Veterinary Microbiology 162 (2013) 288–292

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

Occurrence of ε-proteobacterial species in rabbits (*Oryctolagus cuniculus*) reared in intensive and rural farms

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ARTICLE INFO

Article history:
Received 10 October 2011
Received in revised form 10 August 2012
Accepted 11 August 2012

Keywords:
Campylobacter
Helicobacter
Arcobacter
AFLP
PFGE
Rabbit
Oryctolagus cuniculus

ABSTRACT

In order to investigate the occurrence of *Campylobacter*, *Helicobacter* and *Arcobacter* species in caecal contents of rabbits reared in intensive and rural farms, a total of 87 samples from animals belonging to 29 farms were analysed by both cultural and PCR analyses.

PCR analysis directly from faecal samples detected 100% positive samples for *Campylobacter* genus, 3.4% for *Helicobacter* genus and none for *Arcobacter* genus. 83 out of 87 animals (95.4%) and all the 29 farms were positive for *Campylobacter cuniculorum* as also determined by cultural examination. *Campylobacter coli* and *Campylobacter jejuni* were isolated only from three animals reared in two rural farms. No *Helicobacter* and *Arcobacter* species were isolated. To evaluate a possible genetic variability, one strain of *C. cuniculorum* from each farm was analysed by Pulsed Field Gel Electrophoresis (PFGE) and Amplified Fragment Length Polymorphism (AFLP). Genotyping revealed that *C. cuniculorum* population is heterogeneous among the different sources and no dominant clone has spread in the investigated farms.

This survey highlighted a high presence of *C. cuniculorum* with a high rate of intestinal colonization, low presence of *C. jejuni–coli*, *Helicobacter* spp. and any *Arcobacter* spp. in farmed rabbits.

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1. Introduction

Campylobacter and *Helicobacter* species are frequently found in the gastrointestinal tracts of mammals and birds. *Campylobacter jejuni* and *Campylobacter coli* are worldwide known as major food-borne enteropathogens causing enteric diseases in humans (Engberg et al., 2000) and elicobacter species have been related with gastritis, gastric ulceration, enteric disease and bacteraemia in humans (De Groote et al., 2000; Moyaert et al., 2008). Recently, *Arcobacter* species have been recognized as emerging foodborne pathogens and also detected in livestock animals (Lehner et al., 2005). Although the rabbit (*Oryctolagus cuniculus*) is considered an important source of meat for humans (Kohler et al., 2008) and is markedly gaining in importance as pet animal, microbiological data on the occurrence of ε-proteobacteria in rabbits is limited.

Gastric *Helicobacter* have been detected in the stomach of rabbits (Van den Bulck et al., 2005) and only one study reports the isolation of *Arcobacter butzleri* from rabbit meat (Collado et al., 2009). In addition, few studies have reported low prevalence of *Campylobacter* species (mainly *C. jejuni*), in healthy and diarrheic rabbits as well as from rabbit meat (Prescott and Bruin-Mosch, 1981; Kohler et al., 2008; Little et al., 2008; Piccirillo et al., 2011). Recently a

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http://dx.doi.org/10.1016/j.vetmic.2012.08.009

new *Campylobacter* species, *C. cuniculorum*, was described in rabbit caecal contents (Zanoni et al., 2009), but the occurrence of this *Campylobacter* in rabbits is nowadays unknown. The aim of this study was to evaluate the occurrence of different species of ε-proteobacteria in rabbits caecal content by both cultural and PCR analyses.

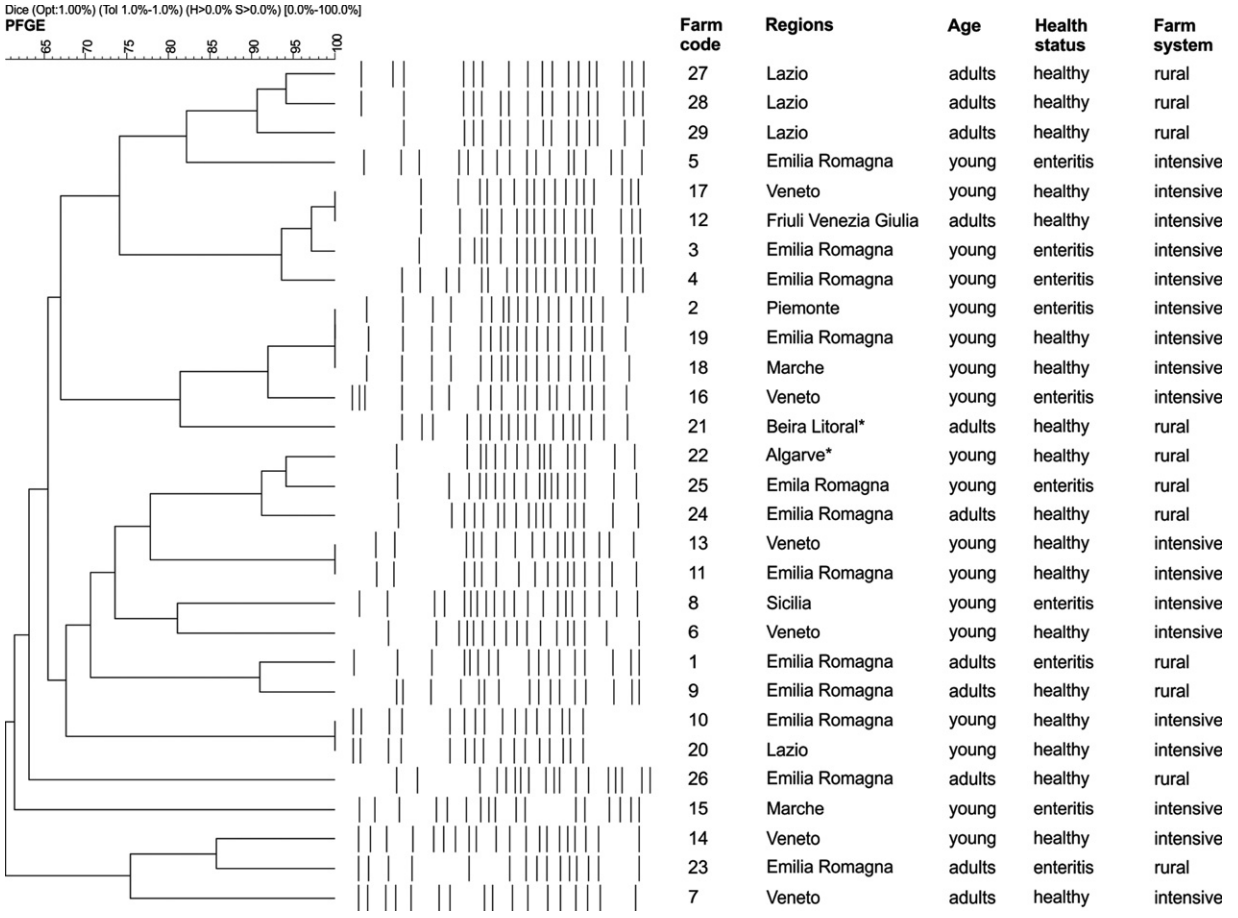
2. Materials and methods

2.1. Sampling

29 farms of rabbits (18 intensive and 11 rural) were sampled from April 2007 to November 2008. Twenty-seven farms (18 intensive and 9 rural) were located in seven different Italian regions, while 2 rural farms were located in Portugal. Data relative to the farms is reported in Fig. 1. Among 87 rabbits (3 animals per farm), 57 were healthy and were sampled at the slaughterhouse (intensive farms) or during private slaughter (rural farms), while 30 animals, with enteritis, were sampled during routine necroscopy. The complete intestinal tract from each rabbit was removed avoiding cross-contamination, collected into a separate sterile plastic bag using fresh disposable gloves, kept at $5 \pm 3\,^{\circ}C$ and examined within 4 h after sampling.

2.2. Cultural examination

Approximately 5 g of caecal content from each gut was diluted into 5 mL of sterile saline and homogenized by vortex mixer. Ten microlitres of the diluted samples were streaked onto plates with the following media: *Campylobacter* blood-free selective agar (mCCDA, Oxoid), Blaser-Wang's Agar (Oxoid), Skirrow's Agar (Oxoid), and C.A.T. Selective Medium (Oxoid). The last three media were prepared using Nutrient Broth N° 2 (Oxoid) with 1.5% of Bacto Agar (Difco) as base media. In addition a modified filter technique of Steele and McDermott was performed as described previously (Zanoni et al., 2007). All plates were incubated at $37 \pm 1\,^{\circ}C$ under a microaerobic atmosphere with hydrogen (Bolton et al., 1992) and examined daily up to 12 days. From each plate different types of colonies of gram



Farm code	Regions	Age	Health status	Farm system
27	Lazio	adults	healthy	rural
28	Lazio	adults	healthy	rural
29	Lazio	adults	healthy	rural
5	Emilia Romagna	young	enteritis	intensive
17	Veneto	young	healthy	intensive
12	Friuli Venezia Giulia	adults	healthy	intensive
3	Emilia Romagna	young	enteritis	intensive
4	Emilia Romagna	young	enteritis	intensive
2	Piemonte	young	enteritis	intensive
19	Emilia Romagna	young	healthy	intensive
18	Marche	young	healthy	intensive
16	Veneto	young	enteritis	intensive
21	Beira Litoral*	adults	healthy	rural
22	Algarve*	young	healthy	rural
25	Emila Romagna	young	enteritis	rural
24	Emilia Romagna	adults	healthy	rural
13	Veneto	young	healthy	intensive
11	Emilia Romagna	young	healthy	intensive
8	Sicilia	young	enteritis	intensive
6	Veneto	young	healthy	intensive
1	Emilia Romagna	adults	enteritis	rural
9	Emilia Romagna	adults	healthy	rural
10	Emilia Romagna	young	healthy	intensive
20	Lazio	young	healthy	intensive
26	Emilia Romagna	adults	healthy	rural
15	Marche	young	enteritis	intensive
14	Veneto	young	healthy	intensive
23	Emilia Romagna	adults	enteritis	rural
7	Veneto	adults	healthy	intensive

Fig. 1. Distribution of *HhoI* digested PFGE patterns of *Campylobacter cuniculorum* (1–29). Information of the origin of the strains (all from Italian regions except those indicated with *, which are from Portuguese regions), age (adults > 6 months; young < 6 months), health status (considered only if animals had or not enteritis) and farm system is presented on the side of the dendongram. The number of rabbit does in the intensive farms ranged from 300 to 700 subjects while in rural from 5 to 15. The numbers on the horizontal axis indicate the percentage of similarities as determined by Dice correlation coefficient and UPGMA clustering.

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