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Campylobacter infection in wild artiodactyl species from southern Spain: Occurrence, risk factors and antimicrobial susceptibility

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ABSTRACT

A cross-sectional study was performed to assess the occurrence of *Campylobacter* species and to identify potential associated risk factors for wild artiodactyl species in southern Spain. *Campylobacter* species were isolated in 55 of 363 (15.2%) faecal samples. *Campylobacter* was identified in faeces from wild boar (49/126; 38.9%), red deer (5/179; 2.8%) and mouflon (1/13; 7.7%) but not from fallow deer (0/45). The isolated *Campylobacter* species were identified as *C. jejuni* (2 isolates; 3.6%), *C. coli* (11 isolates; 20.0%) and *C. lanienae* (37 isolates; 67.3%). Five isolates (9.1%) could not be identified at the species level. This report is the first to describe *C. lanienae* infection in wild ruminant species. Resistance to erythromycin (4.8%), ciprofloxacin (37.5%), tetracycline (52.9%) and streptomycin (55%) were detected. *C. lanienae* presented a significantly higher number of susceptible isolates to ciprofloxacin and tetracycline than *C. coli*. Due to the low number of positive wild ruminants, a Generalised Estimating Equations model was only carried out for wild boar. The model indicated that the risk factors associated with *Campylobacter* infection were the density of wild boar (>10/100 ha) (OR: 3.05; $CI_{95\%}$: 2.2–4.3), the presence of artificial waterholes (OR: 3.67; $CI_{95\%}$: 1.3–10.5) and the winter season (OR: 3.30; $CI_{95\%}$: 1.9–5.8). *Campylobacter* infection is widespread in wild boar populations in southern Spain. These findings suggest that wild artiodactyls, particularly wild boar, constitute a reservoir of *Campylobacter* species, including resistant and multi-resistant strains, which may be of public health concern.

1. Introduction

With 212,064 confirmed cases, campylobacteriosis was the most frequent zoonosis in the European Union in 2010, accounting for over 65% of all reported cases of zoonoses [1]. *Campylobacter* infection in animals is usually asymptomatic, but it is the leading cause of human gastroenteritis around the world. *Campylobacter jejuni* and *Campylobacter coli* are the primary species involved in human cases [2].

The handling and consumption of chicken are considered the most important sources for *Campylobacter* infection in humans [3]. However, even though the prevalence of *Campylobacter* infection in broilers did not exhibit major variations during the last 5 years in the European Union, the number of human cases increased considerably in the same period [1]. Consequently, other risk factors not related to chicken, such as the handling and consumption of meat from other species, contact with both pets and livestock or ingestion of untreated milk and water have been suggested to be involved in the increase of campylobacteriosis in human [3,4].

The role of domestic reservoirs for zoonotic *Campylobacter* species has been thoroughly studied [2,5].

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Although several authors have described *Campylobacter* infections in wild birds [6], the number of studies of this zoonotic agent in wild mammals is still very limited. Enteric disorders in wild ruminants caused by *Campylobacter* species have been previously reported [7].

Wild artiodactyl species are important not only due to their role as natural reservoirs of infectious diseases but also because their zoonotic association with the consumption and handling of wild artiodactyl-derived products [8]. The meat of hunted wild artiodactyls is generally destined for human consumption. Consequently, it could act as source of infection of *Campylobacter* species for humans. Moreover, hunting by shooting causes frequently contamination of the carcass due to breakage of the gut, increasing the risk of transmission of enteric agents such as *Campylobacter* [9].

Differences have been reported in prevalence of *Campylobacter* in wild artiodactyls. Previous studies in deer species reported prevalence ranging between 0% and 6% [10,11]. For wild boar, prevalence ranged between 0% and 66% [11,12]. No studies on risk factors for this agent in wild animals have been found.

Resistance to antimicrobials in *Campylobacter* species has been widely described in humans [13,14], domestic and wild species animals [15,16], and the environment [17]. Calculation of the Minimal Inhibitory Concentration (MIC) by the agar plate dilution method is the technique recommended by the National Committee for Clinical Laboratory Standards (NCCLS) to determine antimicrobial resistance in *Campylobacter* species [18].

To elucidate the role of wildlife as reservoirs of *Campylobacter* species and sources of infection for humans, we conducted a study to investigate the occurrence, risk factors and antimicrobial resistance of this enteropathogen in wild artiodactyl species in southern Spain.

2. Materials and methods

A cross-sectional study was carried out in 18 hunting estates (31,467 ha) located in Córdoba (4°310′ W, 37°250′ N), southern Spain between October 2011 and February 2012. This region is characterised by a typical continental Mediterranean climate with hot, dry summers and mild winters. The habitat is typically Mediterranean, characterised by oak (*Quercus ilex*) forests, scrublands, scattered pastures and small areas with cereal crops. Red deer, fallow deer and wild boar are widely distributed in this region, frequently sharing habitats with domestic ruminants (cattle, sheep and goat) and Iberian pigs. The study area presents one of the highest wild artiodactyl densities in Spain due to intensive big game management [19,20].

A hunting estate is defined as a continuous area of land that can be used for hunting and is declared as such by the competent environmental government at the request of the owner or whoever who owns the hunting rights on the ground. In order to represent the current hunting conditions in southern Spain, eight of the hunting estates were open, and ten were fenced estates.

A total of 363 fresh faecal samples from hunted wild artiodactyl species, including 179 red deer (*Cervus elaphus*), 45 fallow deer (*Dama dama*), 13 mouflon (*Ovis musimon*) and 126 wild boar (*Sus scrofa*) were collected. Between 10 and 46 (mean = 20.2) individuals were randomly selected in each hunting estate. Between 3 and 5 g of faecal content was obtained from the intestine or rectum and introduced into sterile tubes. All samples were refrigerated, transported to the laboratory and processed within 48 h after sample collection. The animals were classified into the following three age groups based on tooth replacement: juveniles (<1 year old), sub-adults (between 1 and 2 years old) and adults (>2 years old) [21].

For each faecal sample, one swab was soaked and streaked onto *Campylobacter* blood-free selective agar (Oxoid®, Basingstoke, Hampshire, United Kingdom) with CCDA (Cefoperazone and Amphotericin B) supplement (Oxoid®). After 24–48 h of incubation at 42 °C in a CO_2-enriched atmosphere (using AnaeroGen sachets, Oxoid®), isolates compatible with *Campylobacter* were phenotypically identified by Gram staining, motility in dark field microscopy and oxidase and catalase testing. The isolates phenotypically classified as *Campylobacter* were stored at −80 °C until required for molecular identification in a semi-solid medium made up of bacteriological agar, nutrient broth, glycerol, pyruvate, yeast extract and *Campylobacter* growth supplement (FBP) (Oxoid® SR 0084). This medium increases the oxygen tolerance of *Campylobacter* isolates. DNA extraction from the positive cultures was performed by heating (80 °C) and then freezing (−20 °C) with phenol and chloroform following the protocol described by Sambrook and Russell [22].

A multiplex PCR assay was performed for molecular identification as previously described [23]. Primers for the *Campylobacter* genus (target gene 16S rRNA) [24], *C. jejuni* (target gene cj0414) [25], *C. coli* (target gen ask) [26], *C. hyointestinalis* (target gen 23S rRNA) [27], *C. lari* (target gen glyA) [25], *C. foetus* (target gen cstA) [23,28] and *C. upsaliensis* (target gene lpxA) [23] were used. Additionally, conventional PCR for *C. ureolyticus* (target gen hsp60) [29] and another conventional PCR for *C. lanienae* (target gene 16S rRNA) [30] were performed.

The MIC was calculated by the agar plate dilution method for *Campylobacter* spp. following the protocol described by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) [31]. Susceptible isolates were determined according the procedures of the EUCAST, using the Ecoff for *C. coli* for all the isolates tested. Ciprofloxacin (CIP), erythromycin (ERY), streptomycin (STR) and tetracycline (TET) were tested on 22 of the isolates obtained in the present study (11 isolates of *C. coli* and 11 of *C. lanienae*). Fourteen of these isolates were obtained from wild boar and 8 from red deer. The reference strain *C. jejuni* ATCC 33,560 was used for quality control. The Chi squared test was used to evaluate differences in resistance between *C. lanienae* and *C. coli*. Additionally, the MIC_{mode} (the most frequent MIC value) and the MIC_{90} and MIC_{50} (MIC value that inhibits the growth of the 90% and 50% of the isolates, respectively) were determined.

Additionally, an epidemiological questionnaire including data on the animals sampled and the hunting estates was completed by direct interview with gamekeepers at