

## OCCURRENCE OF *CAMPYLOBACTER SPP.* IN ITALIAN RABBIT FARMS

Revez J.<sup>1\*</sup>, Rossi M.<sup>1</sup>, Renzi M.<sup>2</sup>, Zanoni R.G.<sup>1</sup>

<sup>1</sup>Department of Veterinary Public Health and Animal Pathology, Alma Mater Studiorum - University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia, Bologna, Italy

<sup>2</sup>Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, via Fiorini 5, 40127 Bologna, Italy

\*Corresponding author: renatogiulio.zanoni@unibo.it

### ABSTRACT

In order to investigate the occurrence of *Campylobacter* spp. in rabbits reared in intensive and rural farms, the caecal contents of 39 animals from 13 different farms (3 rabbits per farm) were collected from April to November 2007. The whole intestinal tract from each rabbit was obtained just after evisceration at the slaughterhouse or during necropsy, and processed within 4 hours. Approximately 5 g of caecal contents were squeezed into 5 ml of sterile saline and shaken in order to obtain a homogenous suspension. Samples were inoculated by streaking 10 µl of each suspension directly onto four different selective fresh media: Blaser-Wang's Agar (Oxoid), Skirrow's Agar (Oxoid), Nutrient Agar N°2 (Oxoid) 5% sheep blood plus CAT Selective Supplement (CAT, Oxoid) and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA, Oxoid). In addition, samples were inoculated on a non selective medium such as Nutrient Agar N°2 (Oxoid) 5% sheep blood using a modified filter technique of Steele & McDermott. All plates were incubated in a jar at 37°C±1 under a microaerobic atmosphere with hydrogen and examined daily for growth up to 12 days. From each sample, 3 colonies showing the same morphotype referable to Gram negative, curved or spiral rod bacteria, were cloned. All the selected colonies were subjected to genus-specific PCR for *Campylobacter*. Positive isolates were submitted to the PCRs specific for *C. jejuni*, *C. coli*, *C. upsaliensis*, *C. helveticus* and *C. lari*. The isolates which resulted negative to the species-specific PCRs were subjected to *rpoB* sequence phylogenetic analysis.

A total of 36 out of 39 animals (92.3%) and all the 13 farms resulted positive for *Campylobacter*. All isolates were positive for *Campylobacter* genus PCR but negative for all the species-specific PCRs tested. Phylogenetic analysis based on the partial nucleotide *rpoB* sequences of 13 isolates (one strain per farm) randomly selected and the reference strains showed that all the rabbit isolates clustered together in a tight clade. This cluster was clearly separated from all the other *Campylobacter* species with high bootstrap values (100), indicating that these isolates may belong to a new species.

This survey allowed reporting the occurrence of a probably new *Campylobacter* species in the caecal contents of farmed rabbits in Italy. Further studies are necessary to describe it and evaluate its possible pathogenic effect on rabbit as well as the eventual zoonotic role.

**Keywords:** *Campylobacter*, New species, Caecum, Rabbits, Italy.

### INTRODUCTION

*Campylobacter* spp. frequently live as commensals in the intestinal tracts of mammals and birds. *Campylobacter jejuni* and *C. coli*, in minor share of cases, are known worldwide as major food-borne enteropathogens causing enteric diseases in humans. Poultry is considered as a major reservoir for transmission to humans. Other members of the genus are known to be responsible for infections both in humans and animals (Vandamme *et al.*, 2005). 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) were isolated from healthy and diarrheic subjects. The aim of this preliminary survey was to investigate the occurrence of *Campylobacter* spp. in rabbits reared in intensive and rural farms in Italy.

## MATERIALS AND METHODS

### Sampling and isolation

From April to November 2007, the caecal contents from 39 rabbits reared on 13 different farms (3 animals per farm), located in 5 Italian regions, were examined. Eighteen samples were collected directly at the slaughterhouse and 21 at the State Veterinary Institute of Lombardia and Emilia-Romagna. Data regarding farming system, age and clinical status of the animal sampled are shown in Table 1.

**Table 1:** Data regarding farming system, age and clinical status of the 39 animals examined

N°	Farm Type	Age	Clinical status
1	Rural	> 1 year	Enteritis
2	Intensive	40 – 50 days	Enteritis
3	Intensive	40 – 50 days	Enteritis
4	Intensive	40 – 50 days	Enteritis
5	Intensive	40 – 50 days	Enteritis
6	Intensive	> 1 year	Pneumonia
7	Rural	> 1 year	Healthy
8	Intensive	> 1 year	Healthy
9	Intensive	> 1 year	Healthy
10	Intensive	75 – 95 days	Healthy
11	Intensive	75 – 95 days	Healthy
12	Intensive	> 1 year	Healthy
13	Intensive	75 – 95 days	Healthy

Samples were collected and processed avoiding cross-contamination. The whole intestinal tract from each rabbit was obtained just after evisceration at the slaughterhouse or during necroscopy at the State Veterinary Institute, packed into a separate plastic bag, kept cool and processed within 4 hours. Approximately 5 g of caecal contents were squeezed into 5 ml of sterile saline and shaken using a vortex mixer in order to obtain a homogenous suspension. Samples were inoculated by streaking 10 µl of each suspension directly onto four different selective media prepared 24 h before use: Blaser-Wang's Agar (Oxoid), Skirrow's Agar (Oxoid), Nutrient Agar N°2 (Oxoid) 5% sheep blood plus CAT Selective Supplement (CAT, Oxoid) and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA, Oxoid). In addition, samples were inoculated on a non selective medium such as Nutrient Agar N°2 (Oxoid) 5% sheep blood using the modified filter technique of Steele & McDermott (Zanoni *et al.*, 2007). All plates were incubated at 37±1°C in a jar under a microaerobic atmosphere with hydrogen obtained by the gas replacement method with anaerobic gas mixture (H<sub>2</sub> 10%, CO<sub>2</sub> 10%, N<sub>2</sub> 80%) (Bolton *et al.*, 1992) and examined daily for growth up to 12 days. From each sample, 3 colonies showing the same morphotype, referable to Gram negative, curved or spiral rod bacteria, were cloned.

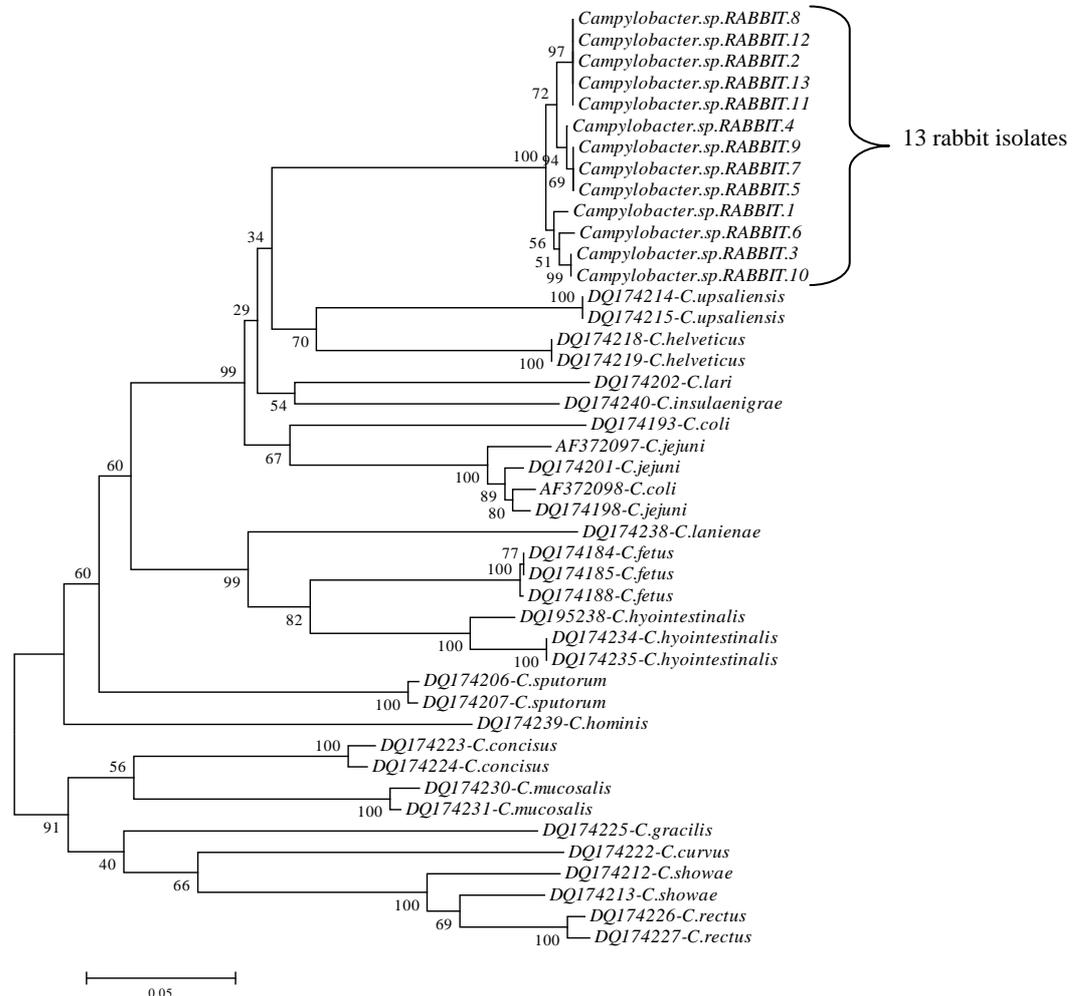
### Identification

All the selected colonies were subjected to genus-specific PCR for *Campylobacter* (Linton *et al.*, 1996) using the REExtract-N-Amp Tissue PCR Kit (Sigma). Positive isolates were submitted to the PCRs specific for the following thermophilic *Campylobacter*: *C. jejuni* - *C. coli* (Denis *et al.*, 1999), *C. upsaliensis* - *C. helveticus* (Lawson *et al.*, 1997) and *C. lari* (Linton *et al.*, 1996). The isolates which resulted negative to the species-specific PCRs above mentioned were subjected to *rpoB* sequence analysis as described by Korczak *et al.* (2006).

## RESULTS AND DISCUSSION

A total of 36 out of 39 animals (92.3%) and all the 13 farms sampled were positive for *Campylobacter*. After 6-8 days of incubation, the first isolation plates of CAT, mCCDA and Nutrient Agar seeded by filter method showed a large number (>50 CFUs) of colonies of Gram-negative spiral-

shaped rods bacteria. Colonies on the same media showed the same morphotype. On Nutrient Sheep Blood Agar after 6 days, colonies appeared greyish-green, flat with rough margins and slightly mucoid-looking; sometimes exhibited a tailing effect along the streak line and resulted haemolytic after subculturing. After 12 days of incubation all the Blaser-Wang's Agar and Skirrow's Agar plates were negative. All isolates were positive for *Campylobacter* genus-specific PCR but negative to the species-specific PCRs tested. A phylogenetic tree based on the partial nucleotide *rpoB* sequences of 13 isolates (1 strain per farm) randomly selected and the reference strains is shown in Figure 1.



**Figure 1:** Neighbour-joining phylogenetic tree of the genus *Campylobacter* based on partial *rpoB* gene sequences. Bootstrap values of 1000 simulations are indicated at major branches. Bar: 0.05 sequence distance value

All the isolates clustered together in a tight clade clearly separated from all the other *Campylobacter* species with high bootstrap values (100), indicating that these isolates may belong to a new species. The sequence similarity within the clade of the rabbit isolates ranged from 98 to 100%, while among this cluster and the other *Campylobacter* species varied from 61 to 81%.

Our preliminary results point out a high prevalence of *Campylobacter* in rabbits, even if *C. jejuni*, *C. coli* and other thermophilic *Campylobacter* were not found. All the farms resulted positive for a *Campylobacter* taxon phenotypically similar to that described by Reynaud *et al.* in 1993. The detection of an elevated number of colonies referable to this *Campylobacter* in the first isolation media suggests that this microorganism, when present, colonizes the caecum at high concentration.

## CONCLUSIONS

This survey allowed reporting the occurrence of a probably new *Campylobacter* species in the caecal contents of farmed rabbits from Italy. Further studies are necessary to describe it and evaluate its possible pathogenic effect on rabbit and eventual zoonotic role.

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