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SHORT COMMUNICATION: ABSENCE OF *CAMPYLOBACTER* SPP. IN INTENSIVE RABBIT FARMING IN EASTERN SPAIN, PRELIMINARY RESULTS

MARÍN C.*, SOTO V.*, MARCO-JIMÉNEZ F.†

*Instituto de Ciencias Biomédicas. Departamento de Producción Animal, Sanidad Animal y Ciencia y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad CEU-Cardenal Herrera, C/Tirant Lo Blanc 7, 46115 Alfara del Patriarca, Valencia, Spain.
†Instituto de Ciencia y Tecnología Animal, Universidad Politécnica de Valencia, C/ Camino de Vera s/n, 46022, Valencia, Spain.

Abstract: Campylobacteriosis and salmonellosis are the most frequently reported zoonoses and among the most common causes of diarrhoeal illness in the European Union and the United States, and their incidence appears to be increasing. *Campylobacter* species are routinely found in poultry, swine, cattle, dairy cows and sheep. So far, there are few descriptions of *Campylobacter* isolation from rabbits. Rabbit meat is a common item in the Mediterranean diet. In this context, the aim of the present study was to investigate the occurrence of *Campylobacter* spp. in healthy rabbits reared in intensive farms in the Alto Palancia region, eastern Spain. Caecal contents from 70 healthy does reared on 7 different farms were collected. Bacteriological culture was performed in accordance with ISO 10272-1:2006. All samples tested negative for *Campylobacter* spp. To our knowledge, this is the first study in which comprehensive monitoring was specifically carried out in order to provide data on the occurrence of thermophilic *Campylobacter* spp. in large intensive rabbit farms in Spain. However, further microbiological studies throughout the Spanish territory are needed to determine the prevalence and risk of other foodborne pathogens in rabbits at farm level.

Key Words: *Campylobacter*, farm level, zoonosis, foodborne pathogen, rabbits.

INTRODUCTION

Campylobacteriosis is the most frequently reported zoonosis in the European Union and one of the most common causes of diarrhoeal illness in the United States, and its incidence appears to be increasing (CDC, 2011; EFSA, 2014). In 2013, the European Food Safety Authority (EFSA) reported a total of 214779 cases of human campylobacteriosis (EFSA, 2015). In the USA, Centers for Disease Control and Prevention (CDC) estimates that each year 845024 cases of human campylobacteriosis occur in the United States (Scallan *et al.,* 2011). According to EFSA (2010), clinical cases of campylobacteriosis are under-reported in the EU (27 member states): "There may be no less than 2 million and possibly as high as 20 million cases of clinical campylobacteriosis per year in the EU 27 member states." Of the 25 *Campylobacter* species validly described to date, *C. jejuni* and *C. coli* are the 2 predominant species causing gastrointestinal infections (Skarp *et al.,* 2016). However, other species such as *C. lari, C. upsaliensis and C. concisus* have also been associated with gastrointestinal disease in humans (Skarp *et al.,* 2016).

Food production animals are considered the primary source of *Campylobacter* infections in humans in developed countries. Transmission occurs primarily through consumption of contaminated food and is most frequently associated with consumption of undercooked products (Humphrey *et al.,* 2007). Poultry and its products are considered the main source of human campylobacteriosis (EFSA, 2014). However, *Campylobacter* spp. are routinely found in cattle, dairy cows, sheep, swine, and birds (Silva *et al.,* 2011; EFSA, 2015). So far, there are few descriptions of *Campylobacter* isolation from rabbits, in particular *C. jejuni* (Prescott and Bruin-Mosch, 1981; Weber *et al.,* 1982) and a *Campylobacter*-like organism (Reynaud *et al.,* 1993; Revez *et al.,* 2008). Moreover, *C. jejuni* has been isolated from wild rabbits (Kwan *et al.,* 2008). European rabbit meat production is approximately 500 thousand tons, corresponding

Correspondence: C. Marín, clara.marin@uchceu.es. Received *April 2016* - Accepted *September 2016.*
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to a 30% share of the world production (Petracci *et al.,* 2009). However, rabbits account for the second highest number of animals slaughtered per year in the European Union-27, with 326.619×1000 head in 2010 (FAOSTAT, 2012). Most production is concentrated in the Mediterranean region, where Italy is the leading producer, with Spain ranking second and France third (FAOSTAT, 2009). Little information is available on rabbit consumption in different countries. Yearly estimates range from 1.5 kg/person in France (Benatmane, 2011) and 1.7 kg/person in Spain (González-Redondo *et al.*, 2012) during 2010, and up to 2.3 kg/person in Italy (Dalle Zotte, 2014). Especially in Mediterranean countries, rabbit meat is a common item in the diet. Rabbits produce white meat that is fine-grained, high in protein, low in fat and cholesterol, and rich in certain minerals and vitamins (Dalle Zotte, 2002). However, due to the low quantities consumed worldwide, this animal is by far the least studied meat production species in terms of foodborne pathogens. In this context, the aim of the present study was to investigate the occurrence of *Campylobacter* spp. in healthy rabbits reared in intensive farms in the Valencian region, eastern Spain.

MATERIALS AND METHODS

All the experimental procedures used in this study were performed in accordance with Directive 2010/63/EU EEC for animal experiments and reviewed and approved by the Ethical Committee for Experimentation with Animals of the Polytechnic University of Valencia, Spain.

Sampling

From February to March 2016, the caecal contents from 70 healthy rabbits reared on 7 different farms (10 animals per farm) located in the Alto Palancia region (Castellón, eastern Spain), were examined. Alto Palancia is a region of large intensive rabbit farms (800-1000 does). On all of these farms, the does were housed in cages made of spot-welded wire and 75×50×30 cm (L×W×H) in size. The photoperiod was set to provide 16 h of light and 8 h of darkness. In all farms from this study, does are inseminated using a reproductive rhythm of 42 d between parturitions. Kits are weaned at 28 d of age and the growing rabbits are slaughtered at the age of 63 d.

Ten healthy breeding does (4th or 5th parity) per farm were euthanised by intravenous injection of barbiturate (Dolethal®; Vétoquinol SA, Lure, France) and caecal contents were collected. All samples collected were packed into an individual sterile plastic container, kept cool and processed within 2 h. Does were selected because they are the only category of animals not receiving massive antimicrobial treatments and are being housed for longer periods than growing rabbits, and could therefore act as potential reservoirs of *Campylobacter* spp. within the farm (Piccirillo *et al.,* 2011).

Detection of Campylobacter spp.

Standard bacteriological culture was performed according to ISO 10272-1:2006 (Annex E) for the detection of *Campylobacter* spp. (ISO, 2006). Moreover, all samples were tested by direct culture; in this case, the caecal samples were processed and cultured as described by Ingresa-Capaccioni *et al.* (2015). Briefly, 0.02 g of caecal sample was homogenised into 2 mL phosphate-buffered saline. Then, 10 µL aliquots of each suspension were plated onto modified charcoal cefoperazone deoxycholate agar (mCCDA, Oxoid, Dardilly, France) and Preston agar (CM0689, Oxoid, Dardilly, France). Then the samples were incubated at 41.5±1°C in a microaerobic atmosphere (84% N_2, 10% CO_2, 6% O_2) for 48 h. Moreover, in accordance with the ISO method, samples were pre-enriched in 1:10 vol/vol Bolton broth (CM0983, Oxoid, Dardilly, France) and then pre-incubated at 37±1°C for 5±1 h. Finally, the pre-enriched broth was incubated at 41.5±1°C for 43±1 h. Afterwards, 100 µL sample was cultured on the 2 selective agar plates (mCCDA and Preston agar) and incubated as described above. Plates were examined for grey, flat, irregular and spreading colonies typical of *Campylobacter*. Campylobacter-like colonies were purified on blood agar and identified to species level on the basis of standard procedures (ISO, 2006). One putative colony was subcultured from each plate onto sheep blood agar for confirmation as *Campylobacter* spp. *Campylobacter* confirmation was performed by a mobility test using a dark field microscope, by oxidase and catalase biochemical test and by streaking at different temperatures and atmospheres on Columbia blood agar (AES Laboratories®R , Bruz Cedex, France), as *Campylobacter* will fail to grow at 25°C in microaerobic atmosphere (84% N_2, 10% CO_2 and 6% O_2) conditions and at 41.5°C in aerobic conditions. Finally, characterisation of the bacterial species was done by hippurate hydrolysis test. The limit of detection for the control strains were 50 CFU/sample for *C. jejuni* and 65 CFU/sample for *C. coli*.

RESULTS AND DISCUSSION

Given the importance of *Campylobacter* infections in humans in developed countries, we focused our study on the occurrence of *Campylobacter* spp. in healthy rabbits reared on intensive farms in eastern Spain. Currently, few data are available on the role of meat rabbits as a potential source of *Campylobacter* spp. in humans (Piccirillo *et al.,* 2011). In this study, no thermophilic *Campylobacter* spp. were isolated from the 70 caecal samples examined. To our best knowledge, *Campylobacter* spp. presence in intensive rabbit farming systems in Spain has not been previously studied. There have been a few studies carried out at the slaughterhouse, but not at farm level where the bacteria have to be controlled. For this reason, more information on the bacterium's epidemiology in primary production is necessary. At the slaughterhouse, Prescott and Bruin-Mosch (1981) reported a carriage rate for *C. jejuni* of 11.3% (14 positives out of 124 samples) from slaughtered healthy rabbits. More recently, Kohler *et al.* (2008) reported a carriage rate for *C. jejuni* of 0.04% at a slaughterhouse in Switzerland. Comparable results were also obtained in studies in Spain (Rodriguez-Calleja *et al.,* 2004; 2006) and in Italy (Cerrone *et al.,* 2004). However, there have been a number of reports on the occurrence of *Campylobacter* spp. in rabbits at farm level in Italy with somewhat contradictory results. Whilst Piccirillo *et al.* (2011) suggest that this pathogen seems to be absent (260 samples), Revez *et al.* (2008) reported a carriage rate for *Campylobacter* of 92.3% (36 positives out of 39 samples). These conflicting results may be explained by a variation in the *Campylobacter* spp. studied or by the different methodologies used. Revez *et al.* (2008) point out a high prevalence of *Campylobacter*, even if *C. jejuni*, *C. coli* and other thermophilic *Campylobacter* were not found. These authors indicate that the strains belong to a novel *Campylobacter* spp. named *Campylobacter cuniculorum* spp. On the other hand, while Revez *et al.* (2008) evaluate caecal contents, Piccirillo *et al.* (2011) examined rectal swabs. Several sampling methods are in use to detect *Campylobacter* in broiler houses, including cloacal swabs (Hansson *et al.,* 2004), faecal samples (Sandberg *et al.,* 2006), caecal contents (Allen *et al.,* 2007; Rosenquist *et al.,* 2009). However, there is not yet an accepted standard method for the detection and isolation of *Campylobacter* spp. at farm level (Vidal *et al.,* 2013). In commercial broiler chickens, *Campylobacter* spp. is found at the greatest levels in the mucosal crypts of the caeca (Beery *et al.,* 1988). After infection, *C. jejuni* rapidly colonises the caeca to a high level and leads to faecal shedding (Shanker *et al.,* 1990). Based on the greater ability of *Campylobacter* spp. to invade the caecum, we analysed the caecal contents dissected on-site. In agreement with Piccirillo *et al.* (2011) in Italy, our study confirms that the carriage rate of *Campylobacter* in rabbits in Spain, if it does occur, may be at undetectable levels. It was hypothesised that, unlike in poultry and pig, rabbit intestinal tracts do not provide an optimal environment supporting the colonisation of *Campylobacter* spp. (Piccirillo *et al.,* 2011). However, Kwan *et al.* (2008) in wild rabbits isolated the 73.7% of the most relevant *C. jejuni* genotypes observed in human infections. These findings immediately lend weight to assess the occurrence of this pathogen in commercially reared rabbits. Unlike poultry and livestock production, rabbits are usually reared in wire mesh flooring cages, which avoid the contact between animals and faeces, thus reducing the risk of oral–faecal transmission of pathogens (Piccirillo *et al.,* 2011). In poultry, the high-level faecal shedding combined with the coprophagic behaviour of chickens means that once the first bird in a broiler flock becomes colonised, the bacterium then is able to rapidly infect the entire flock in just a few days (Chaloner *et al.,* 2014).

CONCLUSIONS

In conclusion, this study was done taking into account that, like other food-producing animals, commercial meat rabbits could have the potential to carry thermophilic *Campylobacter* spp. However, our findings showed that no Campylobacter was present in the animals studied. For this reason, rabbit meat seems not to be a risk for food safety and human health. However, further studies involving a larger number of farms and a different category of animals are required in order to give further insight into the epidemiology of *Campylobacter* spp. in rabbits.

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