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Quantitative assessment of *Yersinia enterocolitica* in raw pork meat using real time PCR (qPCR) technique

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ABSTRACT. The aim of the present study was to develop a highly sensitive and specific TaqMan probe with a set of primers for detection of pathogenic *Yersinia enterocolitica* strains and to eliminate the pre-PCR enrichment step in the real-time PCR (qPCR). A newly developed qPCR assay which is sensitive and specific for quick and reliable quantitative assessment of *Y. enterocolitica* present in artificially contaminated raw pork meat samples is described. This protocol involves the qPCR method with a TaqMan probe. The primers and probe were designed on the base of locus_tag CH49_3099 gene. This protocol appeared to be reliable for both intended applications: 1. identification and quantification of *Y. enterocolitica* in artificially and naturally contaminated raw pork meat and 2. establishment of growth potentials of different serotypes of *Y. enterocolitica* in raw meat at the usually used storage temperatures. This developed method makes it possible to eliminate the pre-PCR enrichment step enabling for the rapid assessment of meat-related consumer exposure to this pathogen.

Introduction

Yersinia enterocolitica is known to constitute a significant pathogen which possesses a huge impact on the pork production and processing industry in Poland. The *Yersinia* genus involves commensal microflora of pig intestines and may be easily transfer into carcasses during the improper and non hygienic slaughtering. *Y. enterocolitica* is considered to be responsible for provoking human diseases which includes self-limiting enteritis, acute mesenteric lymphadenitis, septicaemia, Reiter's syndrome and erythema nodosum. These diseases mainly appear as the result of food-borne transmission. Pigs are believed to be the predominant reservoir of *Y. enterocolitica* strains which are pathogenic to humans. Furthermore, this pathogen can grow at low temperatures (even 0 °C) and survive at a wide pH range (5.0–9.6). Consumption of contaminated meat products constitutes the main route for *Y. enterocolitica* infections (Bolton et al., 2013). In Europe this pathogen is defined as the most common one provoking food-borne yersiniosis (Martinez, 2010). There is more non-pathogenic than pathogenic *Yersinia* strains identified in raw pork meat. Also, there is still a lack of reliable and rapid methods to identify *Y. enterocolitica* since the conventional plating methods are not typically selective for *Yersinia* because they encourage the growth of other members of the family *Enterobacteriaceae*. Moreover, they are usually very laborious and time-consuming (Vázlerová and Steinhauserová, 2006; Van Damme et al., 2010). Real-time polymerase chain reaction (PCR) possesses the potential to cope with the significant limitations of conventional plating methods enabling the elimination of the pre-PCR enrichment step.

The main aim of this study was to develop a highly sensitive and specific TaqMan probe with a set of primers and to eliminate the pre-PCR enrichment step by increasing the reliability of CH49_3099 gene-based real-time PCR (qPCR) used for identification and quantification of enteropathogenic *Y. enterocolitica* strains in raw pork meat. Furthermore, the growth potential in raw pork meat of pathogenic serotypes was assessed at two common storage temperatures.

Material and methods

Bacterial strains and growth conditions

In this study, 16 pathogenic *Yersinia* strains were examined (Table 1). They are considered to be the most frequent serotypes connected with human and animal diseases. Apart from *Y. enterocolitica* strains there were also examined *Yersinia pseudotuberculosis* strains.

These strains were incubated overnight at 30 °C on Brain-Heart Infusion broth (BHI) (Oxoid, Basingstoke, UK) and on Trypton Soya Agar (TSA) (Oxoid, Basingstoke, UK). The total number of viable cells was assessed by preparing 10-fold serial dilutions of bacterial suspension, plating them on BHI agar (Oxoid, Basingstoke, UK), cultivating overnight at 30 °C and counting the colony forming units (CFU).

Table 1. A record of *Yersinia enterocolitica* strains involved in inclusivity assay

Species of *Yersinia*	Original strain no.	Collection
Y. enterocolitica	YE1	Ghent University, Belgium
Y. enterocolitica	YE3	Ghent University, Belgium
Y. enterocolitica	YE5	Ghent University, Belgium
Y. enterocolitica	YE7	Ghent University, Belgium
Y. enterocolitica	YE165	Ghent University, Belgium
Y. enterocolitica	KNG22703	Ghent University, Belgium
Y. enterocolitica	2516-87	Ghent University, Belgium
Y. enterocolitica	8081	Ghent University, Belgium
Y. enterocolitica	WA	Ghent University, Belgium
Y. enterocolitica	W22703	Ghent University, Belgium
Y. enterocolitica subsp. *palearctica*	105.5R(r)	Ghent University, Belgium
Y. enterocolitica subsp. *palearctica*	Y11	Ghent University, Belgium
Y. enterocolitica subsp. *palearctica*	8081	Ghent University, Belgium
Y. pseudotuberculosis	IP32979	Ghent University, Belgium
Y. pseudotuberculosis	IP32981	Ghent University, Belgium
Y. pseudotuberculosis	IP32918	Ghent University, Belgium

The qPCR assay for detection and quantification of enteropathogenic *Yersinia* spp. in naturally contaminated pork meat

A total of 50 raw pork samples taken from pigs from two slaughterhouses located in the eastern part of Poland were examined for the presence of the pathogenic species of *Yersinia* spp. by using locus_tag CH49_3099 gene targeted qPCR. The sampling was carried out according to the European Commission microbiological sampling guidelines (EC, 2006). Pork samples from carcasses were cut aseptically immediately after evisceration, placed into sterile plastic bags and delivered to the laboratory under chilled conditions. Pork samples were aseptically cut into small pieces, and 10 g of samples were put into sterile stomacher bag. Samples were homogenized with 90 ml of tryptic soya broth (Oxoid, Basingstoke, UK) for 2 min in a stomacher (Colworth Stomacher 400, Seward Limited, London, UK). Then they were stored at 4 °C and subjected to DNA isolation within 4 h after collection.

Extraction of genomic DNA from bacterial cells

A 10-ml portion of overnight bacterial growth in BHI was centrifuged at 11 700 *g* for 10 min, washed in 5 ml of 0.1× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate), suspended in 1 ml of 0.01 M sodium phosphate buffer in 20% sucrose (pH 7.0) with lysozyme (2.5 mg · ml^{-1}) and incubated for 45 min at 37 °C. A 9-ml portion of lysis buffer (10 mM Tris-hydrochloride (pH 8.0), 1 mM EDTA, 500 µg of pronase B per ml, 1% sodium dodecyl sulphate) was then added. After additional 30 min at 37 °C, the samples were deproteinized by extraction with phenol and chloroform, and the nucleic acids were precipitated with ethanol. The samples were suspended in 10 mM Tris-hydrochloride (pH 8.0), 1 mM EDTA and stored at 4 °C. The concentration of DNA (ng · µl^{-1}) was measured by spectrophotometer (Evolution 220, Thermo Fisher Scientific, Waltham, MA, USA). The mass (M) of 1 genomic molecule was counted on the base of the equation $M = n \times (1.01 \times 10^{-21}\ g \cdot bp^{-1})$; for *Yersinia* 4.616×10^6 bp. The quantified DNA (number of genomic copies per µl) was serially diluted in water and underwent qPCR according to the conditions specified below.

Inoculating meat samples

Raw pork meat negative for enteropathogenic *Yersinia* was divided into subsamples containing 1 g of raw pork meat and 9 ml of BHI, and inoculated with the tested bacterial strain to a final concentration

of approximately 10^5, 10^4, 10^3 and 10^2 CFU · ml^{-1}. DNA was isolated directly from these samples of meat using Syngen Food DNA Mini Kit (Syngen Biotech, Wrocław, Poland). The growth of *Y. enterocolitica* strains in the contaminated meat samples was studied at two different temperatures: 4 °C and 10 °C for 72 h, then they were subjected to DNA isolation. Two different temperatures were applied to assess the growth rate of *Y. enterocolitica* strains. The qPCR was carried out in the following steps.

DNA isolation from control meat samples and meat samples inoculated with *Yersinia* spp. cells

DNA was isolated from 10 ml of bacterial suspension containing 1 g of raw pork meat and 9 ml of BHI using Syngen Food DNA Mini Kit (Syngen Biotech, Wrocław, Poland). It enabled to achieve 350 µl of DNA extract. DNA concentration was measured by spectrophotometer (Evolution 220; Thermo Fisher Scientific, Waltham, MA, USA).

The qPCR assay

The primer/probe set targeting locus_tag CH49_3099 gene was designed on the base of 50-nucleotide sequence GACGATACCTTGTATAGCAATCTATTTAGCACTGATGTGTCGGTTCCGG specific for *Y. enterocolitica* species. The sequence of the gene was provided by GenBank (www.ncbi.nlm.nih.gov/Genbank/; Accession No. CP009846.1). Sequences unique for *Y. enterocolitica* were compared with those of closely related strains. The primer/probe set was designed using Primer Express Software v3.0 (Applied Biosystems, Foster City, CA, USA). The set was validated using NCBI BLAST (Basic Local Alignment Search Tool: www.ncbi.nlm.nih.gov/blast/). The sequences were as follows: forward primer 5'-GACGATACCTTGGTATAGC-3'; reverse primer 5'-ATAGCTGATGACTTTAT-3'; probe 5'-FAM-CCGGAACCGACACATCAGTGCTAAATAGAT-3'-MGB-NFQ. The amplicon size was 66 bases long. The oligonucleotides were synthesised and purchased from Eurofins Genomics (Ebersberg, Germany).

The PCR mixture contained 1× TaqMan Universal Master Mix (Syngen, Cambridge, UK), 300 nM, 600 nM and 900 nM of reverse and forward primers respectively, and 200 nM of FAM-MGB-NFQ labelled probe and 5 µl of template DNA in total volume of 20 µl. A non-template control (NTC) contained 5 µl of water instead of DNA and was included in each run. The qPCR cycling parameters were the following: 1 cycle of initial denaturation of DNA at 95 °C for 10 min followed by 40 cycles of amplification with denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min. All qPCR assays were performed in the Stratagene Mx3005P thermocycler (Agilent Technologies, Santa Clara, CA, USA).

Statistical analysis

Results of quantitative and qualitative testing were recorded in an Excel programme. The analysis of descriptive data was carried out using Stata 8.0 software (Stata Corporation, College Station, TX, USA). Comparison of enumeration results was performed using Poisson regression.

Results

Inclusivity and exclusivity of newly designed qPCR

With the exception of *Y. enterocolitica* there were not any positive reactions for the probe/primers set with unrelated microorganisms. The assay proved to be in 100% sensitive and specific towards the pathogenic species of *Y. enterocolitica.* For inclusivity and exclusivity tests of the designed sequences, qPCR was carried out on 22 strains (Tables 1 and 2).

Optimization of qPCR conditions in pork meat and assessing the linearity and quantification range

The optimalization of qPCR method conditions was conducted on the basis of the study of Najdenski et al. (2012). The concentrations of forward and reverse primers in the amount of 300 nM, 600 nM and 900 nM together with 200 nM of FAM-MGB-NFQ labelled probe were analysed using genomic DNA isolated from *Y. enterocolitica* strain 8081 serotype O:8 (Accession No. in GeneBank: CP009846.1). The DNA concentration was 10–10^5 of genomic copies per reaction. This strain was used as template. The TaqMan Universal Master Mix (Syngen, Cambridge, UK) was used as PCR mix. The optimal concentration of forward and reverse primers was 900 nM and was applied in the subsequent studies. The standard curve made on the base of these reaction conditions indicated a linear relationship ($R^2 = 0.999$) between log input DNA and the amplification cycle (regression equation $y = -3.73x + 42.92$). Such results indicates that the qPCR has a range of linear quantification from 10 to 10^5 genomic copies per reaction.

Table 2. Partial locus_tag CH49_3099 gene sequence alignment of 6 strains of *Yersinia* with database sequence indicating sequence differences

Strain (GeneBank Accession No.)	Forward primer 5'-3'	Probe 3'-5'	Reverse primer 3'-5'
Y. enterocolitica (CP009846.1)	GACGATACCTTGGTATAGC	ATCTATTTAGCACT GATGTGTCGGTTCCGG	ATAAAGTCATCAGCTAT
Y. kristensenii (CP009997.1)	CGCGCGTTCGGTGTTGGAG	AACTGGGCAACAGTGTCGAGGTGGCGATGA	GGCCACGATGCGCTGGC
Y. intermedia (CP009801.1)	CACTCGTTTGGCACAATTC	TACAGGAAAGGCCATGCAAGGCCAAGACTG	GTTTGCAACATAATTAA
Y. pestis (AE009952.1)	CACCCGCCAATAGCAGTAA	CCCAATAACAGCAG CTCTGCAAACAAGATA	CAACCAATAGAGGGCGA
Y. pseudotuberculosis (CP009786.1)	GGTGCTGGTTTCATGGCTG	CCCAATAACAGCAGCTCTGCAAACAAGATA	CTATTGATTTGTACATG
Y. rohdei (CP009787.1)	TAAAGACAACCAACTGGCA	CT TT CCAACATTCCGGT TT CTGGC AACCTT	GATACTCTCCGCGACAA

DNA isolation from pork meat samples

DNA was isolated from 10 pork meat samples which were *Yersinia*-negative. The isolation was performed according to Syngen Food DNA Mini Kit (Syngen Biotech, Wrocław, Poland). The achieved DNA samples were inoculated with 10^5 genomic copies from *Y. enterocolitica* 8081 DNA. The qPCR with the TaqMan Universal Master Mix (Syngen, Cambridge, UK) showed the quantification cycle values (Cq which is the cycle number where the fluorescence passes the threshold line) being in the range from 22 to 27, whereas the Cq value of 10^5 genomic copies from *Y. enterocolitica* 8081 amounted 22.2. Such results show that the majority of pork meat samples indicated the inhibition of the PCR reaction.

Assessing the efficiency of the TaqMan Environmental Master Mix

The designed primer/probe set targeting locus_tag CH49_3099 gene specific for *Y. enterocolitica* was examined in the qPCR with application of the TaqMan Environmental Master Mix (Syngen, Cambridge, UK). The standard curve created on the base of genomic DNA dilutions indicated a linear relationship ($R^2 = 0.999$) between log input DNA and the quantification cycle (regression equation $y = -3.91x + 43.08$). The Cq values from the DNA coming from 10 pork meat samples inoculated with 10^3 genomic copies of *Y. enterocolitica* 8081 changed not very significantly (30.75–31.43) and were comparable to 10^3 copies of the pure genomic DNA coming from the bacterial culture (31.39). Such results proved that the TaqMan Environmental Master Mix was less sensible to the inhibition caused by meat components in comparison with the TaqMan Universal Master Mix. Due to its high efficiency, the TaqMan Environmental Master Mix was applied in all further studies.

Exclusivity of the assay

The aim of the research was also to confirm the exclusivity of locus_tag CH49_3099 gene base in the qPCR. DNA coming from pure bacterial cultures of 5 *Yersinia* species involving *Y. kristensenii, Y. intermedia, Y. pestis, Y. pseudotuberculosis* and *Y. rohdei* were examined (Table 2). None of qPCR reactions carried out on the above species gave a positive result. This proves the specificity of the designed primer/probe set only for *Y. enterocolitica* species.

Inclusivity of the assay

The inclusivity of the designed primer/probe set with the TaqMan Environmental Master Mix was examined by using bacterial genomic DNA (1×10^4 molecules) coming from *Y. enterocolitica* strains including original strain numbers YE1, YE3, YE5, YE7, YE165, KNG22703, 2516-87, 8081, WA, W22703, 105.5 and Y11 as template spiked into 5 µl of meat DNA. All samples showed positive reactions with Cq values in the range from 27 to 28 which stays in the high correlation with the Cq value (27.68) achieved from 10^4 genomic copies of the standard *Y. enterocolitica* 8081. The negative control gave no positive reaction.

Quantitative assessment of *Y. enterocolitica* in pork meat

The designed primer/probe set for the qPCR was used for raw pork meat samples inoculated with bacterial cells of various serotypes of *Y. enterocolitica* (Table 1). As the infective dose of *Y. enterocolitica* for humans amounts 10^4 CFU, pork meat samples were inoculated with various concentrations of this strain which involved: 1×10^5, 1×10^4, 1×10^3, 1×10^2, 1×10^1 CFU $\cdot$ g^{-1}. The differences between theoretically expected log genome copies and detected log genome copies in 1 g of meat are shown in Table 3.

The amount of theoretically expected DNA copies is based on the real concentration of inoculated bacteria examined after plating of bacterial solution which was applied for inoculating of the pork meat samples. In case when relatively high concentrations of bacteria were inoculated (in the range from 10^3 to 10^5 CFU $\cdot$ g^{-1}), identified DNA copy numbers stayed well with the correlation with the expected number based on plate count. The difference changed from 0.93 to −0.74 log units. The inoculation with

Table 3. Differences between theoretically expected log DNA copies and detected log DNA copies in 1 g of meat after inoculating of meat samples with different concentration of different species of *Yersinia enterocolitica*

Strain of *Yersinia enterocolitica*	Δlog CFU · g^{-1}			
	~5 log/g[b]	~4 log/g[b]	~3 log/g[b]	~2 log/g[b]
Y. enterocolitica YE1	0.12[a]	0.09	0.07	−0.53
Y. enterocolitica YE3	0.93	0.62	−0.28	−0.58
Y. enterocolitica YE5	0.33	0.04	0.18	−0.08
Y. enterocolitica YE7	0	0.03	−0.48	0.10
Y. enterocolitica YE165	0.62	0.59	0.16	0.12
Y. enterocolitica KNG22703	0.55	0.48	0.32	0.15
Y. enterocolitica 2516-87	0.67	0.43	0.38	0.18
Y. enterocolitica 8081	−0.74	−0.47	−0.58	−0.55
Y. enterocolitica WA	0.62	0.36	0.28	−0.28
Y. enterocolitica W22703	0.58	0.43	0.38	−0.46
Y. enterocolitica subsp. *palearctica* 105.5R	0.53	0.34	0.23	−0.28
Y. enterocolitica subsp. *palearctica* Y11	0.6	0.18	0.58	−0.33
Y. enterocolitica subsp. *palearctica* 8081	0.48	0.16	0.51	−0.37

[a] differences (expressed in Δlog CFU · g^{-1} of meat) between theoretically expected log DNA copies and detected log DNA copies in 1 g of meat after inoculating of meat samples with different concentration of different species of *Yersinia enterocolitica* were based on the real concentration of inoculated bacteria examined after plating of bacterial solution which was applied for inoculating of pork meat samples; [b] spiking level based on turbidity

relatively low amount 10^2 CFU · g^{-1} gave a positive qPCR reaction with detected difference of copy numbers in the range from log 0.12 to log −0.58. The Cq changed from 37.9 to 36.3, which stays in correlation with a concentration ranged from 6.9 to 68 genomic copies/g of meat. There was not any amplification found in any of the negative control samples. Such results indicate that the qPCR system possesses the linear quantification in the range from 10^3 to 10^5 CFU · g^{-1} and a detection limit less than 10^2 CFU · g^{-1}.

Growth of *Y. enterocolitica* in raw meat during storage at various temperatures assessed by qPCR

To assess a rate of multiplication of different *Y. enterocolitica* strains which belong to the serotypes O:8, O:9 and O:3, the meat samples were artificially contaminated and stored for 48 h at 4 °C (optimal) and 10 °C (suboptimal) storage regimes in the meat chain. The contamination doses included 1×10^4 and 1×10^1 CFU · g^{-1}. After incubation the number of genomic copies was assessed using qPCR. The multiplication of bacteria stored at 4 °C occurred for all three examined serotypes but was the most significant for the most pathogenic serotype O:8 strain. When meat was inoculated with a high number of cells in amount of 1×10^4 CFU · g^{-1} of this strain, an increase of 3 and 4 log was found after incubation at 4 °C and 10 °C. When meat samples were inoculated with a low number of cells in amount of 1×10^1 CFU · g^{-1} of this strain, an increase of 5 log was found after incubation at 10 °C for 48 h. The weakest growth was assessed for the serotype O:3 strain at 4 °C and 10 °C using an inoculation doses 1×10^4 and 1×10^1 CFU · g^{-1}. No amplification was found in the negative control samples.

Assessment of natural contamination of raw meat with *Y. enterocolitica* by qPCR

A number of 35 raw pork samples were examined for the presence of pathogenic *Y. enterocolitica* by application of the qPCR assay described earlier. Three samples were found to be positive. The detected numbers of pathogenic *Y. enterocolitica* cells amounted 4.2×10^2; 3.3×10^2 and 1×10^2 copies per g of meat. They were positive for locus_tag CH49_3099 gene.

Discussion

Over the last few years many classical microbiological and immunological methods applied to identify pathogenic *Yersinia* have been improved using DNA-based methods. Some of them involve *in vitro* amplification in combination with direct hybridization techniques. There is a number of modifications involving enrichment, filtration, centrifugation and absorption which have been applied to concentrate and separate *Y. enterocolitica* strains in natural samples of meat. However, it is still not possible to directly isolate all the pathogenic serotypes (Wauters et al., 1987; Weynants et al., 1996; Fredriksson-Ahomaa and Korkeala, 2003).

Nowadays PCR is widely used in *in vitro* amplification method because it is able to selectively identify many microorganisms. Conventional PCR with primers coming from the chromosomal *ail* gene have been used for successful identification of *Y. enterocolitica* in food matrices including pork meat, beef meat and cheese (Boyapalle et al., 2001; Thisted Lambertz et al., 2008).

The qPCR assays, especially those which involve TaqMan probes, are more exclusive and less time-demanding in comparison with the conventional PCR. There is still a growing number of newly developed qPCR assays to identify enteropathogenic *Y. enterocolitica* in different food matrices applying a TaqMan probe. These methods enable to obtain the results

for the possible consumer exposures to pathogenic *Y. enterocolitica* and prevent from disease outbreaks (Stenkova et al., 2008; Bonardi et al., 2014).

In our study we developed an optimized, fast, sensitive and specific qPCR assay with the application of a TaqMan probe to identify and qualitatively assess the number of pathogenic *Y. enterocolitica* in contaminated raw meat samples. The protocol possesses practical significance because it involves optimized concentrations of primers, a kit for DNA extraction from meat samples and the TaqMan Environmental Master Mix.

It should be note that there is still a considerable number of recorded cases of food-borne infections provoked by consumption of meat contaminated with pathogenic strains of *Y. enterocolitica*. The ability to quickly identify the pathogenic strains in food matrices is essential for epidemiological assessment. However, there is not a traditional culture method which gives the reliable results. The pathogenic yersiniae constitute a high public health risk as they are able to grow in refrigerated and vacuum-packed meat (Tauxe et al., 1987; Fredriksson-Ahomaa et al., 2007). Typical heat treatment of meat causes the inactivation of *Y. enterocolitica* strains. It means that pathogenic cells of *Y. enterocolitica* can be transferred to people's digestive tract through the consumption of contaminated post-pasteurized meat or contaminated raw meat (Fosse et al., 2008).

In our study we successfully developed a qPCR assay to detect pathogenic *Y. enterocolitica* strains present in contaminated raw meat samples without the need of enrichment procedure for molecular detection. This is a very rapid and sensitive method which enable to detect and quantify pathogenic *Y. enterocolitica* strains present in pork meat. The method is specific and reliable because the examined non-pathogenic *Y. enterocolitica* strains were all negative for locus_tag CH49_3099 gene-targeted qPCR.

The isolation of very little amount of pathogenic *Y. enterocolitica* strains in food products rich in inhibitory contaminants is very hard to carry out and may give false-negative results when the food matrices are examined for the presence of this pathogenic bacteria (Van Damme et al., 2013a). It is known that direct isolation with the application of selective media together with long-lasting enrichment steps requires a lot of time and they are very seldom successful (Thisted Lambertz et al., 2007). In our research, the conventional culturing methods were not successful and did not enable to isolate pathogenic *Y. enterocolitica* strains from meat samples which were positive for locus_tag CH49_3099 gene-targeted qPCR. This is probably caused by the fact that there was not any enrichment step involved or *Yersinia* cells were in a non-cultivable condition. For this reason in our study we applied artificially contaminated meat samples which were treated as a standard to assess the quantitative rates of our investigations.

There is a high number of studies which assess the appearance of pathogenic *Y. enterocolitica* strains in different food products involving meat and meat products. Such studies usually give only information about the qualitative detection of *Y. enterocolitica* in samples taken at slaughter. There is still very little information on the quantitative results relating to this pathogen and the molecular procedures for the quantitative assessment usually involve the enrichment step to achieve a sufficient number of cells to be detected (Van Damme et al., 2013b). The samples, in which quantitative research is carried out, involve pork and pork products, beef and beef products, raw milk, fish, carrots and water (Gürtler et al., 2005; Thisted Lambertz et al., 2008).

As *Y. enterocolitica* is a potential threat for human health because it may grow in the food products to levels sufficient to cause sickness, their presence in food products should be monitored by a quantitative PCR test. Sickness can result not only from the consumption of the food (which in this case appears unlikely as pork meat is normally cooked sufficiently to kill any *Yersinia*), but through the handling of the meat.

The inclusivity and exclusivity tests prove the specificity and reliability of locus_tag CH49_3099 gene-targeted qPCR system. This is the gene which is chromosomally encoded and stably inherited in opposition to the plasmid encoded virulence factors which might be lost during microbiological procedures. Our q PCR assay gave negative results for the presence of *Y. pseudotuberculosis* (Galindo et al., 2011).

Conclusions

This study presents the newly developed TaqMan real-time PCR (qPCR) method which enables to detect and quantify a number of pathogenic *Yersinia enterocolitica* strains in contaminated raw meat samples which were stored at various temperatures. The growth of *Y. enterocolitica* strains proved to stay in close dependence with the serotype, storage temperatures and inoculation dose.

Further investigation is required in order to examine qPCR-positive meat samples with the isolation of pathogenic *Y. enterocolitica* strains. Furthermore, this method will deliver more quantitative results on the presence of *Y. enterocolitica* in meat and meat products. What is more, it will also support with the risk assessment and the establishment of consumer protection policy. However, this test may have some limitations. It should be noted that it will detect DNA of cells which are both viable and dead. Only viable bacteria will be a threat to public health. The other consideration is the cost of such a test for the meat industry. It is regarded to be relatively cost effective for the manufacturer of pork products and such obstacle should also be the subject of thinking.

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