

Prevalence of *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, verotoxigenic *E. coli* in healthy domestic ruminants in Northern Spain

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Introduction: Contamination of foods with bacterial pathogens is a major public health concern worldwide. Livestock acts as an important reservoir of these pathogens, as they colonize asymptotically the gastrointestinal tract. Hence, reducing the prevalence of foodborne pathogens in the farm constitutes a crucial point in the microbiology control of the food chain continuum.

Aims: The aim of this study was to determine the prevalence of the main food-borne bacterial pathogens (*Salmonella* spp., *Campylobacter* spp., verotoxigenic *E. coli* (VTEC), and *Listeria monocytogenes*) in cattle and sheep farms in the Basque Country (Northern Spain) and characterize the isolates obtained.

Methods and Results: As part of a larger on-going study, 189 farms were sampled. Rectal fecal samples were collected from 25 animals per farm and pooled into subsamples for selective enrichment of each organism. Colonies of the four microorganisms were confirmed by specific real-time PCR assays. In addition, for the detection of *E. coli* O157:H7, real-time PCR was used as screening method and positive samples were then submitted to immunomagnetic separation (IMS), chromogenic isolation and PCR confirmation. *Salmonella* isolates were further characterized by serotyping using antisera agglutination. The most prevalent microorganism was *Campylobacter* spp. (86.0%), followed by VTEC (46.7%), *L. monocytogenes* (37.6%) and *E. coli* O157:H7 (15.9%). *Salmonella* spp. was rarely isolated (3.7%) and only one serotype among those commonly associated with human infection (Typhimurium) was identified in a beef cattle farm. In beef cattle, the prevalence of *Salmonella* spp. (4.2%), VTEC (66.7%) and *E. coli* O157:H7 (21.1%) was higher than in dairy or sheep farms. However, dairy cattle presented more cases of *L. monocytogenes* (48.3%) and *Campylobacter* spp. (100%) than beef cattle and sheep. Among the thermophilic campylobacters, the most frequent species was *C. jejuni*, present in 86.8% of cattle farms and 42.1% of sheep farms; *C. coli* was found in 10.5% of cattle and 18.4% of sheep farms. Both species were found in 7.9% of farms. *C. lari* was only found in two dairy cattle farms and in both cases in combination with both *C. jejuni* and *C. coli*. A selection of 172 VTEC isolates were characterized for *stx* and *eaeA* genes by real-time PCR, detecting *stx2* in 59% of them, *stx1* in 42% and *eaeA* in 4%. Whereas in isolates collected from sheep the most common profile was *stx1+stx2* (67%), in cattle *stx2*-only was the most frequent pattern (63%). Conversely, among *E. coli* O157:H7 isolates the combination *stx2+eaeA* was the most frequently found (88%).

Conclusion: Although these are only preliminary results, prevalences found here are slightly higher to those found in the region in previous studies [1-3]. These differences might be due to improvements in the detection techniques used here. Still, no increase has been reported in the number of clinical cases.

Keywords: *Salmonella* spp.; *Campylobacter* spp.; *Listeria monocytogenes*; verotoxigenic *E. coli*; O157:H7; fecal; foodborne; cattle; sheep

References

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