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Short communication

Characterisation of *Yersinia pseudotuberculosis* isolated from animals with yersiniosis during 1996–2013 indicates the presence of pathogenic and Far Eastern strains in Italy

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ABSTRACT

Yersinia pseudotuberculosis is a pathogen that infects both animals and humans worldwide. The epidemiology of infection caused by *Y. pseudotuberculosis* is poorly understood; however, its outbreaks have been traced back to a probable source in wildlife. This study aimed to characterise *Y. pseudotuberculosis* isolates collected from animals with yersiniosis. This study included 90 isolates of *Y. pseudotuberculosis* collected from different animals with yersiniosis between 1996 and 2013 in Italy. The isolates were tested for antimicrobial susceptibility and were biotyped. Genes associated with virulence plasmid pYV and those encoding O-antigen, high pathogenicity island (HPI), and super-antigenic toxin (YPM) were determined by performing PCR. Pulsed-field gel electrophoresis (PFGE) was performed using NotI and SpeI enzymes, and 3 dendrograms were generated. No antibiotic resistance was found. The presence of pYV was shown in 57 out of 90 isolates. Virulence profiles of majority of the isolates indicated that they belonged to O:1a and O:1b serotypes, biotype 1, and genetic type 2. Isolates belonging to O:2a serotype were detected in sheep and cattle and were found to be associated (for the first time) with septicemia in hares. *Y. pseudotuberculosis* isolates belonging to O:5a and O:12–O13 serotypes were also detected in hares. To our knowledge, this is the first study to detect *Y. pseudotuberculosis* isolates belonging to the O:12–O13 serotype from a clinical case in Europe. Results of PFGE indicated that it was a reliable method for investigating the genetic relatedness of *Y. pseudotuberculosis* isolates. Thus, characterisation of *Y. pseudotuberculosis* infection in animals should be considered a possible tool for the surveillance of pseudotuberculosis.

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1. Introduction

Yersinia pseudotuberculosis is a gram negative bacterium that infects various animals and humans (Galindo et al., 2011). Clinical symptoms of yersiniosis in humans vary in different regions around the world. In Europe, yersiniosis usually manifests as a gastrointestinal disorder, with symtoms similar to those of appendicitis. In the Far East, the disease shows systemic manifestations such as fever, scarlatiniform rash and arthritis (Galindo et al., 2011 Yoshino et al., 1995). Among animals, yersiniosis is commonly diagnosed in hares. However, it also affects many domestic and wild animals (both mammals and birds) and is associated with with a variety of clinical manifestations such as enteritis, septicaemia, mastitis and abortion (Giannitti et al., 2007; Juste et al., 2009; Shwimmer et al., 2007; Wuthe et al., 1995). *Y. pseudotuberculosis* is classified into serotypes O:1–O:15 and 10 subgroups (O:1a–c, O:2a–c, O:4a–b, and O:5a–b) (Bogdanovich et al., 2003). *Y. pseudotuberculosis* isolates belonging to the O:1a and O:1b serotypes commonly cause gastroenteritis in humans in Europe and those belonging to the O:1b, O:2b, O:4b and O:5b serotypes commonly infect humans in the Far East (Fukushima et al., 2011; Yoshino et al., 1995). This difference in the worldwide distribution of *Y. pseudotuberculosis* serotypes is not restricted to strains causing diseases in humans because *Y. pseudotuberculosis*

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strains O:6–O:14 have been only isolated from wild birds and from the environment in the Far East (Fukushima et al., 2001). *Y. pseudotuberculosis* strains can be further subdivided according to their pathogenicity, which is associated with the presence of multiple virulence markers. Of these, plasmid pYV, the high pathogenicity island, HPI, and the superantigenic toxin, YPM, are particularly important for characterising *Y. pseudotuberculosis* (Fukushima et al., 2001). *Y. pseudotuberculosis* isolates collected from cases of yersiniosis occurring in different parts of the world do not show the same virulence pattern, which is similar to that observed for *Y. pseudotuberculosis* serotypes. This explains the geographical heterogeneity in the clinical manifestations of this disease. YPM-producing strains have been isolated from cases of systemic yersiniosis in the Far East while HPI-positive and YPM-negative strains have been isolated from cases of yersiniosis in Europe (Fukushima et al., 2001 Yoshino et al., 1995).

Despite its importance in both humans and animals, the epidemiological features of yersiniosis are still not completely understood. The reservoir of this disease has not yet been identified. However, several authors have suggested the role of rodents or wild fauna in the dissemination of this disease (Fredriksson-Ahomaa et al., 2009; Nuorti et al., 2004; Vincent et al., 2008). The association between wildlife and yersiniosis has also been suggested by studies in animals performed in Finland and England. These studies showed that the prevalence of *Y. pseudotuberculosis* was higher in organic pigs than in conventionally reared pigs, suggesting that a contact with wild environment was a risk factor associated of pseudotuberculosis carrier state in swine (Martínez et al., 2011). A study of *Y. pseudotuberculosis* strains causing yersiniosis in animals can provide useful information for preventing this disease in both animals and humans. Thus, the present study aimed to characterise *Y. pseudotuberculosis* isolates from wild and domestic animals with yersiniosis in Italy to determine their antigenic properties, virulence determinants, antibiotic susceptibility, and genetic relatedness.

2. Materials and methods

2.1. Y. pseudotuberculosis isolates

This study included 90 isolates of *Y. pseudotuberculosis* isolates collected from culture collections centres of 5 diagnostic laboratories. These isolates were collected during 1996-2013 and originated from 9 Italian regions: Lombardy (25), Emilia-Romagna (5), Veneto (19), Umbria (27), Tuscany (1), Marche (2) Lazio (2), Sardinia (8), and Campania (1). The origin of these isolates, their species and clinical condition from which they were isolated are described in Table 1. Only 1 isolate from a single animal was included. Two isolates belonging to the atypical O:3 serotype that were collected from the rectal contents of hunted wild boars, which were already described in a previous study (Magistrali et al., 2014), were characterised using PFGE and were included in the present study. All the isolates included in this study were grown at 28 °C for 48 h on blood agar plates (Blood Agar Base, Biolife Italiana Srl, Milan, Italy), supplemented with 5% sheep red blood cells. Strains belonging to the O:1–O:15 serotypes were kindly provided by Prof. M. Skurnik (Haartman Institute, University of Helsinki).

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was assessed using disc diffusion method on Mueller-Hinton agar (Oxoid Ltd, Cambridge, UK), according to the M31-A2 procedure (CLSI, 2002), except that incubation was performed at 30 °C for 24 h. The following commercially available (Oxoid Ltd.) antimicrobial discs were used: amoxycillin/clavulanic acid (20/10 μg), ampicillin (10 μg), cefotaxime (30 μg), ceftazidime (30 μg), cephalexin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), streptomycin (10 μg), trimethoprim/sulphamethoxazole (1.25/23.75 μg) and tetracycline (30 μg). Interpretation of zone diameters was performed using M31-A2 and the M100-S23 tables for Enterobacteriaceae (CLSI, 2002, 2012).

Table 1
distribution of the isolates according to the species of origin, the associated condition and the characterization based upon PCRs directed to O antigen, pYV, YPM and HPI associated genes. The consequent attribution to different genetic types is also shown.

	Serotype							pYV	YPM	High pathogenicity island			Genetic type			
Animal species	Associated condition	*n*	O:1a	O:1b	O:2a	O:5a	others	virF	ypmc	HPI	R-HPI	In-HPI[b]	2	5	6	UK[a]
Hare	Septicemia	61	28	23	8	1	1	38		49		2	49		10	2
Sheep																
	Abortion	9	9					2		8		1	8			1
	Mastitis	2	1	1				2		2			2			
Wild boar	Healthy	3	1				2	3	2	1	2		1	2		
Guinea fowl	Septicemia	2	2					2		2			2			
Cat	Septicemia	2	2					2		2			2			
Turaco	Septicemia	2	2					2		2			2			
Canary	Septicemia	1		1				1		1			1			
Cattle	Mastitis	1			1										1	
Rabbit	Septicemia	1		1				1		1			1			
Deer	Septicemia	1	1					1		1			1			
Roe deer	Septicemia	1	1							1			1			
Cottontail rabbit	Enteritis	1	1					1		1			1			
Goat	Septicemia	1		1				1		1			1			
Parrot	Septicemia	1	1							1			1			
Goldfinch	Septicemia	1		1				1		1			1			
Total (%)		90	49 (54)	28 (31)	9 (10)	1 (1)	3 (3)	57 (63)	2 (2)	74 (82)	2 (2)	3 (3)	74 (82)	2 (2)	11 (12)	3 (3)

[a] UK: unknown: genetic type not already described.
[b] In-HPI an incomplete form of the high pathogenicity island was recorded.

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