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Short communication

Prevalence, bioserotyping and antibiotic resistance of pathogenic *Yersinia enterocolitica* detected in pigs at slaughter in Sardinia

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ABSTRACT

The aims of the present study were to determine *Yersinia enterocolitica* prevalence in finishing pigs and piglets at slaughter and to characterize the isolates in terms of bioserotype, virulence profile, antimicrobial susceptibility and genetic diversity. During the years 2013–2014, nine pig slaughterhouses placed in Sardinia (Italy) were visited twice, in order to collect animal samples and scalding water. Overall, 609 samples respectively of tonsils (126), colon content (161), mesenteric lymph nodes (161) and carcass surfaces (161) were collected from 126 finishing pigs and 35 piglets. Moreover, 18 scalding water samples were collected. Samples were analyzed for the detection of *Y. enterocolitica* according to ISO 10273-2003 standard (with some modifications). With regard to finishing pigs, *Y. enterocolitica* was detected in 11.9% of colon content samples, 3.2% of tonsils and 2.4% of lymph nodes. In piglets, *Y. enterocolitica* prevalence was 8.6% in colon content and 2.8% lymph nodes samples. *Y. enterocolitica* was not detected from carcass surface samples of both finishing pigs and piglets and from scalding water samples. Isolates were bio- and serotyped, tested for the presence of four virulence genes by PCR (*ail*, *ystA*, *ystB* and *inv*) and for antimicrobial resistance by disc-diffusion method. Among 47 confirmed isolates, 33 (70.2%) belonged to bio-serotype 4:O3, 7 (14.9%) to bio-serotype 2/O:5 and 7 (14.9%) to bio-serotype 1A. Bio-serotype 1A was detected only in isolates of piglets' samples. In bio-serotype 4/O:3 isolates the most common virulence genes were *ystA* (97.0%), *ail* (84.8%) and *inv* (78.8%). In bio-serotype 2/O:5, *ail*, *inv* and *ystA* genes were detected in all of the isolates. All bio-serotype 1A isolates were *ystB* positive (lacking *ail*, *inv* and *ystA*).

All isolates were susceptible to cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, nalidixic acid, sulphonamide, tetracycline and trimethoprim-sulphametoxazole. Resistances to ampicillin and cefalothin were the most common (100%), followed by amoxicillin/clavulanic acid (83.0%) and streptomycin (4.3%). Resistance to amoxicillin/clavulanic acid was detected in 57% of bio-serotype 4/O:3 isolates, 71% of bio-serotype 1A and 100% of bio-serotype 2/O:5 isolates. Two bio-serotype 4/O:3 isolates (6%) were resistant to streptomycin.

Thirty-two pathogenic *Y. enterocolitica* isolates were tested by *NotI*-PFGE, which identified 5 patterns among bio-serotype 4/O:3 isolates and 2 patterns among bio-serotype 2/O:5 isolates.

This study provides epidemiological data about human pathogenic *Y. enterocolitica* and highlight the role of pigs as a potential source of infection for the consumers in Sardinia.

1. Introduction

Yersinia enterocolitica causes a human infection known as yersiniosis that in 2016 was the third most commonly reported zoonosis in Europe with 6861 confirmed cases, after campylobacteriosis and salmonellosis (EFSA and ECDC, 2017). Human yersiniosis ensues after ingestion of the microorganisms through contaminated food or water or through blood transfusion (Bottone, 1997). *Y. enterocolitica* causes acute gastroenteritis in humans but more invasive syndromes as mesenteric lymphadenitis, terminal ileitis mimicking appendicitis and septicaemia (in immunosuppressed individuals) can occur (Bottone, 2015).

In Italy, the infection rate is likely underestimated with nine cases (0.015 cases per 100.000 inhabitants) reported in 2016 (EFSA and ECDC, 2017). Pigs are frequently described as the major source of human infection by *Y. enterocolitica* through consumption of raw or undercooked meat or by direct contact with contaminated carcasses (Le

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Guern et al., 2016). Pigs may act as asymptomatic carriers of pathogenic *Y. enterocolitica* mainly in the lymphatic tissues but also in intestines (Fredriksson-Ahomaa et al., 2007). *Y. enterocolitica* may spread to carcasses from infected organs during slaughtering. Since in the European Union there is not a harmonized surveillance program for *Y. enterocolitica*, scarce epidemiological data are available (EFSA and ECDC, 2017). Several studies have been conducted to investigate *Y. enterocolitica* prevalence in pigs at slaughter in different European countries on tonsils, intestines and carcasses samples (Bolton et al., 2013; Bonardi et al., 2013; Bonardi et al., 2016; Fondrevez et al., 2014; Fredriksson-Ahomaa et al., 2010).

In Sardinia, both finishing pigs (210–240 days old) and piglets (30–40 days old) are slaughtered for fresh meat consumption, while finishing pigs are also used for production of meat products with short or medium curing period. Both fresh pig meat and meat products can pose a risk of *Y. enterocolitica* transmission to humans.

Y. enterocolitica is characterized by six biotypes (1A, 1B, 2, 3, 4, 5) and several serotypes. Most strains causing human yersiniosis in Europe belong to bioserotype (BT) 4/O:3 and BT 2/O:9 (EFSA and ECDC, 2017) with pigs and derived meat products being the primary reservoir. Strains of five BTs (1B, 2, 3, 4 and 5) often carry the plasmid for *Yersinia* virulence (pYV), which is essential for full expression of virulence. On the other hand BT 1A strains, although lacking pYV, have been isolated from patients affected by gastroenteritis, causing symptoms indistinguishable from those caused by pathogenic biotypes (Tennant et al., 2003). The most common chromosomally encoded virulence determinants are the *ail* gene (*a*ttachment *i*nvasion *l*ocus), involved in resistance to killing by human serum, the *ystA* and *ystB* genes (*Yersinia* *s*table *t*oxin A and B) that encode for acid heat stable enterotoxins potentially responsible of diarrohoea, which are harboured by pathogenic and byotipe 1A strains, respectively. Finally, the *inv* (invasion) gene, which is important for colonization and internalization of host cells (Bolton et al., 2013).

Y. enterocolitica infection is usually self-limiting and, with the exception of some severe cases, resulting in systemic infections and bacteremia, does not require antimicrobial treatment (Bottone, 2015). Although several antimicrobials are commonly active against *Y. enterocolitica in vivo*, like aminoglycosides, cotrimoxazole, chloramphenicol, tetracycline, third generation cephalosporins and fluoroquinolones, *in vitro* susceptibility to antimicrobials varies among bioserotypes. The microorganism is usually resistant to penicillin, ampicillin and first generation cephalosporins (Fàbrega and Vila, 2012). The resistance to the beta-lactam antibiotics is mediated by the chromosomal genes *blaA* and *blaB*, which encoded constitutively produced beta-lactamases (Bolton et al., 2013).

Epidemiological information on *Y. enterocolitica* epidemiology in Sardinia is to be update (Mazzette et al., 2015). Therefore, the aims of the present work were: *i*) to determine *Y. enterocolitica* prevalence in pigs at slaughter in Sardinia; *ii*) to characterize *Y. enterocolitica* isolates in terms of biotype and serotype; *iii*) to define their virulence profile; *iv*) to determine the antimicrobial susceptibility and evaluate the genetic diversity of the isolates.

2. Materials and methods

2.1. Sampling

The study was performed between June 2013 and July 2014 in nine pig slaughterhouses located in different areas of Sardinia (Italy). Each slaughterhouse was visited on two different days, with a two months' interval between visits. One hundred and twenty-six finishing pigs (slaughtering age of 210–240 days), 35 piglets (slaughtering age of 30–40 days) and 18 scalding water samples were included in the study. The sampling plan is described in Table 1. From each animal, samples of colon content, lymph nodes and carcass surface were collected after evisceration as previously described (Fois et al., 2017). Intestines were collected in plastic bags in a separate room near the slaughterline and at least five lymph nodes in the ileocaecal regions were cut out with a sterile, disposable scalpel. The colon was incised and 25 g of its contents were collected in a sterile plastic bag. The carcass surface of finishing pigs was sampled after evisceration and before chilling, by means of a hydrated-sponge pre-moistened with 10 ml Buffered Peptone Water Broth (BPW, 3 M Health Care, Milan, Italy) at the upper inner part of both the hind legs and at the cut surface area of the abdomen and chest. A total area of approximately 1400 cm^2 of the carcass was sampled. Two sterile sponges were used (one for the upper inner part of the hind legs and one for the surface area of the abdomen) and pooled prior to testing. Piglets carcass surface was sampled as previously described, by using only one sponge (surface area not determined). Tonsils were aseptically collected from finishing pigs only, immediately after evisceration, by using sterile scalpels. Moreover, approximately 100 ml of scalding water were collected using a sterile bottle (Bibby Scientific Limited, Stone, UK).

Table 1
Category, number of sampled piglets/finishing pigs per slaughterhouse and visit and farm origin.

Slaughterhouse	Sampling day	Category (number of sampled pigs)	Farm of origin
SA	*d1*[a]	Piglets (10)	Sardinia
	d2[b]	Finishing pigs (8)	Sardinia
SB	*d1*	Piglets (5)	Sardinia
	d2	Finishing pigs (3)	Sardinia
SC	*d1*	Piglets (10)	Sardinia
	d2	Piglets (10)	Sardinia
SD	*d1*	Finishing pigs (10)	Sardinia
	d2	Finishing pigs (10)	Sardinia
SE	*d1*	Finishing pigs (10)	Sardinia
	d2	Finishing pigs (10)	Sardinia
SF	*d1*	Finishing pigs (10)	Imported (Spain)
	d2	Finishing pigs (10)	Sardinia
SG	*d1*	Finishing pigs (10)	Sardinia
	d2	Finishing pigs (5)	Sardinia
SH	*d1*	Finishing pigs (10)	Imported (Spain)
	d2	Finishing pigs (10)	Sardinia
SI	*d1*	Finishing pigs (10)	Imported (Unavailable data)
	d2	Finishing pigs (10)	Imported (Spain)

[a] First day of sampling.
[b] Second day of sampling.

All the samples were transported to the laboratory at +4°C and processed within 24 h after collection.

Overall, 627 samples from slaughtered pigs and 18 scalding water samples were analyzed.

2.2. Y. enterocolitica detection

Detection of *Y. enterocolitica* was carried out following the ISO 10273-2003 standard, with some modifications. All the samples were suspended 1:10 in Yersinia PSB broth (Biolife, Milan, Italy) and incubated at 25 ± 1 °C for 5 days. Subsequently, 10 µL aliquot was streaked onto Cefsulodin-Irgasan Novobiocin agar (CIN, Biolife, Milan, Italy) plates. From each positive plate, three typical colonies (small, smooth, with a red center and translucent rim) were seeded in Kligler Iron Agar (Oxoid, Milan, Italy) and Christensen's Urea Agar (Biolife, Milan, Italy) for preliminary identification and incubated at 30 ± 1 °C for 48 h. Lactose-negative and urease-positive cultures were submitted to biochemical identification with API® 20 E system, following the manufacturer's instructions (bioMérieux, Marcy l'Etoile, France).

2.3. Y. enterocolitica biotyping and serotyping

Confirmed *Y. enterocolitica* isolates (n.47) were biotyped according