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Pathogenic *Yersinia enterocolitica* O:3 isolated from a hunted wild alpine ibex

S. JOUTSEN[1], E. SARNO[2,3], M. FREDRIKSSON-AHOMAA[1], N. CERNELA[3] AND R. STEPHAN[3]*

[1] *Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland*
[2] *Department of Zootechnical Science and Food Inspection, Faculty of Veterinary Medicine, University of Naples Federico II, Naples, Italy*
[3] *Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Zurich, Switzerland*

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SUMMARY

Occurrence of *Yersinia* spp. in wild ruminants was studied and the strains were characterized to get more information on the epidemiology of enteropathogenic *Yersinia* in the wildlife. In total, faecal samples of 77 red deer, 60 chamois, 55 roe deer and 27 alpine ibex were collected during 3 months of the hunting season in 2011. The most frequently identified species was *Y. enterocolitica* found in 13%, 10%, 4% and 2% of roe deer, red deer, alpine ibex and chamois, respectively. Interestingly, one *Y. enterocolitica* O:3 strain, isolated from an alpine ibex, carried the important virulence genes located on the virulence plasmid (*yad*A and *vir*F) and in the chromosome (*ail*, *hre*P, *myf*A and *yst*A). Most of the *Y. enterocolitica* strains belonged to biotype 1A of which 14 were *yst*B positive. Further studies are needed to clarify the importance of alpine ibex as a reservoir of pathogenic *Y. enterocolitica*.

Key words: Characterization, hunted wild ruminants, *Yersinia enterocolitica*.

INTRODUCTION

Yersiniosis is an important zoonotic disease in humans in Europe [1]. Most of the reported cases are caused by *Y. enterocolitica*. Human enteric yersiniosis is thought to be primarily foodborne [2]. *Y. enterocolitica* has been shown to be transmitted mainly by pork products and *Y. pseudotuberculosis* by contaminated fresh produce. In a *Y. pseudotuberculosis* outbreak in Finland, it was likely that iceberg lettuce were contaminated by irrigation water contaminated with roe deer faeces [3]. In a small study conducted in Germany, raw game (including meat from roe deer, red deer, and chamois) were frequently (38%) contaminated with potentially pathogenic (*ail*-positive) *Y. enterocolitica* when studied by polymerase chain reaction (PCR) [4].

Wild boars were recently shown to be an important reservoir of enteropathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* in Switzerland [5]. Yersiniosis due to *Y. pseudotuberculosis* has also been shown to be a disease of major importance in deer [6, 7]. Moreover, *Y. pseudotuberculosis* has also been reported to be a common finding in clinically healthy farmed deer weaners in New Zealand [8].

* Author for correspondence: Professor R. Stephan, Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Winterthurerstr. 272, CH-8057 Zurich, Switzerland.
(Email: stephanr@fsafety.uzh.ch)

The prevalence of *Y. enterocolitica* and *Y. pseudotuberculosis* in wild deer, however, has so far been very rarely studied [9–12]. In these few studies, both species were isolated from faecal samples of animals free from obvious symptoms of disease. However, all *Y. enterocolitica* strains were considered non-pathogenic, and *Y. pseudotuberculosis* was very rarely isolated from faecal samples. The aim of this work was to study the occurrence of *Yersinia* spp. in wild ruminants in Switzerland and to characterize the strains in order to obtain more information on the epidemiology of enteropathogenic *Yersinia* in the wildlife.

METHODS

Animals

This study was based on investigations carried out during 3 months (September–November) of the hunting season in 2011. The samples originated from shot red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupicapra*), and ibex (*Capra ibex*). The sampled animals were hunted in the central and eastern part of Switzerland. In total, 219 faecal samples (red deer, roe deer, chamois, ibex) were examined. The faecal samples originated from 77 red deer, 60 chamois, 55 roe deer and 27 alpine ibex. State gamekeepers and hunters collected the samples in the field immediately after shooting and evisceration of the wild ruminants. After opening the large intestine, faecal matter (at least 10 g) was collected from the colon, placed into sterile tubes and stored under refrigeration. For each hunted animal, sex, age, and location of hunting were recorded.

Yersinia detection and identification

About 1 g faecal material was mixed in 10 ml PMB [13, 14]. After 2 weeks of cold enrichment at 4 °C, 10 μl of the enrichment was plated on cefsulodin-irgasan-novobiosin (CIN) agar (Oxoid AG, Switzerland). The CIN plates were incubated at 30 °C for 24–48 h. Presumptive positive colonies were subcultured on blood agar and then tested for the urease enzyme. Urease-positive colonies were identified with API 20E and matrix-assisted laser desorption/ionization–time of flight (MALDI–TOF) mass spectrometry [15, 16]. One isolate per sample in a total of 20 strains were biotyped and serotyped. The biotype was determined using pyrazinamidase and Tween activity, esculin hydrolysis, indole production, and salicin, xylose and trehalose fermentation and serotyping was performed with slide agglutination using commercial *Y. enterocolitica* O:1–O:3, O:5, and O:9 antisera (Denka Seiken, Japan).

Further strain characterization

Eight genes were studied by PCR: two virulence genes (*yad*A, *vir*F) located on the virulence plasmid of the pathogenic *Yersinia* spp. (pYV) and five virulence genes (*ail*, *yst*A, *yst*B, *myf*A, *hre*P) and *rfb*C for O:3 serotype located in the chromosome [17–20]. The DNA was released from bacterial colonies by heating at 97 °C for 10 min, and 1 μl of this liquid was added to 19 μl of the mastermix (iQ™ SYBR Green Supermix; Bio-Rad, USA). The fluorescence intensity of SYBR Green and the melting curve analysis were studied using the CFX96 system (Bio-Rad). A threshold cycle (C_t) under 30 and a specific melting temperature (T_m) indicated a positive result.

Antimicrobial susceptibility testing

Antimicrobial resistance analysis was performed by disk-diffusion test according to Clinical and Laboratory Standards Institute (CLSI, 2009). Fourteen antimicrobials were tested: ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), cefalothin (30 μg), cefoxitin (30 μg), cefpodoxim (10 μg), ceftazidim (30 μg), cefuroxime (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), streptomycin (10 μg), tetracycline (30 μg) and trimethoprim/sulfamethoxazole (1·25/23·75 μg) [16]. The reference strain *Escherichia coli* ATCC 25922 was used as the quality control.

RESUTS AND DISCUSSION

The occurrence of *Yersinia* spp. varied between 4% and 13% in wild ruminants being highest in roe (13%) and red deer (12%) (Table 1). The most frequently identified species was *Y. enterocolitica* found in 13%, 10%, 4% and 2% of roe deer, red deer, alpine ibex and chamois, respectively. Surprisingly, no *Y. pseudotuberculosis* was isolated even though cold enrichment in peptone broth supplemented with 1% mannitol and 0·15% bile salts (PMB), which should be favourable for *Y. pseudotuberculosis* [21], was used. The prevalence of *Y. enterocolitica* and *Y. pseudotuberculosis* in wild deer has so far very rarely been studied (Table 2). In Japan, 4% of the

Table 1. *Prevalence of* Yersinia *spp. in faeces of clinically healthy wild ruminants in Switzerland 2011*

Animal species	Animals studied, *n*	*Yersinia*-positive animals, *n* (%)	*Yersinia* spp. (no. of strains)
Cervus elaphus (red deer)	77	9 (12%)	*Y. enterocolitica* (8) *Y. kristensenii* (1)
Rupicapra rupicapra (chamois)	60	3 (5%)	*Y. enterocolitica* (1) *Y. kristensenii* (1) *Yersinia* sp. (1)
Capreolus capreolus (roe deer)	55	7 (13%)	*Y. enterocolitica* (7)
Capra ibex (alpine ibex)	27	1 (4%)	*Y. enterocolitica* (1)
All species	219	20 (9%)	*Y. enterocolitica* (17) *Y. kristensenii* (2) *Yersinia* sp. (1)

Table 2. *Prevalence of* Yersinia *spp. in faeces of clinically healthy wild deer*

Country	Animal species	Animals studied, *n*	*Yersinia*-positive animals, *n* (%)	Identified *Yersinia* spp. (no. of strains)	Ref.
Italy	Red deer	60	14 (23%)	*Y. kristensenii* (13) *Y. enterocolitica* (1)	[10]
	Roe deer	13	1 (8%)		
	Chamois	7	0		
Japan	Sika deer	215	8 (4%)	*Y. pseudotuberculosis* (8)	[11]
New Zealand	Red deer	83	26 (31%)	*Y. enterocolitica* (13) *Y. kristensenii* (1) *Y. intermedia* (1) *Y. frederiksenii* (11)	[9]
	White-tailed deer	40	3 (8%)	*Y. enterocolitica* (2) *Y. frederiksenii* (1)	
Norway	Red deer	170	10 (6%)	*Y. enterocolitica* (13) *Y. mollaretii* (1) *Y. pseudotuberculosis* (1)	[12]

deer were shown to shed *Y. pseudotuberculosis* in faeces [11]. In Norway, the prevalence of *Yersinia* in wild red deer was clearly lower [12]. One reason for the higher prevalence of *Yersinia* in our study could be due to the use of a cold enrichment instead of 2 days enrichment at 21 °C. *Y. enterocolitica* was also the dominant species in Norwegian deer; however, one *Y. pseudotuberculosis* strain was detected in Norway. In Italy and New Zealand, the prevalence of *Yersinia* in red deer was clearly higher (Table 2). In the Italian study, most of the strains isolated were *Y. kristensenii*. One reason for the low isolation rate of *Y. kristensenii* in our study could be that we used CIN agar and *Y. kristensenii* grows very slowly. *Y. enterocolitica* was the dominant species in wild red deer in New Zealand; however, *Y. frederiksenii* was also frequently identified [9]. In the same study, *Y. pseudotuberculosis* was sporadically isolated from clinically healthy farmed deer but not from wild deer. One reason for the low prevalence of *Y. pseudotuberculosis* could be that the carriage status cannot be adequately identified by faecal culture due to either sporadic shedding of this pathogen or due to the localization of this pathogen in the mesenteric or ileocecal lymph nodes [9].

The *Yersinia* spp. strains were identified with MALDI–TOF, API 20E and biotyped (Table 3). Only one of the 20 strains (strain no. 20) could not be identified at species level by MALDI–TOF. By API 20E this strain was identified as *Y. frederiksenii*/*intermedia* with an ID% of 98·5%. The biotype remained unknown for three *Y. enterocolitica* strains (strain nos. 15–17) by MALDI–TOF. One of the *Y. enterocolitica* strains (strain no. 17) was

Table 3. *Identification and characterisation of the* Yersinia *strains isolated from wild ruminants free from obvious symptoms of disease*

Strain no.	MALDI–TOF MS	API 20E		Bio-type	Serotype	Presence of the virulence genes		
		Profile	ID (%)			*ail, yst*A *yad*A, *vir*F	*yst*B	*myf*A, *hre*P
1–6	*Y. enterocolitica*, 1A	1 155 723	98·3	1A	O:5	–	+	V
7–9	*Y. enterocolitica*, 1A	1 155 723	98·3	1A	O:8	–	+	V
10–14	*Y. enterocolitica*, 1A	1 155 723	98·3	1A	NT	–	+	V
15–16	*Y. enterocolitica*, NT	1 155 723	98·3	1A	NT	–	–	–
17	*Y. enterocolitica*, NT	1 114 321	99·6	3 or 5	O:(1,2,)3	+	–	+
18–19	*Y. kristensenii*	1 114 503	89·2	NT	NT	–	V	–
20	*Yersinia* sp.	1 155 733	98·5*	NT	NT	–	–	–

MALDI–TOF MS, Matrix-assisted laser desorption/ionization–time of flight mass spectrometry; NT, biotype not typable; V, the genes were detected in some strains.
* ID for *Y. frederiksenii*/*intermedia*.

Table 4. *Antimicrobial resistance patterns in* Yersinia *strains isolated from wild game*

Antimicrobial agent*	Number of strains							
	YE 1A (16)†		YE 5 (1)		YK (2)		Y sp. (1)	
	I	R	I	R	I	R	I	R
Ampicillin	0	16	0	1	1	1	0	1
Amoxicillin/clavulanic acid	0	16	1	0	2	0	0	1
Cefalothin	0	16	0	1	0	2	0	1
Cefoxitin	8	6	0	0	0	0	1	0
Cefpodoxim	3	0	1	0	2	0	0	0
Cefuroxime	2	0	1	0	1	0	0	0
Kanamycin	2	0	0	0	0	0	0	0
Streptomycin	2	0	1	0	0	0	0	0

YE, *Y. Enterocolitica*; YK, *Y. kristensenii*; Y sp., *Yersinia* species; I, intermediate; R, resistant.
* only antibiotics where intermediate and resistant strains were found are listed.
† Number of strains studied.

regarded as potentially pathogenic because it was pyrazinamidase, esculin and salicin negative. However, it was impossible to clearly differentiate if this strain belongs to biotype 3 or 5. This strain was xylose positive and trehalose negative. A typical strain of biotype 3 should be xylose and trehalose positive, and a typical biotype 5 strain should be xylose and trehalose negative [22]. This strain was also sorbitol negative. *Y. enterocolitica* strains are typically sorbitol positive and *Y. pseudotuberculosis* strains sorbitol negative.

Most (2/17) of the *Y. enterocolitica* strains from wild ruminants belonged to biotype 1A. The majority of the *Y. enterocolitica* strains isolated from food and the environment belong to this biotype and these strains are generally regarded as non-pathogenic because the prerequisite virulence genes are missing [6, 23]. Further, in this study, the most important virulence genes (*ail*, *yad*A, *vir*F) are missing in biotype 1A strains (Table 3). All the 14 strains identified as *Y. enterocolitica* 1A by MALDI–TOF carried the *yst*B gene. Some evidence indicates that YstB plays a role in the pathogenesis caused by *Y. enterocolitica* 1A [23]. Five of the *yst*B-positive strains also carried *hre*P. Two *yst*B-positive strains were also positive for *myf*A. Both *hre*P and *myf*A have sporadically been identified in *yst*B-positive *Y. enterocolitica* 1A strains. However, the impact of *hre*P and *myf*A in virulence

of biotype 1A strains remains unclear [23]. Some of the 1A strains were identified as serotype O:5 or O:8, which are both associated with human disease; however, the role of these O antigens in virulence of this biotype also remains unclear [23].

One *Y. enterocolitica* strain (strain no. 17) that harboured all the important virulence genes was isolated from faeces of a clinically healthy wild alpine ibex (*Capra ibex*) (Table 3). This strain carries the virulence genes *yad*A and *vir*F located on the pYV, and *ail*, *yst*A, *hre*P and *myf*A located in the chromosome. It was identified as serotype O:3 strain with commercial antiserum and PCR targeting the *rfb*C. Furthermore, it agglutinated very weakly with O:1 and O:2 antisera. This pathogenic *Y. enterocolitica* belongs either to biotype 3 or biotype 5. Similar to goats, ibex belong to the genus *Capra* and *Y. enterocolitica*belonging to biotype 5 and serotype O:2,3 has already been isolated from goats in New Zealand [24]. This bioserotype has frequently been associated with *Y. enterocolitica* infections in goat flocks. Young animals, in particular, have been shown to be susceptible to this infection. *Y. enterocolitica* 5/O:2,3 has also been isolated from young emaciated goat and sheep with diarrhoea in Australia [25]. In Europe, bioserotype 5/O:2,3 is reported to be host restricted to hares and thus is known as 'hare type' [26]. Interestingly, *Y. enterocolitica* belonging to biotype 3 and serotype O:1,2,3 has been isolated from chinchillas with lesions associated with pseudotuberculosis in Europe and North America [27]. This type has been assigned as 'chinchilla type'.

All strains were susceptible to ceftazidim, ciprofloxacin, gentamicin, nalidixic acid, tetracycline and trimethoprim/sulfamethoxazole. They were resistant to ampicillin, amoxicillin/clavulanic acid and cefalothin due to the β-lactamase. Intermediate sensitivity occurred sporadically to cefoxitin, cefpodoxim, cefuroxime, kanamycin and streptomycin (Table 4). No multidrug-resistant strain was detected. The resistance patterns of biotype 1A strains of wild ruminants differed slightly from the patterns of human strains belonging to biotype 1A in Switzerland. The human strains were more frequently resistant to cefoxitin and cefpodoxim and some of them were resistant to kanamycin and nalidixic acid [16].

To summarize, clinically healthy wild ruminants are shedding *Y. enterocolitica* biotype 1A in their faeces. An untypical *Y. enterocolitica* O:3 strain carrying the most important virulence genes was isolated from a clinically healthy alpine ibex. More studies are needed to clarify the importance of alpine ibex as a reservoir of pathogenic *Y. enterocolitica* and the significance of this untypical strain in human and animal infections.

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DECLARATION OF INTEREST

None.

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