Veterinary Microbiology 154 (2011) 171–179

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Genetic instability of *Campylobacter coli* in the digestive tract of experimentally infected pigs

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ARTICLE INFO

Article history:
Received 23 January 2011
Received in revised form 13 June 2011
Accepted 4 July 2011

Keywords:
Campylobacter
Genetic instability
Pigs
Experimental infection

ABSTRACT

Campylobacter, a leading cause of food-borne illness worldwide, has a widespread distribution with a broad range of animal hosts and environmental reservoirs. The genetic description of bacterial strains is a powerful tool for epidemiological studies but can be impaired by the high genomic variability of *Campylobacter*. Our study aimed (i) at investigating the genotypic instability of *Campylobacter* generated either *in vitro* by subculturing or after *in vivo* passage on specific pathogen-free pigs and (ii) at evaluating the suitability of typing methods to detect such variation. Pigs were inoculated *per os* with three *Campylobacter* strains (one *C. coli* originating from pig faeces, one *C. jejuni* and one *C. coli* originating from poultry faeces) alone or in mixture and non-inoculated pigs were housed in adjacent pens. Genotypic instability was investigated using both macrorestriction combined with pulsed-field gel electrophoresis analysis (PFGE) and PCR restriction fragment length polymorphism analysis of the *fla*A gene (*fla*A PCR-RFLP). No variability in the genetic profile was observed for the three strains maintained through twenty times subculturing events *in vitro*. Genotypic variability was evidenced *in vivo* only in pigs inoculated with *C. coli* of porcine origin, either alone or in a mix, with both genotyping methods. In our study, for one porcine *C. coli* strain, 13% and 21% of variability were generated in the digestive tract of pigs by PFGE and *fla*A PCR-RFLP typing methods, respectively. This study is a first approach for a better understanding of the genomic instability of *Campylobacter* in pig under field conditions.

1. Introduction

Campylobacter, a leading cause of food-borne illness worldwide, has a widespread distribution with a broad range of animal hosts and environmental reservoirs (Boes et al., 2005; EFSA, 2009; Moore et al., 2005; Ogden et al., 2009). Targeted control of food-borne pathogens generally requires identification of sources and major routes of transmission to determine the most effective place to control infection (Moore et al., 2005). The genetic description of bacterial strains is a powerful tool for epidemiological studies especially because it allows to track individual strains. Several genotyping methods with different intrinsic capacities have been used to describe *Campylobacter* genotypic variability (Guévremont et al., 2004; Knudsen et al., 2005; Ogden et al., 2009; Rivoal et al., 2005; Wassenaar and Newell, 2000). This genomic plasticity of *Campylobacter*

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doi:10.1016/j.vetmic.2011.07.002

and its ability to exchange genetic material with other bacteria has to be taken in consideration in the genetic description of these bacteria (De Boer et al., 2002; Harrington et al., 1997; Meinersmann et al., 2002; Mills et al., 1991; Wang and Taylor, 1990).

Previous studies have investigated the genetic stability of *Campylobacter* both *in vitro* and *in vivo* leading to discrepant results. Nielsen et al. (2001) as well as Ritmeester et al. (2003) reported the *in vitro* genetic stability of *C. jejuni*. On the contrary, *C. coli* porcine isolates were shown to vary after 50 subcultures (On, 1998). Additionally, investigations carried out by *in vivo* passage in chickens demonstrated either genomic instability (Hanel et al., 2009; Ridley et al., 2008; Wassenaar et al., 1998) or stability (Hänninen et al., 1999) of *C. jejuni* strains. The potential for genetic instability is present in *Campylobacter* and appears to reflect different possible mechanisms such as (i) programmed DNA recombination, (ii) uptake of extra-cellular DNA by natural transformation, (iii) recombination within the flagellin genes, and (iv) random recombination on a genomic scale (Harrington et al., 1997; On, 1998; Wassenaar and Newell, 2000). Documenting this genomic instability, especially in a context of experimental infection, is helpful for a better understanding of the data obtained in epidemiological studies under field conditions. To our knowledge, no experimental investigation of the *in vivo* genetic stability of *Campylobacter* has been carried out in pigs until now.

Our study aimed at (i) describing the genotypic instability of *Campylobacter* generated either *in vitro* by subculturing or after *in vivo* passage on specific pathogen-free (SPF) pigs under experimental conditions and (ii) comparing the results obtained with two typing methods. The genotypic instability was investigated over time using both (i) macrorestriction combined with pulsed-field gel electrophoresis analysis (PFGE) and (ii) PCR-restriction fragment length polymorphism analysis of the *flaA* gene (*fla*A PCR-RFLP). Some studies underlined the possible co-existence of different strains in pigs (Guévremont et al., 2004; Hume et al., 2002; Weijtens et al., 1999). For the experimental infection, pigs were inoculated *per os* with three *Campylobacter* strains (one *C. coli* originating from pig faeces, one *C. jejuni* and one *C. coli* originating from poultry faeces) alone or in mixture. The aims were (i) to determine the possible difference between strains in genomic variability *in vivo* and (ii) to assess the effect of co-infection on variability.

2. Materials and methods

2.1. Strains

Three *Campylobacter* field strains were obtained from the French Agency for Food, Environmental and occupational Health and Safety (Anses). One *C. coli* strain was isolated from faeces of pigs collected in a French slaughterhouse, one *C. coli* and one *C. jejuni* strains were obtained from caeca of commercial broilers from two different French slaughterhouses. These three French field-isolated strains, stored at −80 °C in glycerol peptone broth, will be hereafter referred to as "porcine *C. coli*", "poultry *C. coli*", and "poultry *C. jejuni*", respectively. The strains investigated in this study, differing in species and in origin, were selected on the basis of (i) absence of epidemiological linkage, (ii) distinct *Sma*I-*Kpn*I PFGE patterns identified in a previous study (Denis et al., 2008) and (iii) distinct *fla*A PCR-RFLP profiles.

Two reference strains, namely *C. jejuni* NCTC 11168 (National Collection of Type Cultures, Colindale, UK) and *C. coli* CIP 7081 (Collection of the Pasteur Institute, Paris, France), were used as controls to assess the repeatability of the genotyping methods.

2.2. In vitro passage

The three field strains and the two reference strains (*C. jejuni* NCTC 11168 and *C. coli* CIP 7081) were subcultured 20 times every 48 h in duplicate on Karmali agar (Oxoid, Dardilly, France), which corresponds to an assay duration of six weeks. A single colony was picked for subculture each time as in the study of Nielsen et al. (2001). The Karmali plates were incubated for 48 h at 41.5 °C in a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2). For each original strain, ten isolates were randomly picked at the first subculture and at the 20th passage. The 20 isolates per strain were kept at −80 °C in glycerol peptone broth before analysis by genotyping methods (PFGE and *fla*A PCR-RFLP).

2.3. In vivo passage in pigs

2.3.1. Experimental infection of specific pathogen-free (SPF) pigs

For the experimental infection, pigs were inoculated *per os* with three *Campylobacter* strains (one *C. coli* originating from pig faeces, one *C. jejuni* and one *C. coli* originating from poultry faeces) alone or in mixture to assess the effect of co-infection on variability (Leblanc Maridor et al., 2008). As suggested in a previous study (Boes et al., 2005), pigs can be in a farm in contact with other animals including wild birds and mammals, domestic pets or other food production animals, all of them natural reservoir of *Campylobacter*. The three French field-isolated *Campylobacter* strains, differing in species and in origin, were tested under controlled conditions (i) to assess the possible difference in capacity to colonize the digestive tract of pigs and (ii) to determine the possible difference between strains in genomic variability *in vivo*. Twenty-one SPF 7-week-old piglets, obtained from the high-security barn of the Anses at Ploufragan, were distributed into seven groups of three animals. They were housed and treated in accordance with the regulations of the local veterinary office (Direction des Services Vétérinaires des Côtes d'Armor, France). We respected internationally recognized guidelines relative to animal experimentation. All the animals were reared in isolation rooms with controlled air flow and the experiment was carried out in standardized conditions. One group of three piglets was kept as negative controls and placed in a separate unit.

2.3.2. Design of the trial and sample collection

Three groups of three piglets were orally inoculated with 10 mL of tryptone salt medium containing either