed within 36 h. The age of individuals was determined according to physical characteristics, and classified as juveniles (<2 years; $n = 6$), sub-adults (from 2 to 4 years; $n = 54$), and adults (>4 years; $n = 143$). The age of 15 individuals couldn't be determined between sub-adult and adult due to plumage defaults.

2.2. Salmonella and Campylobacter isolation and identification

We performed the isolation and identification of *Salmonella* spp. and *Campylobacter* spp. as previously described by Antilles et al. (2021). Briefly, one swab was used for *Salmonella* isolation using a non-selective pre-enrichment in buffered peptone water (Oxoid, Basingstoke, UK), followed by a selective enrichment in Rappaport-Vassiliadis (Oxoid, Basingstoke, UK) and subculturing onto xylose lysine Tergitol 4 agar (Merck, Darmstadt, Germany). Presumptive colonies were subcultured onto MacConkey agar and confirmed as *Salmonella* with the indole test. *Salmonella* isolates were serotyped at the Laboratori Agroalimentari (Cabrils, Spain. www.bit.ly/3OTqwbi) from the Catalan Governmnet (Departament d'Acció Climàtica, Alimentació i Agenda Rural) according to White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). We used the second swab for *Campylobacter* isolation by streaking it onto modified charcoal cefoperazone desoxycholate agar (CM739 with selective supplement, SR0155E; Oxoid, Basingstoke, UK) and subculturing presumptive colonies onto blood agar plates (BioMérieux, Marcy l'Etoile, France). Identification of *Campylobacter* species (*C. jejuni*, *C. coli* and *C. lari*) was performed by a multiplex PCR using primers targeting the lipid A gene lpxA (Klena et al., 2004). We calculated the occurrence of each pathogen from the proportion of positives to the total number of individuals examined, with Wilson score confidence intervals of 95 %.

2.3. Molecular typing of Salmonella and Campylobacter isolates

We determined the genotypic diversity among bacterial isolates by pulsed-field gel electrophoresis (PFGE), according to the standard operating procedure of PulseNet (www.pulsenetinternational.org). We performed the restriction enzyme digests with enzymes *Xba*I for *Salmonella*, and with enzymes *Sma*I for *Campylobacter* (Roche Applied Science, Indianapolis, IN, USA). We analysed the resulting patterns using Fingerprinting II v3.0 software (Bio-Rad, Hercules, CA, USA), and compared them with previously obtained genotypes from our own database, including isolates from other regions and hosts. Cluster analysis was performed with the unweighted pair group method with arithmetic mean (UPGMA), using the Dice correlation coefficient with a band position tolerance of 1,5 %. We considered PFGE band patterns with a similarity ≥90 % to be the same pulsotype (PFGE type).

2.4. Antimicrobial susceptibility testing of Salmonella and Campylobacter isolates

Antimicrobial susceptibility testing was performed with a minimum inhibitory concentration (MIC)-based broth microdilution using EUVSEC plates for *Salmonella* spp. and EUCAMP2 plates for *Campylobacter* spp. (Sensititre® Susceptibility plates, ThermoFisher Scientific, Spain). For *Salmonella*, the tested antimicrobials were ampicillin (1–64 mg/L), cefotaxime (0.25–1 mg/L), ceftazidime (0.5–8 mg/L), meropenem (0.03–16 mg/L), nalidixic acid (4–128 mg/L), ciprofloxacin (0.015–8 mg/L), gentamycin (0.5–32 mg/L), tetracycline (2–64 mg/L), tigecycline (0,25–8 mg/L), azithromycin (2–64 mg/L), chloramphenicol (8–128 mg/L), colistin (1–16 mg/L) and trimethoprim (0,25–32 mg/L). We used *E. coli* ATCC 25922 as control strain. For *Campylobacter*, we tested the antimicrobial susceptibility to nalidixic acid (1–64 mg/L), ciprofloxacin (0.12–16 mg/L), gentamycin (0.12–16 mg/L), streptomycin (0.25–16 mg/L), tetracycline (0.5–64 mg/L) and erythromycin (1–128 mg/L), and using *C. jejuni* ATCC 33560 as control strain. We followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines to establish the epidemiological cut-off values (ECOFF) to consider an isolate as "wild-type" (hereafter "susceptible") and "non-wild-type" (hereafter "resistant"). We used the ECOFF of *Salmonella enterica* for the *Salmonella* isolates (except in the case of meropenem, tigecycline and colistin where the ECOFF were not available for *Salmonella* spp. and we used those for *E. coli*) and the ECOFF of *C. coli* for *Campylobacter* isolates. We considered an isolate as MDR when showing resistance to three or more nonrelated antimicrobials.

3. Results

Out of the 234 samples analysed, we isolated *Salmonella* from 19 individuals (8.1 %; $CI_{95\%}$: 5.2–12.3) and *Campylobacter* from 11 individuals (4.7 %; $CI_{95\%}$: 2.6–8.2) (see supplementary Table S1 for the monthly occurrence of both pathogens). One individual carried both pathogens. Among the animals sampled more than once, one individual that was negative for both pathogens in December became positive for *Salmonella* in January, one animal that was negative in November and January resulted positive for *Campylobacter* in February and one negative in December became positive to *Campylobacter* in February. The results of age-related occurrences can be found in the supplementary Table S2.

We identified 10 different serotypes of *Salmonella*: monophasic *Salmonella Typhimurium* 4,[5],12:i:- (7/19), Muenchen 6,8:d:1,2 (3/19), Lexington 3,10:z10:1,5 (2/19), Derby 4,12:f,g:- (1/19), Goelzau 15 + 3,15:a:1,5 (1/19), Infantis 6,7:r:1,5 (1/19), Isangi 6,7:d:1,5 (1/19), Meleagridis 3,10:e,h:l,w (1/19), Muenster 3,10:e,h:1,5 (1/19) and Uganda 3,10:l,z13:1,5 (1/19). All *Campylobacter* isolates were identified as *Campylobacter lari*.

All seven monophasic *Salmonella Typhimurium* isolates grouped in five different clusters at >90 % similarity (Fig. 1). Those isolates clustered together with isolates from poultry, swine carcasses and griffon vultures sampled in the same and neighbouring regions, as well as with a human clinical isolate from Catalonia. The three *Salmonella* Muenchen isolates showed the same pulsotype and grouped with an isolate obtained from a kelp gull (*Larus dominicanus*) in Western Cape (South Africa). The two *Salmonella* Lexington isolates showed the same pulsotype. The 11*C. lari* isolates were grouped in six pulsotypes (Fig. 2) and one of them had the same pulsotype as an isolate from a yellow-legged gull (*Larus michahellis*) from the Ebro Delta, 200 km away from our sampling area.

Among *Salmonella* isolates, the most frequent resistance was to tetracycline (52 %) and ampicillin (48 %), followed by trimethoprim (10.5 %), whilst resistance to ciprofloxacin, nalidixic acid, chloramphenicol and gentamicin was found in a single isolate (5.2 %). MDR was present in one monophasic *Salmonella Typhimurium* isolate (Table 1). A 54.5 % (6/11) of

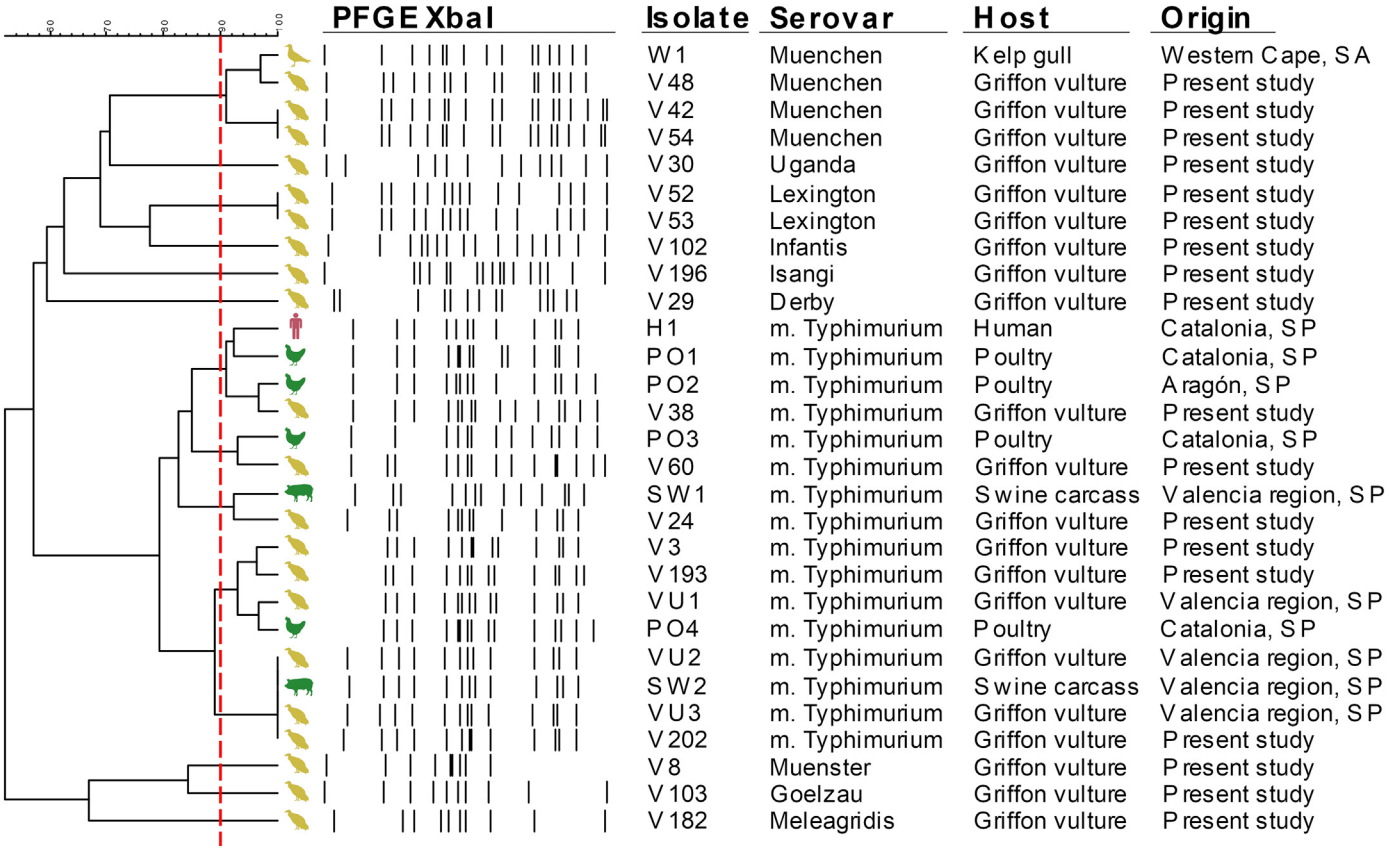

Fig. 1. PFGE dendrogram of *Xba*I patterns of *Salmonella* isolates from Eurasian griffon vultures in north-eastern Spain. Isolates from poultry from Catalonia and Aragón regions (north-eastern Spain, SP), swine carcasses and other vultures from the Valencia region (eastern Spain, SP), a human case from Catalonia, and a kelp gull from Western Cape (South Africa, SA) were also included for comparison purposes; only those isolates sharing pulsotype with vulture isolates are included in the figure. m. Typhimurium: monophasic *Salmonella Typhimurium*.

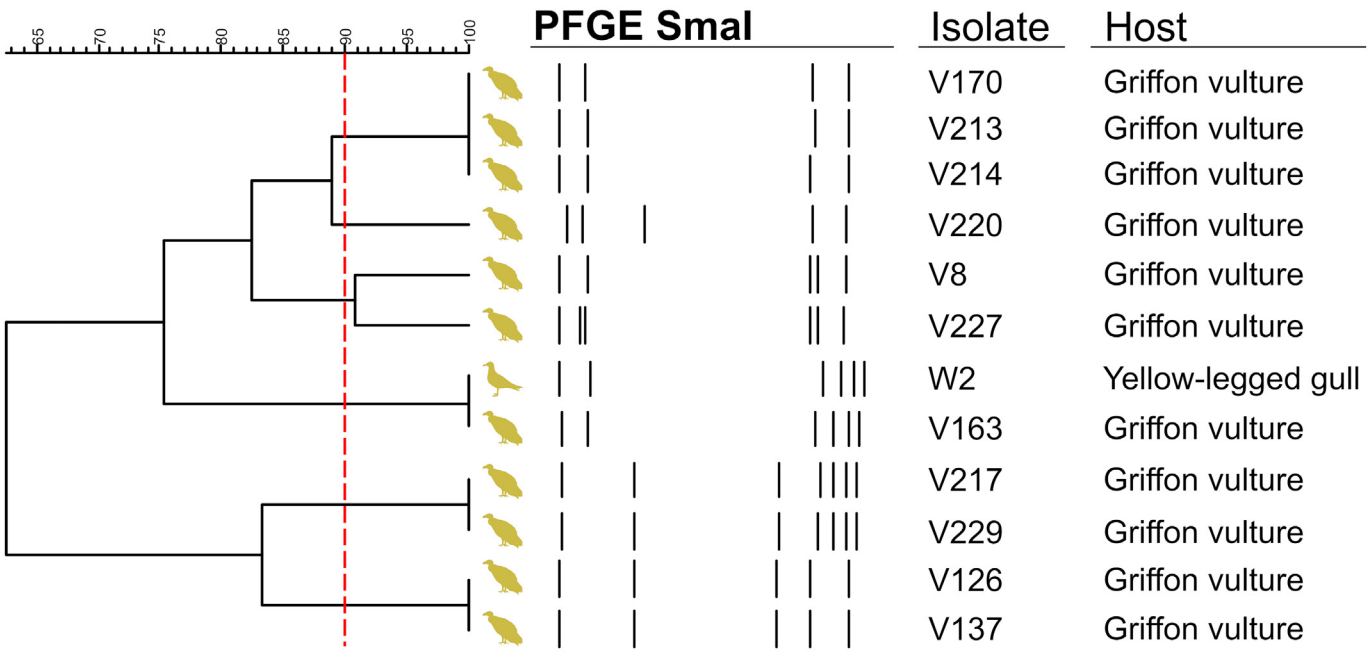

Fig. 2. PFGE dendrogram of *Sma*I patterns of *Campylobacter lari* isolates from griffon vultures in north-eastern Spain. A yellow-legged gull isolate from the Ebro Delta (north-eastern Spain) was also included for comparison purposes.

C. lari isolates showed resistance to ciprofloxacin. The MIC values of the control strains *C. jejuni* ATCC 33560 and *E. coli* ATCC 25922 were within the limits indicated by the EUCAST.

4. Discussion

This study assessed the occurrence of *Salmonella* spp. and *Campylobacter* spp. in Eurasian Griffon vultures from north-eastern Spain. Overall, we detected a relatively low occurrence of both pathogens in faecal swabs. However, the fact that over a half of these isolates showed resistance to at least one antibiotic class is worrying.

We detected a lower occurrence of *Salmonella* isolates than previously reported in Eurasian griffon vultures in Spain, using similar laboratorial methodologies (Blanco and Díaz de Tuesta, 2021; Marin et al., 2014, 2018). Blanco and Díaz de Tuesta (2021) described a seasonal variation in *Salmonella* occurrence, but our sampling encompassed their period of higher occurrence (the pre-breeding period, from December to January). *Salmonella* typically presents an intermittent excretion (Daoust and Prescott, 2007; Marin et al., 2018) that could explain these differences in occurrence between studies in relatively close areas. In accordance with the above-mentioned surveys, monophasic *Salmonella Typhimurium* was also the serovar most frequently isolated in our study. This is not surprising, as more than two thirds of monophasic *Salmonella Typhimurium* isolated from food and animal sources in Europe are associated with pigs (EFSA and ECDC, 2021), which are one of the main foods supplemented to vultures in feeding stations (Donázar et al., 2010). Marin et al. (2018) reported a high similarity between isolates from vultures and pig carcasses, supporting the hypothesis that supplementary feeding stations are an important source of *Salmonella* for vultures. In fact, four vulture isolates from our study showed the same pulsotype as two isolates of pig carcasses from this previous study. We also detected the same profile between vultures and poultry isolates, consistent with the fact that, although to a lesser extent, poultry is also part of the supplemented diet of griffon vultures (Donázar et al., 2010). In contrast, this serovar is scarcely recovered from other wild bird groups or even other raptor species (Botti et al., 2013; Moré et al., 2017; Reche et al., 2003; Troxler et al., 2017). Among the *Salmonella* isolates recovered in our study, 63 % (12/19) are serovars responsible for human salmonellosis cases, including serovars Muenchen, Derby, Infantis and the above-mentioned monophasic *S. Typhimurium* (EFSA and ECDC, 2021). In fact, PFGE analysis showed the same pulsotype in one monophasic *S. Typhimurium* isolate we recovered from a vulture, one strain from a human salmonellosis case in NE Spain, and poultry isolates, suggesting a spill-over from humans or food animals to wildlife. Such events highlight the importance of a One Health approach to gain insight into the epidemiology of zoonoses and antimicrobial resistance (McEwen and Collignon, 2018).

The majority of serovars isolated in our study have been also isolated in other wild species from NE Spain, such as wild boar, red fox, seagulls and raptors (Antilles et al., 2021; Mentaberre et al., 2013; Molina-López et al., 2015; Ramos et al., 2010) but also in pig, poultry and cattle farming (Marin et al., 2018; Ministry of Climate Action, Food and Rural Agenda of Catalonia, personal communication; CESAC-Poultry Health Centre of Catalonia and Aragon, personal communication). The wide variety of hosts with the same *Salmonella* serovars than the ones recovered in our study suggests a low host-specificity and the presence of different sources of *Salmonella* contamination for vultures. As obligate scavengers, vultures feed on a wide variety of species and therefore are more prone to harbour a diversity of serovars. Furthermore, landfill sites —such as the one where the vultures of our study feed regularly— are associated with the presence of *Salmonella*, due to the large quantities of decomposing organic

Table 1
Antimicrobial resistance patterns of *Salmonella* and *Campylobacter* isolates originating from cloacal swabs of Eurasian Griffon vultures in north-eastern Spain.

	AMR profile[a]	Antimicrobial class[b]	Isolates
Salmonella serovar			
monophasic	AMP, NAL, CIP, GEN, TET, CHL,	6	1
Typhimurium	TMP		
	AMP, TET	2	4
	AMP	1	1
	TET	1	1
Muenchen	AMP, TET	2	3
Meleagridis	TET, TMP	2	1
Campylobacter lari	CIP	1	6

[a] β-lactams: AMP (Ampicillin); Quinolones: NAL (nalidixic acid), CIP (ciprofloxacin); Aminoglycoside: GEN (gentamycin); Tetracycline: TET (tetracycline); Phenicol: CHL (Chloramphenicol); Sulfonamide: TMP (Trimethoprim).
[b] Number of different antimicrobial classes per resistance profile.

waste (Plaza et al., 2019). Vultures may ingest the pathogen while feeding there, and these sites may therefore also be an important source of contamination for scavengers. On the other hand, finding the same pulsotypes in vulture isolates from our study and a kelp gull isolate from South Africa was surprising, as the distance between both sampling sites and the movement patterns of both species prevent the existence of a common site of infection, such as a landfill. However, our results may be explained by a cosmopolitan distribution of such serovar (Muenchen), but further research is needed on the epidemiology of such pathogens at global level to figure this out. High throughput techniques, such as whole genome sequencing, could provide complementary and more accurate information on the similarity and phylogeny of strains, and would be helpful to confirm our findings.

The occurrence of *Campylobacter* in wild Eurasian griffon vulture has been scarcely studied and only low prevalences of *C. jejuni* have been reported (Marin et al., 2014; Molina-Lopez et al., 2011). To the best of our knowledge, this is the first report of *C. lari* in an avian scavenger in the northern hemisphere. Despite *C. lari* having been sporadically reported in raptors (Jurado-Tarifa et al., 2016), the great majority of isolates are cultured from Charadriiform species such as sheathbills and auks (Minias, 2020), or brown skuas in the Southern Ocean (Cerdà-Cuéllar et al., 2019). *Campylobacter lari* has been associated with enteritis, urinary tract infections and bacteraemia in humans, but it is currently considered a minor zoonotic pathogen (Igwaran and Okoh, 2019). Finding the same pulsotype in a vulture and a yellow-legged gull suggests a widespread strain or a common source of infection, such as landfills, and reinforce the importance of these sites as a source of contamination for wildlife. Yellow-legged gulls, as vultures, are prone to use landfills as feeding sources (Antilles et al., 2021).

We report that the Eurasian griffon vultures of our study are carriers of antibiotic-resistant zoonotic *Salmonella* serovars and *C. lari*. The presence of resistances to at least one antimicrobial in all the monophasic *S. Typhimurium* isolates is worrying, but also expected. Resistant isolates in griffon vultures have been previously reported by Blanco (2018) and susceptible isolates of monophasic *S. Typhimurium* are rarely reported in food-producing animals in Europe (EFSA and ECDC, 2019). Furthermore, AMR to ampicillin and tetracycline as well as MDR are usually observed at extremely high levels in fattening pigs (EFSA and ECDC, 2019). Concurrently, half of the *C. lari* isolates in this study were resistant to the fluoroquinolone ciprofloxacin. Ciprofloxacin is a critically important antibiotic in human medicine and one of the limited available therapies to treat some serious infections in humans (WHO, 2016). Our results could be due to the partial metabolization of enrofloxacin —an antimicrobial frequently used in veterinary medicine— to ciprofloxacin in livestock, allowing the development of resistant bacteria that are then transmitted to vultures when feeding on carcasses (García Ovando et al., 1999; Schulz et al., 2019). In fact, the prevalence of ciprofloxacin-resistant enteropathogens is very high in food-producing animals, despite this antibiotic not being approved for veterinary medicine in Europe (EFSA and ECDC, 2019).

Vultures have been long time considered as disease mitigators. By removing decomposing organic matter from the environment, they could potentially reduce the spread of pathogens (Ogada et al., 2012). However, even if vultures clearly reduce the availability of rotten organic material, there is still a lack of research to unravel if vultures are effective pathogen regulators (Plaza et al., 2020). At the same time, due to scavenging feeding behaviour, vultures can be colonized by pathogens from a wide variety of species and are usually considered as sentinels of the environmental pathogenic burden. Our study shows that vultures share some *Salmonella* and *Campylobacter* strains with other wildlife and farm animals. The role of vultures, and birds in general, as disseminators of pathogens is also still under study (Smith et al., 2020). Even if our study reports that Eurasian griffon vultures are carriers of AMR zoonotic *Salmonella* and *Campylobacter*, there is still no scientific evidence that vultures play a role in the spread of microorganisms and bacterial resistance to other wildlife, livestock nor humans (Blanco and Díaz de Tuesta, 2021). The impact of carrying AMR bacteria is not clear in wildlife and may be of importance only for individuals that are admitted to recovery centres and require the use of antibiotics (Ramey and Ahlstrom, 2020). Also, scavengers are mostly subclinical carriers of *Salmonella* and *Campylobacter*, and are mainly considered maintenance hosts or reservoirs. However, the direct exposure to antibiotics through the consumption of medicated livestock carcasses may have detrimental effects on vulture health by unbalancing the gut microbiome and enhancing the presence of opportunistic pathogens (Pitarch et al., 2017). In fact, the chronic and indiscriminate consumption of antibiotics could lead to the development of AMR bacteria in the intestinal microbiota of vultures, but this potential problem has not been explored and deserves further investigation.

In conclusion, this study shows that wild Eurasian griffon vultures in north-eastern Spain are carriers of widespread AMR zoonotic *Salmonella* and *Campylobacter*, which in some instances may have an anthropogenic origin, due to their scavenging feeding habits. Many isolates found, such as monophasic *Salmonella Typhimurium*, are of public health concern and the amount of AMR to critically important antimicrobials for human medicine is worrying. However, more in-depth studies, for example combining high throughput genome sequencing with transmission pathways analysis, are still needed to understand the potential risk of spill-over from those wild birds to humans.

CRediT authorship contribution statement

Johan Espunyes: Investigation, Formal analysis, Writing – original draft. **Lucía Illera:** Investigation, Formal analysis, Writing – original draft. **Andrea Dias-Alves:** Investigation, Writing – review & editing. **Lourdes Lobato:** Investigation, Writing – review & editing. **Maria Puig Ribas:** Investigation, Writing – review & editing. **Alicia Manzanares:** Investigation. **Teresa Ayats:** Investigation. **Ignasi Marco:** Writing – review & editing, Conceptualization, Methodology, Validation, Supervision. **Marta Cerdà-Cuéllar:** Writing – review & editing, Conceptualization, Methodology, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.157189.

References

Antilles, N., García-Bocanegra, I., Alba-Casals, A., López-Soria, S., Pérez-Méndez, N., Saco, M., González-Solís, J., Cerdà-Cuéllar, M., 2021. Occurrence and antimicrobial resistance of zoonotic enteropathogens in gulls from southern Europe. Sci. Total Environ. 763, 143018. https://doi.org/10.1016/j.scitotenv.2020.143018.

Ayukekbong, J.A., Ntemgwa, M., Atabe, A.N., 2017. The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrob.Resist.Infect.Control 6, 47. https://doi.org/10.1186/s13756-017-0208-x.

Blanco, G., 2018. Supplementary feeding as a source of multiresistant Salmonella in endangered Egyptian vultures. Transbound. Emerg. Dis. 65, 806–816. https://doi.org/10.1111/tbed.12806.

Blanco, G., Bautista, L.M., 2020. Avian scavengers as bioindicators of antibiotic resistance due to livestock farming intensification. Int. J. Environ. Res. Public Health 17, 3620. https://doi.org/10.3390/ijerph17103620.

Blanco, G., Díaz de Tuesta, J.A., 2021. Seasonal and spatial occurrence of zoonotic Salmonella serotypes in griffon vultures at farmland environments: implications in pathogen pollution and ecosystem services and disservices. Sci. Total Environ. 758, 143681. https://doi.org/10.1016/j.scitotenv.2020.143681.

Blanco, G., López-Hernández, I., Morinha, F., López-Cerero, L., 2020. Intensive farming as a source of bacterial resistance to antimicrobial agents in sedentary and migratory vultures: implications for local and transboundary spread. Sci. Total Environ. 739, 140356. https://doi.org/10.1016/j.scitotenv.2020.140356.

Botti, V., Valérie Navillod, F., Domenis, L., Orusa, R., Pepe, E., Robetto, S., Guidetti, C., 2013. Salmonella spp. and antibiotic-resistant strains in wild mammals and birds in north-western Italy from 2002 to 2010. Vet. Ital. 49, 195–202. 10.12834/VetIt.2013.492.201.208.

Cerdà-Cuéllar, M., Moré, E., Ayats, T., Aguilera, M., Muñoz-González, S., Antilles, N., Ryan, P.G., González-Solís, J., 2019. Do humans spread zoonotic enteric bacteria in Antarctica? Sci. Total Environ. 654, 190–196. https://doi.org/10.1016/j.scitotenv.2018.10.272.

Daoust, P.Y., Prescott, J.F., 2007. Salmonellosis. In: Thomas, N.J., Hunter, D.B., Atkinson, C.T. (Eds.), Infectious Diseases of Wild Birds. Blackwell Publishing Ltd, Oxford, UK, pp. 270–288.

Del Moral, J.C., Molina, B. (Eds.), 2018. El buitre leonado en España, población reproductora en 2018 y método de censo Madrid.

Donázar, J.A., Cortés-Avizanda, A., Carrete, M., 2010. Dietary shifts in two vultures after the demise of supplementary feeding stations: consequences of the EU sanitary legislation. Eur. J. Wildl. Res. 56, 613–621. https://doi.org/10.1007/s10344-009-0358-0.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2019. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. EFSA J. 2019, 17. https://doi.org/10.2903/j.efsa.2019.5598.

EFSA (European Food Safety Authority), ECDC (European Centre for Disease Prevention and Control), 2021. The European Union One Health 2019 zoonoses report. EFSA J. 19 (2), e06406. https://doi.org/10.2903/j.efsa.2021.6406.

Eng, S.-K., Pusparajah, P., Ab Mutalib, N.-S., Ser, H.-L., Chan, K.-G., Lee, L.-H., 2015. Salmonella: a review on pathogenesis, epidemiology and antibiotic resistance. Front.Life Sci. 8, 284–293. https://doi.org/10.1080/21553769.2015.1051243.

García Ovando, H., Gorla, N., Luders, C., Poloni, G., Errecalde, C., Prieto, G., Puelles, I., 1999. Comparative pharmacokinetics of enrofloxacin and ciprofloxacin in chickens. J. Vet. Pharmacol. Ther. 22, 209–212. https://doi.org/10.1046/j.1365-2885.1999.00211.x.

Gómez-Ramírez, P., Blanco, G., García-Fernández, A.J., 2020. Validation of multi-residue method for quantification of antibiotics and NSAIDs in avian scavengers by using small amounts of plasma in HPLC-MS-TOF. Int. J. Environ. Res. Public Health 17, 4058. https://doi.org/10.3390/ijerph17114058.

Grimont, P., Weill, F.-X., 2007. Antigenic Formulae of the Salmonella servovars. 9th edition. WHO Collaborating Centre for reference and research on Salmonella (WHOCC-SALM), Paris.

Igwaran, A., Okoh, A.I., 2019. Human campylobacteriosis: a public health concern of global importance. Heliyon 5, e02814. https://doi.org/10.1016/j.heliyon.2019.e02814.

Jurado-Tarifa, E., Torralbo, A., Borge, C., Cerdà-Cuéllar, M., Ayats, T., Carbonero, A., García-Bocanegra, I., 2016. Genetic diversity and antimicrobial resistance of Campylobacter and Salmonella strains isolated from decoys and raptors. Comp. Immunol. Microbiol. Infect. Dis. 48, 14–21. https://doi.org/10.1016/j.cimid.2016.07.003.

Klena, J.D., Parker, C.T., Knibb, K., Ibbitt, J.C., Devane, P.M.L., Horn, S.T., Miller, W.G., Konkel, M.E., 2004. Differentiation of Campylobacter coli, Campylobacter jejuni, Campylobacter lari, and Campylobacter upsaliensis by a multiplex PCR developed from the nucleotide sequence of the lipid a gene lpxA. J. Clin. Microbiol. 42, 5549–5557. https://doi.org/10.1128/JCM.42.12.5549-5557.2004.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A.K.M., Wertheim, H.F.L., Sumpradit, N., Vlieghe, E., Hara, G.L., Gould, I.M., Goossens, H., Greko, C., So, A.D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A.Q., Qamar, F.N., Mir, F., Kariuki, S., Bhutta, Z.A., Coates, A., Bergstrom, R., Wright, G.D., Brown, E.D., Cars, O., 2013. Antibiotic resistance—the need for global solutions. Lancet Infect. Dis. 13, 1057–1098. https://doi.org/10.1016/S1473-3099(13)70318-9.

Margalida, A., Donázar, J.A., Carrete, M., Sánchez-Zapata, J.A., 2010. Sanitary versus environmental policies: fitting together two pieces of the puzzle of European vulture conservation. J. Appl. Ecol. 47, 931–935. https://doi.org/10.1111/j.1365-2664.2010.01835.x.

Marin, C., Palomeque, M.D., Marco-Jiménez, F., Vega, S., 2014. Wild griffon vultures (Gyps fulvus) as a source of Salmonella and Campylobacter in eastern Spain. PLoS ONE 9, 1–5. https://doi.org/10.1371/journal.pone.0094191.

Marin, C., Torres, C., Marco-Jiménez, F., Cerdà-Cuéllar, M., Sevilla, S., Ayats, T., Vega, S., 2018. Supplementary feeding stations for conservation of vultures could be an important source of monophasic Salmonella typhimurium 1,4,[5],12:i:-. Sci. Total Environ. 636, 449–455. https://doi.org/10.1016/j.scitotenv.2018.04.310.

Matejczyk, M., Płaza, G.A., Nałęcz-Jawecki, G., Ulfig, K., Markowska-Szczupak, A., 2011. Estimation of the environmental risk posed by landfills using chemical, microbiological and ecotoxicological testing of leachates. Chemosphere 82, 1017–1023. https://doi.org/10.1016/j.chemosphere.2010.10.066.

McEwen, S.A., Collignon, P.J., 2018. Antimicrobial resistance: a one health perspective. Microbiol.Spectr. 6, 255–260. https://doi.org/10.1128/microbiolspec.ARBA-0009-2017.

Mentaberre, G., Porrero, M.C., Navarro-Gonzalez, N., Serrano, E., Domínguez, L., Lavín, S., 2013. Cattle drive Salmonella infection in the wildlife-livestock interface. Zoonoses Public Health 60, 510–518. https://doi.org/10.1111/zph.12028.

Minias, P., 2020. Contrasting patterns of Campylobacter and Salmonella distribution in wild birds: a comparative analysis. J. Avian Biol. 51. https://doi.org/10.1111/jav.02426 jav.02426.

Molina-Lopez, R.A., Valverdu, N., Martin, M., Mateu, E., Obon, E., Cerda-Cuellar, M., Darwich, L., 2011. Wild raptors as carriers of antimicrobial-resistant Salmonella and Campylobacter strains. Vet. Rec. 168, 565. https://doi.org/10.1136/vr.c7123.

Molina-López, R.A., Vidal, A., Obón, E., Martín, M., Darwich, L., 2015. Multidrug-resistant Salmonella enterica Serovar Typhimurium monophasic variant 4,12:i:- isolated from asymptomatic wildlife in a Catalonian Wildlife Rehabilitation Center, Spain. J. Wildl. Dis. 51, 759–763. https://doi.org/10.7589/2015-01-019.

Moore, J.E., Barton, M.D., Blair, I.S., Corcoran, D., Dooley, J.S.G., Fanning, S., Kempf, I., Lastovica, A.J., Lowery, C.J., Matsuda, M., McDowell, D.A., McMahon, A., Millar, B.C., Rao, J.R., Rooney, P.J., Seal, B.S., Snelling, W.J., Tolba, O., 2006. The epidemiology of antibiotic resistance in Campylobacter. Microbes Infect. 8, 1955–1966. https://doi.org/10.1016/j.micinf.2005.12.030.

Moré, E., Ayats, T., Ryan, P.G., Naicker, P.R., Keddy, K.H., Gaglio, D., Witteveen, M., Cerdà-Cuéllar, M., 2017. Seabirds (Laridae) as a source of Campylobacter spp., Salmonella spp. and antimicrobial resistance in South Africa. Environ. Microbiol. 19, 4164–4176. https://doi.org/10.1111/1462-2920.13874.

Musson, S.E., Townsend, T.G., 2009. Pharmaceutical compound content of municipal solid waste. J. Hazard. Mater. 162, 730–735. https://doi.org/10.1016/j.jhazmat.2008.05.089.

Ogada, D.L., Torchin, M.E., Kinnaird, M.F., Ezenwa, V.O., 2012. Effects of vulture declines on facultative scavengers and potential implications for mammalian disease transmission. Conserv. Biol. 26, 453–460. https://doi.org/10.1111/j.1523-1739.2012.01827.x.

O'Neill, J., 2016. Tackling Drug-resistant Infections Globally: Final Report and Recommendations.

Pitarch, A., Gil, C., Blanco, G., 2017. Oral mycoses in avian scavengers exposed to antibiotics from livestock farming. Sci. Total Environ. 605–606, 139–146. https://doi.org/10.1016/j.scitotenv.2017.06.144.

Plaza, P.I., Blanco, G., Madariaga, M.J., Boeri, E., Teijeiro, M.L., Bianco, G., Lambertucci, S.A., 2019. Scavenger birds exploiting rubbish dumps: pathogens at the gates. Transbound. Emerg. Dis. 66, 873–881. https://doi.org/10.1111/tbed.13097.

Plaza, P.I., Blanco, G., Lambertucci, S.A., 2020. Implications of bacterial, viral and mycotic microorganisms in vultures for wildlife conservation, ecosystem services and public health. Ibis 162, 1109–1124. https://doi.org/10.1111/ibi.12865.

Ramey, A.M., Ahlstrom, C.A., 2020. Antibiotic resistant bacteria in wildlife: perspectives on trends, acquisition and dissemination, data gaps, and future directions. J. Wildl. Dis. 56, 1. https://doi.org/10.7589/2019-04-099.

Ramos, R., Cerda-Cuellar, M., Ramirez, F., Jover, L., Ruiz, X., 2010. Influence of refuse sites on the prevalence of Campylobacter spp. and Salmonella serovars in seagulls. Appl. Environ. Microbiol. 76, 3052–3056. https://doi.org/10.1128/AEM.02524-09.

Reche, M.P., Jiménez, P.A., Alvarez, F., García de los Ríos, J.E., Rojas, A.M., de Pedro, P., 2003. Incidence of Salmonellae in captive and wild free-living raptorial birds in Central Spain. J. Vet. Med. Ser. B 50, 42–44. https://doi.org/10.1046/j.1439-0450.2003.00623.x.

Schulz, J., Kemper, N., Hartung, J., Janusch, F., Mohring, S.A.I., Hamscher, G., 2019. Analysis of fluoroquinolones in dusts from intensive livestock farming and the co-occurrence of fluoroquinolone-resistant Escherichia coli. Sci. Rep. 9, 5117. https://doi.org/10.1038/s41598-019-41528-z.

Sevilla, E., Marín, C., Delgado-Blas, J.F., González-Zorn, B., Vega, S., Kuijper, E., Bolea, R., Mainar-Jaime, R.C., 2020. Wild griffon vultures (Gyps fulvus) fed at supplementary feeding stations: potential carriers of pig pathogens and pig-derived antimicrobial resistance? Transbound. Emerg. Dis. 1–11. https://doi.org/10.1111/tbed.13470.

Smith, O.M., Snyder, W.E., Owen, J.P., Sciences, B., Box, P.O., 2020. Are We Overestimating Risk of Enteric Pathogen Spillover From Wild Birds to Humans ?. 95, pp. 652–679. https://doi.org/10.1111/brv.12581

Sun, H., Wan, Y., Du, P., Bai, L., 2020. The epidemiology of monophasic Salmonella Typhimurium. Foodborne Pathog. Dis. 17, 87–97. https://doi.org/10.1089/fpd.2019.2676.

Troxler, S., Hess, C., Konicek, C., Knotek, Z., Barták, P., Hess, M., 2017. Microdilution testing reveals considerable and diverse antimicrobial resistance of Escherichia coli, thermophilic Campylobacter spp. and Salmonella spp. isolated from wild birds present in urban areas. Eur. J. Wildl. Res. 63, 68. https://doi.org/10.1007/s10344-017-1125-2.

Vittecoq, M., Godreuil, S., Prugnolle, F., Durand, P., Brazier, L., Renaud, N., Arnal, A., Aberkane, S., Jean-Pierre, H., Gauthier-Clerc, M., Thomas, F., Renaud, F., 2016. Antimicrobial resistance in wildlife. J. Appl. Ecol. 53, 519–529. https://doi.org/10.1111/1365-2664.12596.

World Health Organization, 2016. Critically Important Antimicrobials for Human Medicine: Ranking of Antimicrobial Agents for Risk Management of Antimicrobial Resistance Due to Non-human Use Geneva.

World Health Organization, 2017. WHO Guidelines on Use of Medically Important Antimicrobials in Food-producing Animals. World Health Organization.