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Yersiniosis in France: overview and potential sources of infection

Anne-Sophie Le Guern ^{*}, Liliane Martin, Cyril Savin, Elisabeth Carniel

Yersinia Research Unit and National Reference Laboratory, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris cedex 15, France

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SUMMARY

Objectives: The aim of this study was to exploit the extensive database on strains of *Yersinia* collected over more than 50 years in France in order to gain an overview of yersiniosis and potential sources of contamination in this country.

Methods: The 19 670 strains of *Yersinia* of human, animal, environmental, and food origin isolated in France were grouped by species, biotype, and serotype.

Results: Most human strains (59%) were pathogenic, with a marked predominance of *Yersinia enterocolitica* bioserotype 4/O:3 (66.8%), followed by *Y. enterocolitica* 2/O:9 (23.8%) and *Yersinia pseudotuberculosis* (6.1%). Pigs and pork meat were the nearly exclusive sources of *Y. enterocolitica* 4/O:3. Other pathogenic strains were rarely isolated from food or environmental samples (0.2%). The major source of pathogenic *Yersinia* was the animal reservoir, with a remarkable association between *Y. enterocolitica* 4/O:3 and pigs, *Y. pseudotuberculosis* and wildlife, *Y. enterocolitica* 2/O:9 and grazing farm animals, *Y. enterocolitica* 5/O:2,3 and hares, and *Y. enterocolitica* 3/O:1,2,3 and chinchillas.

Conclusions: The frequency of human infection caused by certain *Yersinia* subgroups might be related to the frequency of exposure to specific animal sources. In contrast, non-pathogenic *Yersinia* were commonly isolated from foodstuffs and the environment, most probably accounting for the abundance of non-pathogenic *Yersinia* recovered from human stools.

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1. Introduction

The genus *Yersinia* is composed of 18 species, of which three are well-recognized human pathogens: *Yersinia pestis*, the causative agent of plague, and the two enteropathogenic species *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*.¹ The pathogenicity of the recently described species *Yersinia wautersii* awaits further analyses.² The 14 other species are *Yersinia albovæ*, *Yersinia aleksiae*, *Yersinia bercovieri*, *Yersinia entomophaga*, *Yersinia frederiksenii*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia massiliensis*, *Yersinia mollaretii*, *Yersinia nurnii*, *Yersinia pekkanenii*, *Yersinia rohdei*, *Yersinia ruckeri*, and *Yersinia similis*.^{3–5}

In most countries, enteric yersiniosis is caused primarily by *Y. enterocolitica*, and much less frequently by *Y. pseudotuberculosis*.⁶ *Yersinia* are the third cause of bacterial diarrhoea in Europe.⁷ Infection occurs predominantly in children under 10 years of age. Clinical manifestations include diarrhoea, fever, and abdominal pain that can mimic appendicitis.⁸ Systemic forms are also

observed, particularly in elderly persons and in patients with underlying conditions such as iron overload, cirrhosis, diabetes, and cancer.⁹

All pathogenic *Yersinia* strains harbour the pYV virulence plasmid. *Y. pseudotuberculosis* strains are divided into serotypes, and all strains are potentially pathogenic for humans and various animal species, whatever their serotype. In contrast, not all *Y. enterocolitica* strains are pathogenic. This species is divided into six biotypes (BT), of which BT1A does not harbour the pYV and corresponds to non-pathogenic strains. The putative pathogenicity of some BT1A strains has been suggested based on the presence of certain potential virulence genes in their genomes; however recent studies have concluded that evidence for a role of BT1A in human infection is still lacking.^{10,11} All other biotypes (BT1B, 2 to 5) harbour the pYV and are pathogenic.¹² The species is also divided into more than 70 serotypes, but there is a strong association between pathogenic BTs and some serotypes, while non-pathogenic BT1A strains have a wide array of serotypes.¹³

Sources of *Yersinia* contamination are seldom identified because most cases are sporadic and no investigations of the contamination source are performed. Yersiniosis outbreaks have been reported in some countries such as Japan,¹⁴ Russia,¹⁵

* Corresponding author. Tel.: +33 145688329; fax: +33 145688954.

E-mail address: anne-sophie.le-guern@pasteur.fr (A.-S. Le Guern).

Norway,¹⁶ and the USA,¹⁷ but not in France. The consumption of raw or undercooked pork meat is a well-established mode of *Y. enterocolitica* contamination.¹⁸ Pigs are healthy carriers of *Y. enterocolitica* and the contamination of pig carcasses in slaughterhouses has been largely documented in several countries.^{19,20} Other potential reservoirs of pathogenic *Yersinia* exist but are much less documented.

The French Yersinia National Reference Laboratory (YNRL) has been in charge of the surveillance of human yersiniosis since the 1960s, but cases of enteric yersiniosis are not notifiable in France. The YNRL has received more than 20 000 *Yersinia* strains isolated in France for characterization (species, BT, and serotype) over more than 50 years. These strains were isolated from clinical cases, but also from sick animals or during specific food, environmental, or veterinary surveys. The aim of this study was to benefit from the extensive YNRL database to gain an overview of yersiniosis in France and to analyze the distribution of the various *Yersinia* species and subgroups according to their sources of isolation, thus highlighting potential reservoirs and human infections.

2. Materials and methods

2.1. Strain origins

The *Yersinia* strains were isolated in France between 1931 and 2013. They were identified as *Yersinia* by peripheral laboratories (medical, veterinary, etc.) and sent to the YNRL for further characterization (determination of the species, BT, and serotype). An epidemiological form indicating the place, date, sample, and source of the strain was sent with the isolate.

Clinical strains were isolated from 1951 to 2013 and sent on a regular basis and with a voluntary approach by 840 medical laboratories located throughout France. The majority of these strains were recovered from patients with clinical symptoms.

The animal isolates were collected from 1931 to 2013 during epizootics (particularly in pseudotuberculosis in lagomorphs in the 1950s, 60s, and 70s), sporadic animal infections, surveys of potential reservoirs, or investigations in herds with positive brucellosis serology because of known cross-reactions with *Y. enterocolitica* serotype O:9.

Food strains were isolated from 1966 to 2011, and environmental strains from 1975 to 2006 during specific surveys or occasional surveillance of possible sources of contamination.

2.2. Phenotypic characteristics of the *Yersinia* strains registered in the database

Species were assigned using metabolic tests: API20E and API50CH strips (bioMérieux, Marcy l'Etoile, France), pyrazinamidase,²¹ and Tween esterase activity²² performed at 28 °C. *Y. enterocolitica* strains were further biotyped according to the biogrouping scheme of Wauters et al.¹² The strains were serotyped with a set of 47 O:antigen-specific rabbit antisera.

2.3. Database and data analysis

Software designed specifically to accommodate the data of the YNRL (Lagon, Epicentre, France) was used to enter the phenotypic characteristics and epidemiological data for each strain. These data were analyzed using Excel Pivot Tables (Microsoft, Redmond, WA, USA).

The strength of association between pathogenic BTs and specific reservoirs was determined by 2 × 2 contingency table analysis using GraphPad Prism 5. The Chi-square with Yates correction was calculated. A p-value of <0.01 was considered statistically significant.

3. Results

3.1. Characteristics of the data analyzed

The database of the YNRL contains information on the source (human, animal, environment, or food) and bacteriological characteristics (species, serotype, and for the *Y. enterocolitica* species, also BT) of 20 216 *Yersinia* strains isolated in France between 1931 and 2013. Among these strains, 451 were not included in the analysis because their identification was not complete, and 95 because their source of isolation was unknown. Thus, 19 670 characterized French strains were retained for the study (Table 1). No strains belonging to the species *Y. aleksiciae*, *Y. entomophaga*, *Y. massiliensis*, *Y. nurnmii*, *Y. pekkanenii*, *Y. wautersii*, *Y. similis*, or *Y. pestis* were isolated in France and sent to the YNRL for characterization. Most of the strains were isolated from humans ($n = 12\,526$), because the main assignment of the YNRL is the epidemiological surveillance of human yersiniosis in France, followed by food ($n = 3249$), animals ($n = 2994$), and the environment ($n = 901$).

Table 1
Source of isolation of the various *Yersinia* species

Species	Humans		Animals		Food		Environment		Total	
	Number	%	Number	%	Number	%	Number	%	Number	%
Pathogenic										
<i>Y. pseudotuberculosis</i>	453	3.6	1813	60.6	0	0.0	2	0.2	2268	11.5
<i>Y. enterocolitica</i>	6935	55.4	437	14.5	96	3.0	3	0.3	7471	38.0
Sub total	7388	59.0	2250	75.1	96	3.0	5	0.5	9739	49.5
Non-pathogenic										
<i>Y. enterocolitica</i> 1A	4162	33.2	496	16.6	1883	58.0	380	42.2	6921	35.2
<i>Y. aldovae</i>	1	0.0	0	0.0	0	0.0	0	0.0	1	0.0
<i>Y. bercoieri</i>	208	1.7	10	0.3	212	6.5	19	2.1	449	2.3
<i>Y. frederiksenii</i>	430	3.4	65	2.2	262	8.1	175	19.4	932	4.7
<i>Y. intermedia</i>	122	1.0	92	3.1	543	16.7	284	31.6	1041	5.3
<i>Y. kristensenii</i>	154	1.2	67	2.2	227	7.0	35	3.9	483	2.5
<i>Y. mollarettii</i>	56	0.5	14	0.5	26	0.8	0	0.0	96	0.5
<i>Y. rohdei</i>	5	0.0	0	0.0	0	0.0	0	0.0	5	0.0
<i>Y. ruckeri</i>	0	0.0	0	0.0	0	0.0	3	0.3	3	0.0
Sub total	5138	41.0	744	24.9	3153	97.0	896	99.5	9931	50.5
Total	12 526	100.0	2994	100.0	3249	100.0	901	100.0	19 670	100.0

3.2. Characteristics of the *Yersinia* clinical strains in France

The majority of the strains isolated from humans were pathogenic (59%) (Table 1), with a marked predominance of *Y. enterocolitica* (93.9%) over *Y. pseudotuberculosis* infections. In the *Y. enterocolitica* species, bioserotype 4/O:3 was by far the most frequent (71.1%), followed by bioserotype 2/O:9 (25.4%) (Table 2). Only two *Y. enterocolitica* strains of BT1B and none of BT5 were isolated from humans (Table 2). *Y. pseudotuberculosis* infections represented only 6.1% of clinical yersiniosis cases. Most of these cases were caused by serotype I strains (76.4%), followed by serotypes II and III (Table 2). Of note, non-pathogenic *Yersinia* species were frequently isolated from humans (41% of clinical strains). The majority of these non-pathogenic isolates (81%) were *Y. enterocolitica* BT1A strains (Table 1).

3.3. Characteristics of the *Yersinia* strains isolated from animals in France

In contrast to humans, *Y. pseudotuberculosis* was the predominant pathogenic species isolated from animals (Table 1). A wide variety of animal species were infected with *Y. pseudotuberculosis* (Table 3). Lagomorphs (hares and rabbits) were the major source of *Y. pseudotuberculosis* strains (75.5%), due to pseudotuberculosis epizootics in these animal species. Other frequent sources of *Y. pseudotuberculosis* strains were birds and rodents (Table 3). As in humans, strains of serotype I were the most common (66.5%), but serotype II represented a significant proportion (24.3%) of the *Y. pseudotuberculosis* strains of animal origin (Table 2). No association between a specific serotype and a given animal species was observed: the predominant serotype I was isolated from most *Y. pseudotuberculosis*-infected animal species, and serotype II was also largely distributed in the animal reservoir (Table 3).

Y. enterocolitica represented only 19.4% of the pathogenic animal strains. Within this species, there was a clear association between BT and animal sources. The well-known link between BT4 *Y. enterocolitica* and pigs was confirmed ($p < 0.0001$), while only nine *Y. enterocolitica* strains of BT2 or BT3 were isolated from pigs (Table 3). A few BT4 strains were also isolated from canines, lagomorphs, monkeys, and rodents, but in a low proportion, indicating that pigs are the major animal source of BT4 *Y. enterocolitica* in France. There was a statistically

significant association between BT2 *Y. enterocolitica* and bovines ($p < 0.0001$), sheep ($p < 0.0001$), and goats ($p < 0.0001$), but not with pigs. The few remaining BT2 strains came from one dog and four monkeys (Table 3). BT5 *Y. enterocolitica* were found on rare occasions in cattle (three bovines and two sheep), but were significantly associated with lagomorphs ($p < 0.0001$). Finally, BT3 *Y. enterocolitica* was mainly associated with rodents ($p < 0.0001$), most of them being chinchillas. A single BT1B *Y. enterocolitica* was of animal origin, and was isolated from a rodent (Table 3).

The comparison of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* distribution in animals showed a clear difference in habitat: farm animals (bovines, goats, pigs, and sheep) were a major source of *Y. enterocolitica* strains (68.2% of pathogenic animal *Y. enterocolitica* strains), while only 1.7% of *Y. pseudotuberculosis* animal strains were recovered from these farm animals (Table 3). Conversely, wild animals (birds, deer, lagomorphs, rodents) were the major source of *Y. pseudotuberculosis* isolation (91.9% of *Y. pseudotuberculosis* animal strains), whereas only 29.1% of *Y. enterocolitica* strains were isolated from these wild animals (Table 3). Zoo animals (felines, monkeys) were also more frequently infected with *Y. pseudotuberculosis* (61 strains) than with *Y. enterocolitica* (seven strains). The status 'live' or 'dead' of the animals from which a pathogenic strain was recovered was available for 464 isolates (Table 4), although the status 'sick' or 'healthy' was not available for live animals. Nevertheless, most *Y. pseudotuberculosis* strains (97%) were isolated from dead animals, while nearly all pathogenic *Y. enterocolitica* strains (98%) were recovered from live animals, emphasizing the higher pathogenicity of *Y. pseudotuberculosis* for their animal hosts. Non-pathogenic *Yersinia* represented only 24.9% of the animal strains (Table 1) and were isolated from a variety of animal species (Table 3). Wild (rodents, birds) and farm (pigs, bovine) animals were the major sources (Table 3). BT1A *Y. enterocolitica* were the non-pathogenic strains most frequently isolated from animals (66.6%), followed by *Y. intermedia* (12.4%) (Table 1).

3.4. Sources and characteristics of the *Yersinia* strains isolated from foodstuffs

Yersinia strains isolated from food were almost exclusively non-pathogenic (97%; Table 1). These strains were recovered from

Table 2
Biotype and serotype distribution of pathogenic *Yersinia* strains isolated from different sources

Species	Biotype	Serotype	Humans		Animals		Food Number	Environment Number
			Number	%	Number	%		
<i>Y. enterocolitica</i>								
Sub total	1B	0:8	1	0.0	1	0.2	0	0
2	0:21	1	0.0	0	0	0.0	0	0
		0:5,27	87	1.3	6	1.4	0	0
	0:9	1760	25.4		160	36.6	1	2
		Other ^a	7	0.1	2	0.5	0	0
3	O:1,2,3	18	0.3		34	7.8	0	0
		0:5,27	123	1.8	9	2.1	0	0
	Other ^a	4	0.1		0	0.0	0	0
4	O:3	4932	71.1		141	32.3	95	1
	Other ^a	2	0.0		0	0.0	0	0
5	O:2,3	0	0.0		84	19.2	0	0
		6935	100.0		437	100.0	96	3
<i>Y. pseudotuberculosis</i>								
Sub total	NA	I	346	76.4	1205	66.5	0	2
	NA	II	35	7.7	440	24.3	0	0
	NA	III	18	4.0	22	1.2	0	0
	NA	IV	1	0.2	3	0.2	0	0
	NA	V	7	1.5	38	2.1	0	0
	NA	Other	46	10.2	105	5.8	0	0
			453	100.0	1813	100.0	0	2

NA, not applicable.

^a Not typeable or self-agglutinating.

Table 3Animal species from which *Yersinia* strains were isolated

Animal	<i>Y. pseudotuberculosis</i>						Pathogenic <i>Y. enterocolitica</i>					Pathogenic strains Total	Non-pathogenic strains Total	Total strains	
	Total	ST I	ST II	ST III	ST IV	ST V	Total	BT 1B	BT 2	BT 3	BT 4	BT 5			
Birds	197	138	40	0	0	2	0	0	0	0	0	0	197	120	317
Bovines	4	2	0	1	0	0	97	0	92	2	0	3	101	118	219
Canines	5	4	1	0	0	0	5	0	1	1	3	0	10	6	16
Cervidae	8	6	1	0	0	1	0	0	0	0	0	0	8	1	9
Felines	14	10	2	1	0	0	0	0	0	0	0	0	14	2	16
Fish	2	0	2	0	0	0	0	0	0	0	0	0	2	7	9
Goats	8	5	0	3	0	0	43	0	43	0	0	0	51	3	54
Horses	0	0	0	0	0	0	0	0	0	0	0	0	0	7	7
Lagomorphs	1369	900	351	9	3	33	96	0	0	8	9	79	1465	34	1499
Monkeys	47	29	13	2	0	0	7	0	4	0	3	0	54	9	63
Pigs	7	5	0	2	0	0	133	0	6	3	124	0	140	184	324
Rodents	92	66	16	3	0	2	31	1	0	28	2	0	123	225	348
Sheep	12	8	2	1	0	0	25	0	22	1	0	2	37	17	54
Unknown	48	32	12	0	0	0	0	0	0	0	0	0	48	11	59
Total	1813	1205	440	22	3	38	437	1	168	43	141	84	2250	744	2994

ST, serotype; BT, biotype.

Table 4

Species isolated according to the condition of the source animal

Species	Animal condition			Total
	Live Number	Dead Number	Unknown Number	
Pathogenic				
<i>Y. pseudotuberculosis</i>	6	225	1582	1813
<i>Y. enterocolitica</i>	120	2	315	437
Non-pathogenic	73	38	633	744
Total	199	265	2530	2994

a wide variety of foodstuffs (meat, dairy products, eggs, vegetables, aquatic animals) that were either processed or unprocessed, with a marked predominance in vegetables (42.5% of non-pathogenic food strains, Table 5). *Y. enterocolitica* BT1A strains were the most frequently isolated from food (59.7%), followed by *Y. intermedia* (17.2%), and then by *Y. frederiksenii*, *Y. kristensenii*, and *Y. bercovieri* (Table 1).

Pathogenic *Y. enterocolitica* represented only 3% of the *Yersinia* strains isolated from food (Table 1). These pathogenic strains were almost exclusively isolated from pork meat (97.9%) and they were of bioserotype 4/O:3. The other, but very minor, sources of isolation of pathogenic *Y. enterocolitica* strains were vegetables (Table 5): one bioserotype 4/O:3 strain was isolated from potatoes

and one bioserotype 2/O:9 strain from carrots. No *Y. pseudotuberculosis* strains were recovered from foodstuffs.

3.5. Sources and characteristics of the *Yersinia* strains isolated from the environment

Almost all strains isolated from the environment (99.5%) were non-pathogenic (Table 1). *Y. enterocolitica* BT1A were once again the most frequent non-pathogenic strains (42.4%), followed by *Y. intermedia* (31.7%) and *Y. frederiksenii* (19.5%). Their main sources of isolation were sewage (53.2%) and water (42.2%) (Table 6).

Only five *Yersinia* strains (0.5%) pathogenic for humans were recovered from the environment (Table 1). The two *Y. enterocolitica* bioserotype 2/O:9 strains were isolated from grass in a farm during a veterinary survey of the environment of infected animals. This suggests that this grass was contaminated by the faeces of the infected animals, and also served as a source of contamination for other farm animals. One *Y. enterocolitica* bioserotype 4/O:3 strain was isolated from sewage collected during a systematic survey. The two *Y. pseudotuberculosis* strains, both of serotype I (Table 2), were recovered at the same time and in the same area from superficial water (Table 6).

Three strains of *Y. ruckeri*, a species pathogenic for fish, were recovered from water samples collected from the same outdoor rearing tank of a fish farm during a routine monthly sampling that lasted for 1 year.

Table 5Distribution of *Yersinia* strains in foodstuffs

Food	Total	Non-pathogenic strains	Pathogenic strains		% pathogenic strains/type of food
			<i>Y. enterocolitica</i> 2/O:9	<i>Y. enterocolitica</i> 4/O:3	
Fish	24	24	0	0	0.0
Shellfish	54	54	0	0	0.0
Vegetables	1341	1339	1	1	0.1
Eggs	36	36	0	0	0.0
Milk	583	583	0	0	0.0
Cheese	188	188	0	0	0.0
Ready-made meals	45	45	0	0	0.0
Pork products	175	175	0	0	0.0
Beef meat	26	26	0	0	0.0
Pork meat	116	22	0	94	81.0
Poultry	215	215	0	0	0.0
Other food	256	256	0	0	0.0
Unknown	189	189	0	0	0.0
Total	3249	3153	1	95	3.0

Table 6Distribution of *Yersinia* strains from environmental sources

Environment	Total	Non-pathogenic strains	Pathogenic strains			% pathogenic strains/source
			<i>Y. enterocolitica</i> 2/O:9	<i>Y. enterocolitica</i> 4/O:3	<i>Y. pseudotuberculosis</i>	
Sewage	478	477	0	1	0	0.2
Water ^a	380	378	0	0	2	0.5
Ground ^b	34	32	2	0	0	5.9
Other ^c	9	9	0	0	0	0.0
Total	901	896	2	1	2	0.5

^a Rivers, streams, lakes, ponds, and wells.^b Soil, grass, and silage.^c Farm equipment, brooder, table, mixer, and slicer.

4. Discussion

The *Yersinia* strains and data collected over more than 50 years by the YNRL in France provided a unique opportunity to gain a reliable overview of yersiniosis and sources of contamination in this country. Although the reporting of human cases of yersiniosis is not compulsory in France, clinical laboratories send *Yersinia* strains on a regular basis to the YNRL for characterization, thus allowing the determination of the most common species and subgroups of *Yersinia* responsible for human infections. The analysis of more than 12 000 clinical *Yersinia* strains showed that, as in most countries worldwide, human *Y. pseudotuberculosis* infections are rare,^{6,23} accounting for only 6.1% of clinical cases in France. *Y. enterocolitica* is by far the most common enteropathogenic species causing human infections. Within this species, bioserotype 4/O:3 strains largely prevail, followed by bioserotype 2/O:9 strains. This is also the case in most countries worldwide,²⁴ with some exceptions such as England where *Y. enterocolitica* bioserotype 2/O:9 is the most common cause of human yersiniosis.²⁵ Other subgroups of *Y. enterocolitica* rarely cause human cases in France. While *Y. enterocolitica* BT1B infections occur from time to time in the USA,¹⁷ and regularly since 2004 in Poland,²⁶ only two *Y. enterocolitica* 1B strains were recovered from humans in France over the 50-year period. No *Y. enterocolitica* BT5 strain was isolated from clinical samples in this survey, and there is no report of human infection caused by this BT worldwide.

Non-pathogenic *Yersinia* strains were frequently isolated from human stools, representing almost half of the clinical strains. Among them, *Y. enterocolitica* BT1A predominated. However, most clinical laboratories do not determine the BT of the isolated strains.²⁷ Some of them perform serotyping, but the serotype is not a fully reliable indicator of virulence, as some *Y. enterocolitica* strains are non- or self-agglutinating, and BT1A strains sometimes harbour serotypes usually associated with virulent BT (O:3, O:5, O:8, O:13, etc.). This means that 33% of human patients in France might have received unnecessary antibiotic treatment if the BT of the *Y. enterocolitica* strain isolated from their stools was not determined, re-emphasizing the importance of biotyping these strains.

Non-human *Yersinia* isolates were not collected on a regular and continuous basis, but during temporary surveys or epizootics. It is thus not possible to draw conclusions about the respective importance of each contaminated source as a reservoir of *Yersinia*. However, when a sample was found to be contaminated by a *Yersinia* strain, there was no bias in the determination of the species, BT, and/or serotype of the strain isolated. Therefore, the data collected are usable to evaluate the relative distribution of the different species and subgroups of *Yersinia* in various sources, and to identify potential reservoirs for the different types of strains causing human infections.

Y. pseudotuberculosis strains were never isolated from food-stuffs, and almost never from the environment in France. This is in contrast with Russia²⁸ and Finland,²⁹ where this pathogen has often been isolated from vegetables and other environmental samples. Animals were the major source of *Y. pseudotuberculosis* in France. This bacterium causes severe and sometimes fatal infections in susceptible animal species, in particular hares during epizootics and zoo animals (monkeys, felines). *Y. pseudotuberculosis* was also isolated during specific surveys from more resistant hosts such as birds and rodents, which usually develop milder or asymptomatic forms of infection. These more resistant animals represent a major reservoir of *Y. pseudotuberculosis* because they spread the bacteria in the environment, which is the source of infection of susceptible species. Of note, very few farm animals were found to be infected with this species in France, while cattle are a major source of *Y. pseudotuberculosis* infection in some South American countries.³⁰ The fact that the vast majority of the *Y. pseudotuberculosis* strains were isolated from wild animals, which have no close contact with humans, may explain the low frequency of clinical cases of pseudotuberculosis in France. An increase in these animal populations may raise the risk of transmission to humans, as occurred during the winter of 2004–2005, when cases of *Y. pseudotuberculosis* infection suddenly increased throughout France. The sources of infection were multiple and were associated with a transient increase in the population of small wild mammals.³¹ *Y. pseudotuberculosis* strains of serotype I were the major cause of infection in both humans and animals. However, it is worth noting that serotype II was much more frequently isolated from animals (24.3% of *Y. pseudotuberculosis* animal strains) than from humans (7.7% of *Y. pseudotuberculosis* clinical strains). These serotype II strains were mostly isolated from lagomorphs and birds, but in proportions similar to those of serotype I strains, suggesting that the difference in distribution of these two serotypes in animals and humans may be due to a lower pathogenic potential of serotype II *Y. pseudotuberculosis* for humans. This may be consistent with the fact that the high pathogenicity island, which favours the occurrence of severe forms of pseudotuberculosis, is present in serotype I but not in serotype II *Y. pseudotuberculosis* strains.³²

Pigs and pork meat were the major sources of non-human *Y. enterocolitica* 4/O:3 strains in France, confirming the well-known association between this *Y. enterocolitica* bioserotype and pigs.^{33,34} Fattening pigs are recognized healthy carriers of *Y. enterocolitica*. They host the bacteria in their oral cavity (tongue and tonsils) and lymph nodes. The carcass and the pluck set become readily contaminated at slaughter after removal of the tongue and the tonsils, and then by carcass splitting.³⁵ The capacity of *Y. enterocolitica* to grow at +4 °C and to multiply in meat products during refrigerated storage increases the risk of transmission to humans. Thus, the consumption of raw or undercooked pork meat is considered to be the main route of

transmission of pathogenic *Y. enterocolitica* to humans. The fact that in many European countries pigs are contaminated with *Y. enterocolitica* 4/O:3 argues for a specific adaption of this subgroup of *Y. enterocolitica* to pigs. A recent comparative genomic analysis of pathogenic *Y. enterocolitica* biotypes showed that *Y. enterocolitica* 4/O:3 harbours some biotype-specific genes, one of them encoding an *N*-acetyl-galactosamine phosphotransferase system. Since the gut of pigs is rich in *N*-acetyl-galactosamine-containing mucin, it may represent a favourable environment for the growth of *Y. enterocolitica*, allowing efficient colonization and long-term persistence in this natural reservoir host.³⁶ However, bioserotype 2/O:9 strains predominate in English pigs²⁵ and bioserotype 3/O:3 in Chinese pigs.³⁷ This suggests that, more than being bioserotype-dependent, the association between subgroups of *Y. enterocolitica* and pigs might be country-dependent, due to specific eating and cultural habits. Outside the pig source, it was found that *Y. enterocolitica* 4/O:3 strains were almost never isolated from the environment (only one out of 901 *Yersinia* strains), never from foodstuffs, and very rarely (seven strains) from animal species other than pigs. Since most human cases were caused by this *Y. enterocolitica* subgroup, this indicates that pigs and pork meat represent by far the predominant source of human yersiniosis in France.

Animals were the main source of isolation of non-human *Y. enterocolitica* 2/O:9 strains in France. Almost all non-human *Y. enterocolitica* 2/O:9 strains were recovered from farm animals (bovines, goats, sheep) other than pigs. These animals may share the same pasturelands, allowing contamination of the grass through infected faeces and the ingestion of the bacteria by other grazing animals. Indeed, the only two 2/O:9 strains of environmental origin were isolated from grass in the pastureland of infected cattle. Since pigs are usually kept away from grazing animals, they are less exposed to *Y. enterocolitica* 2/O:9 contamination. Curiously, no *Y. enterocolitica* 2/O:9 strains were isolated from meat products. The carriage of the bacteria in the intestine of livestock rather than in their oral cavity and a different processing of the animal carcasses at slaughterhouses³⁸ could be reasons why beef, goat, and sheep meats are less frequently infected with pathogenic *Y. enterocolitica* than pork meat. This rare contamination of meat products may thus explain why *Y. enterocolitica* 2/O:9 strains are less frequently responsible for human infections than 4/O:3 strains in France. A possible means of human contamination with *Y. enterocolitica* 2/O:9 could be through direct contact with infected animals or their close environment.

Other pathogenic subgroups of *Y. enterocolitica* (BT1B, bioserotypes 5/O:2,3, 2–3/O:5,27, and 3/O:1,2,3) were never isolated from food or environmental samples in France, but they were recovered from various animal species. *Y. enterocolitica* 5/O:2,3 were essentially isolated from lagomorphs, and on rare occasions from farm animals (cows and sheep), suggesting an adaptation of these strains to hares. The absence of human cases caused by this bioserotype may be due to their lower pathogenicity for humans, but another more likely explanation is that contacts between infected hares and humans are scarce. Most of the *Y. enterocolitica* 3/O:1,2,3 strains were isolated from chinchillas, suggesting here again an adaptation of this subgroup of *Y. enterocolitica* to this animal host. The very low number of human infections caused by 3/O:1,2,3 strains in France (18 cases over 50 years) could therefore also be attributed to very limited interactions between chinchillas and humans. *Y. enterocolitica* bioserotypes 2–3/O:5,27 were isolated from various wild (rodents) and farm (pigs, sheep and cows) animal species, on rare occasions. Of note, they were isolated in similar proportions from animals (3.4% of pathogenic *Y. enterocolitica* animal strains) and from humans (3% of pathogenic *Y. enterocolitica* human strains), suggesting that the low frequency

of 2–3/O:5,27 infections in humans is due to the infrequent carriage of these strains by animal reservoirs.

Non-pathogenic *Yersinia* species were abundantly isolated from food and environmental samples, showing that the low number of pathogenic strains found in these samples was not due to an inability to recover *Yersinia* strains from these sources, or to a low number of samples analyzed (the information about the proportion of *Yersinia*-positive samples per total number of samples analyzed was not available in the database). In all of these sources, and in particular in foodstuffs, *Y. enterocolitica* BT1A was by far the most common non-pathogenic *Yersinia* isolated, thus explaining its high recovery rate from human stools. Among the other non-pathogenic species, *Y. bercovieri*, *Y. frederiksenii*, *Y. intermedia*, and *Y. kristensenii* were the most commonly isolated from non-human sources as well as from human stools, further demonstrating that these bacteria are abundant in nature and may thus frequently be found in transit in the human gut.

In conclusion, the analysis of the large database provided an unprecedented overview of yersiniosis in France. It showed that food (except pork meat) and environmental samples are colonized essentially by non-pathogenic *Yersinia*, which may be ingested and excreted in human stools, but that they are a very minor source of human contamination. As in many countries worldwide, *Y. enterocolitica* 4/O:3 is the major cause of clinical yersiniosis in France, with pigs and pork meat being by far the most common sources of human infections. These data also clearly demonstrated that animals are the major reservoir of pathogenic *Yersinia* strains and suggest an adaptation of bacterial subgroups to specific hosts: *Y. pseudotuberculosis* to wild animals, *Y. enterocolitica* 2/O:9 to grazing farm animals, *Y. enterocolitica* 5/O:2,3 to hares, and *Y. enterocolitica* 3/O:1,2,3 to chinchillas. The frequency of contacts between these various animal sources and humans could thus reflect the risk of infection caused by the different groups of pathogenic *Yersinia*.

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