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Short communication

Food-borne zoonotic pathogens and antimicrobial resistance of indicator bacteria in urban wild boars in Barcelona, Spain

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ABSTRACT

Wildlife is increasingly abundant in urban environments, but little is known about the zoonotic pathogens carried by these populations. Urban wild boars are of particular concern because this species is well-known as a pathogen reservoir, and thus, we studied selected zoonotic pathogens in urban wild boars in Barcelona, Spain ($n = 41$). *Salmonella enterica* was found in 5.00% (95% CI 0.61–16.91) and *Campylobacter coli* in 4.88% (95% CI 0.6–16.53) of the animals. *E. coli* O157:H7 and *C. jejuni* were not found. Other thermophilic *Campylobacter* were moderately prevalent (19.51%, 95% CI 8.82–34.87). Additionally, we screened for antimicrobial resistance in indicator bacteria: resistance was most frequent in *Enterococcus faecium* (95% of the isolates were resistant to at least one antimicrobial agent), followed by *Enterococcus faecalis* (50%) and *Escherichia coli* (10%). For the first time resistance to linezolid in bacteria carried by wildlife is reported. These findings pose a concern for public health, and thus, further research is needed on wildlife in urban environments.

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1. Introduction

Urbanization of natural areas is one of the key factors driving disease emergence (Daszak et al., 2000) and in recent decades urban and suburban landscapes have been infiltrated by numerous animal species previously considered intolerant of human activity, especially songbirds but also squirrels and other small to medium-sized mammals (Ditchkoff et al., 2006). Thus, new interactions between animal hosts, zoonotic pathogens and humans may occur due to the increasing urbanization of wild areas.

Wild boar (*Sus scrofa*) is a worldwide distributed species that can thrive in areas that are heavily influenced by human activity (Schley and Roper, 2003). The Western European population has increased over the past several decades and in the USA feral hogs are also in expansion (Massei et al., 2011). Wild boar habituation to urban areas has happened in certain cities in countries as diverse as Spain, Germany and Poland (Cahill et al., 2010) and because of this a public health concern may arise. In the case of Barcelona (Spain), groups of wild boars feed and defecate in public parks, private gardens and other green areas, and drink from fountains and swimming-pools. This underscores the need for knowledge about this population, since pathogens can be transmitted either through direct contact or indirectly via infected urine or faeces in places frequented by people or pets. Also, transmission may occur from people to wild boars through the consumption of domestic rubbish and other uncontrolled waste. In fact, artificial feeding (either directly by people or indirectly from bins) is according to Cahill et al. (2010) the main reason for habituation of this wild boar population.

Jansen et al. (2007) found leptospirosis among the urban wild boar population in Berlin; however, wild boars

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in other cities have not been studied for this or other pathogens. In the current work, we aim to contribute to the knowledge of the health status of wild boars from Collserola Natural Park (Barcelona, Spain). Due to its zoonotic importance in the European Union (EFSA, 2013), the selected pathogens were *Salmonella enterica*, *Escherichia coli* O157:H7, *Campylobacter coli*, *Campylobacter jejuni* and other thermophilic *Campylobacter* species. Indicator bacteria tested for antimicrobial susceptibility were *Escherichia coli*, *Enterococcus faecium* and *Enterococcus faecalis.*

2. Materials and methods

2.1. Study area

The study area is the Serra de Collserola Natural Park in Barcelona (NE Spain), a massif that rises over the Barcelona Metropolitan Area. This is an 8000 ha protected area where forest predominates and is widely frequented by the huge human population (around 3,225,000) inhabiting the metropolitan area.

2.2. Animal sampling

Forty-one wild boars from the metropolitan area of Barcelona were necropsied in the facilities of the Veterinary Medicine Faculty (Universitat Autònoma de Barcelona, Spain) from September 2010 to August 2011. Faeces were taken directly from the rectum and stored in a sterile container. Containers were placed in refrigeration and sent to the laboratory within the subsequent 24 h.

2.3. Microbiological analyses

2.3.1. Salmonella enterica

Cultures of *Salmonella* were performed according to ISO 6579:2002 Annex D (ISO, 2007), which is the method recommended by the European Union Reference Laboratory for *Salmonella* in faecal and environmental samples. See Navarro-Gonzalez et al. (2012) for more details on the identification and serotyping of *Salmonella enterica*.

2.3.2. Thermophilic Campylobacter

For isolation and detection of thermophilic *Campylobacter* from the samples under investigation, direct plating of stool samples was performed as described in Ugarte-Ruiz et al. (2012). Further identification using conventional PCR was also performed as described by these authors.

2.3.3. E. coli O157:H7

Faecal samples were processed according to the ISO 16.654:2001 protocol to obtain *E. coli* O157 (ISO, 2001). One suspected colony per sample was confirmed by PCR as *E. coli* O157:H7 as described previously (Desmarchelier et al., 1998).

2.3.4. Indicator E. coli

In total 25 g of faeces were diluted in buffered peptone water (225 ml). Once diluted, one loop was cultured on MacConkey agar (direct plating) at 37 °C for 18–20 h. One compatible colony per plate was selected and confirmed by PCR (Heininger et al., 1999). This confirmed colony of indicator *E. coli* (i.e. one clon per animal) was tested for antimicrobial susceptibility.

2.3.5. Enterococcus spp.

Enterococcus spp. were cultured in M-Enterococcus agar and one colony per sample was selected. Confirmation was based on PCR for *Enterococcus* (Dutka-Malen et al., 1995).

2.3.6. Antimicrobial susceptibility testing

2.3.6.1. Escherichia coli and Salmonella enterica. Antimicrobial susceptibility of *Salmonella enterica* was tested as reported in Navarro-Gonzalez et al. (2012). The same methodology was used for *E. coli*, however, cut-off values differ for some agents: amoxicillin-clavulanate (cut-off value = 17 mm), cefoxitin (19 mm), amikacin (18 mm), imipenem (24 mm), aztreonam (27 mm), sulfamethoxazole (64 μg/ml), cefotaxime (0.25 μg/ml), ceftazidime (0.5 μg/ml), kanamycin (8 μg/ml), colistin (2 μg/ml). We have preferentially used those values reported by the EFSA (2012), and those from EUCAST when the former was not available.

2.3.6.2. Enterococcus faecium and Enterococcus faecalis. The broth micro-dilution method was performed to determine susceptibility to the following agents with the epidemiological cut-off values: chloramphenicol (32 μg/ml), florphenicol (4 μg/ml), ampicillin (4 μg/ml), trimethoprim (8 μg/ml), ciprofloxacin (4 μg/ml), vancomycin (4 μg/ml), erythromycin (4 μg/ml), quinupristin-dalfopristin (*Ent. faecalis*: 16 μg/ml, *Ent. faecium*: 1 μg/ml), tetracycline (4 μg/ml), linezolid (4 μg/ml), penicillin (8 μg/ml), and the high-level resistance to gentamicin (500 μg/ml) and streptomycin (2000 μg/ml). We have preferentially used those values reported by the EFSA (2012), and those from EUCAST when the former was not available.

2.3.6.3. Statistical analysis. The 95% confidence interval was calculated with R Software (R Development Core Team, 2013), specifically with package epiR (Stevenson et al., 2012).

3. Results and discussion

3.1. Zoonotic pathogens

Results are shown in Table 1. The *Salmonella enterica* isolates were identified as serotype Anatum and Corvallis, and were susceptible to all antimicrobial agents tested. Remarkable findings are the presence of *Salmonella* (although at relatively low prevalence), *Campylobacter coli* and other thermophilic *Campylobacter*. The detection of *Campylobacter* spp. and *Salmonella enterica* in urban wild boars poses a concern for public health since these are the most frequent zoonoses in the European Union (EFSA, 2013). In urban wildlife, prevalence of diseases is sometimes greater than that found in rural habitats, due to a greater food availability leading to higher survival, increased density and aggregation (Ditchkoff et al.,

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