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Original article

Description and sources of contamination by *Campylobacter* spp. of river water destined for human consumption in Brittany, France

Description et origines de contamination par Campylobacter *spp. d'eau de rivières destinée à la consommation humaine en Bretagne, France*

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ABSTRACT

Presence or absence of *Campylobacter* spp. in water of five rivers upstream from an intake point for drinking water production was investigated, and isolates genetically compared with human, pig and poultry isolates in order to determine their source. River water and drinking water obtained from these rivers were sampled one time per month, over a period of one year, and tested for *Campylobacter*. Isolates were typed by PFGE. *Campylobacter* was not detected in treated drinking water, but 50% of the river samples were contaminated. Contamination was observed on the four seasons. In total, 297 *Campylobacter* isolates were collected and generated 46 PFGE profiles. *Campylobacter jejuni* was the most frequently detected species in samples (74.1% of the isolates), followed by *Campylobacter coli* (17.8%) and *Campylobacter lari* (8.1%). Forty-two of the 46 PFGE profiles were unique. Only one genotype was detected three times in a river during the year and four genotypes in two different rivers. When compared to animal and human *Campylobacter* PFGE profiles, 14, 11 and one *Campylobacter* genotypes from water were genetically closed to human, poultry, and pig *Campylobacter* genotypes, respectively. The *Campylobacter* population displayed a high level of genetic diversity, suggesting that contamination originated from various origins. Human, poultry and pig were sources of contamination of the river by *Campylobacter*. Finally, no *Campylobacter* were detected in drinking water, indicating that the risk of outbreaks due to consumption of drinking water is low.

Mots clés:
Campylobacter
Eau
Volaille
Porc
Humain
PFGE

RÉSUMÉ

La présence ou l'absence de *Campylobacter* spp. dans l'eau de cinq rivières en amont d'un point de pompage pour la production d'eau potable a été considérée, et les isolats génétiquement comparés à des isolats d'origine humaine, porcine et aviaire pour identifier leur origine. L'eau de rivière et l'eau de consommation obtenue à partir de ces rivières ont été prélevées une fois par mois pendant un an en vue de rechercher *Campylobacter*. Les isolats ont été génotypés par PFGE. *Campylobacter* n'a pas été détecté dans l'eau de consommation, mais 50 % des échantillons d'eau de rivière étaient contaminés. La contamination a été observée sur les quatre saisons. Au total, 297 isolats de *Campylobacter* ont été collectés et ont généré 46 profiles PFGE. *Campylobacter jejuni* était l'espèce la plus retrouvée (74,1 % des isolats), suivi de *Campylobacter coli* (17,8 %) et *Campylobacter lari* (8,1 %). Quarante-deux des 46 profils génétiques étaient uniques. Seul un génotype a été détecté trois fois dans une rivière sur l'année et quatre génotypes dans deux rivières différentes. Quand comparés aux profils PFGE des isolats d'origine animale et humaine, 14, 11 et un génotypes de *Campylobacter* de l'eau étaient génétiquement proches respectivement des génotypes de *Campylobacter* humains, porcins et aviaires. La diversité génétique des *Campylobacters* issus de l'eau est très élevée, indiquant plusieurs sources de contamination. Les humains, la volaille et les porcs sont sources de contamination des rivières par *Campylobacter*. Aucun *Campylobacter* n'a été détecté dans l'eau traitée destinée à la consommation, indiquant que le risque de campylobactérioses par cette voie est faible.

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1. Introduction

The main source of human *Campylobacter* infections, as highlighted by several epidemiological studies, is the consumption of contaminated food, particularly raw or insufficiently cooked poultry products [1,2]. The consumption of untreated water [3–5], rain water [6], and water from wells, lakes and streams [7,8] has also been identified as a source of outbreaks of *Campylobacter* infection in human. Outbreaks due to the consumption of treated water supplies are rare and generally linked to a lack of chlorination [9–12] or to contamination of drinking water supply by surface water or effluent or rainwater [13–18]. Several studies have reported contamination of surface water such as lakes, rivers and streams with *Campylobacter* [19–21], and have described seasonal variation levels of *Campylobacter* contamination [14,20].

Brittany is a region of large-scale poultry and pig production in France (36% of the French poultry production and 58% of the French pig production). Local stream water is the source of 75% of the water destined for human consumption in this region. Our primary objective was to describe, monthly, *Campylobacter* contamination (1) of rivers before water treatment, and (2) of drinking water after processing at water treatment plants. Our secondary objective was to determine, by Pulsed Field Gel Electrophoresis typing (PFGE), whether poultry, pigs and humans contributed to the contamination of river with *Campylobacter*.

2. Materials and methods

2.1. Water samples

This study was carried out over a period of one year in 2006 in the neighbourhoods of Saint-Brieuc in Brittany (Fig. 1). Water was sampled, on time monthly, upstream from water treatment plants (R1, R2, R3, R4 and R5) from five rivers, and from public drinking water taps from the five water treatment plants (R1T, R2T, R3T, R4T and R5T). Taps were localized in the water treatment plants (WTP).

The treatment of the water entailed the following steps in the WTP. These steps were coagulation, flocculation, decantation, filtration, post-ozonization, denitratation, and chlorination in WTP1; nano-filtration and chlorination in WTP2; chlorination, decantation, filtration, post-ozonization and chloration in WTP3; ozonization, filtration and chlorination in WTP4; ozonization, decantation, filtration, post-ozonization, and chloration in WTP5.

River water was collected in sterile flasks. Water samples from public taps were collected in sterile flasks containing thiosulfate to neutralize free chlorinate. The end of the tap was flamed and the water left to run for five minutes before collecting the sample.

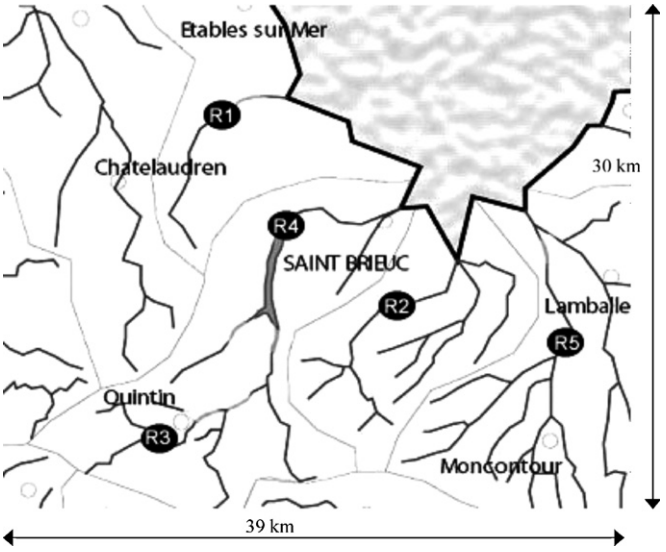

Fig. 1. Localization of the river sampling in the neighbourhoods of Saint-Brieuc in Brittany, France. Rx: sample in river before water treatment plant.

2.2. Bacteriological analysis

Five hundred millilitres of river water samples were diluted in 500 ml of 2X Bolton broth (Oxoid) supplemented with Bolton antibiotics (Oxoid). For water samples from public taps, 1000 ml of water were filtered into sterile flasks using a 0.45 μm filter and the filter was then placed in 100 ml of Bolton Broth with Bolton antibiotic supplement. *Campylobacter* was cultured at 37 °C in a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2).

Broths were incubated for 48 hours and then streaked on mCCDA plates (Oxoid). *Campylobacter* colonies (maximum of 20 per positive sample) were subcultured and streaked onto blood agar. Blood agar plates were incubated at 37 °C for 24 hours.

For each isolate, a few colonies from the bacterial culture were suspended in 100 μl TE buffer (10 mmol/l Tris-HCl, 1 mmol/l EDTA, pH 7.6) for analysis by PCR. The remaining colonies were used for genotyping by PFGE, as described below.

2.3. Identification of species

We used multiplex-PCR [22] to identify *Campylobacter jejuni* and *Campylobacter coli*. The remaining unidentified strains were then analyzed by multiplex-PCR as described by Wang et al. [23], using primers for the identification of *Campylobacter lari* and *Campylobacter fetus* only.

2.4. Pulsed-field gel electrophoresis (PFGE) and electrophoretic pattern analysis

DNA preparation, restriction endonuclease digestion and PFGE were carried out as described by Rivoal et al. [24]. We used two endonucleases in PFGE typing as recommended by Eyles et al. [25]. Two profiles, corresponding to the restriction profiles obtained with *Sma1* and *Kpn1*, were obtained for each isolate.

Electrophoretic patterns were compared using BioNumerics® (Applied Maths, Sint-Martens-Latem, Belgium). Similarities between profiles, based on band positions, were determined by calculating the Dice correlation coefficient, with a maximum position tolerance of 1%. A dendrogram based on the combined results for *Kpn1*- and *Sma1*-digested DNA (KS) 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) [26].

The Simpson's index (D) determined as follows [27], with a 95% confidence interval, as described by Grundmann et al. [28], to assess the genetic diversity of the *Campylobacter* populations:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} nj(nj-1)$$

N: number of isolates tested; S: number of different genotypes; nj: number of isolates belonging to type j.

Isolates with high similarity levels were considered to be derived from the same parental strain and were clustered using a threshold of 80% [29].

2.5. Campylobacter PFGE profile collection

The genotypes of *Campylobacter* isolates from water were compared with the genotypes of strains from humans, poultry and pigs (363 from humans, 867 from poultry, and 98 from pigs). These genotypes were obtained from *Campylobacter* isolates collected in Brittany from the years 2003 to 2006 and typed by PFGE in our laboratory. The PFGE profiles are stocked in our *Campylobacter* BioNumerics data base. Our collection contained (1): 291 human and 550 poultry PFGE profiles for the species *C. jejuni*; (2) 68 human, 317 poultry and 98 pig PFGE profiles for the species *C. coli*, and (3) four human PFGE profile for the species *C. lari*. Human isolates were obtained from the French National Reference Laboratory.

3. Results

3.1. Campylobacter in water

In total, 60 river samples upstream from the WTP and 60 samples of drinking water were collected. *Campylobacter* was not detected in drinking water but 30 of the river samples (50%) were positive for *Campylobacter*. Over the 12 months of the study, *Campylobacter* was detected five, seven, seven, one and 10 times during the year in rivers R1, R2, R3, R4 and R5, respectively (Table 1). At least one sample over the five realised per month was positive for *Campylobacter*. Fifteen samples were done per season, seven (46.6%) were positive for *Campylobacter* in winter and spring, and eight (53.3%) in summer and in fall (Table 2).

In total, 297 *Campylobacter* isolates were collected from river water over the year (Tables 1 and 2). *C. jejuni* was the most

Table 1
Number of positive samples, *Campylobacter* isolates and *Kpn1-Sma1* genotypes per river.

River	No. of positive samples	No. of isolates	No. of isolates per species			No. of genotypes
			C. jejuni	*C. coli*	*C. lari*	
R1	5/12	42	3	28	11	8
R2	7/12	63	62	1	–	8
R3	7/12	63	40	10	13	10
R4	1/12	15	15	–	–	1
R5	10/12	114	100	14	–	23
Total	30/60	297	220	53	24	50*

Notes: *50 instead of 46 because four genotypes were found in different rivers.

Table 2
Number of *Campylobacter* spp. and *Kpn1-Sma1* profiles of the isolates per river and month.

River	Month											
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
R1			K69S52-8l K71S57-1l K72S55-2l				K6S3-2j[a]	K10S7-11c K32S23-1c	K6S24-1j			K18S14-16c
R2		K1SND-16j[a]	K29S1-1j			K5SND-7j[a]	K7S4-11j		K44S30-1c		K16S12-17j K33S12-1j	K19S15-9j
R3	K42S29-5j[a] K43S29-1j		K72S55-1l	K2S1-8j[a] K3S1-4j[a] K22S1-5j		K69S52-12l		K11S8-8c		K14S10-17j	K46S32-2c	
R4				K4S2-15j								
R5	K1SND-7j[a] K50S40-2c		K20S16-1c K21S17-12j	K1SND-20j[a]	K23S18-17j	K24S19-1j K25S20-5j K26S21-1j K27S20-1j	K1SND-1j[a] K5SND-2j[a] K9S1-5j K30SND-1j K31S22-1j K8S5-1j[a] K9S6-1j	K13SND-2j[a] K12S9-3j K12S1-6j		K15S4-10j K15S11-3j K45S31-2c[a]	K17S13-7c	K51S41-2c

KxSx: code of *Kpn1-Sma1* profiles; ND: not digested; j, c, l: *jejuni*, *coli*, *lari* respectively.
[a] PFGE profile from water isolate clustered with poultry *C. jejuni* genotypes or pig *C. coli* genotype.

frequently detected species in samples (74.1% of the isolates), followed by *C. Coli* (17.8%) and *C. lari* (8.1%). *C. jejuni*, *C. coli* and *C. lari* were respectively detected in 20, 10 and three samples over the 30 samples tested positives. The five rivers were contaminated at least once during the year by *C. jejuni*; R1, R2, R3 and R5 were contaminated at least once by *C. coli*, and R1 and R3 were contaminated at least once by *C. lari* (Table 1). *C. jejuni* was isolated in each of the four seasons. *C. coli* was not detected in spring and *C. lari* was detected only in winter and spring (Table 2).

3.2. Genetic diversity of Campylobacter

Forty-six PFGE profiles were obtained from the 297 *Campylobacter* isolates; 32 for *C. jejuni*, 11 for *C. coli*, and three for *C. lari*. *C. jejuni* was more diverse ($D = 0.92_{CI95\%}$ [0.91–0.94]) than *C. coli* ($D = 0.83_{CI95\%}$ [0.82–0.85]). *Kpn1-Sma1* profiles are indicated by month and by river in Table 2. Four genotypes were coded SND (ND for not digested) because the genome of the corresponding isolate was not successfully digested with the *Sma1*.

The *Campylobacter* population in water samples was genetically highly diverse. Forty-two genotypes were identified only once during the year in the rivers. Only one *C. jejuni* PFGE profile, K1SND, was detected three times in the year, in the same river (R5). Two *C. jejuni* genotypes (K1SND, K5SND) were obtained from R2 and R5 and two *C. lari* genotypes (K69S52, K72S55) were obtained from R1 and R3.

The number of *Kpn1-Sma1* profiles observed per river in the year was variable: eight PFGE profiles in rivers R1 and R2, 10 in river R3, 23 in river R5 and only one in river R4. Seventeen PFGE profiles were obtained in summer, 12 in spring and, 11 in fall and also in winter.

With a cut-off value of 80%, 39.1% of the water PFGE profiles (18/46) were grouped into eight clusters coded W on the dendrogram (Fig. 2). In five clusters (W1, W3, W4, W6 and W8), the genotypes were from the same river and, in three clusters (W2, W5, and W7), the genotypes were found in two different rivers. Clusters did not tend to represent one season but tended to include isolates from most seasons.

3.3. Genetic relationship to animal and human Campylobacter isolates

The 46 *Campylobacter* genotypes obtained from water were analyzed with 965 *Campylobacter* genotypes of animal origin and 363 genotypes of human origin using Dice correlation coefficient and UPGMA method (dendrograms in Figs. 3 and 4). Nine *C. jejuni* isolates obtained from river water (from R2 and R5) had a profile (K5SND) identical to those of human *Campylobacter* isolate and two poultry isolates. Over the 46 *Campylobacter* genotypes from water, 14, 11 and one genotypes were genetically closed to human, poultry, and pig *Campylobacter* genotypes, respectively (indicated by ● in dendrogram). Water *C. lari* PFGE profiles were genetically distant from those of humans, to which they were only 51.6% genetically similar (Fig. 5).

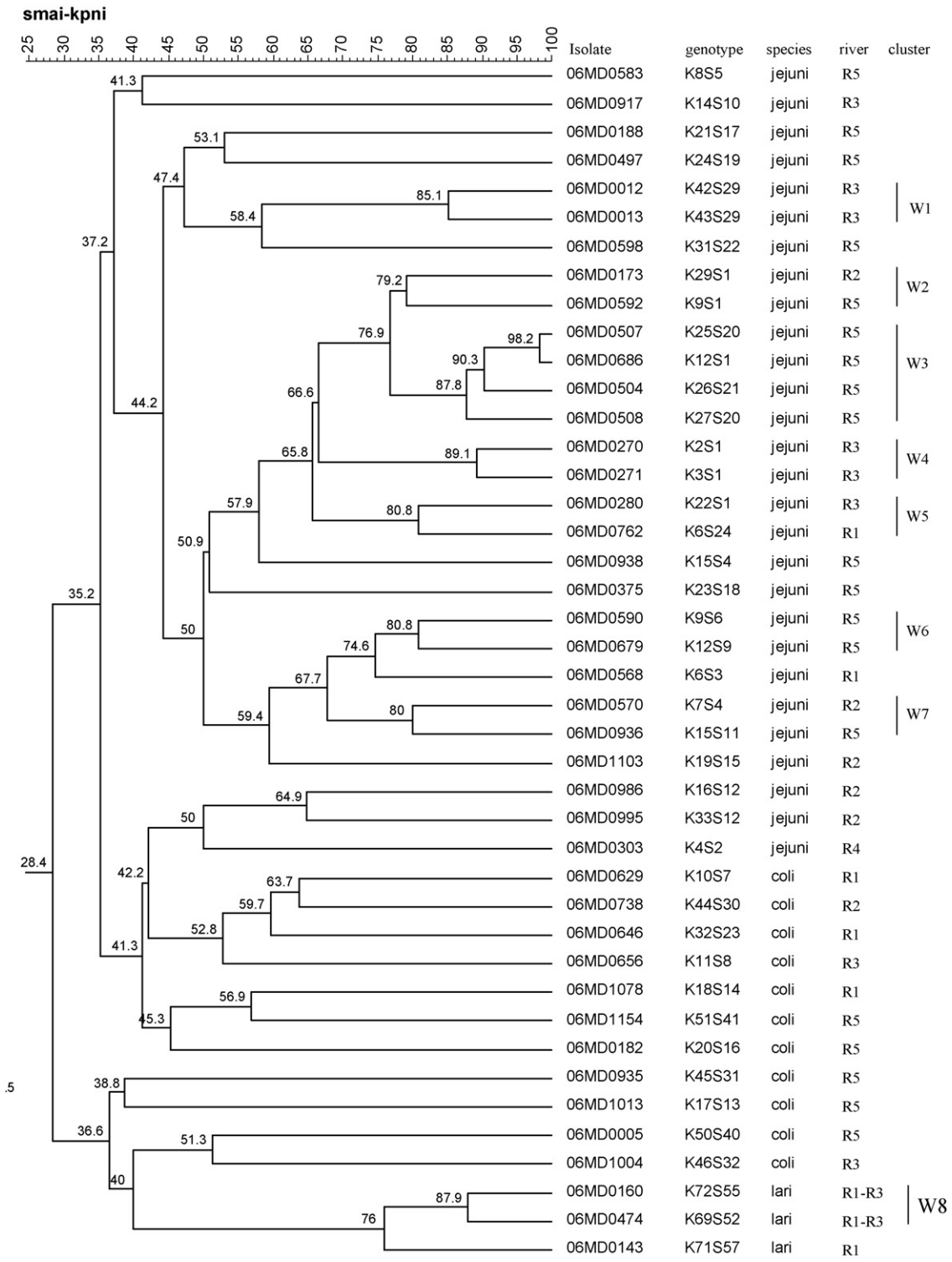

Fig. 2. Dendrogram of the *Kpn1-Sma1* profiles of water *Campylobacter* spp. isolates.

4. Discussion

Surface water has been implicated in transmission of *Campylobacter* [30,31]. In our year-long study, surface water appeared to be an important reservoir of *Campylobacter*; with 50% of river water samples testing positive for *Campylobacter*. The prevalence of *Campylobacter* in surface water is highly variable with reported isolation rates of 0% [32], 12% [33], 17.1% [34], 17.3% [35], 53.3% [36], 70% [37], 82.1% [38,39] and 87.5% [19]. This difference between these studies could be related to the method used for detecting *Campylobacter* from water.

Several studies have indicated that the rate of *Campylobacter* detection in surface water is variable and depends on sampling season. *Campylobacter* isolation rates from surface water are highest in the late fall and winter and lowest in spring and summer, according Carter et al. [40], Obiri-Danso and Jones [14] and Daczkowska-Kozon and Brzostek-Nowakowska [41]. Eyles et al. [20] reported larger number of positive samples during winter and

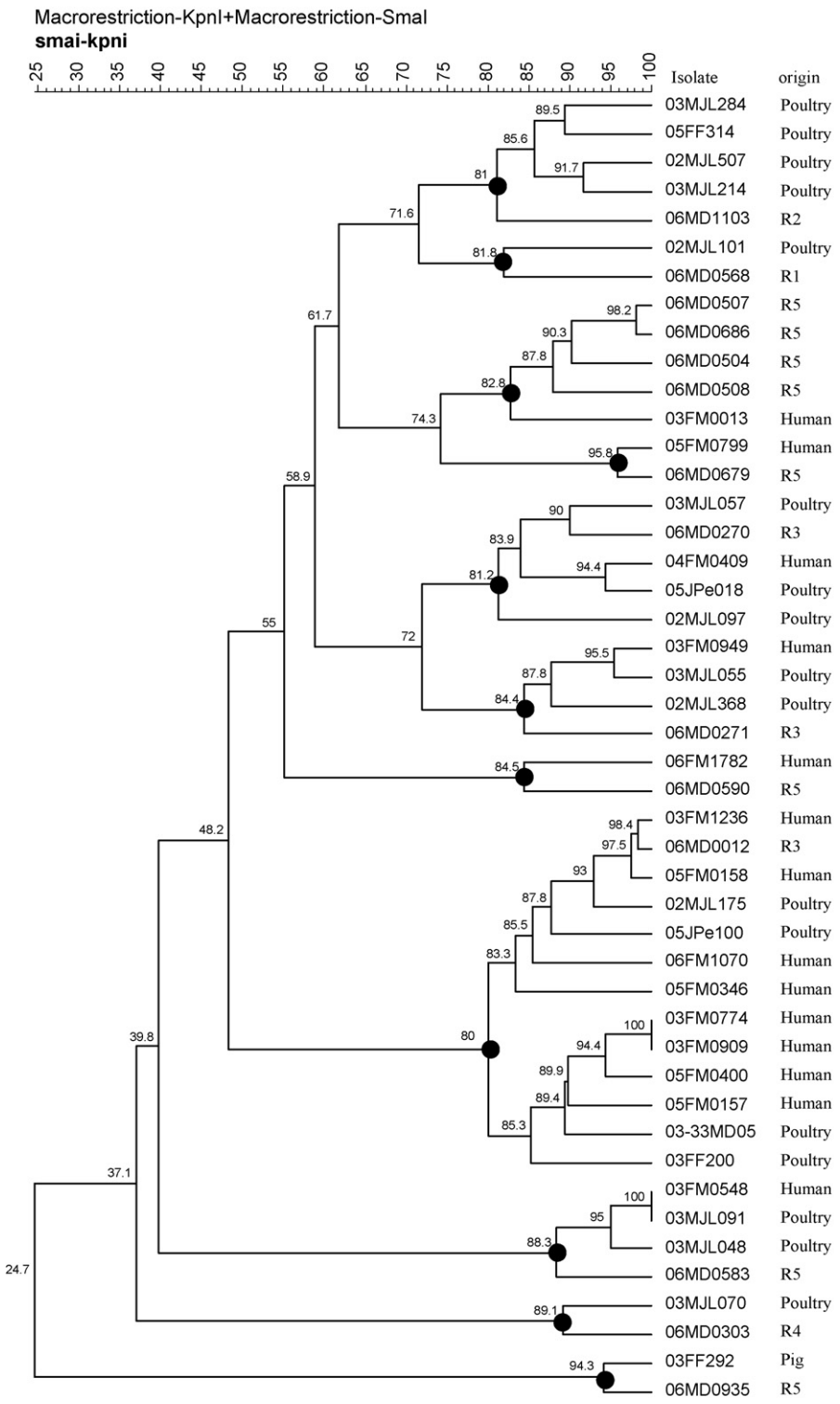

Fig. 3. Dendrogram of the *Kpn1-Sma1* profiles of water *Campylobacter* spp. isolates clustered with poultry, pig and human *Campylobacter* isolates (●).

summer, whereas Close et al. [33] reported smaller number of positive samples in winter. We observed no seasonal effects on *Campylobacter* isolation. For the five rivers considered together, 46.6% to 53.3% of the samples collected during each season were contaminated with *Campylobacter.*

One of the rivers tested positive for *Campylobacter* only once, possibly due to the location of the sampling site just after a barrage, resulting in the sedimentation of particles.

In this study, 66.6% of positive river water samples taken upstream from water treatment plants contained *C. jejuni* and 33.3% contained *C. coli. C. jejuni* represented 76.1% of the isolates. *C. jejuni* was also the major species in the river samples analysed by Daczkowska-Kozon and Brzostek-Nowakowska [41]. Hörman et al. [35] reported a higher percentage of *C. jejuni* (45.8%) than of *C. coli* (4.2%) in populations from the surface water of lakes and rivers. A similar situation was reported by Close et al. [33] in their analysis

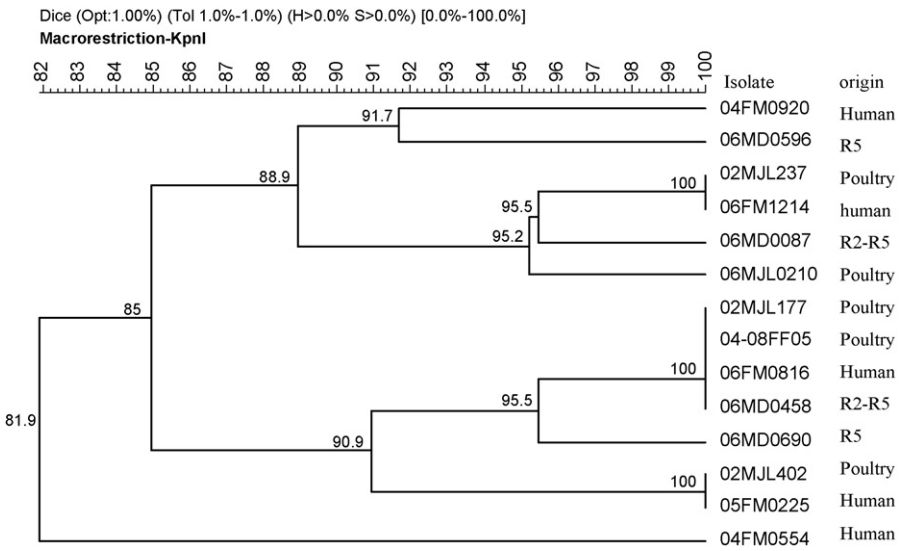

Fig. 4. Dendrogram of the *Kpn1* profiles of water *Campylobacter* spp. isolates clustered with *Kpn1* profiles of poultry and human *Campylobacter* isolates. These isolates had all a non-digested genome by *Sma1*.

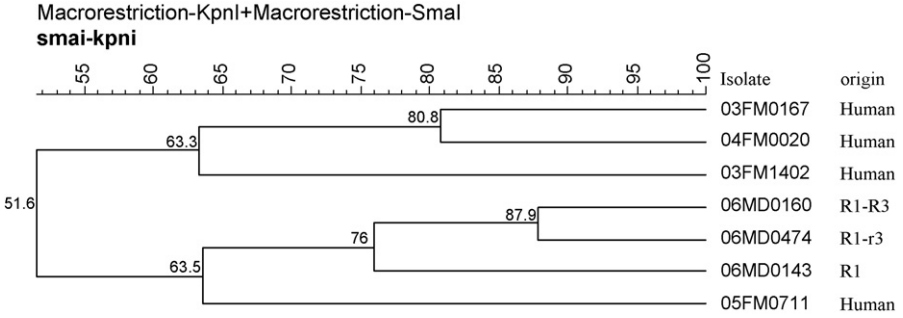

Fig. 5. Dendrogram of the *Kpn1-Sma1* profiles of water and human *C. lari* isolates.

of groundwater samples. By contrast, *C. coli* was the major species (44%), closely followed by *C. jejuni* (34.6%) in a similar study by Rosef et al. [36].

In this study, *C. lari* was detected in 10% of the positive water samples and accounted for 8.1% of isolates. Other studies have also reported the presence of *C. lari* in surface water. Daczkowska-Kozon and Brzostek-Nowakowska [41] detected *C. lari* in 3.6% of positive surface water samples. This *Campylobacter* species was also detected in 4.2% and in 5% of the water samples analyzed by Hörman et al. [35] and Brown et al. [42], respectively. *C. lari* accounted for 14.7% of the *Campylobacter* isolates collected by Rosef et al. [36] from groundwater.

Our results showed that the PFGE profiles of *Campylobacter* in river were highly diverse, with most profiles (91.3%) unique. Only four genotypes were detected on several occasions during the year, in the same or in different rivers. Moreover, only 39% of the water *Campylobacter* PFGE profiles was grouped into clusters; so a few of the genetic profiles were genetically similar. Lévesque et al. [43] described also an important genetic diversity for their *C. jejuni* isolates collected from environmental water in Quebec, Canada.

This variability in *Campylobacter* genotype during the year is probably linked to the presence, at particular times, of animals and agricultural activities around the rivers, rather than to seasonal effects. Thomas et al. [44] pointed out that reported variations in the rate, type and seasonality of *Campylobacter* of surface water contamination are not unexpected, given the multitude of factors potentially influencing this contamination, including rainfall, temperature, the indigenous fauna, and flow rates. Close et al. [33] reported a higher rate of *Campylobacter* detection during the irrigation season.

The sampling site R5 was the one with the highest number of positive samples and the highest number of genotypes. These results are probably due to its geographical localization; after the junction of several rivers coming from different valleys which increases the possibilities to be contaminated by *Campylobacter* from multiple sources.

Our study confirms that poultry may be a source of water contamination by *C. jejuni*; indeed, 34.4% of the water genotypes clustered with poultry genotypes. *C. jejuni* is known to be the predominant species in poultry production systems in Brittany, France [24,45,46]. Some of the *C. jejuni* isolates from water were also closely related to human isolates suggesting than human could be a source of contamination of river by *Campylobacter*. But our findings cannot show unequivocally that cases of *Campylobacter* infection in humans are due to contamination from chickens or water, but they do show that isolates from both these sources are indistinguishable from isolates capable of producing disease in humans. Only one *C. coli* genotype isolated from R5 water in October could be associated with pig in this study. No *C. coli* from water clustered with *C. coli* isolates from poultry and/or humans. This result suggests that there must be other sources of *C. coli*.

Transmission from animals and birds to water may occur through direct contamination, or indirectly, through contamination of the catchment area, with subsequent drainage into water reservoirs [47,48]. Runoff from agricultural land, particularly

during periods of heavy rainfall and flooding, may introduce *Campylobacter* into surface waters [41].

In our study, 73.9% of the *C. jejuni* and *C. coli* genotypes from water were not of poultry or pig or human origin. They may have come from strains infecting wild animals and birds, or from other farms animals. *C. jejuni* is the predominant species in birds [49,50], ruminants and poultries [42,45,48,49,51–53]. Thermophilic *Campylobacter* species were prevalent in all of the wild animals analyzed by Wahlström et al. [54]. Moreover, French et al. [55] indicate that isolates from wildlife feces were of the same sequence types as surface water isolates. Kwan et al. [56] reported similar findings for isolates from birds, rabbits and water, and also showed restricted exchange of *C. jejuni* between cattle and the environment.

The *C. lari* in water in our study here may result from contamination by birds. Brown et al. [42] detected *C. lari* in birds and water from the same area. *C. lari* was also detected in migrating birds by Waldeström et al. [57]. Although *C. lari* has been isolated from poultry in Belgium [53], this species has not been detected in poultry flocks in Brittany, France [24,45,46].

In this study, *Campylobacter* was not detected in drinking water sampled after passage through the five WTP. The treatment processes at all five treatment plants included a final chlorination step and *Campylobacter* is susceptible to chlorination [58]. The consumption of ground water without disinfection was identified as a source of outbreaks of waterborne *Campylobacter* infection in Finland [11,59], and failure of the chlorination system has been identified as a cause of waterborne outbreaks [10,12]. Our findings indicate that the risk of outbreaks due to consumption of drinking water is low in the neighbourhoods of Saint-Brieuc, but may exist in the absence of chlorination.

In conclusion, the rivers of Brittany, France, tested displayed almost continual *Campylobacter* contamination and the *Campylobacter* population was found to be highly genetically diverse during this study, consistent with multiple origins of contamination. Human, poultry and pig were implied in the contamination of river by *Campylobacter*. Finally, no *Campylobacter* was detected in drinking water indicating that the risk of outbreaks due to consumption of drinking water is low.

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