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Evaluation of the ISO 10273:2003 method for the isolation of human pathogenic *Yersinia enterocolitica* from pig carcasses and minced meat

Inge Van Damme [a,*], Dirk Berkvens [b], Nadine Botteldoorn [c], Katelijne Dierick [c], Julie Wits [d], Brigitte Pochet [d], Lieven De Zutter [a]

[a] Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[b] Department of Animal Health, Prince Leopold Institute of Tropical Medicine, B-2000 Antwerp, Belgium
[c] Scientific Service Food-borne pathogens, Scientific Institute of Public Health, B-1050 Brussels, Belgium
[d] Federal Agency for the Safety of the Food Chain (FASFC), B-1000 Brussels, Belgium

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ABSTRACT

Pig carcass swabs ($n = 254$) and minced meat samples ($n = 82$) were examined for pathogenic *Yersinia enterocolitica* using different routinely used enrichment protocols. All samples were obtained in the context of the official *Yersinia* monitoring program in Belgium. In total, 28 carcasses (11.0%) were contaminated with *Y. enterocolitica* bioserotype 4/O:3 and one (0.4%) with bioserotype 2/O:9. Four minced meat samples (4.9%) tested positive for *Y. enterocolitica* bioserotype 4/O:3. Using the ISO 10273:2003 method, eight out of the 29 *Yersinia*-positive carcasses (27.6%) and none of the contaminated minced meat samples (0.0%) were detected. Reducing the enrichment time in PSB from 5 to 2 days increased the number of positive samples. Overall, enrichment in PSB at 25 °C recovered more positive carcasses and minced meat samples than selective enrichment and cold enrichment. As the exclusive use of the ISO 10273:2003 method results in a strong underestimation of *Y. enterocolitica* positive carcasses and minced meats, efforts are needed to optimize the current version of the ISO method. In addition, isolation of pathogenic *Y. enterocolitica* requires experience and the use of a stereomicroscope to avoid false negative results.

1. Introduction

Yersiniosis is the third most frequently reported zoonosis in Europe, which is mainly caused by the species *Yersinia enterocolitica* (EFSA, 2012). This species is subdivided in six biotypes and several serotypes (Wauters et al., 1987), with bioserotype 4/O:3 being the predominant combination to cause clinical infections in Europe. Infections are usually sporadic and mostly affect young children under five years of age. The most prevalent clinical manifestation is acute enterocolitis, with diarrhoea, abdominal pain, and fever as most common symptoms (EFSA, 2012; Rosner et al., 2010; Verhaegen et al., 1998).

Humans get infected with *Y. enterocolitica* primarily through the consumption of raw or undercooked pork (Ostroff et al., 1994; Tauxe et al., 1987) and slaughter pigs are considered to be the principal animal reservoir for pathogenic strains of *Y. enterocolitica*, mainly bioserotype 4/O:3 (Bucher et al., 2008; Fredriksson-Ahomaa et al., 2006). At time of slaughter, many pigs harbour pathogenic *Y. enterocolitica* in their tonsils, and to a lesser extent also in their intestines and lymph nodes (Gürtler et al., 2005; Nesbakken et al., 2003). Since pigs are asymptomatic carriers, positive animals are not recognised during veterinary inspection. As such, pathogens may spread from infected organs and contaminate the carcass surface during slaughter (Borch et al., 1996; Laukkanen et al., 2009). Moreover, chilling does not reduce the prevalence of pathogenic *Y. enterocolitica* (Nesbakken et al., 2008) and the psychrotrophic character of this organism even allows growth at refrigerated temperatures. Fredriksson-Ahomaa et al. (2012) demonstrated that despite low initial contamination in packaged pork cheeks, numbers of pathogenic *Y. enterocolitica* may exceed 10^4 CFU/g after 12 days of storage at 6 °C.

The limited number of pathogens combined with the occurrence of an excessive background flora can complicate the recovery of pathogenic *Y. enterocolitica* from naturally contaminated food matrices (Fredriksson-Ahomaa and Korkeala, 2003) and result in false-negative findings. Therefore, the choice of an adequate testing

* Corresponding author. Tel.: +32 (0)9 264 74 51; fax: +32 (0)9 264 74 91.
E-mail addresses: inge.vandamme@ugent.be, vandammeinge@hotmail.com (I. Van Damme).

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procedure strongly influences the effectiveness of epidemiological studies. For monitoring and survey purposes of pathogenic *Y. enterocolitica*, the European Food Safety Authority recommends the use of ISO 10273:2003 (EFSA, 2007), which is used in Belgium for the official monitoring of pathogenic *Y. enterocolitica* on pig carcasses and minced meat. Nevertheless, the current ISO method is laborious and has already been shown to be ineffective for isolation of pathogenic *Y. enterocolitica* from pig tonsils, intestines, and various lowly contaminated foods (Fredriksson-Ahomaa et al., 2008; Laukkanen et al., 2010a; Thisted Lambertz et al., 2007; Van Damme et al., 2010). Therefore, this study evaluates the ISO 10273:2003 method and other routinely used isolation methods for the recovery of human pathogenic *Y. enterocolitica* from pig carcasses and minced meat. In addition, the effect of co-incubation of the sponge on the recovery of *Y. enterocolitica* from carcass swabs was assessed after artificial inoculation of pork skin samples.

2. Materials and methods

2.1. Recovery of human pathogenic Y. enterocolitica from naturally contaminated pig carcasses and minced meat samples

2.1.1. Sample collection

In total, 254 pig carcasses and 82 minced meat samples (including pure pork, mixed pork with beef, and pork with veal) were analysed for the presence of pathogenic *Y. enterocolitica*. All samples were taken in the context of the official monitoring program of zoonotic agents in pig carcasses and minced meat in Belgium during 2011. Carcasses were sampled before chilling by meat inspectors from the Federal Agency for the Safety of the Food Chain (FASFC). The following sites of one carcass halve were swabbed: (i) the inner side of the ham (100 cm^2); (ii) the pelvic duct (100 cm^2); (iii) the sternum and neck along the incision line (300 cm^2); and (iv) the foreleg (100 cm^2) (Korsak et al., 2003) using a cellulose sponge (3M, Diegem, Belgium) prehydrated with 10 ml buffered peptone water (BPW). Samples were kept at 4 °C until analysis (1–6 days, median 2 days).

2.1.2. Isolation of human pathogenic Y. enterocolitica from naturally contaminated samples

Samples were enriched in three different broths (Fig. 1): irgasan–ticarcillin–potassium chlorate (ITC) broth [ITC Broth Base (Fluka) supplemented with 1 mg/l ticarcillin (Sigma–Aldrich, Steinheim, Germany) and 1 g/l $KClO_3$ (Merck, Darmstadt, Germany)], peptone–sorbitol–bile (PSB; Fluka, Steinheim, Germany) broth, and phosphate-buffered saline supplemented with 1% mannitol and 0.15% bile salts (PMB) broth. Carcass swabs and 10 g of minced meat were homogenized after the addition of 20 ml and 90 ml of 0.1% peptone water (PW), respectively, using a stomacher apparatus (Colworth Stomacher 400, Seward Ltd, London, UK) for two minutes. For minced meat samples, 10 ml homogenate was transferred into 90 ml of each of the three enrichment broths. For carcass swabs, 10 ml of homogenate was transferred into 90 ml of ITC and PMB, and 90 ml of PSB broth was added to the remaining homogenate (10 ml) with sponge.

ITC and PSB were incubated at 25 °C for 2 days, after which a loopful (10 µl) was streaked onto a cefsulodin–irgasan–novobiocin (CIN; Bio-Rad, Marnes-la-Coquette, France) plate. Additionally, 100 µl was streaked onto another CIN agar plate after alkali treatment (0.5 ml of culture mixed with 4.5 ml 0.5% KOH in 0.5% NaCl solution for 20 s). After ITC enrichment, an extra *Salmonella–Shigella*–desoxycholate–calcium chloride agar (SSDC; Conda, Madrid, Spain) was inoculated using a loop. For PSB, the same procedure was repeated after 5 days enrichment, with the difference that after alkali treatment a loopful instead of 100 µl was streaked onto a CIN agar plate.

PMB broth was incubated at 4 °C and a loopful was plated on CIN agar plates after 7 and 14 days. Additionally, 0.5 ml of the enriched PMB culture was transferred into 4.5 ml of 0.25% KOH in 0.75% NaCl solution for 20 s, after which 100 µl was streaked onto a CIN agar plate.

All agar plates were incubated at 30 °C for 24 h and examined for *Yersinia* colonies using a stereomicroscope with Henry illumination. From each plate, one to five suspected *Yersinia* colonies were streaked on Plate Count Agar (PCA) for pure culture.

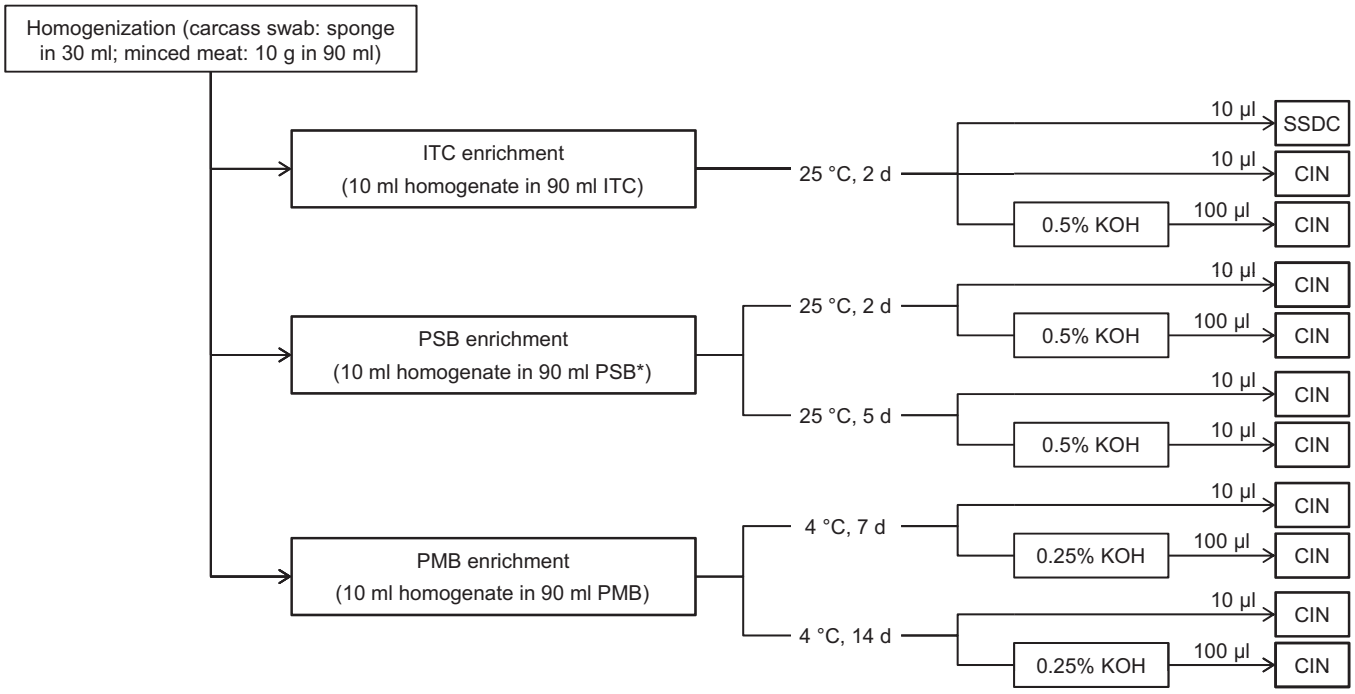

Fig. 1. Schematic overview of the isolation protocols for recovery of pathogenic *Y. enterocolitica* from carcass swabs and minced meat samples. *For carcass swabs: 90 ml PSB was added to the remaining homogenate (10 ml) with sponge.