Epidemiol. Infect. (2017), **145**, 2197–2203. © Cambridge University Press 2017
doi:10.1017/S095026881700108X

Thirty years of human infections caused by *Yersinia enterocolitica* in northern Spain: 1985–2014

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Received 2 January 2017; Final revision 3 May 2017; Accepted 4 May 2017; first published online 5 June 2017

SUMMARY

Yersinia enterocolitica infection is a zoonosis with worldwide distribution, gastroenteritis being by far the most common clinical manifestation of human infection. In Gipuzkoa, northern Spain, human *Y. enterocolitica* infections increased from the mid-1980s to the beginning of the 21st century (from 7·9 to 23·2 annual episodes per 100 000 population) to decrease to 7·2 annual episodes per 100 000 population in the last years of the study. The hospital admission rate due to yersiniosis during the last 15 years of the study was 7·3%. More than 99% of isolates were serotype O:3. Infection affected mainly children under 5 years of age (average rate: 140 episodes per 100 000 population). The incidence in adults was low but hospitalisation increased with age, exceeding 50% in people over 64 years old.

Key words: Epidemiology, *Yersinia enterocolitica*, zoonoses.

INTRODUCTION

Yersinia enterocolitica infection is a zoonosis with worldwide distribution. The most common clinical manifestation of this infection is acute gastroenteritis, especially in young children; in older children and adults, other frequent clinical presentations of infection are mesenteric adenitis, mimicking appendicitis or terminal ileitis [1,2]. Reactive complications after *Y. enterocolitica* infection, such as reactive arthritis and erythema nodosum, have also been described [3]. *Y. enterocolitica* can colonise many different domestic and wild animals; the main reservoir are pigs, which are considered the leading source of human infections [3]. The infection is generally acquired through the oral route after consumption of contaminated food or drinks, although person-to-person transmission has also been reported, even through blood transfusions [3].

Six different *Y. enterocolitica* biotypes (biotypes 1A, 1B, 2, 3, 4 and 5) have been established on the basis of their different metabolic capacities. Biotype 1A is considered non-pathogenic to humans and most human infections are caused by the other biotypes [4]. *Y. enterocolitica* has been differentiated serologically, based on the antigenic diversity of polysaccharide antigens (O antigens), as they are the principal antigenic factor responsible for the serologic reaction. Although more than 50 different *Y. enterocolitica* serotypes have been described, only a few (O:3, O:5,27, O:8 and O:9) are mainly associated with human infections [5]. Of them, biotype 4 serotype O:3 (4/O:3) and, to a lesser extent,

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2/O:9 are the most prevalent in Europe [6–10]. In the USA, *Y. enterocolitica* 1B/O:8 was traditionally considered the most prevalent serotype in human infections [11], but since the 1990s serotype O:3 has become the most prevalent serotype [2,12], both in the USA and in other American countries, such as Brazil [13].

In Spain and elsewhere in Europe, *Y. enterocolitica* is currently the third most commonly reported bacterial pathogen causing acute gastroenteritis after *Campylobacter* and *Salmonella* [14]. However, few studies have analysed the incidence of yersiniosis in Spain or elsewhere in Europe. The aim of the present study was to determine the incidence and evolution of yersiniosis in a 30-year period between 1985 and 2014 in the area of San Sebastián, Basque Country, northern Spain and to describe the clinical and microbiological characteristics of *Y. enterocolitica* infections.

METHODS

The study included all *Y. enterocolitica* infections detected at the Microbiology Department of the Donostia University Hospital, located in the city of Donostia-San Sebastián, Basque Country, northern Spain between 1985 and 2014. During the 30 years of the study, the Donostia University Hospital served an average population of about 400 000 people (range 392 707 people in 1991 and 417 347 in 2014) according to official data (EUSTAT – Basque Statistics Institute). An episode was defined as the isolation of a *Y. enterocolitica* recovered from normally sterile clinical samples (e.g., blood, joint fluids) from inpatients and from stool samples of hospitalised or ambulatory patients with gastroenteritis. Isolates recovered from stool samples within 3 months following the date of the first isolation were considered as belonging to the same episode [2].

The medical records of hospitalised patients infected during the last 15 years were revised to analyse the complications of infections, length of admission and antimicrobial therapy, if applied. The Ethics Committee for Clinical Research of the Health Area of Gipuzkoa approved the publication of the results of this study.

Isolates

Y. enterocolitica detection in stool samples was performed after direct stool culture in cefsulodin–irgasan–novobiocin (CIN) agar plates and incubation under aerobic conditions at 37 °C for 24 h and at room temperature for the next 24 h. Colonies showing morphologic characteristics of *Y. enterocolitica* (small pink lactose-negative colonies surrounded by a translucent zone) were identified at the species level using the API 20-E (BioMérieux, Marcy l'Etoile, France) miniaturised identification system according to the manufacturer's instructions and incubated for 24 h at 28 °C. From 2012 onwards, isolates were identified by using the Matrix-Assisted Laser Desorption Time-Of-Flight (MALDI-TOF) system according to the manufacturer's guidelines (Autoflex II, Bruker Daltonics, Germany). Non-stool samples were cultured on appropriate media, according to each sample, and the Gram-negative bacteria grown were identified at the species level using the API20-E or the MALDI-TOF system as detailed above.

In 2013, the strategy of diagnosis from traditional stool culture was supplemented with a commercial multiplex PCR (Fast-Track diagnosis) that included the detection of *Y. enterocolitica* among other enteric bacterial and viral pathogens. Total nucleic acids were extracted using the automated extraction-purification system NucliSens EasyMag (BioMérieux, Marcy l'Etoile, France).

Biotyping and serotyping

Y. enterocolitica biotyping was performed using the scheme described by Wauters *et al.* [15]. Biotyping was routinely performed until 1998, with sporadic typing thereafter (*between 8 and 12 randomly selected isolates per year*). Biotype 1A isolates (considered non-pathogenic) were not included in the study. Serotypes were established using specific O:3, O:5,27, O:8 and O:9 antisera (Bio-Rad, Marnes-la-Coquette, France).

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed by the disk-diffusion method (Kirby–Bauer) and the broth microdilution method according to CLSI (Clinical and Laboratory Standards Institute) guidelines [16].

Statistical analysis

Incidence rates were calculated by using the official population data for 1986, 1991, 1996, 2001, 2006 and 2011, which are available at the EUSTAT webpage (http://www.eustat.es). All incidence figures are expressed as episodes per 100 000 population.

https://doi.org/10.1017/S095026881700108X Published online by Cambridge University Press

RESULTS

From January 1985 to December 2014, 1417 episodes of *Y. enterocolitica* infection were detected: 1413 patients had gastrointestinal disturbances (mainly acute gastroenteritis) and four patients had extraintestinal infections: there was one case of endocarditis in a 77-year-old man with aortic aneurysm, two cases of primary bacteraemia in a 78-year-old man with cirrhosis and a 49-year-old man with lymphoma and one prosthetic knee infection in an 81-year-old women with an isolate collected from synovial fluid. In two patients (aged 1 month and 15 years old), enteritis were also bacteraemic and the *Y. enterocolitica* isolate included in the study was obtained from the blood-culture. The remaining 1411 isolates from the gastrointestinal episodes were obtained from the 208 887 samples sent for stool culture. All *Y. enterocolitica* detected by multiplex-PCR from stools were also detected by culture in CIN plates.

Overall, 694 patients (49%) were women. By age, most patients with yersiniosis were children under 5 years of age (871, 61·5%), while 179 were under 1 year of age (Table 1).

Except five isolates 2/O:9 and one isolate 1B/O:8, all other isolates were serotype O:3. All serotype O:3 isolates that were biotyped ($n = 687$) were biotype 4. All nine non-stool isolates were *Y. enterocolitica* 4/O:3.

The overall annual incidence (per 100 000 population) of *Y. enterocolitica* infection increased from 7·9 episodes in 1985–1987 to 23·2 episodes in 2000–2002 and then decreased to 7·2 episodes in 2012–2014 (Table 1). The average incidence rate of *Y. enterocolitica* infection in children under 5 years old was 139·9 with children aged 1–2 years old being the age group with the highest incidence rate (average annual incidence 376·3 episodes).

The seasonal distribution of episodes during the year was quite regular, with no clear seasonality and with percentages of isolates ranging from 21% (305 episodes) in summer (June–August) to 23·6% in spring (March–May) and to 27·5% (389 episodes) in autumn (September–November) and winter (December–February).

Admission and treatments

In the last 15 years of the study (2000–2014), 55/750 (7·3%) patients with *Y. enterocolitica* diseases were admitted to hospital. The average admission length was 7·5 days (range 1–47 days) for the entire sample

Table 1. *Episodes of* Y. enterocolitica *infection distributed by age groups and period of study in Gipuzkoa, northern Spain (1985–2014)*

	Annual incidence per 100 000 inhabitants (number of episodes)										
Age (years)	1985–1987	1988–1990	1991–1993	1994–1996	1997–1999	2000–2002	2003–2005	2006–2008	2009–2011	2012–2014	Average (total) 1985–2014
<1	119·0 (13)	172·3 (17)	158·9 (14)	73·1 (7)	267·0 (27)	375·6 (40)	167·2 (19)	182·2 (22)*	98·4 (12)	65·0 (8)	165·9 (179)
1–2	172·8 (19)	214·6 (22)	399·8 (38)	247·4 (23)	866·6 (87)	918·2 (99)	358·0 (41)	305·1 (37)	122·8 (15)	235·8 (29)	376·3 (410)
2–4	58·3 (23)	52·0 (18)	100·7 (30)	74·4 (21)	159·6 (47)	176·0 (54)	103·5 (34)	65·7 (23)	38·6 (14)	47·9 (18)	84·4 (282)
5–14	6·1 (12)	12·1 (21)	16·7 (25)	11·7 (13)	64·5 (67)	48·5 (47)	27·5 (27)	47·1 (47)	29·7 (32)	18·2 (21)	24·9 (312)
15–64	0·9 (7)	1·1 (9)	1·0 (8)	0·7 (6)	3·4 (28)	3·6 (30)*	2·5 (21)	2·3 (19)	2·0 (16)	1·5 (12)*	1·9 (156)
>64	1·4 (2)	0·7 (1)	0·0 (0)	0·0 (0)	0·5 (1)	0·5 (1)*	1·4 (3)*	1·3 (3)*	0·4 (1)	0·8 (2)	0·7 (14)
Unknown	– (18)	– (11)	– (5)	– (20)	– (7)	– (3)	– (0)	– (0)	– (0)	– (0)	– (64)
Total	7·9 (94)	8·3 (99)	10·2 (120)	7·6 (90)	22·3 (264)	23·2 (274)	12·1 (145)	12·3 (151)	7·4 (90)	7·2 (90)	11·8 (1417)

* One of the episodes corresponds to isolates from normally sterile clinical samples.

https://doi.org/10.1017/S095026881700108X Published online by Cambridge University Press

and 6·2 days in patients with gastroenteric infections (range 1–24 days). During this 15-year period, hospitalisation due to *Y. enterocolitica* infections in patients younger than 5 years decreased with increasing age: 11·9% (12/101), 5% (11/221) and 0·7% (1/143) in children aged <1 year, 1–2 years and 3–4 years, respectively. In contrast, in older children and adults, hospitalisation rates increased with age: 2·3% (4/174), 21·4% (21/98) and 60% (6/10) in patients aged 5–14 years, 15–64 years and >64 years, respectively.

The reason for hospitalisation due to *Y. enterocolitica* infection was acute gastroenteritis in all 24 children <5 years and 17 (70·8%) required intravenous rehydration. Of these 24 children, four (16·7%) did not receive antibiotic therapy, 14 received trimethoprim–sulfamethoxazole and five, all of them under 1 year of age, received cefotaxime. In one patient, the antibiotic therapy data were not recorded.

In patients aged 5–14 years, four episodes required hospitalisation: two were diagnosed of mesenteric adenitis (one of them with acute diarrhoea), one with abdominal pain and reactive arthritis and one acute ileitis. Two of them received cefotaxime (the one with mesenteric adenitis without diarrhoea and the other with reactive arthritis) and the other two did not receive antibiotics during admission.

In hospitalised adolescents and adults aged 15–64 years, the most common clinical picture was terminal ileitis in 13/21 (61·9%) without ($n = 9$) or with ($n = 4$) mesenteric adenitis. Six patients were admitted because of acute gastroenteritis and the other two patients had primary bacteraemia. Ten patients received ciprofloxacin, two trimethoprim–sulfamethoxazole and one cefotaxime; seven did not receive antibiotic therapy. In one patient this datum was not available.

Among the six patients older than 64 years who required hospitalisation, three had extraintestinal infection: two had bacteraemia (an endocarditis and a primary bacteraemia in a cirrhotic patient), both of whom were treated with ceftriaxone, and one patient had infection of a prosthetic knee as well as *Y. enterocolitica* isolated from stools, treated with trimethoprim–sulfamethoxazole and ciprofloxacin. Of the three remaining patients, two had gastroenteritis and one terminal ileitis, and all of them received ciprofloxacin as antibiotic therapy.

Antimicrobial susceptibility

The antimicrobial susceptibility was available for 975 isolates (≈100 isolates in each 3-year period). All isolates were resistant to ampicillin and first-generation cephalosporins but all showed susceptibility to cefotaxime (minimum inhibitory concentration (MIC) < 1 mg/l). Although all isolates showed susceptibility to ciprofloxacin (MIC < 2 mg/l), resistance to nalidixic acid (MIC > 64 mg/l) was frequent and increased from 26% in the period 2000–2002 to 47% in 2012–2014. Only two isolates (0·2%) were gentamicin resistant. Chloramphenicol resistance (MIC > 16 mg/l) increased from 25% in 1985–1987 to rates equal to or above 65% in each 3-year period between 2000 and 2014. Most (92%) isolates showed resistance to sulphonamides (MIC > 256 mg/l), without significant changes across the study periods, but most of these isolates showed susceptibility to trimethoprim–sulfamethoxazole (MIC < 4/76 mg/l). Resistance rates to trimethoprim–sulfamethoxazole across the different study periods ranged from 3% to 9%.

DISCUSSION

There are few data on the current incidence of *Y. enterocolitica* infection in southern Europe [17]. The European Centre for Disease Prevention and Control (ECDC) have reported annual notification rates ranging from 1·59 to 2·98 cases per 100 000 inhabitants for the overall Europe and European Economic Area (EU/EEA) estates and rates ranging from 1·75 to 3·13 cases per 100 000 inhabitants for Spain between 2007 and 2014 [17]. According to the ECDC, and based on the statements of national agencies [14,17], Germany and other countries of northeastern Europe surpassed Spain in incidence rates, but the rates ranging from 7·2 to 12·3 cases per 100 000 inhabitants found in the present study during the same recent period were about three times those of both the overall EU/EEA and Germany. The incidence of *Y. enterocolitica* infection per 100 000 population reported by FoodNet in the USA was much lower than that in Europe, ranging from 1·03 in 1996 to 0·28 in 2014 [18].

Because of differences in requests for diagnostic tests, isolation methods and report types, it is difficult to compare incidence data between regions. The main limitation of our work was due to dependence on general practitioners to request a microbiological diagnosis in patients with suspected infection.

Nevertheless, physicians' criteria for requesting gastroenteritis diagnostic tests did not remarkably change over time allowing for the detection of changes in the incidence of laboratory-confirmed infections in our region over the

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past three decades. The high incidence rate recorded in this study, an average rate of 11·8 cases per 100 000 inhabitants, was revealed by the large number of requests for diagnostic tests carried out (more than 200 000 faecal samples were processed), which also explains the low percentage of hospitalisations observed.

In Gipuzkoa, in northern Spain, the incidence of yersiniosis increased from 1985 to 1987, until reaching a maximum in 2000–2002, and then decreased until 2012–2014. Consumption of poorly cooked pork is considered the main source of human yersiniosis [3]; however data of changes in eating habits or in the origin of the pork consumed in our region between 1985 and 2002 were not found, so that we could not find an explanation for the high increase in yersiniosis rates observed until 2000–2002. A decreasing trend in the rate of yersiniosis has also been observed in most countries of the EU/EEA and in the USA in the past few years [17,18]. Because most episodes were detected among children younger than 5 years, the decrease in the incidence was even more evident in this age group [17,19,20]. The high incidence in children can probably be explained by their greater susceptibility to acquiring the infection (the infective dose required is much lower than for adults), by a stronger likelihood of the development of symptomatic illness and by the greater demand for medical care for mild illnesses in this age group than in adults. Few studies conducted in Europe have reported *Y. enterocolitica* incidence rates, and the only study that has specified the rates in age groups among children less than 5 years, performed in Germany [9], also found the highest incidence in children aged between 1 and 2 years old. The higher incidence rates of *Y. enterocolitica* infections in children between 1 and 2 years old *vs.* those in infants can partly be explained by their different eating habits. In contrast, in Nordic countries, and especially in Finland, which has the highest incidence rates reported by the ECDC (just over 10 cases per 100 000 inhabitants), most of the cases were observed among adult (older than 14 years) population [17].

As in other European studies [9,17], in our region the seasonal distribution of *Y. enterocolitica* gastroenteric infections was very homogeneous throughout the year.

With very few exceptions, most isolates were serotype O:3 (99%), as were the isolates obtained in other studies performed in Spain [21–23]. Although the majority of human episodes reported in Europe were caused by serotype O:3 [6–10,24,25], the O:3 rate found in the present study was somewhat higher.

In both the USA and in Europe, the major reservoir of pathogenic *Y. enterocolitica* are pigs; consequently, the higher incidence of yersiniosis in European countries than in the USA could be due to differences both in the prevalence of *Y. enterocolitica* in pigs as well as to differences in the populations' eating habits in each region. In our region, with one of the highest incidence rates of *Y. enterocolitica* infection in Europe, eating pork or pork derivatives such as cold meat cuts is highly frequent, which could partly explain the high incidence of *Y. enterocolitica* O:3 infections. In the USA, the highest rates of *Y. enterocolitica* gastroenteritis were observed among infants younger than 1 year [19,20], suggesting another mechanism of transmission in addition to the alimentary route.

Pigs and pork foods consumed in our region (northern Spain) had a varied origin, coming not only from Spain but also from other European countries. We do not know the real prevalence of *Y. enterocolitica* in pigs from Spain throughout the study period; however, the data found [26–28] support the hypothesis that changes in the incidence of human yersiniosis largely depended on the prevalence of *Y. enterocolitica* in pigs. The prevalence of *Y. enterocolitica* detected in pig tonsil differed depending on the screening method, time of the study and region. *Y. enterocolitica* was detected in 37·5% of pigs from Gipuzkoa (northern Spain) slaughterhouses in 1988 [27], in 93% of pigs from southeast Spain (44% in pigs from Belgium) from 2005 to 2006 [28], and in 38·7% of pigs from different regions of Spain in 2013 [26]. On all occasions, all the strains detected in pigs were serotype O:3.

In the last 15 years of the study, we found that 7·3% of patients with *Y. enterocolitica* infection (51 with gastroenteric infection and the four with extraintestinal infection) were admitted to hospital with an average admission length of 6·2 days in patients with gastroenteric infection. This hospitalisation rate is very low compared with rates in most other studies, with percentages above 25% and even >50% not being uncommon [17, 25,29,30]. The low incidence rate together with a high hospitalisation rate observed in other studies probably reflects low monitoring of mild gastrointestinal infections.

Bacteraemia is reported in the literature as a rare complication of *Y. enterocolitica* infection representing 0·4–9% infections [6,31] as a consequence of dissemination of the pathogen from the gastrointestinal tract in patients with predisposing factors such as diabetes mellitus, chronic renal failure, cirrhosis,

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malignancies and iron overload, with high mortality. In our series, bacteraemia represented only 0·35% of episodes with no deaths, probably due to rapid and adequate treatment.

As in most studies, this study found that *Y. enterocolitica* gastroenteric infections were sporadic and no outbreak was detected, although foodborne outbreaks have been reported in other countries [32]. Diarrhoea caused by *Y. enterocolitica* tends to be self-limiting, usually resolving spontaneously, the most important treatment being rehydration.

In conclusion, *Y. enterocolitica* infections in our region sharply increased from 7·9 episodes in 1985–1987 to 23·2 episodes in 2000–2002 and then rapidly decreased to 12·1 episodes in 2003–2005 and to 7·2 episodes in 2012–2014. Nevertheless, nearly all *Y. enterocolitica* infections were limited to the gastroenteric tract, most of them being non-serious. The high incidence rates observed in our region can probably be explained by eating habits and high rates of *Y. enterocolitica* pig colonisation.

ACKNOWLEDGEMENT

This study was partly supported by a grant from the Education Department of the Basque Country Government to the Basque Country University (UPV/EHU) (IT656-13).

DECLARATION OF INTEREST

None.

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