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Prevalence of Shiga toxin-producing *Escherichia coli*, *Salmonella* spp. and *Campylobacter* spp. in large game animals intended for consumption: Relationship with management practices and livestock influence

S. Díaz-Sánchez [a,*], S. Sánchez [b], S. Herrera-León [b], C. Porrero [c], J. Blanco [d], G. Dahbi [d], J.E. Blanco [d], A. Mora [d], R. Mateo [a], I. Hanning [e], D. Vidal [f]

[a] *Instituto de Investigación en Recursos Cinegéticos IREC, Ronda de Toledo s/n (CSIC-UCLM-JCCM), 13005 Ciudad Real, Spain*
[b] *Laboratorio de Enterobacterias, Servicio de Bacteriología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, 28220 Madrid, Spain*
[c] *Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Servicio de Vigilancia de Zoonosis de Transmisión Alimentaria y Resistencia a Antibióticos, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain*
[d] *Laboratorio de Referencia de E. coli (LREC), Departamento de Microbiología y Parasitología, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain*
[e] *Department of Food Science and Technology, University of Tennessee, Knoxville, TN, USA*
[f] *Departamento de Ciencias Médicas, Facultad de Medicina, Edificio Polivalente, Universidad de Castilla-La Mancha, Camino de Moledores s/n, 13071 Ciudad Real, Spain*

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ABSTRACT

Although wild ruminants have been identified as reservoirs of Shiga-toxin producing *Escherichia coli* (STEC), little information is available concerning the role of *Salmonella* spp. and *Campylobacter* spp. in large game species. We evaluated the presence of these pathogens in faeces ($N = 574$) and carcasses ($N = 585$) sampled from red deer ($N = 295$), wild boar ($N = 333$) and other ungulates (fallow deer, mouflon) ($N = 9$). Animal sampling was done *in situ* from 33 hunting estates during two hunting seasons. *Salmonella* spp. and *Campylobacter* spp. strains associated with human campylobacteriosis were infrequently detected indicating that both pathogens had a limited zoonotic risk in our study area. The overall STEC prevalence in animals was 21% (134/637), being significantly higher in faeces from red deer (90 out of 264). A total of 58 isolates were serotyped. Serotypes O146:H- and O27:H30 were the most frequent in red deer and the majority of isolates from red deer and wild boar were from serotypes previously found in STEC strains associated with human infection, including the serotype O157:H7. The STEC prevalence in red deer faeces was significantly higher with the presence of livestock ($p < 0, 01$) where high densities of red deer ($p < 0.001$) were present. To the best of our knowledge, this is the first study reporting the occurrence of *Salmonella* spp. and STEC in carcasses of large game animals. Furthermore, this study confirmed by pulsed-field gel electrophoresis (PFGE) that cross contamination of STEC during carcass dressing occurred, implying the likelihood of these pathogens entering into the food chain.

1. Introduction

Bacterial food-borne zoonotic infections are the most common cause of human intestinal disease in many countries. Three major bacterial food-borne agents (*Salmonella* spp., *Campylobacter* spp. and *Escherichia coli*)

* Corresponding author. Tel.: +34 926 295450; fax: +34 926 295451.
E-mail addresses: sandra.diaz@uclm.es, sandra.dsan@gmail.com, sdiazsan@utk.edu (S. Díaz-Sánchez).

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command the most research and surveillance attention from government agencies and the most awareness from the food industry (Newell et al., 2010).

Domestic livestock are recognised as reservoirs of *Salmonella* spp. and *Campylobacter* spp. infection (Newell et al., 2010), and healthy domestic ruminants are considered to be a major natural reservoir of Shiga toxin-producing *E. coli* (STEC) infection (Sánchez et al., 2009). Nowadays, it has been also recognised that livestock and wildlife share diseases, and thus large game animals, could act as reservoirs of some zoonoses (Gortázar et al., 2007).

Meanwhile there is scarce information available regarding *Salmonella* spp. and *Campylobacter* spp. occurrence in large game animals, different studies revealed the presence of O157:H7 and non-O157 STEC in wild ruminants (García-Sánchez et al., 2007) and in wild boars (Sánchez et al., 2010; Mora et al., 2012), that confirmed the role of large game animals as reservoir of STEC and a potential source of human infection.

In spite of the current development in Spain of hunting practices, almost all the information available regarding to the occurrence of these three foodborne pathogens in large game animals is mainly descriptive. The aim of this study is to offer a wide view over the prevalence and distribution of these three foodborne pathogens in faeces and carcasses of large game animals, in order to provide more clues about their potential risk to public health including individual and management-related factors that can influence on its prevalence.

2. Materials and methods

2.1. Sampling sites

Samples were collected in 33 different hunting estates distributed in four geographic areas in South-central Spain (Table 1). Through a personal interview, some information about the presence of livestock in the hunting estate, species of livestock and fencing of the estate was obtained from game keepers, estates owners and veterinarians. According to all data recovered, the hunting estates were classified as open (open hunting areas) or fenced (fenced hunting areas) and classified into three density categories, that is, low (<5 animals/km^2), medium (wild boar 5–10 animals/km^2; red deer 5–15 animals/km^2) and high (wild boar >10 animals/km^2; red deer >15 animals/km^2) (Vicente et al., 2004).

2.2. Animals

Samples were collected from 637 large game animals killed during the 2009–2010 and 2010–2011 hunting seasons (from mid October to mid February) (Table 1). On each hunting event, samples from 15 animals of each species were collected when possible, as sampling was based on hunter success. The harvested individuals were grouped by sex and classified into five age groups based on the dentition eruption patterns, as previously described (Saenz de Buruaga et al., 1991).

2.3. Sample collection

On each sample occasion, and following carcass dressing, one sample of rectal faeces and one carcass swab were collected per animal. The carcass swabbing was carried out by taking samples of about 1 dm^2 from the inside of the retroperitoneal area and the thoracic cavity, from both the left and right sides. Collection of faeces and carcass swabs from the same individual was not possible on some occasions due to the poor conditions and difficulties at the moment of sampling *in situ* on the hunting estate.

We analysed a total number of 585 carcass samples and 574 faecal samples (Table 1). Samples were obtained aseptically, transported to the laboratory under refrigeration in Amies transport medium (Deltalab, Barcelona, Spain) and placed in culture media within 24 h at 37 °C.

2.4. Salmonella spp. screening

Detection and isolation of *Salmonella* spp. were performed according to ISO 6579:2002, using Rappaport-Vassiliadis broth (Merck, Madrid, Spain) as enrichment broth (48 h at 42 °C), followed by culture onto XLT4 agar (Scharlab, Barcelona, Spain) as selective plating medium (24 h at 37 °C). The resulting isolates were confirmed biochemically as *Salmonella* spp. using the API 20E system (BioMérieux, Marcy L'Étoile, France). The serotyping of isolates was performed by the Kauffmann–White scheme using commercial antisera (Bio-Rad, Hemel Hempstead, United Kingdom).

2.5. Campylobacter spp. screening

Only faecal samples were analysed for *Campylobacter* spp. detection (samples from red deer were tested only during the first hunting season). Detection and isolation of *Campylobacter* spp. were performed according to ISO 10272-1:2006 by plating the fresh faeces onto CCDA medium (Oxoid, Madrid, Spain) as selective agar (48 h at 42 °C) under microaerophilic conditions (Campygen™, Oxoid). Presumptive *Campylobacter* colonies were identified by their morphological characteristics and confirmed by a multiplex PCR assay with specific primers for *Campylobacter. coli*, *Campylobacter jejuni* and *Campylobacter lari* (Yamazaki-Matsune et al., 2007).

2.6. STEC screening

Faecal samples were plated directly onto MacConkey agar (MAC) (Scharlab) and carcass swabs were plated onto MAC after pre-enrichment in buffered peptone water (24 h at 37 °C). Following overnight incubation, bacterial growth from the first streaking area of the culture plate was tested for the genes encoding Stx1 and Stx2 toxins (*stx1* and *stx2* genes) by PCR as previously described (Sánchez et al., 2010). For each PCR-positive culture, up to 50 *E. coli*-like colonies were tested for *stx1* and *stx2* genes to obtain the STEC isolates for further characterisation. For detection and isolation of *E. coli* O157:H7, samples were specifically processed according to ISO 16654:2001 and presumptive