INVITED REVIEW

Salmonella importance and current status of detection and surveillance methods

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Abstract

Salmonella, a genus within Enterobacteriaceae, remains as an important human pathogen and it has been reported to be the most common food-borne bacterial disease in the world. Although majority of the *Salmonella* cases are sporadic, outbreaks occur frequently. *Salmonella* can be associated with many kinds of foods and the presence of *Salmonella* in ready-to-eat foods is considered significant regardless of the level of the contamination. Therefore isolation is carried out by enrichment culture of a defined weight or volume of the food (normally 25 g). The traditional and time-consuming detection and isolation of *Salmonella* spp. from food and feed utilizes a multistep protocol with nonselective pre-enrichment, followed by a selective enrichment step, isolation on selective agar media and a preliminary biochemical and serological confirmation. Several rapid methods have been developed to speed up the detection of *Salmonella*. This paper aims to give an overview of the occurrence and current status of *Salmonella* detection and surveillance methods.

Introduction

Salmonella, a genus within Enterobacteriaceae, are mesophilic, chemoorganotrophic, facultatively anaerobic Gram-negative rod-shaped bacteria. The cells are typically 0.7–1.5 µm by 2–5 µm. They grow at 7–48 °C with an optimum growth temperature at 37 °C and at pH 4.0–9.5 with an optimal growth at pH 6.5–7.5. *Salmonella* grows optimally at a water activity of 0.995 (Ellermeier & Slauch, 2006).

The nomenclature of the genus *Salmonella* has been variable, since two systems of nomenclature have been used (Tindall *et al.*, 2005). Recently it was agreed that the genus *Salmonella* includes only two species, namely *Salmonella enterica* and *S. bongori*. The type species *Salmonella enterica* is divided into six subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*) and most *Salmonella* belong to the subspecies *Salmonella enterica* subsp. *enterica* (Tindall *et al.*, 2005, http://www.bacterio.cict.fr). Members of this subspecies have usually been named based on the original isolation location of the serovar or serotype, e.g. *S.* Typhimurium. More accurate name for this serotype is

Salmonella enterica subsp. *enterica* serovar Typhimurium. The genus *Salmonella* is extremely heterogenous, with more than 2500 currently recognized serovars (Popoff, Bockemühl & Gheesling, 2004). In addition, O-antigen and flagellar antigens are used to classify *Salmonella* strains.

Salmonella, although being intestinal bacteria, are widespread in the environment and are commonly found in farm effluents and in any material subjected to faecal contamination (Liebana *et al.*, 2003; Martinez-Urtaza *et al.*, 2004). Salmonellosis is an infectious disease of humans and animals caused by living cells of the two species of *Salmonella* (*S. enterica* and *S. bongori*). *S. enterica* subsp. *enterica* inhabit warmblooded animals, whereas *S. enterica* subspecies and *S. bongori* are commensals of cold-blooded animals and only rarely infect humans (Humphrey, 2004; Ellermeier & Slauch, 2006). *S. enterica* is a facultatively intracellular pathogen that preferentially resides inside macrophages, although it requires both antibodies and a cellular immune response for clearance (Kaufmann, Raupach & Finlay, 2001).

Salmonella infection of the host often leads to a self-limiting gastroenteritidis. However, the nature of the

pathogenic action of *Salmonella* varies with the serovar, the strain, the infectious dose, the nature of the contaminated food and the host status (Chiu, Su & Chu, 2004; Humphrey, 2004). Infants and immunosuppressed patients are more susceptible to *Salmonella* infection than healthy adults (Tauxe, 2002; Voetsch *et al.*, 2004). Infections with *Salmonella* are initiated when the pathogen invades the gastrointestinal epithelium (Merrell & Falkow, 2004). *Salmonella* infections have been reported to result either in fatal bacteremia when unrestricted, or in the generation of neutrophil and mononuclear-rich microabscesses that lead to bacterial clearance when successfully controlled (Merrell & Falkow, 2004). In some cases bacteria persist in the gall bladder of asymptomatic carriers, who contribute substantially to the dissemination of disease by providing a constant source of infectious bacteria (Raupach & Kauffman, 2001).

Virulence of *Salmonella* requires multiple factors and genes (Groisman & Ochman, 1997; Marcus *et al.*, 2000). Although some virulence genes are found on virulence plasmid common to many *Salmonella* serovars, majority of the virulence genes are encoded within *Salmonella* pathogenicity islands in the chromosome (Marcus *et al.*, 2000). Lipopolysaccharide (LPS) is the major virulence factor of Gram-negative bacteria (Alexander & Rietschel, 2001; Raetz & Whitfield, 2002; Trent *et al.*, 2006). LPS forms the outermost layer of Gram-negative bacteria and protect the cell from the environment (Raetz & Whitfield, 2002). O-antigen capsules produced by *Salmonella* strains potentiate their survival in the environment (Gibson *et al.*, 2006). Pathogenic *Salmonella* have evolved many strategies in adapting to the hostile environment of the phagosome (Raupach & Kauffman, 2001). In addition, survival and propagation of *Salmonella* in the environment are genetically defined and enhanced by the wideranging adaptation ability to various stress responses (Humphrey, 2004; Anriany *et al.*, 2006).

Sources and epidemiology of *Salmonella*

Salmonellosis has been reported to be the most common food-borne bacterial disease in the world (Herikstad, Motorjemin & Tauxe, 2002; Plym-Forshell & Wierup, 2006). In the United States it has been estimated that 1.4 million non-typhoidal *Salmonella* infections with 400 deaths occur annually (Voetsch *et al.*, 2004). However, it is possible that under-reporting of salmonellosis is common. Of food-borne diseases, salmonellosis accounts for 26% of hospitalizations and 31% of deaths in the United States (Voetsch *et al.*, 2004). In 2006, salmonellosis remained the second most frequent zoonosis with 160 649 reported human cases in the European

Union (European Food Safety Authority [EFSA], 2007). In 2006 there was a 7.6% decrease of incidence in salmonellosis in EU from 2005, *S.* Enteritidis and *S.* Typhimurium being the most frequently reported serovars associated with human illness. Human *S.* Enteritidis cases have often been associated with the consumption of contaminated eggs (Guard-Bouldin *et al.*, 2004; Guard-Petter, 2001, Cogan *et al.*, 2004) and broiler meat, whereas *S.* Typhimurium cases have typically been associated with the consumption of contaminated pig, poultry and bovine meat (Humphrey, 2004).

S. Typhi, a host-restricted human pathogen, remains an important health threat for mankind with more than 22 million cases and 220 000 deaths annually world-wide (Crump, Luby & Mintz, 2004; Zhang, Tunje Jeza & Pan, 2008). Typhoid fever caused by *S.* Typhi is a disease that usually results from overcrowding and poor sanitary conditions. Hence, the incidence of this disease is highest in developing countries with poor hygienic conditions and inadequate clean water supplies and sewage systems (Gasem *et al.*, 2002; Vollaard *et al.*, 2004). Non-Typhi *Salmonella* has been reported to be a major cause of morbidity and mortality throughout the world, specifically among children under 5 years old (Graham, 2002). Furthermore, the emergence of antibiotic-resistant *Salmonella* strains, e.g. due to previous uncontrolled use of antimicrobials in animal feeds and increased therapeutic use in other areas, is an increasing problem globally (Humphrey, 2001; Sørum & L'Abée-Lund, 2002; Su *et al.*, 2004).

Salmonella can be associated with many kinds of foods (Humphrey, 2004). Contamination of meat (cattle, pigs, poultry) may originate from animal salmonellosis, but most often it results from the contamination of meat with intestinal contents during evisceration of animals, washing, and transportation of carcasses (al-Saigh *et al.*, 2004). Likewise, vegetables and fruits may carry *Salmonella* if contaminated with fertilizers of faecal origin, or when washed with polluted water (Duffy *et al.*, 2005; Das, Gürakan & Bayindirh, 2006). Although majority of the *Salmonella* cases are sporadic, outbreaks occur frequently. In June 2008 in the US Food and Drug Agency (FDA) issued a warning about outbreak of *Salmonella* serotype Saintpaul, which had been linked to consumption of some raw tomatoes (http://www.fda.gov/oc/opacom/hottopics/tomatoes.html). Recently a *Salmonella* Typhimurium outbreak caused by peanut butter and peanut products was reported in the United States (http://www.fda.gov/oc/opacom/hottopics/salmonellatyph.html).

Although proper heat treatment of the foods will kill *Salmonella* in food, caution should be taken to avoid cross-

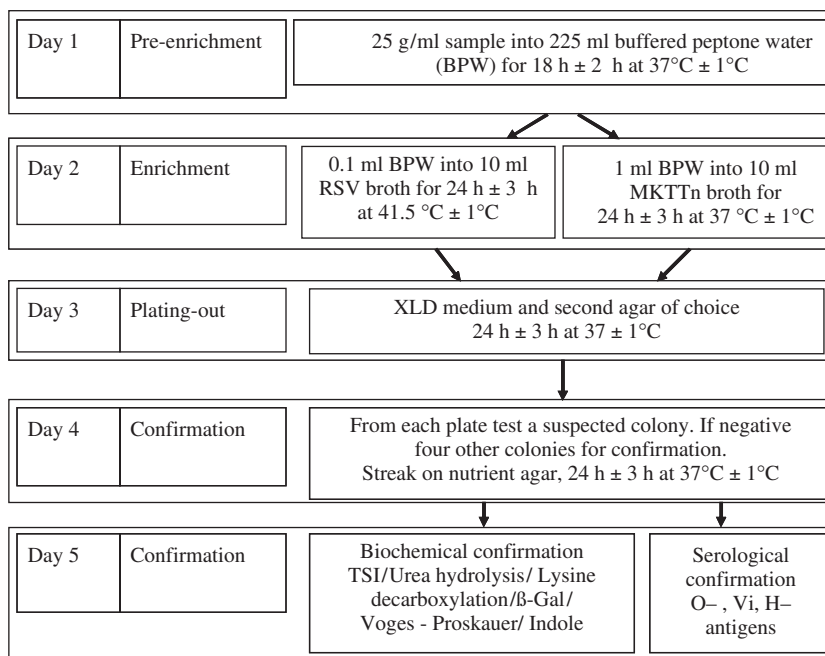| Day 1	Pre-enrichment	25 g/ml sample into 225 ml buffered peptone water (BPW) for 18 h ± 2 h at 37°C ± 1°C	
Day 2	Enrichment	0.1 ml BPW into 10 ml RSV broth for 24 h ± 3 h at 41.5 °C ± 1°C	1 ml BPW into 10 ml MKTTn broth for 24 h ± 3 h at 37 °C ± 1°C
Day 3	Plating-out	XLD medium and second agar of choice 24 h ± 3 h at 37 ± 1°C	
Day 4	Confirmation	From each plate test a suspected colony. If negative four other colonies for confirmation. Streak on nutrient agar, 24 h ± 3 h at 37°C ± 1°C	
Day 5	Confirmation	Biochemical confirmation TSI/Urea hydrolysis/ Lysine decarboxylation/ß-Gal/ Voges - Proskauer/ Indole	Serological confirmation O– , Vi, H– antigens

Figure 1   Scheme of the *Salmonella* detection according to ISO 6579:2002 (Horizontal standard for the detection of *Salmonella*).

contamination (Reij & Den Aantrekker, 2004; van Asselt & Zwietering, 2006). Other essential elements in the prophylaxis of salmonellosis are consumer education (especially the improvement of hygiene), correct storage temperatures (preventing multiplication of *Salmonella* in food), and the use of pasteurization (e.g. for milk) or sterilization whenever possible (Humphrey, 2004).

Brief review of methodologies

International Standards for the microbiological analysis of foods are vital in order to obtain reliable and comparable results. Earlier several international standards were available for different food products. Harmonization and standardization of vertical methods have been conducted in the International Organization for Standardization (ISO) working group subcommittees in cooperation with meat and dairy sectors, e.g. International Dairy Federation (Lombard, 2006). Critical steps in the detection of *Salmonella*, as in other microbiological analyses, are sampling, storage, and handling of the samples. ISO 7218:2007 standard contains information about general requirements and guidance for microbiological examinations, whereas ISO 6579:2002 (Microbiology of food and animal feeding stuffs—Horizontal method for the detection of *Salmonella* spp.) contains information about the detection of *Salmonella* spp. In addition ISO 6579:2002/Amd 1:2007 Annex D deals with

the detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. The Nordic Committee on Food Analysis (NMKL) method NMKL 71 (*Salmonella* detection in foods) has been reviewed by Nordic experts to be equivalent with ISO 6579 method.

The presence of *Salmonella* in ready-to-eat foods is considered significant regardless of the level of contamination. Therefore isolation is carried out by enrichment culture of a defined weight or volume of food (normally 25 g). The traditional detection and isolation of *Salmonella* spp. from food and feed products utilizes a multi-step protocol with nonselective pre-enrichment, followed by a selective enrichment step, isolation on selective agar media, and a preliminary biochemical and serological confirmation. Scheme of the detection according to ISO 6579:2002 is presented in Figure 1. From the pre-enrichment broth two selective enrichment broths are inoculated. The ISO 6579 method uses Rappaport Vassiliadis Soya peptone (RVS) broth, which is highly effective for the recovery of *Salmonella* from foods with a high level of background contamination. In addition, this method replaces the formerly used selenite broth with Muller–Kauffmann tetrathionate, novobiocin broth (MKTTn) for the isolation of serotypes of *Salmonella* that are inhibited by constituents by RVS broth. From the enrichment broths two selective solid isolation media are inoculated. In ISO 6579:2002 method xylose lysine deoxycholoate (XLD) agar isolation media is

specified. For the other selective media any other solid selective agar, e.g. brilliant green agar can be used. For samples where *S*. Typhi and *S*. Paratyphi are specifically sought selenite cystine (SC) broth and additional selective media are recommended (Health Protection Agency, 2007).

Several chromogenic and fluorogenic growth media have been developed in order to speed up the detection and diagnostics of *Salmonella* (Manafi, 2000; Maciorowski *et al.*, 2006; Schönenbrücher, Mallinson & Bülte, 2008). The conventional media for the detection of *Salmonella* in some cases have poor specificity and false positives (such as *Citrobacter*, *Proteus*) hinder identification of positive *Salmonella* colonies. In addition, examination of potential *Salmonella* colonies growing on conventional media is time-consuming. Chromogenic media provide a rapid, accurate means of isolating and enumerating target microbes based on the detection of specific enzymatic activities (reviewed by Manafi, 2000). Several commercially available chromogenic and fluorogenic growth media for *Salmonella* are on the market: e.g. SM-ID agar (bioMèrieux SA, Marcy l'Etoìle, France), Rambach agar (Merck KGaA, Darmstadt, Germany), MUCAP-test (Biolife, Milan, Italy), BBL^TM CHROMagar^TM *Salmonella* (BD, Franklin Lakes, NJ, USA), Rainbow Salmonella agar (Biolog, Hayward, LA, USA), Brilliance^TM salmonella agar (Oxoid, Basingstoke, UK), Chromogenic *Salmonella* esterase agar (PPR Diagnostics Ltd, London, UK), Compass *Salmonella* agar (Biokar diagnostics, Allonne, France), Harlequin *Salmonella* ABC (Lab M Ltd, Bury, UK), RAPID'Salmonella (BioRad, Hercules, CA, USA). Specificity and selectivity of the agars vary and in many cases combination of different methods is essential for the detection of *Salmonella* strains.

Currently, traditional detection methods of *Salmonella* provide information 'presence or absence of *Salmonella* in a test portion of product'. However, to identify critical contamination points and to provide quantitative data for risk analysis, cost-effective methods for the enumeration of *Salmonella* are also needed. Therefore, the ISO and European Committee for Standardization have recently decided to include enumeration of *Salmonella* in their agenda and a new ISO standard is being developed by TC34/SC9 members (Malorny *et al.*, 2008). The protocol will be based on modified semisolid Rappaport–Vasiliadis (MSRV) medium in microwell plate scale (Fravalo *et al.*, 2003).

Since traditional culture-based methods are time-consuming, labour intensive, and relatively slow for the needs of food industry several rapid methods have been developed for the faster detection of *Salmonella*. Approved rapid methods should be validated by a third party according to

EN/ISO 16140 standard and be certified according to Association Francaise de Normalisation (AFNOR), Nordic System for Validation of Alternative Microbiological Methods (NordVal), or Association of Official Analytical Chemist (AOAC). MicroVal is a European certification organization for the validation and approval of alternative methods for the microbiological analysis of food and beverages (http://www.microval.org). In several EU countries the use of rapid methods is approved as in-house control but not in the examination of samples belonging to national *Salmonella* control program. Several rapid and automated methods have been developed, validated, and are on market for the detection of *Salmonella*. Approved list of validated rapid methods can be found, e.g. from the www-pages of NordVal (http://www.nmkl.org/NordVal/METHODS.pdf), AFNOR (http://www.afnor-validation.com/afnor-validation-validated-methods/validated-methods.html), and AOAC (http://www.aoac.org/testkits/testedmethods.html#Micro biological). Table 1 summarizes examples of the rapid methods. In contrast to the conventional standard culture method, which requires 3 working days to generate a negative result and 5 working days for a confirmed positive

Table 1 Examples of rapid methods for detection and identification of *Salmonella*.

Method	Manufacturer
Immunoenzymatic tests (ELISA-based)	
Vidas *Salmonella* (SLM, easy SLM)	bioMèrieux SA
Transia system	Biocontrol Systems (Bellevue, WA, USA)
Bioline Selecta, Bioline Optima	Bioline, Vejle, Denmark
Rapidyme *Salmonella*	BIO ART SA/NV, Sint-Katelijne-Qaver, Belgium
Tecra Unique *Salmonella*	Tecra International, Frenchs Forest, NSW, Australia
Tecra Ultime *Salmonella*	
Ridascreen *Salmonella*	R-Biopharm AG, Darmstadt, Germany
Patharix, (RIMS) re-circulator immuno-magnetic-separation	Matrix MicroScience Ltd, Golden, CO, USA
PCR-based methods	
BAX Salmonella PCR (BAX System)	DuPont Qualicon
iQ-Check *Salmonella*	BioRad Laboratories
TagMan^® *Salmonella*	Applied Biosystems, Foster City, CA, USA
LightCycler *Salmonella* detection kit	Roche Diagnostics, Manheim, Germany
Foodproof *Salmonella* detection kit	Merck KGaA
Assurance CDS *Salmonella*	Biocontrol Systems
Identification and immunological tests	
VITEK^® 2 Gram-negative card	bioMèrieux SA
Oxoid *Salmonella* rapid test (ORST)	Oxoid

result, rapid methods can provide positive results in less than 24 hours.

Currently most rapid methods are based on metabolic and enzymatic properties, antibodies, nucleic acids, or filtration. The most common tools used for pathogen detection have been polymerase chain reaction (PCR) as well as immunology-based methods (Croci *et al.*, 2004; Mozola, 2006; Hagren *et al.*, 2008). Critical steps in rapid technologies are the capture of the target agent (microbial cells/or some molecules) and specific detection of the target agents (Noble & Weisberg, 2005). Immunomagnetic separation (IMS), as a pre-treatment and/or pre-concentration step, can be used to capture and extract the targeted pathogen from the bacterial suspension by means of para-magnetic beads coated with an appropriate antibody for the target microbe (Warren, Yuk & Schneider, 2006). Afterwards IMS can be combined with other detection methods, e.g. immunological techniques or real-time PCR (Hagren *et al.*, 2008). Immunological technique, e.g. enzyme-linked im-munosorbent assay (ELISA) test is a well established technique and there are several commercial validated ELISA kits for the detection of *Salmonella* (Maciorowski *et al.*, 2006) (e.g. Transia plate Salmonella Gold (Biocontrol Systems) is a sandwich type ELISA assay on a microtiter plate format). In addition, VIDAS SLM (bioMèrieux SA), an automated enzyme immunoassay for the detection of *Salmonella* in foods and agricultural products, is a widely used in-house method in the food industry. Presumptive-positive results obtained with rapid systems need to be confirmed by culture isolation and identification of viable *Salmonella* from the selective enrichment and post-enrichment cultures involved in the commercial system (McMahon, Schultz & Johnson, 2004). Warren *et al.* (2006) reported that flow-through immunocapture (FTI), using Pathatrix devices, followed by plating on XLD agar (FTI-XLD) or analysis by real-time PCR (RTPCR) (FTIPCR), resulted in the detection of *Salmonella* in food matrix within 8 h.

To speed up *Salmonella* analysis, PCR and RTPCR, have been applied at different stages of diagnostics: confirmation of suspected colonies, analysis of enrichment broths, and direct analysis of suspected foods. PCR has been standardized by ISO and development of new PCR- and RTPCR-based methods should be performed according to ISO 20838: 2006 and ISO/DIS 22119 standards. Many targeted sequences and detection protocols using commercial kits and non-patented methods have been developed for *Salmonella*, all with different specificities, sensitivities, accuracies, and detection limits (Löfström *et al.*, 2004; Hein *et al.*, 2006; Wolffs *et al.*, 2006; Malorny, Bunge & Helmuth, 2007; Malorny *et al.*, 2008). In majority of the PCR-based rapid methods PCR is performed from the pre-enrichment broth. Several automated commercial PCR-based systems, like BAX system (DuPont Qualicon, Wilmington, DE, USA), TagMan$^{®}$ *Salmonella* (Applied Biosystems, Foster City, CA, USA), or BioRad's iQCheck^{TM} *Salmonella* kit, are based on RTPCR, where specific gene(s) of *Salmonella* are amplified and detected simultaneously by an automated system. In assays an internal control is present and in each PCR reaction, validating the presence or absence of inhibitory factors, and ensuring reliability of negative results. Dunbar & Jakobson (2007) reported use of quantitative, multiplex detection of *Salmonella* and other pathogens by Luminex xMAP suspension array (Luminex, Austin, TX, USA).

PCR-based methods have been reported to detect cell concentration of $10^4 ml^{-1}$ after enrichment whereas sensitivity of the immunoassays have been reported to be 10^4–$10^5 ml^{-1}$ after enrichment. However, the background microbiota and sample matrix can have a significant effect on the sensitivity and specificity of the methods, e.g. during DNA isolation (Cheung, Kwok & Kam, 2007; D'Aoust *et al.*, 2007; Malorny *et al.*, 2008). Croci *et al.* (2004) reported that ELISA coupled with flow injection analysis (ELISA-FIA) and PCR method using ST11 and ST15 primers for detecting of *Salmonella* allowed detection of *Salmonella* from meat contaminated with a low number of microbes (1–10 colony-forming units [CFU] per 25 g) after only 5 h of incubation of pre-enrichment. Detection limits for the methods being 5×10^3 cells/g for ELISA-FIA and 10^3 cells/g for PCR method. Wolffs *et al.* (2006) combined two-step filtration and RT-PCR for the direct quantification and detection of *Salmonella* in biological samples without enrichment or DNA extraction and were able to detect levels as few as 220 CFU of *Salmonella* in 100 ml chicken rinse samples. Ultrafiltration-based techniques have been applied for water samples for the detection of low amounts of pathogens (Polaczyk *et al.*, 2008). Fluorescence *in situ* hybridization (FISH) can be combined, e.g., with epifluorescence microscopy or flow cytometry for the detection of specific pathogen (Fang *et al.*, 2003; Kutter, Hartmann & Schmid, 2006). Benefit of the traditional enrichment methods is that low numbers of *Salmonella* can be detected from large sample matrix (25 g). In addition, viable cells are obtained for biological confirmation tests and typing.

Currently PCR-based detection does not discriminate between dead and live cells. To exclude detection of non-viable organisms, DNA-based techniques may be combined with enrichment step (Juste, Thomma & Lievens, 2008). An alternative is to use certain chemicals like ethidium mono-azide (EMA) or propidium monoazide (PMA) to

differentiate between live and dead cells (Nocker & Camper 2006; Nocker, Cheung & Camper, 2006). PMA is highly specific in penetrating only into bacterial cells with compromised membrane integrity (which generally occur in dead cells) but not in cells with intact cell membranes/walls. Upon intercalation in the DNA of dead cells, the photo-inducible azide group allows PMA to be covalently cross-linked by exposure to bright light. This process renders the DNA insoluble and it is therefore removed during DNA extraction (Nocker et al., 2006). Specificity of EMA treatment is weaker as EMA is proven to penetrate living cells of some bacterial species, leading to substantial loss of DNA (Nocker et al., 2006). Hein et al. (2007) reported that EMA and PMA additions suppressed the unspecific background signal without affecting the RT-PCR reaction.

An important step in the epidemiology of *Salmonella* cases is proper identification of the isolates. *Salmonella* are serotyped based on the antigenic structure of the O-antigen (heat stable somatic antigen), the H-antigen (flagellar), and the Vi-antigen (capsule) (Ellermeier & Slauch, 2006) and designated according to the Kauffmann–White scheme (Popoff, 2001). However, *Salmonella* isolates having rough LPS or thick capsules cannot be serotyped (Hoorfar, Baggesen & Porting, 1999; Ellermeier & Slauch, 2006). Large variation in antigens can occasionally weaken sensitivity of ELISA-based detection methods (Maciorowski et al., 2006). *Salmonella* strains within a given serotype can be further differentiated using classical and molecular techniques (Ellermeier & Slauch, 2006; Malorny et al., 2007). Several phenotypic, serotypic, and molecular techniques like biotyping, phage typing, ribotyping, pyrosequencing, IS2000 typing, plasmid typing, RT-PCR targeting, e.g., at *Salmonella* spp. *inv*A gene, pulsed-field gel electrophoresis, and nucleic acid hybridization have been developed for differentiation of *Salmonella* isolates (Rodríguez-Lázaro et al., 2003; Lukinmaa et al., 2004; Ellermeier & Slauch, 2006; Hopkins, Arnold & Threlfall, 2007). Phage typing is one of the oldest and most sensitive methods used in epidemiological studies. PCR based on detection of bacteriophages has also been studied as an alternative detection method for *Salmonella* (Kuhn et al., 2002; Hagens & Loessner, 2007).

Detection of pathogens in food chain is challenging due to the high variety in the sample materials. Foods as well as feeds and environmental and faecal samples from primary production represent complex matrixes and the target microbes are often present in low numbers, which affects the accuracy of the results (Malorny et al., 2008). In addition, the intrinsic background microbiota may hinder isolation and identification of the pathogens. These issues cause problems in all types

of methodologies, in conventional culturing as well as in rapid methods. The presences of nonculturable but viable *Salmonella* cells are in addition problematic in the detection of *Salmonella*. Studies suggest that the recovery of stressed but otherwise nonculturable *Salmonella* cells can be increased by adding supplements like siderophore ferrioxamine E, antioxidant, or enterobacterial 'autoinducer' into the growth medium (Reissbrodt et al., 2002).

The presence of large number of beneficial microbes (e.g. probiotics and starters) in the product may interfere with the detection of *Salmonella* and mask the presence of contaminants (Joosten, Bidlas & Garofalo, 2006). Joosten et al. (2006) suggested that this problem can be overcome by modification of the pre-enrichment broth, i.e. by adding antimicrobial compounds to selectively suppress the growth and/or metabolic activity of the probiotic bacteria.

New, innovative methods and methodological principles

Development of new PCR and RT-PCR-based methods should be performed according to ISO 20838: 2006 and ISO/DIS 22119 standards. Future development of RNA (ribonucleic acid)-based methods can facilitate detection of viable *Salmonella* cells. In addition, developments in quantitative multiplex assays can improve detection of *Salmonella* in various applications. A method combining the specificity of nucleic acid-based tests with the rapidity, simplicity, and matrix-independent robustness of an antibody-binding assay would be a valuable tool for *Salmonella* detection. Lantz, BrehmStecher & Armstrong (2008) recently reported that combined capillary electrophoresis and DNA-FISH (CE-FISH) was a rapid promising molecular tool for molecular diagnostic of *Salmonella*. The availability of complete genome sequences has increased understanding of the evolution and ecology of *Salmonella* (McClelland et al., 2001; Baker & Dougan, 2007). The availability of genomic data will facilitate the molecular characterization and typing of isolates as well as the development of improved diagnostic tools (Baker & Dougan, 2007). Microarrays have been suggested as efficient methods to screen *Salmonella* isolates for the presence of various antimicrobial and virulence genes (Chen et al., 2005) as well for typing of *S. enterica* serovars (Scaria et al., 2008). However, at the moment these methods cannot yet replace the traditional typing methods.

Biosensors are one line of rapid methods that has been studied during recent years. Many biosensors rely on either specific antibodies or DNA probes to provide specificity (recently reviewed by Lazcka, Del Campo & Xavier

Muñoz, 2007). For example, surface plasmon resonance and magnetoelastic biosensors have been studied for the specific detection of *Salmonella* from food materials (Datta Mazumdar *et al.*, 2007; Guntupalli *et al.*, 2007). However, further studies are needed until these new technologies can function as alternative validated methods for the detection of *Salmonella*.

Legal situation and surveillance

Food products of animal origin are considered to be the major source of human *Salmonella* infections and *Salmonella* infections of production animals play an important role in public health and in food safety (Plym-Forshell & Wierup, 2006). Surveillance and monitoring of zoonotic agents should therefore cover the whole food chain from primary production to the consumption of the food. Since feed contaminated with *Salmonella* is a potential source of contamination of farm livestock, investigation of feed and feed raw materials for *Salmonella* is also essential (Plym-Forshell & Wierup, 2006). The European parliament has prepared several regulations for the control and surveillance of zoonotic agents (http://eur-lex.europa.eu/). The regulation (EC) No 2160/2003 of the European parliament and council on the control of *Salmonella* and other specific foodborne zoonotic agents aims to ensure that proper and effective measures are taken to detect and control *Salmonella* and other zoonotic agents at all relevant stages of production, processing, and distribution, particularly at the level of primary production, including in feed, in order to reduce their prevalence and risk they pose to public health. This regulation contains information, e.g., about the establishment of national control programmes and control methods.

In the European Union, the Zoonoses Directive 92/117/EC requires collection of information on zoonosis and zoonotic agents in humans, animals, foods, and feeds as well as monitoring of, e.g., breeding flocks for *Salmonella* (EFSA, 2007). EFSA recently reported that *Salmonella* infection in slaughter pigs has the potential to translate into *Salmonella* contamination of pig meat and ultimately lead to human disease (EFSA, 2008).

Community Reference Laboratory for *Salmonella* (CRL-*Salmonella*) conducted on behalf of the European Commission is situated in the Netherlands. It was established in 1992 according to the EU Directive 92/117/EC (http://www.rivm.nl/crlsalmonella). The main tasks of the CRLSalmonella are to harmonize methods for the detection and typing of *Salmonella* and to evaluate the performance of the National Reference Laboratories (NRLs). This is achieved, e.g., by informing of NRLs and organization of annual interlaboratory comparison studies on the bacteriological detection of *Salmonella* in the presence of competitive microbes (Korver *et al.*, 2003; Berk *et al.*, 2007). According to Council Directive 92/117/EC 882/2004, each EU Member State shall designate approved NRLs for the zoonoses and zoonotic agents.

Due to increasing global trade and travelling the global surveillance of *Salmonella* is crucial. Hence, several international surveillance networks for *Salmonella* monitoring have been established. A global *Salmonella* surveillance and laboratory support project of the World Health Organization (Global SalmSurv) project maintains a database where national institutions report data on *Salmonella* serotypes isolated from human and non-human sources (WHO Global Salm-Surv Country Databank, http://www.who.int/salmsurv/activities/en/). Besides offering information about the global perspective of *Salmonella* epidemiology WHO Global Salm-Surv also conducts training.

Enter-net, funded by European Union, is an international surveillance network for human gastrointestinal infections serving both the EU countries and several countries outside the EU (http://www.hpa.org.uk/hpa/inter/enter-net_menu.htm). PulseNet-Europe is a multi-disciplinary network of food, public health, and veterinary laboratories dedicated to the molecular surveillance of food-borne infections, e.g. *Salmonella* (http://www.pulsenet-europe.org). Spreading of excellence and improvement of the understanding, prevention, and control of zoonotic diseases in EU is also done through large research projects like, Med-Vet-Net network of excellence (http://www.medvetnet.org/pdf/Reports/AnnualReportYr2.pdf).

The Rapid Alert System for Food and Feed (RASFF, legal basis regulation (EC) No. 178/2002) has been in place since 1979 (http://ec.europa.eu/food/food/rapidalert/index_en.htm). The network involves Member States, the Commission and the EFSA. Also Norway, Liechtenstein, and Iceland are longstanding members of the RASFF. Whenever a member of the network has any information related to existence of a serious risk to human health, the information is immediately notified to the Commission under the RASFF and the Commission immediately transmits this information to the members of the network.

Conclusions and future perspectives

As food trade is becoming more global and consumers prefer more fresh produce and uncooked ready-to-eat foods, the microbiological risks of imported foods have increased.

The volume of global trade is increasing and food and feed stuffs are moving faster from one country to another. In addition, people are travelling more and further away than before. Hence, collaboration between various global networks/programmes needs to be reinforced. There is a need for the harmonization of methods, training, and organization of inter-laboratory comparison studies as well as for on-line reporting of new zoonotic cases. Since the globalization of trade means that food/feed presenting a risk to human health may have a worldwide distribution, a project of a worldwide RASFF has been included in the Commission's financial perspectives for 2006-2013 (RASFF, 2006). Currently ISO standardization group is discussing about semi-quantitative detection of *Salmonella* (Malorny *et al.*, 2008). The semi-quantitative data obtained can be used in quantitative microbial risk assessment of *Salmonella* (Malorny *et al.*, 2008).

The fast adaptation ability of *Salmonella* enhances their survival in various processes and environments. Besides the development of efficient, reliable, fast, and cost-effective detection methods efforts should also be invested in *Salmonella* prevention. For example, efficient *Salmonella* control programs in broiler production have been established in Finland and Sweden for decades (Kangas *et al.*, 2007). This includes all the steps in the 'from farm to fork' chain and has proven to be an effective way in the prophylaxis of *Salmonella*. Competitive exclusion used in the poultry industry is an additional way to control *Salmonella* and has been applied successfully in Finland and Sweden for many years (Schneitz *et al.*, 1992; Schneitz & Renney, 2003). In addition, vaccination has been used efficiently to restrict *Salmonella* among poultry (van Immerseel *et al.*, 2005). Resources should also be allocated to consumer education and information about proper handling of raw food materials in order to prevent cross-contamination.

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