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Short Communication

Investigation of an unusual increase in human yersinioses in Creuse, France

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SUMMARY

Objectives: To investigate an unusual cluster of *Y. enterocolitica* 4/O:3/VIII human infections that occurred in Creuse (France) during the summer 2008, and to perform retrospective and prospective analyses of yersiniosis cases to get a better view of the general trend.
Methods: 33 pathogenic *Y. enterocolitica* strains isolated between 2008 and 2010 in Creuse were subjected to phenotypic and molecular typing. The database of the *Yersinia* National Reference Laboratory was used to compare the number of human cases over 23 years in Creuse and at the national level.
Results: The 33 isolates had three distinct phenotypes and a high genetic diversity, ruling out a unique source of contamination. A long-term analysis of yersiniosis cases in Creuse showed a progressive increase over years, with a peak in 2008 and a subsequent decrease. This trend contrasted with the national cases that showed an opposite pattern.
Conclusions: Local environmental conditions were most likely responsible for a transient expansion of pathogenic *Y. enterocolitica* strains in Creuse.

1. Introduction

Enteropathogenic *Yersinia enterocolitica* are the third leading cause of bacterial diarrhoea in France[1] and Europe.[2] These bacteria have an animal reservoir and are transmitted by the faecal-oral route. The real incidence of *Yersinia* infections is unknown because reporting of human yersiniosis cases is not compulsory in France. Based on the number of strains sent to the French *Yersinia* National Reference Laboratory (YNRL), the rate of *Yersinia* isolation has been estimated to be 2.1/100,000.[3] This number is probably largely underestimated because the recovery of these bacteria from stools is difficult. In France and in Europe yersinioses usually occur as sporadic cases,[4] although a few outbreaks have been described.[5,6]

Between July 1st and September 27, 2008, two clinical pathology laboratories in Creuse (central France), 83 kilometres distant, isolated 8 *Y. enterocolitica* strains from diarrhoeal patients. The YNRL confirmed that they were pathogenic strains. The very uncommon isolation of pathogenic *Y. enterocolitica* from this region suggested a possible common source of contamination. An in depth analysis of *Y. enterocolitica* strains isolated from this area and a temporal follow up of human yersinioses cases was performed.

2. Methods and materials

All strains were subjected to phenotypic analyses (biotyping, serotyping, and for biotype 4 isolates phage typing).[7] For Pulsed Field Gel Electrophoresis (PFGE) of biotype 4 isolates, genomic DNA was prepared in agarose plugs and digested with *Pme*I. The macrorestriction fragments were resolved with a CHEF-DRIII apparatus (Bio-Rad Laboratories) in 1% agarose gels at 14 °C, using an electric field of 6 V/cm and an angle of 120°. For Multiple-Locus Variable tandem repeat Analysis (MLVA), genomic DNA was extracted with the Gentra Puregen kit (Qiagen), and the six repeats previously selected[8] were subjected to PCR amplification. The sizes

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of the PCR products were determined by capillary electrophoresis with an ABI Prism 310 genetic analyzer (Applied Biosystems).

3. Results and discussion

All 8 strains isolated in Creuse during summer 2008 were pathogenic *Y. enterocolitica* biotype 4, serotype O:3 and phage type VIII (4/O:3/VIII) (Table 1, highlighted in grey), suggesting a possible exposure to a common source of contamination. However, an epidemiological investigation launched at the regional level did not identify any links between the cases. A PFGE analysis yielded 3 distinct restriction profiles for these 8 strains (Table 1), arguing against a unique source of contamination. This was confirmed by MLVA typing, which identified 5 different profiles (Table 1). Three strains had the same MLVA profile and pulsotype 2, and a fourth strain also of pulsotype 2 differed from the other 3 at a single MLVA locus. This suggested that despite the absence of identified epidemiological links between the clinical cases, half of the patients might have been exposed to the same pathogenic strain. However, since unrelated *Y. enterocolitica* 4/O:3 strains were isolated from the four other cases, it nevertheless indicated an unusual increase in the circulation of different pathogenic strains in Creuse.

A retrospective analysis of the database of the YNRL showed that between 1991 and 2001, the average number of pathogenic *Y. enterocolitica* isolated by these two laboratories was 1.5/year. This trend increased between 2002 and 2007, with an average of 4.7 strains/year (Figure 1). A prospective study of the strains isolated in Creuse during the entire year 2008 and over the following two years confirmed the increasing trend, with a total of 33 pathogenic *Y. enterocolitica* strains isolated between 2008 and 2010 (average of 11 strains/year). Most of these strains were of bioserotype 4/O:3 (26 strains) and had the common phage type VIII (24 strains), but two had the infrequent phage type IXa (Table 1). The seven remaining pathogenic strains were of bioserotype 2/O:9 (Table 1), which is the second most common bioserotype in France.[7] Therefore, the increased number of human yersiniosis cases in Creuse was caused by at least three phenotypically distinct pathogenic *Y. enterocolitica* strains.

To further evaluate the diversity of the 4/O:3/VIII strains isolated between 2008 and 2010 in Creuse, a PFGE analysis was carried out. Eight pulsotypes were found among the 24 strains. Pulsotype 2 predominated in 2008, but was not found later on. Pulsotypes 1 and 3 persisted, but in low noise, while pulsotype 4, which was not detected during the summer 2008 episode, became the most frequent (8 strains) in the following years (Table 1). However, only 2 of the 8 strains of pulsotype 4 shared the same MLVA profile. They were isolated in November by the same laboratory, suggesting a possible common source of contamination for the two patients. This could also hold true for the two patients infected with *Y. enterocolitica* 4/O:3/IXa isolates that displayed identical MLVA profiles and were isolated at one day interval (Table 1). With these few exceptions, all other 4/O:3 and 2/O:9 strains exhibited distinct MLVA profiles. The pathogenic strains responsible for human yersinioses in Creuse were therefore remarkably diverse.

Altogether our data indicate an unusual increase in the isolation rate of pathogenic *Y. enterocolitica* in Creuse since the 2000s, with a

Table 1
Characteristics of 33 pathogenic *Y. enterocolitica* strains isolated in Creuse between 2008 and 2010

Laboratory	Year	Month	IP number	Gender	Age (year)	Phage type	Pulsotype	MLVA profile
Bioserotype 4/O:3 strains								
Aubusson	2008	Jan 19	29401	M	77	VIII	1	10-3-10-6-13-5
Aubusson	2008	July 01	29604	M	30	VIII	2	9-6-12-4-5-6
Aubusson	2008	July 07	29610	M	23	VIII	3	10-5-9-10-7-6
La Souterraine	2008	July 09	29621	M	8	VIII	1	10-3-10-6-15-5
Aubusson	2008	Aug 01	29681	F	35	VIII	2	12-6-12-7-6-8
Aubusson	2008	Aug 04	29639	M	19	VIII	2	12-6-11-7-6-8
Aubusson	2008	Sept 06	29684	M	14	VIII	2	12-6-12-7-6-8
Aubusson	2008	Sept 16	29691	M	5	VIII	2	12-6-12-7-6-8
Aubusson	2008	Sept 27	29706	M	<1	VIII	1	11-3-11-5-X-5
La Souterraine	2008	Oct 21	29735	F	81	VIII	1	11-3-9-5-13-5
La Souterraine	2008	Nov 04	29747	M	08	VIII	5	13-6-10-3-6-6
La Souterraine	2008	Nov 05	29746	M	56	VIII	4	8-5-10-9-4-6
La Souterraine	2008	Nov 18	29756	F	68	VIII	4	8-5-10-9-4-6
Aubusson	2009	July 24	29990	M	3	VIII	4	9-3-9-11-12-5
La Souterraine	2009	Aug 21	33485	F	12	VIII	4	3-6-9-4-7-6
La Souterraine	2009	Sept 07	33503	M	2	VIII	4	8-6-11-7-6-7
Aubusson	2009	Sept 09	33505	F	1	VIII	4	19-7-10-9-13-3
La Souterraine	2009	Nov 06	33545	M	60	IXa	ND	7-6-14-3-X-9
La Souterraine	2009	Nov 07	33544	F	36	IXa	ND	7-6-14-3-X-9
La Souterraine	2009	Nov 21	33556	M	11	VIII	1	13-3-7-5-9-5
La Souterraine	2009	Nov 21	33563	M	40	VIII	4	10-3-14-11-16-4
La Souterraine	2010	Apr 03	33672	F	6	VIII	3	10-3-12-7-10-5
La Souterraine	2010	July 13	33769	F	73	VIII	6	6-6-7-5-6-6
La Souterraine	2010	Aug 19	33826	M	05	VIII	4	12-3-11-7-20-4
La Souterraine	2010	Sept 04	33849	M	UN	VIII	7	10-6-14-3-6-5
Aubusson	2010	Nov 24	33923	F	67	VIII	8	4-8-5-9-13-4
Bioserotype 2/O:9 strains								
Aubusson	2008	May 29	29545	M	3	ND	ND	2-2-4-7-5-4
La Souterraine	2009	March 06	29835	M	54	ND	ND	2-2-9-8-3-5
La Souterraine	2009	May 23	29913	M	69	ND	ND	4-2-9-7-4-5
La Souterraine	2009	July 21	29986	F	63	ND	ND	6-2-7-7-7-9
La Souterraine	2009	Nov 26	33564	M	66	ND	ND	4-2-7-7-7-5
La Souterraine	2010	Jan 06	33580	F	71	ND	ND	5-2-6-5-9-5
La Souterraine	2010	Jan 25	33589	M	54	ND	ND	4-2-6-9-8-5

All strains were isolated from stools. Strains in a grey background correspond to those that were isolated during the summer 2008 episode.
IP: Institut Pasteur, MLVA: Multiple-Locus Variable tandem repeat Analysis, M: male, F: female, UN: unknown, ND: not determined. X in the MLVA profile means that the number of repeats could not be determined.

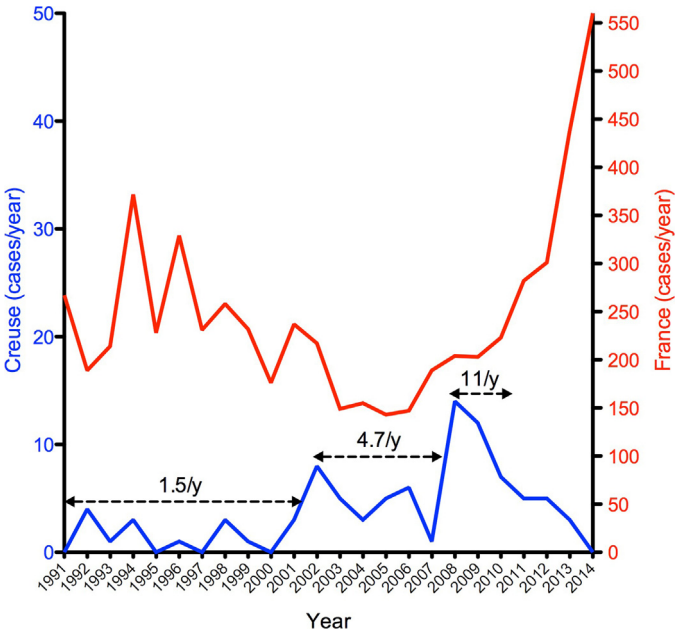

Figure 1. Number of pathogenic *Y. enterocolitica* strains isolated in Creuse (left axis) or over the whole French territory (right axis) between 1991 and 2014.
Numbers above the dashed arrowed lines indicate the average of pathogenic *Y. enterocolitica* strains isolated from human cases in Creuse during the given period. These figures correspond to the number of strains that were sent to the YNRL for epidemiological surveillance and phenotypic characterization.

peak in 2008. No changes in laboratory practices, staff, or data reporting to the YNRL occurred in the two laboratories. The high diversity of the pathogenic isolates over several years in two different laboratories demonstrate that these cases were not caused by a common source of contamination or by the expansion of a specific *Y. enterocolitica* clone. Rather, this phenomenon is reminiscent of the sudden onset of *Y. pseudotuberculosis* infections in France during the winter 2004-2005 that was most likely due to environmental conditions favourable for the expansion of rodent reservoirs.[9] After the peak of 2008 (14 strains), the number of pathogenic *Y. enterocolitica* isolated in Creuse remained above the average in 2009 (12 strains) and 2010 (7 strains), but then severely declined (Figure 1), suggesting that the conditions that favoured the occurrence of human yersinioses in Creuse did not persist. Since pigs are the main reservoir of *Y. enterocolitica* 4/O:3, changes in pig farming might have had an influence on the occurrence of yersiniosis in Creuse. However, arguing against this hypothesis was the observation that 2/O:9 strains also were isolated in higher numbers during the years 2008-2010 although they usually have other animal reservoirs (cattle). Human *Y. enterocolitica* infections occur all year round in France, but with a peak during the warmest months. This was the case for the cluster of strains isolated during summer 2008. However, of the 33 human cases that occurred during the 3 years follow up study, only 18 (54.4%) of them happened during the warmest months (Table 1). Moreover, no major differences in maximal and minimal temperatures or in pluviometry were observed in Creuse between years with low and high numbers of *Y. enterocolitica* isolation.

A comparison with the national data collected over the same period (1991-2014) showed opposite trends in the isolation of pathogenic *Y. enterocolitica*: the period with lower numbers of isolates in France (2002-2009) was the one with higher numbers in Creuse (Figure 1). Therefore, the increase in human yersinioses observed in Creuse in the 2000s was transient and was most likely caused by local environmental conditions that favoured the expansion/transmission of pathogenic *Y. enterocolitica*.

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