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ScienceDirect
Journal of Microbiological Methods 70 (2007) 245–251
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

A real-time PCR for the detection of *Salmonella* Enteritidis in poultry meat and consumption eggs

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Received 6 March 2007; received in revised form 20 April 2007; accepted 20 April 2007
Available online 29 April 2007

Abstract

A robust duplex 5′ nuclease (TaqMan) real-time PCR was developed and in-house validated for the specific detection of *Salmonella enterica* subspecies *enterica* serovar Enteritidis in whole chicken carcass rinses and consumption eggs. The assay uses specifically designed primers and a TaqMan probe to target the Prot6e gene located on the *S.* Enteritidis specific 60-kb virulence plasmid. As an internal amplification control to monitor *Salmonella* DNA in the sample, a second primer/TaqMan probe set detects simultaneously the *Salmonella* specific *invA* gene. The assay identified correctly 95% of the 79 *Salmonella* Enteritidis strains tested comprising 19 different phage types. None of the 119 non-Enteritidis strains comprising 54 serovars was positive for the Prot6e gene. The assay detection probability was for 10^2 or more genome equivalents 100% and for 10 equivalents 83%. A pre-PCR sample preparation protocol including a pre-enrichment step in buffered peptone water, followed by DNA extraction was applied on low levels of artificially contaminated whole chicken carcass rinses and eggs from hens as well as 25 potentially naturally contaminated chickens. The detection limit was less than three CFU per 50 ml carcass rinse or 10 ml egg. The sensitivity and specificity compared to the traditional culture-based detection method and serotyping were both 100%. Twenty-five potentially naturally contaminated chickens were compared by the real-time PCR and the traditional cultural isolation method resulting in four *Salmonella* positive samples of which two were positive for the Prot6e gene and serotyped as *S.* Enteritidis. We show also that *Salmonella* isolates which have a rough lipopolysaccharide structure could be assigned to the serovar Enteritidis by the real-time PCR. This methodology can contribute to meet the need of fast identification and detection methods for use in monitoring and control measures programmes.

Keywords: Real-time PCR; *Salmonella* Enteritidis; Detection; Food

1. Introduction

Salmonella enterica subspecies *enterica* serovar Enteritidis (*S.* Enteritidis) is the world-leading cause of salmonellosis and is often implicated in over 60% of cases of human salmonellosis in Europe (Thorns, 2000). The reservoir for *S.* Enteritidis is mainly poultry often adopting asymptomatic infection (Bäumler et al., 2000; Guard-Petter, 2001). The pandemic of *S.* Enteritidis might have started in the mid of 1980s (Ward et al., 2000) and involves interactions of the pathogen with the environment, especially hen house conditions, the birds, the eggs as well as the human host (Guard-Petter, 2001). Through the food production chain they can consequently pass to humans. Several outbreaks have been reported where eggs were the source of human infection (Crespo et al., 2005; Berghold et al., 2003). Especially undercooked or raw eggs and poultry meat are a high risk for humans (Gillespie et al., 2005). In chicken it has been shown that both *S.* Typhimurium and *S.* Enteritidis infect the reproductive tract and contaminate forming eggs but *S.* Enteritidis persists after eggs are laid (Keller et al., 1995, 1997). PT4 was in the 1990s formerly the predominant phage type within *S.* Enteritidis (Gillespie et al., 2005). However, between 1998 and 2003 a dramatic shift in the proportion of phage types affecting humans in Western Europe was recognized (Fisher, 2004). PT4 is replaced by non-PT4 (mainly PT8, PT14b and PT21) phage types. It is possible that these other phage types are replacing the biological niche previously occupied by PT4, although this requires verification.

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doi:10.1016/j.mimet.2007.04.013

Table 1
Salmonella strains used for selectivity real-time PCR tests and results

Serovar (phage type)	No. of strains	Result	
		Prot6e	*invA*
Enteritidis (PT1)	7	+	+
Enteritidis (PT1)	1	−	+
Enteritidis (PT4)	29	+	+
Enteritidis (PT4)	1	−	+
Enteritidis (PT5)	1	−	+
Enteritidis (PT6)	3	+	+
Enteritidis (PT7)	5	+	+
Enteritidis (PT8)	8	+	+
Enteritidis (PT9)	1	+	+
Enteritidis (PT11)	1	−	+
Enteritidis (PT12)	1	+	+
Enteritidis (PT13)	2	+	+
Enteritidis (PT14)	4	+	+
Enteritidis (PT21)	8	+	+
Enteritidis (PT23)	1	+	+
Enteritidis (PT24)	1	+	+
Enteritidis (PT25)	1	+	+
Enteritidis (PT31)	1	+	+
Enteritidis (PT32)	1	+	+
Enteritidis (PT34	1	+	+
Enteritidis (PT36)	1	+	+
Agona	3	−	+
Albany	1	−	+
Bareilly	1	−	+
Bere	1	−	+
Berta	1	−	+
Bessi	1	−	+
Blockley	3	−	+
Bovismorbificans	4	−	+
Braenderup	2	−	+
Brandenburg	3	−	+
Bredeney	3	−	+
Cerro	2	−	+
Corvallis	1	−	+
Derby	5	−	+
Dresden	1	−	+
Dublin	6	−	+
Falkensee	1	−	+
Fischerstrasse	1	−	+
Gallinarum	5	−	+
Hadar	3	−	+
Havanna	1	−	+
Heidelberg	3	−	+
Hull	1	−	+
Idikan	1	−	+
Indiana	1	−	+
Infantis	3	−	+
Javiana	1	−	+
Kiambu	1	−	+
Litchfield	1	−	+
Livingstone	4	−	+
Mbandaka	1	−	+
Meleagridis	1	−	+
Minnesota	1	−	+
Montevideo	3	−	+
Moreo	1	−	+
Moualine	1	−	+
Muenchen	1	−	+
Newport	3	−	+
Nima	1	−	+
Offa	1	−	+
Ohio	1	−	+
Paratyphi B dT+	4	−	+
Pullorum	2	−	+
Reading	1	−	+
Saintpaul	3	−	+
Sandiego	2	−	+
Thompson	1	−	+
Typhi	2	−	+
Typhimurium	16	−	+
Uganda	1	−	+
Urbana	1	−	+
Vinohady	1	−	+
Virchow	4	−	+
Welikade	1	−	+

In Europe, a baseline study conducted in 2005 on the prevalence of *Salmonella* in egg-laying flocks has been shown that at the global EU-level 20.3% of the large-scale laying hen holdings are bacteriologically positive for *S.* Enteritidis. In some countries the prevalence was higher than 80% (European Food Saftey Authority, 2006). A portion of *Salmonella enterica* subspecies *enterica* isolates could not be further serotyped, because of its rough lipopolysaccharide (LPS). These strains were not further investigated to assign them to a certain serovar.

S. Enteritidis harbours in its genome a unique 60-kb virulence plasmid (Chu et al., 1999) with a high prevalence (Helmuth and Schroeter, 1994). The plasmid possesses a gene, called Prot6E gene encoding probably a unique surface fimbriae specific to *S.* Enteritidis (Clavijo et al., 2006). The role of Prot6e is not clear but it is believed that it alters its interaction with egg albumen components.

Real-time PCR for the specific detection of pathogens in foods is increasingly being used as a rapid and reliable tool for the control of contaminated samples along the food production chain. It offers an advantage in rapidity, avoiding of cross contamination compared to conventional PCR and the possibility for automation. For the detection of *S.* Enteritidis in pooled eggs it was recently published a specific real-time PCR based on the *sefA* target gene (encoding fimbrial antigen SEF14) (Seo et al., 2004). However, *sefA* is also present in other serogroup D *Salmonella enterica* subspecies *enterica* strains which can lead to false positive results. In the assay an internal amplification control is also not included to identify possible false-negative samples (Malorny et al., 2003a).

We have selected the Prot6e gene to develop a fast and sensitive real-time PCR method for the identification of the serotype Enteritidis from pure cultures or directly from *Salmonella* contaminated poultry and egg samples. An internal amplification control is included indicating the presence of *Salmonella* DNA. The selectivity of the real-time PCR assay was tested. The accuracy of the method was evaluated by comparing it with the traditional culture method with use of artificially inoculated or potentially naturally contaminated samples from poultry meat, chicken carcass rinses and eggs of laying hens. *Salmonella enterica* subspecies *enterica* strains isolated in Germany during the European egg-laying flock baseline study which could be not