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Selenite enrichment broth to improve the sensitivity in molecular diagnostics of *Salmonella*

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ABSTRACT

Selenite enrichment broth (SEB) is used to optimize the recovery of *Salmonella enterica* subspecies *enterica* from stool samples. Compared to a direct culture approach, it enhances culture yield by reducing growth of faecal coliforms and faecal streptococci. Over the course of seven years from 2000 to 2017, 47,235 faecal samples were tested with a *Salmonella* PCR. We investigated the added value of using SEB in combination with faeces for DNA extraction, in order to improve the sensitivity of molecular diagnostics for detection of *Salmonella*. A *Salmonella enterica* subspecies *enterica* strain was tested for growth characteristics, with and without incubation in SEB, to determine the impact of Selenite enrichment in the *Salmonella* PCR.

Retrospectively, a total of 102 *Salmonella enterica* subspecies *enterica* PCR positive faecal samples were re-analysed. DNA extraction was performed with the EasyMag® and MagNaPure96® system using three different input volumes of faeces and SEB.

Prospectively, 114 *Salmonella* PCR positive faecal samples were retested within 2 days using five different input volumes for DNA extraction.

Retrospectively, PCR that used SEB as part of input in the DNA extraction, 7/102 (7%) *Salmonella* PCR positive samples were additionally detected compared to no use of SEB. Of these, *Salmonella enterica* subspecies *enterica* serovariation Thompson, Enteritidis, 9,12:l.v and Senftenberg have been outbreak related in the past.

Prospectively results were combined in collaboration with another microbiology laboratory, 15/114 (13.2%) additional specimens were detected with the *Salmonella* PCR, including processing Selenite enrichment broth.

In conclusion, of the total 47,235 feacal samples, with SEB the prevalence of a positive PCR for Salmonella is 2.2%. Of these 2.2% positive Salmonella PCRs, 0.4% was not detected in culture. By using SEB an improved detection of Salmonella diagnostics could be realized and a substantial part of 13,2% additional *Salmonella* cases could be detected.

1. Introduction

Non-typhoidal *Salmonella* is considered one of the leading causes of foodborne disease worldwide. The WHO estimated that the annual median number of non-typhoidal salmonellosis is 78.7 million foodborne illnesses with over 59,000 deaths (Havelaar et al., 2015).

Typhoidal *Salmonella enterica* subspecies *enterica* serovariation Typhi (*S.* Typhi) and Paratyphi A (*S. paratyphi* A), are human-restricted pathogens transmitted by faeco-oral ingestion. The ensuing disease, enteric fever (or 'typhoid fever'), is a non-specific febrile illness which

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affects an estimated 12–27 million people worldwide each year, resulting in 129,000–223,000 deaths (Crump and Mintz, 2010; Buckle et al., 2012; Mogasale et al., 2014). Despite a dramatic reduction in incidence over the last century in most high-income countries, continuing inadequate access to clean water and increasing intercontinental spread of multiple antibiotic-resistant strains hampers disease control efforts, especially in resource-limited settings (Mogasale et al., 2014; Arndt et al., 2014; Darton et al., 2014).

In 2016, 28 Member States (MS) of the European Union reported to the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) a total of 4786 food-borne outbreaks, including waterborne outbreaks. Bacteria, in particular *Salmonella* (22.3% of all outbreaks) and *Campylobacter* (9.6% of all outbreaks), were the most commonly detected causative agents in food-borne outbreaks (33.9% of all outbreaks). A total of 96,039 salmonellosis cases were reported by 28 EU MS for 2016, with 94,530 confirmed cases resulting in an EU notification rate of 20.4 cases per 100,000 (EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2017). In the Netherlands, with a population of 16.5 million, the annual gastroenteritis (GE) incidence is approximately 4.5 million (van Pelt et al., 2003). It is the second causative agent in food-borne outbreaks (laboratory confirmed cases) with an estimated incidence of 10.7 cases per 100,000 inhabitants in 2016. The number of patients that were admitted to hospital due to GE was estimated to be 20,796 in 2016. The total costs of illness was estimated at €430 million in 2016, the estimated costs of *Salmonellosis* were €21million (Mangen et al., 2016). Although most episodes of GE are brief and do not require medical attention, the economic and social burden of GE is significant (van den Brandhof et al., 2004; Mangen et al., 2015; Roberts, 2000). These costs include primarily healthcare costs and productivity losses due to absence of patients who were either ill themselves or have to take care of a sick child. Once an outbreak is identified public health authorities conduct investigations to determine the pathogen and the source, and if required, implement control measures to prevent further spread, also leading to substantial costs (Suijkerbuijk et al., 2017).

Besides clinical purposes, gastroenteritis diagnostics should be sensitive enough to detect all *Salmonella* strains in case of potential outbreaks. Culture techniques, enrichment broths and for the last 10 years also realtime PCR, are the diagnostics tools. *Salmonella enterica* is detected in faeces by PCR or solid and liquid culture. SEB as a liquid culture was originally introduced by Leifson (2) and shows good recovery of *Salmonella* and reduced growth of faecal coliforms and faecal *Streptococci*. Other Enrichment broths used for optimization of culture yield for *Salmonella* including components as brilliant green or desoxycholate citrate agar are usually highly selective and will inhibit certain serotypes of *Salmonella* in their growth. In gastroenteritis diagnostics the goal for SEB is to provide a better growth and recovery for all *Salmonella* isolates (Fries and Steinhof, 1997; Sparbier et al., 2012; Moriñigo et al., 1993). In this study we aimed at assessing the impact of using Selenite Enrichment broth on sensitivity of PCR diagnostics for *Salmonella*, compared to using only stool as input in the DNA extraction.

2. Materials and methods

The Laboratory for Medical Microbiology and Public Health, LabMicTA in Hengelo performs diagnostics for approximately 950,000 inhabitants in the eastern part of the Netherlands, with approximately > 6000 PCRs for *Salmonella* annually. All 47,235 *Salmonella* PCR's performed including selenite enrichment broth from the years 2010 to 2017. Positive Salmonella PCR's were checked for their culture results on the Hektoen Enteric Agars plates(Thermo Scientific™, Oxoid Deutschland GmbH, Am Lippeglacis 4–8, D-46483 Wesel, Germany) in comparison to the Crossing point value (Cp) of the PCR. We performed a combined retro and a prospective study, in collaboration with the National Institute for Public Health and the Environment (RIVM), department of Disease burden of food-related pathogens in the Netherlands, and Certe-Laboratory for Infectious Diseases, Groningen, the Netherlands.

A *Salmonella enterica* subspecies *enterica* (*S. enterica*) strain was tested for growth characteristics, with and without incubation in SEB. A negative stool sample was spiked with a *S. enterica* strain and serial diluted (10^5 till 10^1 CFU/ml), followed by a 18–24 h incubation in O_2 at 37 °C. Before and after incubation all dilutions were analysed under identical pre- processing conditions and analysed with a specific *Salmonella* PCR assay that also detect *S. bongori*, targeting the *ttr* gene (Malorny et al., 2004).

2.1. Study population

Retrospectively, DNA extraction was performed on 102 Salmonella PCR positive faecal samples, and stored at −80 °C. DNA extraction was performed with the EasyMAG® and MagNA Pure 96® system with three different input volumes for SEB and feaces, i.e. 100 µl faeces lysate together with 50 µl Selenite enrichment broth (routine protocol), 50 µl Selenite enrichment broth only, or100 µl of faeces lysate only.

One hundred randomly chosen serovars of *Salmonella enterica* strains isolated in the Netherlands provided by the RIVM, department of Disease burden of food-related pathogens in the Netherlands, were tested with and without use of SEB (Table 1). After DNA extractions on the EasyMAG® system, the *Salmonella* PCR assay was performed.

Prospectively, 114 faecal samples PCR positive for *Salmonella enterica* subspecies *enterica* were re-analysed with and without SEB. Analysis was repeated within 2 weeks both with and without Selenite enrichment broth. DNA extraction was performed with the EasyMAG® system using five different input volumes for Selenite enrichment broth and faeces, i.e. 100 µl faeces lysate together with 50 µl Selenite enrichment broth (routine protocol), 50 µl Selenite enrichment broth only, 100 µl of faeces lysate only, as well as a 1:10 and a 1:100 dilution of the faeces lysate.

2.2. Real-time PCR

The PCR reactions at LabMicTA for *Salmonella* were performed in a multiplex PCR, format Molecular Faeces Panel (MFP1) also targeting *Campylobacter jejuni*, *Campylobacter coli* and *Synechococcus* that was used as an internal control (Table 2). PCRs positive for Salmonella are confirmed by culture and the strain is checked for antibiotic resistants.

The total reaction volume of the multiplex PCR MFP1 consisted of 20 µL reaction mix and 10 µL of DNA extract. The reaction mix contained 3 µg bovine serum albumin (Invitrogen, Breda, the Netherlands) which was added to 15 µL of Roche Probes Master (Roche Diagnostics Nederland BV, Almere, the Netherlands) and 2 µL Molecular Grade Water (Roche Diagnostics Nederland BV, Almere, the Netherlands), with primers and probes forming a total reaction volume of 20 µL. The assay was performed with the LightCycler480 (Roche diagnostics Nederland BV, Almere, the Netherlands) using the following PCR profile: 45 cycles of 95 °C during 15 s and 60 °C during 60 s. The molecular methods used at Certe laboratory were described previously (de Boer et al., 2010a).

The performance of the used multiplex assays was extensively tested regarding sensitivity and specificity, using analytical panels from quality control programs as well as clinical materials (Table 3).

3. Results

Over a period of 7 years (2010–2017) a total of 47,235 *Salmonella* PCR's were performed at our laboratory. In total 2.2% (1058 faecal samples) were PCR positive for *Salmonella* PCR. Fig. 1 shows the distribution of Cp values of the *Salmonella* positive PCR results and for conventional culture outcome. Out of the 1058 positive *Salmonella* PCRs 18.1% (191) of the faecal samples were culture negative while all

Table 1
One hundred randomly chosen serotypes of *Salmonella enterica* subspecies *enterica* strains isolated in the Netherlands.

"O" antigens	Serovar	Phase 1 "H" antigens	*N*	"O" antigens	Serovar	Phase 1 "H" antigens	*N*
B	Abony		1	C4	Tennessee		1
	Schwarzengrund		1		Potsdam		1
	Paratyphi B variatie Java		1		Rissen		1
	Brancaster		1		Jerusalem		1
	Typhimurium		1		Mikawasima		1
		1, 4, 5, 12:i-	1		Braenderup		1
	Agama		1	D1	Berta		1
		4, 12:i-	1		Dublin		2
		4, [5], 12:b:-	1		Enteritidis		1
	Banana		1		Panama		1
	Agona		1			9, 12: l, v:-	1
	Brandenburg		1		Goettingen		1
	Bredeney		1		Napoli		1
	Derby		1		Javiana		1
	Heidelberg		2	E_1	Anatum		1
	Saintpaul		1		Give		1
	Kingston		1		London		1
	Chester		1		Meleagridis		1
	Indiana		1		Lexington		1
	Sandiego		1		Amsterdam		1
	Haifa		1		Muenster		1
	Coeln		1		Orion		1
	Arechavaleta		1	E_4	Senftenberg		1
	Stanley		1			1,3,19:i-	1
C_1	Isangi		1	F	Leeuwarden		1
	Ohio		1		Abaetetuba		1
	Colindale		1	G	Poona		1
	Livingstone		1	G2	Putten		1
	Mbandaka		1		Worthington		1
	Bareilly		1		Kedougou		1
	Infantis		1		Idikan		1
	Virchow		1		Adjame		1
	Montevideo		1		Bracknell		1
	Oranienburg		1	H	Havana		1
	Thompson		10		Fischerkietz		1
	Irumu		1		Madelia		1
C_2	Bovis morbificans		1	K	Cerro		1
	Newport		1		Langenhorn		1
	Kottbus		1	m	Nima		1
	Muenchen		1		Kitenge		1
	Litchfield		1	O	Adelaide		1
	Manhattan		1				
	Goldcoast		1				
	Hadar		1				
C3	Albany		1				
	Emek		1				
	Kentucky		1				
	Corvallis		1				

Table 2
specific primers and probes for MFP1.

Gastro-enteritis multiplex PCR				
Panel	target	Primers/probe	Primers/probe 5'à 3'	Reference
MFP1	*Salmonella*	SE-ttr-6F	CTC ACC AGG AGA TTA CAA CAT GG	(Malorny et al., 2004)
		SE-ttr-4R	AGC TCA GAC CAA AAG TGA CCA TC	
		SE-ttr-5TP	CAC CGA CGG CGA GAC CGA CTT T	
	Campylobacter jejuni	CJ-mapA-F	CTG GTG GTT TTG AAG CAA AGA TT	(Best et al., 2003)
		CJ-mapA-R	CAA TAC CAG TGT CTA AAG TGC GTT TAT	
		CJ-mapA-MGB	AAT TCC AAC ATC GCT AAT G	
	Campylobacter coli	Cc-ceuE-fw	AAG CTC TTA TTG TTC TAA CCA ATT CTA ACA	(Best et al., 2003)
		Cc-ceuE4-re	TCC ATG TGT GCC TAC TTT TAC ATT	
		Cc-ceuE-pr-FAM	TTG GAC CTC AAT CTC GCT TTG GAA TCA TT	
	Synechococcus (IC)	Cyano-fw	CAT TCT TGA CAA GTT AAC CAG TTA GCT G	(Dullaert-de Boer et al., 2018)
		Cyano-re	CAA GGT TCT GCT GAC ATT CAA ACA	
		Cyano-pr-670	TCT CCA GGG CAG CAT TGA ATC CAG	

Table 3
efficiency of the four targets in the MFP1 multiplex panel.

Multiplex	Target	Filter	Efficiency PCR
MFP 1	Synechoccus (IC)	CY5	2274
	C. coli	FAM	2368
	C. jejuni	HEX	1981
	Salmonella	RED 610	1978

other samples were culture positive. Of these 191 culture negatives for *Salmonella enterica*, 43.5% (83) of the faecal samples had a PCR result with a Cp < 30. The Cp for negative culture results ranges between Cp 13 and Cp 35. The point where cultures resulted in more negative as positive ranges between Cp 29 and 30. Above Cp 35 the total positivity incidence of *Salmonella* decreases.

The effect of serial dilution of a specific *Salmonella enterica* strain in SEB in the PCR for *Salmonella enterica* was studied. These dilutions were cultured in SEB. DNA was then extracted from the enriched broth and used as input in the *Salmonella enterica* PCR. The results of the SEB of the *Salmonella enterica* strain revealed after incubation an increase in Cp value in the *Salmonella enterica* PCR (Fig. 2).

A total of 102 *Salmonella* PCR positive faecal samples were tested retrospectively with and without SEB, using the MFP1 multiplex PCR to study the influence of SEB in the Salmonella PCR. An additional 6.8% (EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2017) of the samples was detected *Salmonella* positive after enrichment, using the EasyMAG® DNA extraction system. Using the MagNA Pure 96® system this percentage was 9.4% (van Pelt et al., 2003) (Table 4).

All *Salmonella* PCR positive feacal materials were subsequently cultured and obtained isolates were typed at the RIVM. The 7 *S. enterica* feacal samples, which were detected additionally after enrichment in the MFP1 PCR, three of the four *S. enterica* serotype Thompson, two of the two *S. Enteritidis*, and one S. 9,12:l.v and one *S. Senftenberg* were additionally detected. The feacal samples containing *S. typhymurium* and *S. nordrhein* showed a strong increase in Cp value and were additional detected when the EasyMag® extraction system was used instead of the MagNaPure96®. On the MagNaPure96® DNA isolation system, 8 *Salmonella* feacal samples were detected additionally after enrichment in the MFP1 PCR. The 8 *Salmonella* serovars were two of the four *S. enterica* serotype *Thompson*, two of the two *S. enteritidis*, and one S. 9,12:l.v, one *S. senftenberg,* one *S. typhymurium* and one *S. nordrhein*. These retrospective results from our own region were obtained from a limited number of randomly chosen Salmonella PCR positive feacal samples.

To study the dynamics of SEB for *Salmonella enterica* serovars, one hundred randomly chosen *S. enterica* strains from the RIVM were diluted in feaces and tested in the MFP1 PCR. All one hundred *S. enterica* strains with a different subtype revealed the same trend, showing a strong increase in sensitivity of the *Salmonella enterica* PCR when using SEB for input in the DNA extraction (range: 7–12 Cp-values lower).

A total of 114 *S. enterica* PCR positive feacal samples were prospectively re-analysed with and without use of SEB for input in de DNA extraction, after routine gastroenteritis diagnostics was performed to investigate the additional value of SEB. When using SEB, an additional 13.2% (15/114) of *S. enterica* PCR positive samples was detected. A 10 Cp increase was seen in 20% of the samples that were retested with use of SEB (Table 5). At LabMicTA, without use of SEB, 9/65 (13.8%) positive *S. enterica* faecal samples were not detected by PCR. Furthermore, the *Salmonella enterica* genotypes *Enteritidis*, *Typhimurium*, *diarizonae and Infantis* could not be detected without SEB. Certe retested 49 *Salmonella* PCR positive samples; 6/49 (12.2%) positive *Salmonella enterica serovars* faecal samples were not detected if the SEB was not used as input in the DNA extraction. The six *Salmonella enterica* genotypes *S. enteritidis*, *S. typhimurium*, *S. mbandaka*, *S. salamae* could not be detected without use of Selenite enrichment broth.

Furthermore, of the 9 (13.8%) additionally detected *S. enterica* PCR positive samples when using SEB we investigated the clinical relevance of these results. Two patients had other infections. For one of these patients the treatment was altered due to the *Salmonella* infection. Five patients were not treated with antibiotics. In total three patients were treated with antibiotics and had no complaints after treatment. The decision to treat these patients was based on the severity of their gastrointestinal symptoms (diarrhea > 10 bowel movements/day) and their vulnerability due to comorbidity (immunocompromised after recent chemotherapy, celiac disease in remission and a severe pneumonia). All patients were hospitalized. No formal follow up was undertaken concerning all these 9 patients. However, no further complaints were

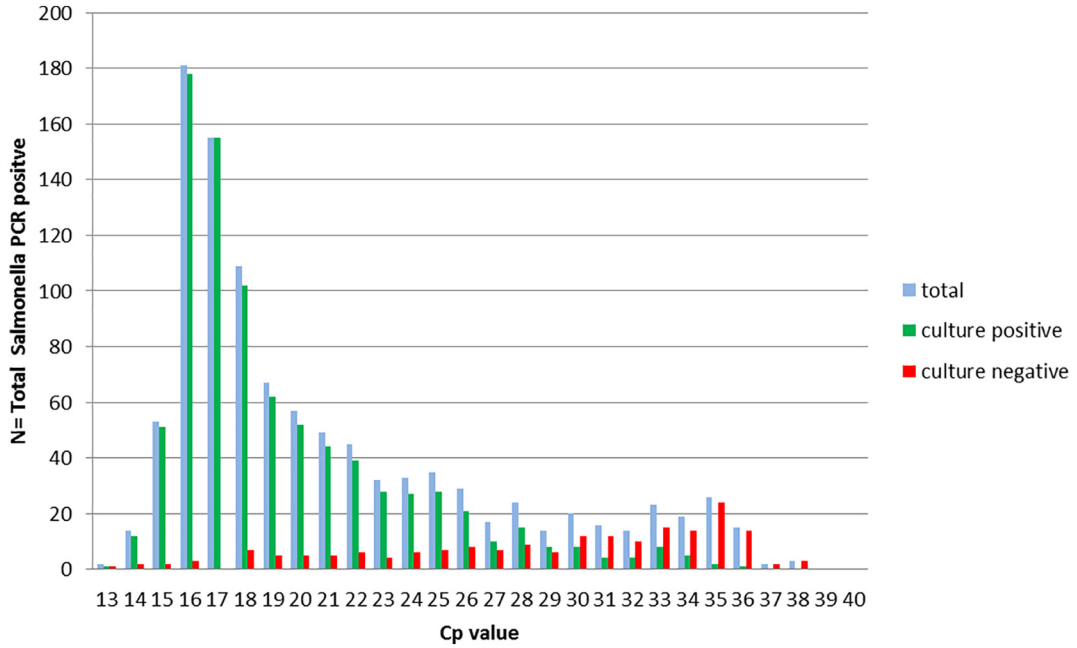

Fig. 1. Culture positive and negative results in comparison with the *Salmonella* PCR results. On the x-axle the specific Cp value of the *Salmonella* PCR is depicted and on the y-axle the amount of faecal samples positive for *Salmonella* PCR and *Salmonella* culture is shown.

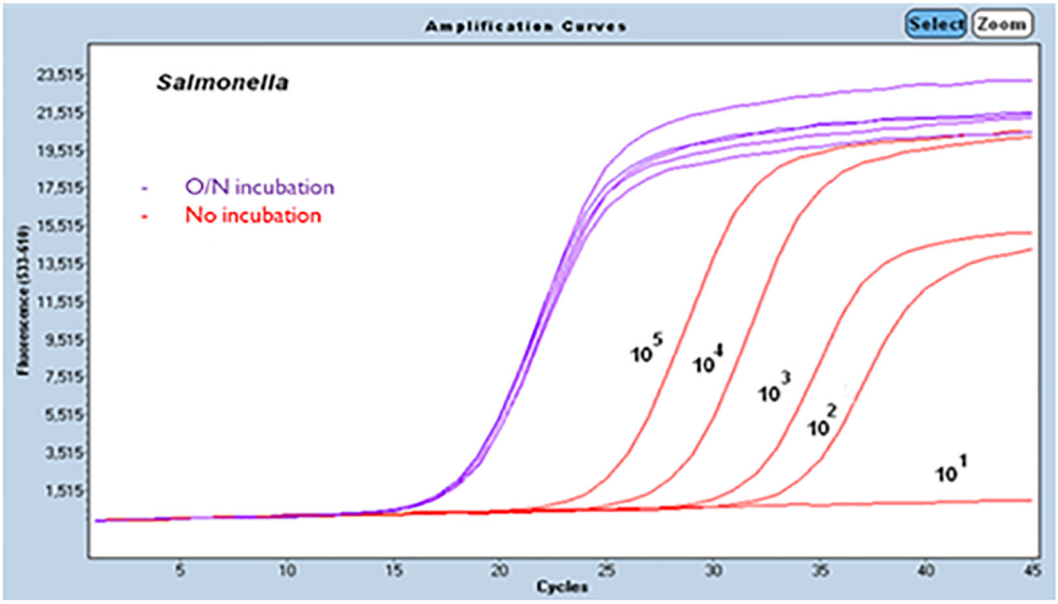

Fig. 2. Amplification curves of *Salmonella enterica* dilutions with selenite enrichment broth overnight incubation (O/N) (purple) and without selenite enrichment broth (red) in the MFP1 PCR. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Retrospective outcome of *Salmonella enterica* PCR's with and without Selenite enrichment broth performed with EasyMag® and MagNaPure96® DNA isolation system. Were > 10 Cp, 5–10 Cp and lower than 5Cp. The differences between using Selenite Enrichment broth and without using Selenite Enrichment broth in the pre-proces of the DNA extraction are dispicted as > 10Cp, 5-10Cp and < 5 Cp values.

	EasyMag® N(%)	MagNaPure96® N(%)
Total	102 (100)	85 (100)
Additional detected	**7 (6.8)**	**8 (9.4)**
> 10 Cp	25 (24.5)	14 (16.5)
5–10 Cp	21 (20.6)	22 (25.9)
< 5 Cp	49 (48.0)	41 (48.2)

Bold indicates additional detected Salmonella's.

Table 5
Prospective results of two laboratories of *S. enterica* PCR's in which *S. enterica* is additional detected or with an increase of > 10 Cp's in sensitivity with Selenite enrichment broth.

	LabMicTA		Certe		Total	
Total	65	100%	49	100%	114	100%
Additionally detected	9	13.80%	6	12.24%	15	13.16%
> 10 Cp increase	11	16.90%	12	24.49%	23	20.18%

registered in the patient files after the initial admittance in the hospital or visit at their general practitioner.

4. Discussion

In this study we retrospectively and prospectively investigated the additional value of using SEB for enhancing the sensitivity of molecular diagnostics for *Salmonella* enterica in feacal samples. Using enriched selenite broth in combination with feaces as input in the DNA extraction increased the sensitivity of PCR in comparison to only use faeces as input material; additional positive samples were detected and Cp values of PCR positive samples were on average considerably lower. Historically, SEB was added to the faecal culture of *Salmonella* to get a higher sensitivity and because the confirmation of the culture will be faster. For the growth of Salmonella in SEB 12–16 h incubation is needed, the same counts for normal culture. When molecular techniques became available for detection of *Salmonella*, this process was not changed because of the added value of the SEB.

The 1058 *Salmonella* PCR positive results of the past 7 years showed that 191 samples PCR-guided culture remained negative. For a total of 83 (43%) of these PCR positive/culture negative samples, the detected Cp value < 30. This suggests that the culture technique for detection of *Salmonella* lacks sensitivity for example by the use of antibiotics in de clinic, technical failure in culture diagnostic or the condition of the bacteria in the sample that will be delivered at the laboratory (de Boer et al., 2010a, 2010b; Schuurman et al., 2007). In general, a PCR-guided culture will result in an isolation of Salmonella, if the detected Cp value < 30 cycles, as the amount of viable *Salmonella* bacteria present is high enough (de Boer et al., 2010a).

Retrospectively, without use of Selenite enrichment broth, 7 of the 102 (7%) positive *Salmonella enterica's* were not detected by PCR. *Salmonella* Thompson, *Enteritidis*, 9,12:l.v and *Senftenberg* are outbreak related serovars that were not always detected.

To obtain a clearer insight in the dynamics of using SEB for enrichment of *Salmonella*, one hundred randomly selected *S. enterica* isolates were diluted in feaces and tested in the MFP1 PCR. In comparison to using no enrichment as input in the DNA extraction, an enrichment with SEB resulted in significantly lower Cp values for all isolates. Hence, an increase in sensitivity of the *Salmonella enterica* PCR. These findings suggest that the increased sensitivity when using SEB is not *Salmonella* serotype specific. Moreover it could result in a clinical important infection or could be part of a cluster of *S. enterica* infections.

Prospectively, in total 13% is additionally detected with use of Selenite enrichment broth. At LabMicTA 9/65 (13.8%) positive *Salmonella* faecal samples were additionally detected by PCR. At Certe 6/49 (12.4%) positive *Salmonella* feacal samples were additionally detected with use of SEB. This implies that difference in PCR setup does not change the effect of SEB.

The clinical relevance of detecting low viable loads of *Salmonella enterica serovars* in faeces of patients with a suspicion for gastroenteritis, is debatable. Most of the patients with a positive *Salmonella enterica* serovar are not treated with antibiotics conform national guidelines. For a sub selection of the prospectively acquired results, we investigated the clinical relevance of the additional detected Salmonella PCR positive results (9/65), when using SEB (Table 5). Three patients

(43%) were treated by their specialist in the hospital and had no complaints after treatment. This indicates clinical relevance of accurate detection of Salmonella infections, especially in selected cases.

In the European Union more people were affected and ill due to *Salmonella enterica* outbreaks than *Campylobacter* related outbreaks (EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2017). If during an outbreak situation *Salmonella enterica* serovars are not detected, the disease burden and associated costs might increase due to more cases and a larger outbreak. In 2012 a large *Salmonella* Thompson outbreak affected 1149 people, reported and laboratory confirmed, caused by smoked salmon. Twenty percent of the cases was hospitalized and four cases were reported to be fatal. Taking underestimation of 13,8% into account the total number of persons having an infection was estimated at 21,123. Total outbreak costs were estimated at €6.8 million of which costs of laboratory tests of the reported cases were 128,738. In outbreak situations, the use of Selenite enrichment broth in laboratory diagnostics can be of great value at low costs, estimated at €0,40 cents per faecal sample including additional handling of the samples by a labtechnician (Suijkerbuijk et al., 2017; Friesema et al., 2012, 2014).

5. Conclusion

Over the course of seven years we tested 47,235 faecal samples for *Salmonella by* PCR. When using SEB 2.2% of the faecal samples were additionally detected Salmonella PCR positive. In conventional culture 0.4% of the total *Salmonella* positive faecal samples were not detected. Without use of SEB 13.8% of *Salmonella enterica* containing faecal samples would not be detected. By using SEB in combination with the faecal sample as input in DNA extraction procedures the sensitivity of *Salmonella* molecular diagnostics could be improved. This can be of important value in (individual) patient diagnostics, as well as for optimal detection of patients in outbreak situations. This study has shown the improvement of *Salmonella* detection at low costs by using SEB. This could potentially influence healthcare costs, patient/family costs and costs in other sectors, especially in outbreak settings.

References

Arndt, M.B., Mosites, E.M., Tian, M., et al., 2014. Estimating the burden of paratyphoid a in Asia and Africa. PLoS Negl. Trop. Dis. 8, e2925.

Best, E.L., Powell, E.J., Swift, C., Grant, K.A., Frost, J.A., 2003. Applicability of a rapid duplex real-time PCR assay for speciation of Campylobacter jejuni and Campylobacter coli directly from culture plates. FEMS Microbiol. Lett. 229 (2), 237–241. Available at. http://www.ncbi.nlm.nih.gov/pubmed/14680705, Accessed date: 23 January 2015.

de Boer, R.F., Ott, A., Kesztyüs, B., Kooistra-Smid, A.M., 2010 Nova. Improved detection of five major gastrointestinal pathogens by use of a molecular screening approach. J. Clin. Microbiol. 48 (11), 4140–4146 (Epub 2010 Sep 22).

de Boer, R., Peters, R., Gierveld, S., Schuurman, T., Kooistra-Smid, M., Savelkoul, P., 2010 Feb. Improved detection of microbial DNA after bead-beating before DNA isolation. J. Microbiol. Methods 80 (2), 209–211. https://doi.org/10.1016/j.mimet.2009.11.009. (Epub 2009 Dec 6. PMID: 19995580).

Buckle, G.C., Walker, C.L., Black, R.E., 2012. Typhoid fever and paratyphoid fever: systematic review to estimate global morbidity and mortality for 2010. J. Glob. Health 2, 10401.

Crump, J.A., Mintz, E.D., 2010. Global trends in typhoid and paratyphoid fever. Clin. Infect. Dis. 50, 241–246.

Darton, T.C., Blohmke, C.J., Pollard, A.J., 2014. Typhoid epidemiology, diagnostics and the human challenge model. Curr. Opin. Gastroenterol. 30, 7–17.

van den Brandhof, W.E., de Wit, G.A., de Wit, M.A.S., van Duynhoven, Y.T.H.P., 2004. Costs of gastroenteritis in the Netherlands. Epidemiol. Infect. 132, 211–221.

Dullaert-de Boer, M., Akkerman, O.W., Vermeer, M., Hess, D.L.J., Kerstjens, H.A.M., Anthony, R.M., et al., 2018. Variability and cost implications of three generations of the Roche LightCycler® 480. PLoS One 13 (1), e0190847.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J. 15 (12). https://doi.org/10.2903/j.efsa.2017.5077. (5077, 288 pp).

Fries, R., Steinhof, U., 1997. Growth kinetics of Salmonella in mixed cultures incubated in Rappaport–Vassiliadis medium. Food Microbiol. 14 (5), 505.

Friesema, I.H., Lugnér, A.K., van Duynhoven, Y.T., GEops Working Group, 2012. Costs of gastroenteritis in the Netherlands, with special attention for severe cases. Eur. J. Clin. Microbiol. Infect. Dis. 31 (8), 1895–1900 Epub 2012 Jan 8.

Friesema, I., de Jong, A., Hofhuis, A., Heck, M., van den Kerkhof, H., de Jonge, R., Hameryck, D., Nagel, K., van Vilsteren, G., van Beek, P., Notermans, D., van Pelt, W., 2014. Large outbreak of Salmonella Thompson related to smoked salmon in the Netherlands, August to December 2012. Euro Surveill. 19 (39) (pii = 20918).

Havelaar, A.H., Kirk, M.D., Torgerson, P.R., et al., 2015. World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLoS Med. 12, e1001923.

Malorny, B., Paccassoni, E., Fach, P., Bunge, C., Martin, A., Helmuth, R., 2004. Diagnostic real-time PCR for detection of Salmonella in food. Appl. Environ. Microbiol. 70 (12), 7046–7052.

Mangen, M.J., Bouwknegt, M., Friesema, I.H., et al., 2015. Cost-of-illness and disease burden of food-related pathogens in the Netherlands, 2011. Int. J. Food Microbiol. 196, 84–93.

Mangen, M.J., et al., 2016. National Institute for Public Health and the Environment, Disease Burden of Food-Related Pathogens in the Netherlands. (RIVM report 2017-0097).

Mogasale, V., Maskery, B., Ochiai, R.L., et al., 2014. Burden of typhoid fever in low-income and middle-income countries: a systematic, literature based update with risk-factor adjustment. Lancet Glob. Health 2, e570–e580.

Moriñigo, M.A., Muñoz, M.A., Martinez-Manzanares, E., Sánchez, J.M., Borrego, J.J., 1993. Laboratory study of several enrichment broths for the detection of Salmonella spp. particularly in relation to water samples. J. Appl. Bacteriol. 74, 330–335.

van Pelt, W., de Wit, M.A.S., Wannet, W.J.B., Ligtvoet, E.J.J., Widdowson, M.A., van Duynhoven, Y.T.H.P., 2003. Laboratory surveillance of bacterial gastroenteric pathogens in the Netherlands, 1991–2001. Epidemiol. Infect. 130, 431–441.

Roberts, J.A., 2000. Economic aspects of food-borne outbreaks and their control. Br. Med. Bull. 56, 133–141.

Schuurman, T., de Boer, R., Patty, R., Kooistra-Smid, M., van Zwet, A., 2007 Dec. Comparative evaluation of in-house manual, and commercial semi-automated and automated DNA extraction platforms in the sample preparation of human stool specimens for a Salmonella enterica 5′-nuclease assay. J. Microbiol. Methods 71 (3), 238–245 (Epub 2007 Sep 15).

Sparbier, K., Weller, U., Boogen, C., Kostrzewa, M., 2012. Rapid detection of Salmonella sp. by means of a combination of selective enrichment broth and MALDI-TOF MS. Eur. J. Clin. Microbiol. Infect. Dis. 31 (5), 767.

Suijkerbuijk, Anita W.M., Bouwknegt, Martijn, Marie-Josee, J., Mangen, G. Ardine de Wit, van Pelt, Wilfrid, Bijkerk, Paul, Friesema, Ingrid H.M., 2017 Apr 1. The economic burden of a Salmonella Thompson outbreak caused by smoked salmon in the Netherlands, 2012–2013. Eur. J. Public Health 27 (2), 325–330.