Genetic comparison of *Campylobacter coli* resulting from pigs and poultry with isolates resulting from human campylobacteriosis..

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Abstract

133 isolates of *Campylobacter coli* isolated from Brittany in France and collected in 2003 were analysed by RFLP/PFGE. They came from pig (65), poultry (56) and human campylobacteriosis (12). No pulsotype common to the 3 origins could be detected but the analysis of the genetic similarity at 80% of the isolates made it possible to build 19 groups of similarity. In 3 cases, poultry isolates were found in groups containing human isolates. Neverthless, the pig isolates were always in groups different from the poultry isolates and the human ones. These results tend to indicate that the two animal productions would have their own genotype and that the campylobacters from pigs are rarely responsible of human campylobacteriosis.

Introduction

Campylobacter sp. is one of the most frequent causes of human gastro-enteritis. The poultry meat is mainly accused; it would be responsible for at least 40% of the human campylobacteriosis (Vellinga and Van Loock, 2002). In France, *C.jejuni* species represents 76% of the human isolates against 17% for *C coli* (Gallay *et al*, 2005) and pigs are known to be frequently infected with *C coli* (Magras *et al*, 2004). It was thus interesting to genetically compare *C. coli* from human campylobacteriosis with *C. coli* isolates resulting from pig and poultry productions in order to estimate the importance of these animal productions in the human infections at *Campylobacter coli*.

Material et methods

Isolates

The isolates of *Campylobacter coli* analyzed in this work were collected in Brittany in France and during the year 2003. Twelve *C. coli* of human origin were provided by Pr F Mégraud of the CNR-CH of Bordeaux. Each isolate comes from an analysis carried out on a patient presenting gastro-enteritis. The 121 isolates of animal origin (56 poultry *C. coli* and 65 pig *C. coli*) come from samples collected in farm, slaughter-house, and supermarket. Only one isolate was retained by analyzed sample.

Methods

The typing of the isolates was carried out by RFLP/PFGE as described by Rivoal *et al.*, (2005). Two enzymatic profiles were obtained by isolate : a *Kpn*1profile and a *Sma*1 profile. The combined profile resulting from the 2 enzymes was coded KS.
Electrophoretic patterns were compared by BioNumerics® (Applied Maths). Similarities between profiles, based on band positions, were derived from the Dice correlation coefficient with a maximum position tolerance of 1%. A dendrogram of the analysis of the combined *Kpn*1- and-*Sma*1-digested DNA was constructed to reflect the similarities between the strains in the matrix. Strains were clustered by the Unweighted Pair-Group Method using the Arithmetic Mean (UPGMA) (Struelens, 1996). Isolates with high similarity were considered as deriving from the same parent strain (Tenover *et al.*, 1995). In this study, clusters were defined for a genetic similarity equal or superior to 80%.
The index of Simpson (Hunter, 1990) was calculated to estimate the diversity of the sample.

Results

Table 1 gives the number of genetic profiles per enzyme, and origin on the number of isolates, as well as the Index of Simpson.

Table 1 : number of genetic profiles per enzyme, and origin

enzyme	Origin		
	Human	Poultry	Pig
Kpn 1	11 / 12	52 / 56	62 / 65
Sma 1	11 / 12	47 / 56	60 / 65
KS	**11 / 12**	**53 / 56**	**64 / 65**
Simpson index	0,984	0,998	0,999

Eleven, 53 and 64 combined KS profiles were obtained respectively for 12, 56, and 65, human, poultry and pig isolates.
For the 3 origins, the index of Simpson is very close to 1 what indicates that our sampling has a very great diversity.

Table 2 : number of isolates per cluster and origin

clusters	N° isolates per origin			N° isolates per cluster
	pig	poultry	Human	
C1	2			2
C2	2			2
C3	2			2
C4		2		2
C5		2		2
C6		2		2
C7		**1**	**1**	**2**
C8	12			12
C9			3	3
C10		3		3
C11		**4**	**1**	**5**
C12		4		4
C13		**6**	**2**	**8**
C14		2		2
C15		4		4
C16		2		2
C17		2		2
C18	2			2
C19	2			2
Total	22	34	7	63

No pulsotype common to the 3 origins could be detected but the analysis of the genetic similarity at 80% of the isolates made it possible to build 19 groups of similarity coded clusters C1 to C19 (table 2) which contained 47,6 % of the total isolates.

In 3 cases (in fat in the table), poultry isolates are in the same clusters containing human isolates. On the other hand, the pig isolates are always in clusters different from those containing poultry and / or human isolates.

The figure 1 represents the dendrogram obtained from the analysis of KS profiles by BioNumerics®.

Discussion

The genetic comparison by RFLP/PFGE of *Campylobacter coli* from pig and poultry productions with isolates resulting from the human campylobacteriosis showed genetically close isolates between the poultry production and the human cases. In spite of the importance of the sampling of the isolates from the pig production and its diversity, it was not possible in our study to highlight identical or very close isolates between this animal production and the human isolates. In addition, the *C coli* from pigs are always in clusters different from those containing poultry and / or human isolates.
This result consolidates other studies which show the implication of the poultry in the human campylobacteriosis (Steinhauserova *et al*, 2002; Nadeau *et al*, 2002; Kärenlampi *et al*, 2003; Michaud *et al*, 2005). Genetic separation between *C. coli* from poultry and *C. coli* from pig were described by Hopkins *et al*., (2004) and by Siemer *et al*., (2005). The latter, moreover, showed that *C. coli* resulting from the poultries are in the same genetic groups as the isolates resulting from human campylobacteriosis.

This result is in agreement with the results of Guévremont *et al.*. (2004). For the same period and the same geographical area in Canada, Guévremont compared 660 isolates resulting from feces of pigs taken in slaughter-house with 24 isolates resulting from patients. No isolate genetically identical and commun to the two origins was found.

Conclusions

These results tend to indicate that the two animal productions would have their own genotypes and that the campylobacters resulting from the pig would not be implied in the human campylobacteriosis. The probability of human *Campylobacter* contamination by pig thus seems very weak.

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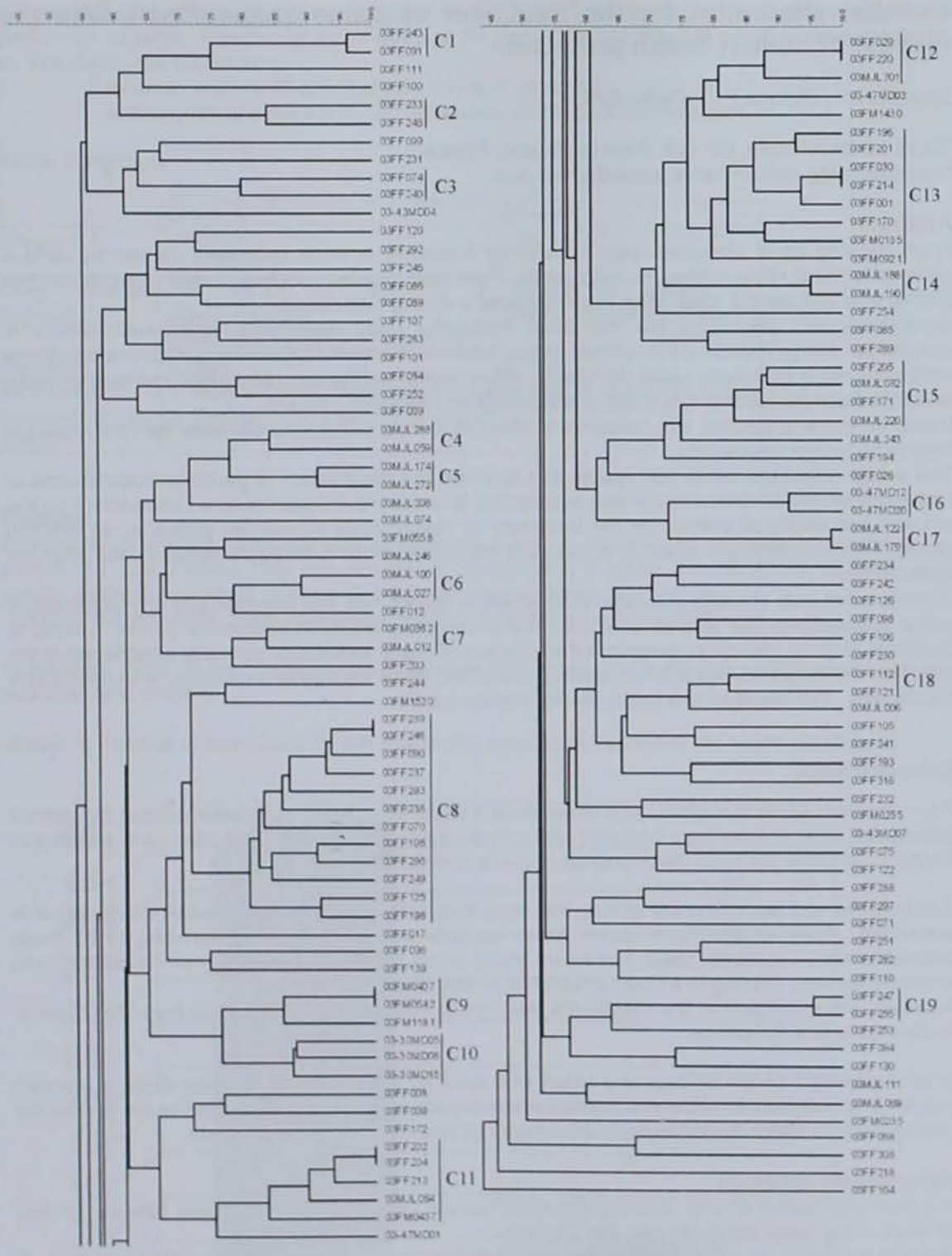

Figure 1 : dendrogram obtained from the analysis of KS profiles by BioNumerics®.
C1 to C19 : clusters defined for 80% of similarity

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