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Genetic diversity and antimicrobial resistance of *Campylobacter* and *Salmonella* strains isolated from decoys and raptors

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ABSTRACT

Infections caused by thermotolerant *Campylobacter* spp. and *Salmonella* spp. are the leading causes of human gastroenteritis worldwide. Wild birds can act as reservoirs of both pathogens. A survey was carried out to determine the prevalence, genetic diversity and antimicrobial resistance of thermotolerant *Campylobacter* and *Salmonella* in waterfowl used as decoys and wild raptors in Andalusia (Southern Spain). The overall prevalence detected for *Campylobacter* was 5.9% (18/306; $\text{CI}_{95\%}$: 3.25–8.52) in decoys and 2.3% (9/387; $\text{CI}_{95\%}$: 0.82–3.83) in wild raptors. Isolates were identified as *C. jejuni*, *C. coli* and *C. lari* in both bird groups. *Salmonella* was isolated in 3.3% (10/306; $\text{CI}_{95\%}$: 2.3–4.3) and 4.6% (18/394; $\text{CI}_{95\%}$: 3.5–5.6) of the decoys and raptors, respectively. *Salmonella* Enteritidis and Typhimurium were the most frequently identified serovars, although *Salmonella* serovars Anatum, Bredeney, London and Mikawasima were also isolated. Pulsed-field gel electrophoresis analysis of isolates showed higher genetic diversity within *Campylobacter* species compared to *Salmonella* serovars. *Campylobacter* isolates showed resistance to gentamicin, ciprofloxacin and tetracycline, while resistance to erythromycin and tetracycline was found in *Salmonella* isolates. The results indicate that both decoys and raptors can act as natural carriers of *Campylobacter* and *Salmonella* in Spain, which may have important implications for public and animal health.

1. Introduction

Campylobacteriosis and salmonellosis are the most prevalent zoonotic diseases, with 236,851 and 88,715 human cases reported in 2014 in the European Union, respectively [1]. Thermotolerant *Campylobacter* and *Salmonella* cause enteric disorders in humans characterized by fever, diarrhea, abdominal pain, nausea and vomiting. These zoonotic bacteria are mainly transmitted to humans through the consumption of contaminated meat, water, milk, eggs or by cross-contamination [2]. Wild birds are considered one of the main reservoirs for both pathogens, being implicated in the contamination of water and the environment through their faeces. Moreover, birds play an important role in the geographical spread of these bacteria due to their ability to cover long distances during their foraging and migration movements [3,4].

Decoys are domestic anatid species, including those from the orders Anseriformes and Charadriiformes, which are reared in backyards to use as lures for small game purposes. Hunting of waterfowl using live decoys is a traditional activity of cultural, social and economic importance particularly in some countries from the Mediterranean Basin [5]. In Spain, more than 167,000 anatids are annually hunted, with an economic value exceeding 1.3 million euros [6]. Hunting decoys is carried out in wetlands which are stopovers for migratory anatids through their flyways during the hunting season (autumn and winter periods). During this period, decoys share habitat with wild waterfowl and therefore there may be a risk of pathogens transmission between both groups of birds. Since the direct interaction between decoys and wild birds is limited, the risk of pathogens transmission is mainly indirect by water and environment contaminated through their faeces. Moreover, because of the direct contact between decoys and hunters, this species may also be implicated in the zoonotic pathogens transmission.

Raptors are on the top of the food chain, being considered usefull species to monitor pathogens in the environment [7,8]. This

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group of birds became infected by *Campylobacter* and *Salmonella* mainly through direct eating of prey [9]. Even though raptor species are considered asymptomatic carriers of both zoonotic bacteria [8,10,11], the number of studies on these host species is still very limited. On the other hand, the antimicrobial resistances in domestic and wild birds as well as the spread of these strains within different ecosystems are considered a human and veterinary medicine threat [12].

The main goal of the present study was to assess the role of two groups of bird species, hunting decoys and wild raptors, in the epidemiology of thermotolerant *Campylobacter* and *Salmonella,* which may be of animal and public health concern. To achieve this aim, the prevalence, genetic diversity and antimicrobial resistance of both pathogens were determined.

2. Material and methods

2.1. Sampling

Based on the number of decoys in Andalusia (2319) (36°N–38°60′N, 1°75′W–7°25′W), an estimated prevalence of 50% (which provides the highest sample size in studies with unknown prevalence [13]), a desired precision of ±5% and a confidence level of 95%, the aim was to sample 330 decoys. A total of 306 birds, including 227 greyland geese (*Anser anser*) and 79 mallard ducks (*Anas platyrhynchos*), were finally collected during the hunting season 2011–2012 (from October to January) (Fig. 1). The sampling was stratified by provinces according to the number of decoy flocks in each province (Table 1). The flocks were selected using a simple random sampling from the census of flocks obtained from the Regional Government of Andalusia. Within each flock one to 24 anatids (median = 9) were sampled. Samples were collected using swabs placed in tubes containing Amies transport medium (Deltalab, Rubí, Spain).

Additionally, 394 wild raptors of 22 different species (15 diurnal and 7 nocturnal raptors) (Table 2) were sampled between 2012 and 2014. A convenience sampling was carried out from raptors admitted in different wildlife rehabilitation centres of Andalusia. The raptors analyzed died before or after their admission in the centre, or were euthanized because of humanitarian reasons (no raptor was sacrificed just to be included in our study). Raptors were necropsied and aproximately two grams of cecal and rectal contents were collected whenever it was possible. Due to the small size of some species, about 5 g of a pool of cecum, colon and rectum contents were used for the microbiological analysis. Data of the individual identification, species, sampling date, location and census of decoys' flock were recorded. Individual data related with raptors (species, sex, age, type of raptor (diurnal and nocturnal), reason for admission, municipalities and province where they were rescued) were provided by the wildlife rehabilitation centres. Any of the sampled birds showed clinical signs compatible with campylobacteriosis or salmonellosis.

2.2. Bacterial isolation and identification

Cloacal swabs from decoys and fecal contents from raptors were streaked onto *Campylobacter* Blood-Free Selective Agar Base (Oxoid®, Basingstoke, Hampshire, United Kingdom) with CCDA Selective Supplement (Oxoid®SR0155, Basingstoke, UK). After 48 h of incubation at 42 °C in a CO_2-enriched atmosphere achieved with AnaeroGen sachets (Oxoid®, Basingstoke, UK), colonies that exhibited morphology compatible with *Campylobacter* were cultured on blood agar and incubated under the same conditions as described above. These selected isolates were confirmed by biochemical

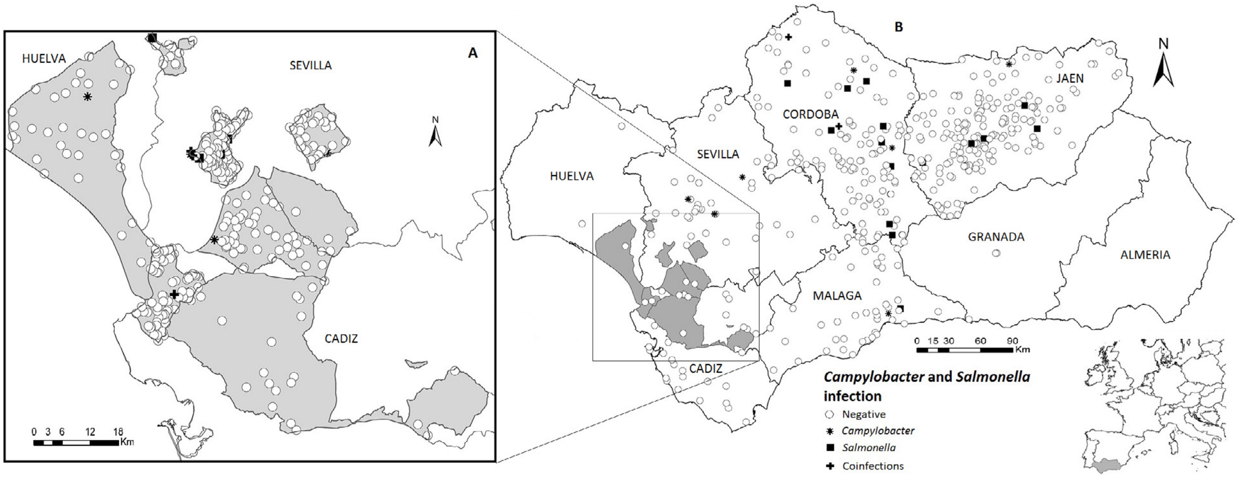

Fig. 1. Map of Andalusia (Southern Spain), showing the spatial distribution of *Campylobacter* spp. and *Salmonella* spp. in decoy flocks (A) and raptors (B). White dots indicate negative results to both *Campylobacter* spp. and *Salmonella* spp. infection. Black squares, black crosses and black asterisks indicate positive results to *Campylobacter* spp. and *Salmonella* spp. and coinfections, respectively.

Table 1
Flock and individual prevalences of *Campylobacter* spp. and *Salmonella* spp. in decoys in Andalusia (Southern Spain).

Provinces	Flock census[a]	Decoy census[a]	*Campylobacter* spp.		*Salmonella* spp.	
			Flock prevalence (No. positive/No. examined)	Individual prevalence (No. positive/No. examined)	Flock prevalence (No. positive/No. examined)	Individual prevalence (No. positive/No. examined)
Huelva	11	198	50.0% (1/2)	23.5% (8/34)	0.0% (0/2)	0.0% (0/34)
Seville	94	1553	29.4% (5/17)	4.4% (8/183)	35.3% (6/17)	4.4% (8/183)
Cadiz	38	568	9.0% (1/11)	2.2% (2/89)	9.0% (1/11)	2.2% (2/89)
Total	143	2319	23.3% (7/30)	5.8% (18/306)	23.3% (7/30)	3.2% (10/306)

[a] Total flock and decoy census in Andalusia.