Evaluation of different target genes for the detection of Salmonella sp. by loop-mediated isothermal amplification

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Significance and impact of the study: This study evaluates six specific target regions in the salmonella genome using the loop-mediated isothermal amplification technique. Primers were designed targeting five regions, which had not previously been studied intensively, and the invA gene, which is frequently used in biomolecular detection of Salmonella sp. Most primer sets were not able to amplify DNA of all tested salmonella strains, revealing deficiencies in inclusivity of the corresponding target regions. These findings highlight the differing suitability of the investigated target regions to identify Salmonella sp. and underline the importance of selecting appropriate sequences for providing reliable detection methods.

Keywords
bcfD gene, gene62181533, invA gene, LAMP, phoP gene, salmonella, siiA gene, ttrRSBCA locus.

Abstract

The loop-mediated isothermal amplification (LAMP) technique was used to investigate six salmonella-specific sequences for their suitability to serve as targets for the pathogen identification. Sequences selected for designing LAMP primers were genes invA, bcfD, phoP, siiA, gene62181533 and a region within the ttrRSBCA locus. Primers including single nucleotide polymorphisms were configured as degenerate primers. Specificity of the designed primer sets was determined by means of 46 salmonella and 32 other food- and waterborne bacterial reference species and strains. Primers targeting the ttrRSBCA locus showed 100 % inclusivity of target and exclusivity of other test species and strains. Other primer sets revealed deficiencies, especially regarding Salmonella enterica subsp. II–IV and Salmonella bongori. Additionally, primers targeting the siiA gene failed to detect S. enterica subsp. enterica serotypes Newport and Stanley, whereas bcfD primers did not amplify DNA of S. enterica subsp. enterica serotype Schleissheim. TtrRSBCA primers, providing short detection times and constant melting temperatures of amplification products, achieved best overall performance.

Introduction

Salmonellosis represents one of the major foodborne zoonoses of great public concern worldwide. The World Health Organization reported that particularly non-typhoidal Salmonella enterica was involved in a total of 230 000 deaths caused by foodborne diarrhoeal disease agents in 2010 (World Health Organization 2015). As a contribution to food safety improvement, different loop-mediated isothermal amplification (LAMP) assays for the detection of Salmonella sp. have been developed. In 2000, a Japanese research group introduced LAMP as a novel technique for identifying nucleic acids (Notomi et al. 2000). It soon became seen as a robust, sensitive and rapid alternative to the polymerase chain reaction (PCR) and was widely applied within the scope of diagnostic and scientific issues (Mori and Notomi 2009). The first LAMP assay for the detection of Salmonella sp. was developed in 2005. Primer design was based on host cell invasion gene invA (Hara-Kudo et al. 2005). Most of the
subsequently established LAMP assays followed this example and focused on further validation of *invA*-based detection, various equipment for visualization of LAMP products and general method improvement by merging LAMP with additional technologies and providing multiplex approaches (Yang et al. 2018). One drawback of using *invA* as a target gene is that some serotypes including *S. enterica* subsp. *enterica* serotypes Kentucky, Senftenberg and Litchfield are known to occasionally lack this sequence and therefore cannot be detected using nucleic acid amplification techniques (Ginocchio et al. 1997; Turki et al. 2012). To supply optimal properties regarding inclusivity of *Salmonella* sp. and exclusivity of non-salmonella strains, several species-specific alternative target genes were tested in new LAMP assay development (see below). Since those corresponding sequences were not investigated in detail in subsequent studies, using them as targets for LAMP-based salmonella detection lacks verification regarding specificity. Therefore, in the present study, *invA* (Hara-Kudo et al. 2005) and four alternative target genes, including *bcfD* (Zhuang et al. 2014), *phoP* (Li et al. 2009), *siiA* (Zhao et al. 2017) and gene62181533 (Li et al. 2016), were tested for their suitability regarding LAMP detection. Additionally, the *ttrRSBCA* locus, which has so far only been published as a target in connection with a real-time PCR protocol (Malorny et al. 2004), was investigated. The aim of these analyses was to provide further information about target gene specificity and to point out suitable and more specific alternatives to the *invA* gene.

**Results and Discussion**

In the present study, six LAMP primer sets were designed, targeting a region within the *ttrRSBCA* (*ttr*) locus and genes *invA, bcfD, phoP, siiA* and gene62181533 (*g62*). All sequences have been reported to be exclusively present in *Salmonella* sp. in the literature. A total of 78 bacterial strains were used to determine specificity and to evaluate overall performance of the different primer sets. LAMP primers were configured as degenerate primers to ensure inclusivity of salmonella strains in case of single nucleotide polymorphisms within the target genes. Inclusivity was 97.8% for *invA*, 93.5% for *bcfD*, 97.8% for *phoP*, 87.0% for *siiA*, 95.7% for *g62* and 100% for *ttr* primers. Except for *g62* primers that occasionally showed unspecific reactions after 40 min, all primer sets revealed exclusivity of 100% towards all tested non-salmonella strains (Table 1). *invA* (*x* = 10.28 min) and *ttr* primers (*x* = 10.60 min) provided the shortest detection times (Fig 1; for supporting information also see Fig S1). Melting temperatures showed the greatest range (*R* = 1.8°C) when *invA* primers were used (Fig 2).

### Table 1 Positive (PPV), negative (NPV) predictive values and accuracy obtained by different primer sets

|            | *invA* | *bcfD* | *phoP* | *siiA* | *g62* | *ttr* |
|------------|--------|--------|--------|--------|-------|-------|
| PPV        | 1      | 1      | 1      | 1      | 0.917 | 1     |
| NPV        | 0.970  | 0.914  | 0.970  | 0.842  | 0.933 | 1     |
| Accuracy (%) | 98.7   | 96.2   | 98.7   | 92.3   | 92.3  | 100   |

Except for *ttr* primers, there were deficiencies mainly concerning inclusivity of *S. enterica* subsp. II–IV and *S. bongori* for all tested primer sets. Corresponding target genes were not intensively applied and studied for constant occurrence in these rather rarely appearing salmonella species and subspecies. LAMP-associated investigations of *siiA, bcfD, g62* and the widely used *invA* sequences focused on *S. enterica* subsp. I and IIIa (Hara-Kudo et al. 2005; Zhuang et al. 2014; Li et al. 2016; Zhao et al. 2017). In the present study, DNA of *S. enterica* subsp. IV could not be amplified by *invA* primers, while this subspecies was reliably identified during a multicentre validation study for an *invA*-based salmonella PCR (Malorny et al. 2003). Primers targeting genes *phoP, bcfD, siiA* and *g62* also failed to detect that strain. In a previous LAMP study, *phoP* was shown to be included in *S. enterica* subsp. IV (Li et al. 2009), whereas corresponding data for genes *bcfD, siiA* and *g62* are not available. In contrast to the findings of Li et al. (2016), primers for *g62* were not able to amplify DNA of *S. enterica* subsp. IIIa. In previous studies, appearance of gene *siiA* was proven in *S. enterica* subsp. II and IIIa, whereas *bcfD* was additionally shown to be inclusive in *S. bongori* (Zhuang et al. 2014; Zhao et al. 2017). These results do not comply with the findings in our study. The *siiA* sequence could not be amplified in one strain of *S. enterica* subsp. II and *S. enterica* subsp. IIIa–IIIb. Furthermore, *S. enterica* subsp. *enterica* serotypes Newport and Stanley gave negative results. Although salmonella strains of serotype Newport were successfully tested within an *siiA*-based PCR study, there are no data available for *S. enterica* subsp. IIIb and *S. Stanley* strains (Ben Hassena et al. 2015). In contrast to the findings of Zhuang et al. (2014), no amplification occurred when *bcfD* primers were used for detection of *S. bongori*. In addition, *S. enterica* subsp. *enterica* serotype Schleissheim could not be identified and was not included in previous studies either.

Since *invA, phoP, g62, siiA* and *bcfD* primers showed positive results for most of the tested salmonella strains, this implies that the corresponding sequences may not be genetically stable in all *Salmonella* sp. or were presumably absent in unidentifiable strains. Besides the possibility of completely lacking sequences, point mutations
within the gene regions can explain the inability of primers to bind to otherwise complementary sequences. As a common characteristic, genes invA, phoP, siiA and bcfD encode for different virulence factors regarding cell invasion (Galán et al. 1992; Wille et al. 2014), formation of fimbria (Yue et al. 2012) and transcriptional regulation of virulence-associated genes (Groisman 2001). The sequence of g62 is characterized as protein encoding but

**Figure 1** Distribution of detection times using different LAMP primer sets.

**Figure 2** Distribution of melting temperatures using different LAMP primer sets.
not specified in detail (Kong et al. 2013). It can be assumed that the expression of these genes is not necessary for the survival of certain Salmonella sp. lineages. Thus, their sequences are likely to be rather susceptible to genetic variation or to be even missing in the salmonella genome. In contrast, the ttr locus is required for tetrathionate respiration in anaerobic environments and is likely to be significant within the life cycle of Salmonella sp. (Hensel et al. 1999). Malorny et al. (2004) concluded that the ttr genes should be genetically stable in all salmonella strains and emphasized the advantages of ttr-based salmonella detection. The assumption that a correlation exists between the constant presence of genes and their importance for survival is sustained by the fact that, except for ttr primers, weaknesses in primer specificity mainly occurred in S. enterica subsp. II–IV and S. bongori. These strains are primarily associated with cold-blooded animals and known as opportunistic pathogens with low human virulence. However, they should not be neglected since cold-blooded animals play an increasing role as pet animals or as a food source for human consumption in various countries. Although non-entérica subspecies are less virulent, they can induce extraintestinal infections causing a broad spectrum of serious diseases (Lamas et al. 2018).

It was shown that all primer set-specific LAMP products are characterized by different melting temperatures (Fig. 2; for supporting information also see Fig S2). Except for invA-based amplification products, no wide-ranging measurements were apparent. Since melting temperatures depend on sequence content and should not differ more than ±1°C in specific amplification products, divergent measurements indicate unspecific amplification of non-target species. Wide-ranging measurements as shown in invA-based LAMP products can complicate the evaluation of results. When using different sample processing techniques, additional impacts of matrices could lead to increased variability in measured melting temperatures.

In the present study, the ttr locus was identified as most suitable target for reliable detection of all salmonella species and subspecies by LAMP. Ttr primers showed the best overall performance, providing short detection times and constant melting temperatures. Deficiencies in other primer sets involved poor inclusivity of cold-blooded animal-associated strains. This reveals the importance of choosing essential target genes for the reliable nucleic-acid-based detection of Salmonella sp. However, further validation of the established LAMP assays is necessary to evaluate their suitability for salmonella detection in food matrices. Validation should include artificial contamination experiments and investigation of naturally contaminated products (Feldsine et al. 2002).

Close attention should be given to predestined foodstuffs and also to exotic meat sources as suggested by the results of this study.

Materials and methods

Primer design

Numerous sequence data used for primer design were obtained from the DNA sequence database GenBank of the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/). For creating the six gene-specific LAMP primer sets, the same GenBank accession numbers were used as in the aforementioned target gene-related publications. Therefore, the primer design for the invA gene was based on S. enterica subsp. enterica serotype Typhimurium (GenBank accession no. M90846), for the befD gene on S. enterica subsp. enterica serotype Newport (GenBank accession no. JX026810), for the phoP gene on S. enterica subsp. enterica serotype Paratyphi A ATCC 9150 (GenBank accession no. NC006511), for the siiA gene on S. enterica subsp. enterica serotype Typhimurium str. LT2 (GenBank accession no. NC_003197), for the g62 gene on S. enterica subsp. enterica serotype Choleraesuis str. SC-B67 (GenBank accession no. NC006905) and for the ttr locus on S. enterica subsp. enterica serotype Typhimurium str. LT2 (GenBank accession no. AF282268), whereby the ttr target region was limited to 701 bp including parts of the ttrC and ttrA gene. All primer sequences were configured by the software LAMP Designer (Premier Biosoft, San Francisco, CA) and subsequently submitted to basic local alignment search tool (BLAST) analysis (NCBI) against 53 salmonella whole-genome sequences obtained from GenBank (NCBI). Polymorphisms within the primer sequences were marked in accordance with the nucleotide code of the International Union of Pure and Applied Chemistry (IUPAC) and considered by ordering appropriate degenerate primers via Eurofins Genomics GmbH (Ebersberg, Germany). Primer sequences are shown in Table 2.

Bacterial strains and DNA extraction

A total of 46 salmonella and 32 non-salmonella strains were used to determine the specificity of the LAMP primers (Table 3). Salmonella strains were selected to represent all known S. enterica subspecies including various epidemiologically important serotypes, and S. bongori. Non-salmonella strains were chosen because of the close relation to salmonella or because they are found in the same environment and grow under the same conditions. Generally, isolates were cultured aerobically on
Table 2 Primer sequences used for determining specificity of different target genes

| Primer | Sequence (5’–3’) | Position |
|--------|----------------|----------|
| GenBank accession no. JX026810 | bcfD-F3 | ACCGATTACGCACTGTA | 107–126 |
| bcfD-B3 | GCCATGCGCACTGTA | 113–1139 |
| bcfD-FIP | GCCATGCGCACTGTA | 113–1139 |
| bcfD-LoopF | GCCATGCGCACTGTA | 113–1139 |
| bcfD-LoopB | GCCATGCGCACTGTA | 113–1139 |
| GenBank accession no. NC006905, Region 3144912 | g62-LoopB | GCCATGCGCACTGTA | 113–1139 |
| g62-LoopF | GCCATGCGCACTGTA | 113–1139 |
| g62-LoopB | GCCATGCGCACTGTA | 113–1139 |
| GenBank accession no. NC006511, Region 1680130 | invA-LoopF | GCCATGCGCACTGTA | 113–1139 |
| invA-LoopB | GCCATGCGCACTGTA | 113–1139 |
| GenBank accession no. NC006511, Region 1680130 | invA-F3 | GCCATGCGCACTGTA | 113–1139 |
| invA-B3 | GCCATGCGCACTGTA | 113–1139 |
| invA-FIP | GCCATGCGCACTGTA | 113–1139 |
| invA-LoopF | GCCATGCGCACTGTA | 113–1139 |
| invA-LoopB | GCCATGCGCACTGTA | 113–1139 |
| GenBank accession no. NC006511, Region 1680130 | invA-F3 | GCCATGCGCACTGTA | 113–1139 |
| invA-B3 | GCCATGCGCACTGTA | 113–1139 |
| invA-FIP | GCCATGCGCACTGTA | 113–1139 |
| invA-LoopF | GCCATGCGCACTGTA | 113–1139 |
| invA-LoopB | GCCATGCGCACTGTA | 113–1139 |
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| invA-FIP | GCCATGCGCACTGTA | 113–1139 |
| invA-LoopF | GCCATGCGCACTGTA | 113–1139 |
| invA-LoopB | GCCATGCGCACTGTA | 113–1139 |

Table 3 Strains and isolates used for determining specificity of different LAMP primers

| Strain | No. of tested strains | No. of positive results in LAMP |
|--------|----------------------|--------------------------------|
| Salmonella enterica subsp. enterica serotype | Agona | 1 | 1 | 1 | 1 | 1 | 1 |
| | Bovismorbificans | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | Braenderup | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Brandenburg | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Chester | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Derby | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | Dublin | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Enteritidis | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Essen | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Hadar | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Haifa | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Hato | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Heidelberg | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Infantis | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Kentucky | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Kisangani | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Coeln | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Mglulani | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Muenchen | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Napoli | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Newport | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Oranienburg | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Paratyphi B | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | Saintpaul | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | Schlesseheim | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Stanley | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Tennessee (CCUG 12658) | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | Typhimurium (DSM 19587) | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| | Virchow | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | S. enterica subsp. I (not serotyped) | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | S. enterica subsp. II | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | S. enterica subsp. IIIa | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | S. enterica subsp. IIb | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | S. enterica subsp. IV | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | S. enterica subsp. V | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | S. borgori | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Non-salmonella strains | Aeromonas | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| | hydrophila (DSM 30187) | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Arcobacter butzleri (DSM 8739) | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Arcobacter cryaerophilus (DSM 7289) | 1 | 0 | 0 | 0 | 0 | 0 | 0 |

(continued)
Columbia agar with 5% sheep blood (Oxoid Deutsch-
land GmbH, Wesel, Germany) for 24 h at 37°C. Differ-
ning from this, *Campylobacter* sp., *Arcobacter* sp. and 
*Helicobacter pylori* were cultured for 48 h under 

microaerophilic conditions. DNA was isolated from 
5–10 colonies from each agar plate using the DNeasy 
Blood & Tissue Kit (Qiagen GmbH, Hilden, Germany) 
in accordance with the manufacturer’s recommenda-
tions. Subsequently, DNA concentration in the eluate 
was determined spectrophotometrically by NanoDrop 
2000c (Thermo Fisher Scientific GmbH, Dreieich, Ger-
many). Measurements were performed in triplicate.

**LAMP assay**

LAMP reactions were carried out using the real-time fluo-
rometer Genie® II (OptiGene Ltd, Horsham, UK). Each 
reaction mixture with a total volume of 25 µl consisted of 
15 µl GspSSD isothermal master mix ISO-001 (Opti-
Gene), 2/C1 5 µl primer mix containing 5 pmol F3 and B3 
primer, 20 pmol FIP and BIP primer and 10 pmol LoopF 
and LoopB primer, 2/C1 5 µl nuclease-free water (Qiagen) 
and 5 µl DNA template (5 pg). Runs took place at 65°C 
and lasted 60 min. Melting temperatures of the LAMP 
products were determined using a ramp rate of 0.05°C/s 
within temperatures ranging from 98 to 80°C. In each 
run, one reaction mixture with 5 pg DNA of *S.
enterica* subsp. *enterica* serotype Typhimurium DSM 19587 and 
one reaction mixture containing 5 µl nuclease-free water 
instead of template DNA served as positive and negative 
control, respectively.

**Data analyses**

Raw data analysis was conducted using the software Genie 
Explorer (OptiGene) and processed using Microsoft Excel 
2016 (Microsoft Corporation, Redmond, WA). Positive 
predictive values were calculated as (number of true posi-
tives)/(number of true positives + number of false posi-
tives) and negative predictive values were calculated as 
(number of true negatives)/(number of true negatives + 
number of false negatives). The accuracy was cal-
bulated as (number of true positives + number of true 
negatives)/(number of isolates) × 100.

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**Conflict of Interest**

The authors declare that there is no conflict of interest.
References

Ben Hassena, A., Barkallah, M., Fendri, I., Grosset, N., Ben Neila, I., Gautier, M. and Gdoura, R. (2015) Real time PCR gene profiling and detection of Salmonella using a novel target: the siiA gene. J Microbiol Methods 109, 9–15.

Feldsine, P., Abeyta, C. & Andrews, W.H. (2002) AOAC International methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. J AOAC Int, 85, 1187–1200.

Galán, J.E., Ginocchio, C. and Costeas, P. (1992) Molecular and functional characterization of the Salmonella invasion gene invA: homology of InvA to members of a new protein family. J Bacteriol 174, 4338.

Ginocchio, C.C., Rahn, K., Clarke, R.C. and Galán, J.E. (1997) Naturally occurring deletions in the centisome 63 pathogenicity island of environmental isolates of Salmonella spp. Infect Immun 65, 1267.

Groisman, E.A. (2001) The pleiotropic two-component regulatory system PhoP-PhoQ. J Bacteriol 183, 1835–1842.

Hara-Kudo, Y., Yoshino, M., Kojima, T. and Ikedo, M. (2005) Loop-mediated isothermal amplification for the rapid detection of Salmonella. FEMS Microbiol Lett 253, 155–161.

Hensel, M., Hinsley, A.P., Nikolaus, T., Sawers, G. and Berks, B.C. (1999) The genetic basis of tetrathionate respiration in Salmonella Typhimurium. Mol Microbiol 32, 275–287.

Kong, X., Lu, Z., Zhai, L., Yao, S., Zhang, C., Lv, F. and Bie, X. (2013) Mining and evaluation of new specific molecular targets for the PCR detection of Salmonella spp. genome. World J Microbiol Biotechnol 29, 2219–2226.

Lamas, A., Miranda, J.M., Regal, P., Vazquez, B., Franco, C.M. and Cepeda, A. (2018) A comprehensive review of non-enterica subspecies of Salmonella enterica. Microbial Res 206, 60–73.

Li, J., Zhai, L., Bie, X., Lu, Z., Kong, X., Yu, Q., Lv, F., Zhang, C. et al. (2016) A novel visual loop-mediated isothermal amplification assay targeting gene62181533 for the detection of Salmonella spp. in foods. Food Control 60, 230–236.

Li, X., Zhang, S., Zhang, H., Zhang, L., Tao, H., Yu, J., Zheng, W., Liu, C. et al. (2009) A loop-mediated isothermal amplification method targets the phoP gene for the detection of Salmonella in food samples. Int J Food Microbiol 133, 252–258.

Malorny, B., Hoorfar, J., Bunge, C. and Helmuth, R. (2003) Multicenter validation of the analytical accuracy of Salmonella PCR: towards an international standard. Appl Environ Microbiol 69, 290–296.

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

Mori, Y. and Notomi, T. (2009) Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. J Infect Chemother 15, 62–69.

Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. and Hase, T. (2000) Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 28, e63.

World Health Organization (2015) WHO Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007-2015. Geneva, Switzerland: World Health Organization.

Turki, Y., Ouzari, H., Mehri, I., Aissa, R.B. and Hassen, A. (2012) Biofilm formation, virulence gene and multi-drug resistance in Salmonella Kentucky isolated in Tunisia. Food Res Int 45, 940–946.

Wille, T., Wagner, C., Mittelstädt, W., Blank, K., Sommer, E., Malengo, G., Döhler, D., Lange, A. et al. (2014) SiiA and SiiB are novel type I secretion system subunits controlling SPI 4-mediated adhesion of Salmonella enterica. Cell Microbiol 16, 161–178.

Yang, Q.R., Domese, K.J. and Ge, B.L. (2018) Loop-mediated isothermal amplification for Salmonella detection in food and feed: current applications and future directions. Foodborne Pathog Dis 15, 309–331.

Yue, M., Rankin, S.C., Blanchet, R.T., Nulton, J.D., Edwards, R.A. and Schifferli, D.M. (2012) Diversification of the Salmonella fimbiae: a model of macro- and microevolution. PLoS One 7, e38596.

Zhang, L., Gong, J., Li, Q., Zhu, C., Yu, Y., Dou, X., Liu, X., Xu, B. et al. (2014) Detection of Salmonella spp. by a loop-mediated isothermal amplification (LAMP) method targeting bcfD gene. Lett Appl Microbiol 59, 658–664.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Amplification curves after LAMP reaction with 5 pg DNA of Salmonella Typhimurium DSM 19587 obtained by primer sets targeting different salmonella-specific genes.

Figure S2. Melting curves after LAMP reaction with 5 pg DNA of Salmonella Typhimurium DSM 19587 obtained by primer sets targeting different salmonella-specific genes.