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Detection, isolation and enumeration of *Yersinia enterocolitica* from raw pork

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Abstract

The methods available for the isolation of *Yersinia enterocolitica* from foods are generally considered to be less than optimal, and methods for estimation of numbers are lacking. Such methods are needed to understand better the significance of foodborne yersiniosis and to provide data for exposure assessment. We describe a method for the detection and enumeration of *Y. enterocolitica* containing the pYV virulence plasmid (*Ye*P+) in samples from pork surfaces. The method uses a multiplex PCR targeting the *ail* and *virF* genes to detect *Y. enterocolitica* after incubation of surface swabs in *Yersinia* enrichment broth according to Ossmer. Enumeration was achieved by adapting the enrichment to a most probable number (MPN) method format. A presumptive result was available within 24 h of sample receipt, and *Ye*P+ isolates were confirmed within four days. The presence/absence and MPN methods were evaluated in a pilot survey of 34 packs of raw pork meat purchased from retail outlets in Christchurch, New Zealand. *Ye*P+ was detected by PCR on meat from 32% of the packs, and *Ye*P+ isolates were obtained from 18% of the samples. *Ye*P+ were present at numbers ranging from 0.30 to 5.42 MPN/cm^2. This improved method for the detection and enumeration of *Ye*P+ from meat samples can be used for microbiological surveys to obtain data for assessments of consumer exposure to virulent *Y. enterocolitica*, and in outbreak investigations.

Keywords: *Yersinia enterocolitica*; Detection method; Enumeration method; Survey; Pork; pYV plasmid

1. Introduction

The genus *Yersinia* comprises 12 species, of which three are known to be human pathogens (*Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*), while the role of other species (e.g. *Y. frederiksenii* and *Y. kristensenii*) in human disease is still under investigation (Sulakvelidze, 2000). *Y. enterocolitica* is divided into subgroups according to biochemical activities (biogroups) and O antigens (serotypes). The serotype most frequently implicated in human disease worldwide is O:3 and almost all are biogroup 4 (Weynants et al., 1996). Other biogroups associated with human infection include 1B (serotypes O:8 and O:4), biogroup 2 (O:9, O:5,27), biogroup 3 (O:5,27, O:1,2,3) and biogroup 5 (O:2,3). Isolates belonging to biogroup 1A are regarded as avirulent or 'environmental', although they may be opportunistic pathogens (Bhagat and Virdi, 2007). In New Zealand biogroup 4 strains account for over 90% of cases of yersiniosis (Fenwick and McCarthy, 1995; Wright, 1996).

The current incidence of yersiniosis in New Zealand is 13.0 cases per 100,000 people, making it the third most frequently reported, possibly foodborne, enteric disease (ESR, 2007). Little is known about the epidemiology of foodborne yersiniosis in New Zealand, but a case control study identified pork consumption as the only food-related risk factor for yersiniosis (Satterthwaite et al., 1999). This observation is consistent with data from other countries where, for example, pork consumption was a risk factor for sporadic yersiniosis in a Norwegian case control study (Kapperud et al., 1995) and identified as the source of an outbreak of yersiniosis (Grahek-Ogden et al., 2007).

Generally, detection methods involve cold enrichment for up to three weeks using non-selective and/or selective broths, followed by plating onto a selective agar (Fredriksson-Ahomaa et al., 1999). Cold enrichment may be replaced by more rapid

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methods using selective media that exploit the resistance of *Yersinia* to certain antimicrobials such as irgasan (Toora et al., 1994). The organism's tolerance of high pH may also be used to inhibit growth of or inactivate competing organisms (Aulisio et al., 1980). These relatively rapid culture methods have the advantage of being better suited to outbreak investigations than procedures involving cold enrichment. Pathogenic isolates contain plasmid pYV which mediates phenotypic responses useful for identifying them (Foultier and Cornelis, 2003). In particular the low calcium response results in pinhead sized colonies on low calcium agar, and in addition the cells are able to take up the dye congo red. A low-calcium congo red-containing agarose (CRBHO) medium has been formulated to distinguish these characteristics (Bhaduri et al., 1991). The presence of the plasmid also presents an opportunity for pathogen-specific genes to be detected by PCR so that pathogenic but not environmental strains are detected.

However, the detection, isolation and enumeration of *Y. enterocolitica* remain problematic. Consequently there is a paucity of data on the prevalences of the organism in foods (Fredriksson-Ahomaa and Korkeala, 2003) and a lack of any quantitative data. The contemporary quantitative risk assessment (QRA) approach to managing food safety risks requires the input of adequate data quality and quantity, so the application of QRA to yersiniosis is currently not possible. Data on the occurrence of *Y. enterocolitica* in New Zealand foods are needed to identify the extent to which food contributes to yersiniosis, but we are aware of only one published survey of foods in New Zealand, in which *Y. enterocolitica* was isolated from 3.4% of 203 samples of ready-to-eat fleshfoods (Hudson et al., 1992). Only one of the seven isolates tested was of a human pathogenic type. Therefore, to facilitate quantitative surveys we sought to identify a reliable and sensitive PCR-based method for recovering and enumerating *Y. enterocolitica* from pork surfaces, and to test the method in a small pilot survey of raw retail pork.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Yersinia isolates were obtained from the New Zealand Reference Culture Collection, Medical Section (NZRM) (http://www.esr.cri.nz/competencies/communicabledisease/nzrcc.htm). Serotype O:3 isolates with the prefix "Z" were also kindly supplied by Dr. Stan Fenwick of Massey University, Palmerston North, New Zealand (Table 1). Other bacteria used for PCR validation (Table 1) were those held by ESR's Christchurch Public Health Laboratory. *Y. enterocolitica* NZRM 3596 was used as a positive control for all pork analyses. Inocula were prepared by inoculating 2–3 colonies into 5 ml volumes of trypticase soy broth (TSB; Becton, Dickinson and Company, Sparks, USA) which were incubated at 24 °C for 18 h. These cultures were serially diluted in 0.1% peptone water and the numbers were determined from plate counts of colonies on trypticase soy agar (TSA; 15 g agar/L TSB) incubated for 24 h at 37 °C.

Table 1
Detection of the *ail* and *virF* genes by PCR in isolates of *Yersinia enterocolitica*, *Yersinia* spp. and other bacteria

Isolate	Isolate	*ail* (356 bp)	*virF* (231 bp)
Yersinia enterocolitica, serotype 1(2a,3)	NZRM 767	+	−
Y. enterocolitica, serotype 3	NZRM 1000	+	−
Y. enterocolitica, serotype 9	NZRM 1001	+	−
Y. enterocolitica, serotype O:8	NZRM 2603 (ATCC 9610)^T	−	−
Y. enterocolitica, serotype O:3	NZRM 3596	+	+
Y. enterocolitica, serotype O:3, pig isolate	z22	+	+
Y. enterocolitica, serotype O:3, pig isolate	z26	+	−
Y. enterocolitica, serotype O:3, pig isolate	z27	+	+
Y. enterocolitica, serotype O:3, pig isolate	z28	+	−
Y. enterocolitica, serotype O:3, pig isolate	z31	+	+
Y. enterocolitica, serotype O:3, pig isolate	z32	+	+
Y. enterocolitica, serotype O:3, pig isolate	z39	+	+
Y. enterocolitica, serotype O:3, pig isolate	z52	+	+
Y. enterocolitica, serotype O:3, pig isolate	z53	+	+
Y. enterocolitica, serotype O:3, pig isolate	z57	+	+
Y. enterocolitica, serotype O:3, pig isolate	z77	+	+
Y. pseudotuberculosis	NZRM 768	−	−
Y. frederiksenii	NZRM 2534	−	−
Y. kristensenii	NZRM 2535	+	−
Y. intermedia	NZRM 2604	−	+?
Arcobacter butzleri	NZRM 4017	−	−
Bacillus cereus	NZRM 5	−	−
B. subtilus	NZRM 143	−	−
B. thuringiensis	NZRM 3610	−	−
Brochothrix campestris	NZRM 3569	−	−
B. thermosphacta	NZRM 3320	−	−
Campylobacter coli	NZRM 2607	−	−
C. fetus	NZRM 2398	−	−
C. hyointestinalis	NZRM 3676	−	−
C. jejuni	NZRM 2397	−	−
C. jejuni subsp. *doyley*	NZRM 3516	−	−
C. lari	NZRM 2622	−	−
C. showae	ATCC 51146	−	−
C. upsaliensis	NZRM 3675	−	−
Carnobacterium divergens	NZRM 3572	−	−
C. gallinarum	NZRM 3575	−	−
C. mobile	NZRM 3576	−	−
C. piscicola	NZRM 3571	−	−
Clostridium perfringens	NZRM 20	−	−
Enterococcus faecalis	NZRM 1106	−	−
Enterobacter aerogenes	NZRM 798	−	−
Escherichia coli	NZRM 480	−	−
E. coli O157:H7	NZRM 3614	−	−
Klebsiella pneumoniae	NZRM 482	−	−
Listeria grayi	NZRM 1088	−	−
L. innocua	NZRM 3024	−	−
L. ivanovii	NZRM 797	−	−
L. monocytogenes	NZRM 2597	−	−
L. seeligeri	NZRM 3287	−	−
L. welshimeri	NZRM 3286	−	−