Detecting non-typhoid *Salmonella* in humans by ELISAs: a literature review

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Non-typhoid salmonellosis is one of the most common causes of foodborne illness throughout the world. Serological methods for the diagnosis of *Salmonella* infections vary widely and the most commonly used test is limited by high running costs as well as low sensitivity and specificity. Fast and reliable immunoassays which detect subunit antigens for *Salmonella enterica* subsp. *enterica* serovar Typhi are commercially available but at present there is no international consensus on similar tests for non-typhoid salmonellosis. In contrast to the veterinary and food sectors, most immunoassays for non-typhoid human *Salmonella* diagnosis are developed in-house and used in-house for research or surveillance purposes, rather than for routine diagnostics. Considering the current burden of disease, the development of a validated and standardized, commercially available antibody assay for diagnosing non-typhoid human salmonellosis could be of great benefit for diagnostic and surveillance purposes throughout the world.

Introduction

Infections with *Salmonella enterica* are one of the most important sources of human gastroenteritis throughout the world. A distinction is made between typhoid and non-typhoid salmonellosis, where the former can produce a serious and potentially fatal systemic illness known as typhoid fever. Typhoid fever is caused by *S. enterica* subsp. *enterica* serovar Typhi (S. Typhi) and S. Paratyphi A, B and C and is transmitted between humans through contaminated water or food. In contrast, non-typhoid salmonellosis is due to infection by a large variety of different zoonotic serovars and is primarily (though not exclusively) transmitted by ingestion of contaminated food. The predominant clinical manifestation is acute self-limiting gastroenteritis, but serious post-infection complications such as septicaemia, reactive arthritis or aortic aneurysms may be observed (Locht *et al.*, 2002; Ternhag *et al.*, 2008; Townes, 2010). In Europe, the serovars Enteritidis and Typhimurium are the second and third most common causes of bacterial gastroenteritis, with *Campylobacter* species being the most common (EFSA, 2010).

The gold standard for diagnosing a *Salmonella* infection is bacteriological culture, usually from faeces. However, viable bacteria may sometimes be present for a few days only or too much time may have passed since infection to use detection by PCR. In such cases, serological antibody assays are usually applied to provide evidence of infection; for instance when complications are suspected. In areas with a high incidence of typhoid fever, fast and reliable serological assays are highly desirable as facilities for bacterial culture are often not available (Narayanappa *et al.*, 2010). Serological diagnosis of *Salmonella* infections has classically been performed using the Widal tube agglutination test, which has formed the basis of typhoid serodiagnosis for more than a century (Widal, 1896). Today, however, it is well known that the diagnostic value of the Widal test, although specific, is limited by poor sensitivity, an inability to discriminate between different antibody classes and cross-reactivity with other *Salmonella* species (Sack & Sack, 1992). Furthermore, it is time-consuming and thus expensive in practice. In a study of sera from patients with culture-confirmed *Salmonella* infections, the sensitivity of the Widal test in detecting S. Enteritidis and S. Typhimurium infection was only 44% and 8%, respectively (Strid *et al.*, 2007).

At present, the beneficial impact of ELISA on health-care systems in general is considered to be almost unsurpassable. Lequin (2005) estimated that during 2001–2005, almost 50 000 articles quoting ELISA (and/or EIA, enzyme immunoassay) as a keyword were published. Considering the need for rapid screening of patients with suspected typhoid salmonellosis, the ELISA technique was an optimal candidate for serological diagnosis of *Salmonella* infections and early attempts to use the outer-membrane LPS in ELISAs showed promising results for detecting antibodies in...
Table 1. Non-typhoid *Salmonella* antibody assays identified from a literature search

NR, Not reported; NA, not applicable; S. Ent, *Salmonella* Enteritidis; S. Tm, *Salmonella* Typhimurium; ETEC, enterotoxigenic *E. coli*; ReA, reactive arthritis.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Antigen</th>
<th>Study population</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Svenungsson <em>et al.</em> (1979)</td>
<td>LPS from S. Ent, S. Tm</td>
<td>Cases: 26 patients with stool culture-confirmed <em>S. Ent</em> infection, part of an outbreak Controls: 45 patients with other bacterial infections, 38 healthy blood donors</td>
<td>92 %</td>
<td>NR</td>
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<tr>
<td>Hirschl <em>et al.</em> (1983)</td>
<td>LPS from S. Typhi, S. Tm</td>
<td>Cases: 50 patients with culture-confirmed <em>Salmonella</em> (21 S. Typhi, 10 other group D serotypes, 19 group B serotypes) Controls: 44 control sera</td>
<td>100 %</td>
<td>90 %</td>
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<tr>
<td>Isomäki <em>et al.</em> (1989)</td>
<td>Commercially available LPS of S. Tm and S. Ent (Sigma), used separately or pooled</td>
<td>Cases: 130 patients with stool culture-confirmed <em>Salmonella</em> infection (20 S. Ent, 88 S. Tm, 22 other serotypes) Controls: 100 healthy blood donors, 46 patients infected with <em>Yersinia enterocolitica</em>, <em>Shigella</em> spp., <em>Campylobacter jejuni</em>, or <em>Chlamydia trachomatis</em>, 19 patients with viral infections</td>
<td>S. Tm 92 %</td>
<td>S. Ent 85 %</td>
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<td>Jertborn <em>et al.</em> (1990)</td>
<td>LPS from a group B and a group C S. Newport strain, used separately</td>
<td>Cases: 9 patients with stool culture-confirmed <em>Salmonella</em> infection of group B and C serovars (not specified) Controls: 64 patients with negative stool cultures, 3 with ETEC infections and 4 with <em>Campylobacter jejuni</em></td>
<td>78 %</td>
<td>94 %</td>
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<td>Thomson <em>et al.</em> (1994)</td>
<td>LPS from the S. Ent strain that caused the outbreak (only IgA was measured)</td>
<td>Cases: 29 patients with a history of acute gastroenteritis (including 11 with ReA), part of an outbreak of S. Ent Controls: 18 patients with rheumatoid arthritis, 15 other patients</td>
<td>NR</td>
<td>Antibody titres higher in ReA (vs non-ReA) patients and in outbreak cases (vs controls)</td>
</tr>
<tr>
<td>Dalby <em>et al.</em> (2005)</td>
<td>Commercially available LPS of S. Tm and S. Ent (Sigma-Aldrich) used separately</td>
<td>Cases: 303 patients with stool culture-confirmed <em>Salmonella</em> infection (153 S. Ent, 150 S. Tm) Controls: 170 healthy blood donors, 109 patients with other gastroenteric infections</td>
<td>S. Tm 77 %</td>
<td>S. Ent 70 %</td>
</tr>
<tr>
<td>Seuri <em>et al.</em> (2005)</td>
<td>Same as Isomäki <em>et al.</em> (1989)</td>
<td>Cases: 79 wastewater treatment plant workers with chronic abdominal complaints and negative <em>Salmonella</em> stool cultures Controls: 79 healthy blood donors</td>
<td>NA</td>
<td>Antibody titres in cases lower than in controls</td>
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human sera (Svenungsson et al., 1979). As an alternative to LPS, flagellar Salmonella antigens have also been used, but these tests had much lower sensitivity and specificity as well as higher cross-reactivity than the LPS-based assay (Dalby et al., 2005). Follow-up studies of salmonellosis patients have demonstrated the persistence of LPS-specific IgG for up to 12 months after infection, while IgM and IgA disappear after 2–4 months in most patients (Isomäki et al., 1989; Strid et al., 2007). Thus it has been argued that an LPS-based ELISA which detects IgG, IgM and IgA responses provides a highly specific, sensitive, fast, easy and reliable assay for routine analyses of human sera (Strid et al., 2007).

At present, a large number of commercially available Salmonella-specific ELISAs are routinely used in the veterinary and food sectors across Europe and in the States. The veterinary tests are not used for diagnosis of infection in individual animals but rather applied as tools in control and surveillance programmes (Wegener et al., 2003). Common for all these tests is that they cannot be directly applied to human samples. In South-East Asia, several assays exist for fast diagnosis of typhoid salmonellosis; however, these are primarily specific to S. Typhi and will not detect zoonotic Salmonella species. Considering the new developments in the field, the potential for using ELISAs to diagnose human infection with non-typhoid Salmonella is promising, but knowledge about the current availability and application of such tests is scarce.

In this paper, we applied a broad literature search to identify existing ELISAs for non-typhoid human salmonellosis with the aim of reviewing these tests and identifying possible candidates and methods that can be used at a broad international level. We choose to focus on non-typhoid Salmonella serotypes as these are responsible for the highest morbidity and mortality, with an estimated annual 200 million–1.3 billion cases and 3 million deaths compared to 16–20 million cases and 200 000 deaths for typhoid fever (Boyle et al., 2007; Coburn et al., 2007; Sethuraman & Kamat, 2007).

**Table 1. cont.**

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<tr>
<td>Strid et al. (2007)</td>
<td>Same as Dalby et al. (2005) Used separately or pooled</td>
<td>Cases: 303 patients with stool culture-confirmed Salmonella infection (same as Dalby et al., 2005) Controls: 164 healthy blood donors, 132 patients with other gastroenteric infections (33 each Escherichia coli, Yersinia enterocolitica, Campylobacter spp., Helicobacter pylori)</td>
<td>S. Tm 89 %</td>
<td>S. Tm 94 %</td>
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**Documented use of ELISAs to detect Salmonella antibodies in human sera**

In order to identify all possible variants of *Salmonella* ELISAs, a literature search was performed in the Scopus database (http://www.info.sciverse.com/scopus/), which contains MEDLINE and other literature sources. A search was performed in the ’title’, ’abstract’ or ’keywords’ using different combinations of the words serolog*, antibod*, salmonella, infectio*, patient*.

The resulting abstracts were manually screened for relevance and selected for inclusion in this review using the following criteria: non-typhoid *Salmonella* species only and relevance to analysis of human sera.

The literature search resulted in 206 hits of which eight publications (4 %) were relevant for the current review (Table 1).

All reported assays used LPS from a single *Salmonella* serovar (mostly *S. Enteritidis* or *S. Typhimurium*) separately, while Isomäki et al. (1989), Seuri et al. (2005), Dalby et al. (2005) and Strid et al. (2007) also report results for a mixture of *S. Enteritidis* and *S. Typhimurium* LPS. Six studies reported the assay sensitivity (the proportion of positives correctly identified as positive) while four indicated specificity (the proportion of negatives correctly identified as negative); sensitivity ranged from 78 to 100 % and specificity ranged from 90 to 97 %. All studies applied ELISA to culture-confirmed *Salmonella* cases as well as to a group of controls (either healthy or with another infection/illness). The total number of patients and controls examined ranged from 62 (Thomson et al., 1994) to 599 (Strid et al., 2007), with five out of eight studies having a study population of fewer than 200 persons. The largest study (Strid et al., 2007) examined 303 patients, 164 healthy controls and 132 patients with other gastroenteric infections. In two cases (Svenungsson et al., 1979; Thomson et al., 1994), the assay was used for outbreak investigation purposes.

Cross-reactivity with *Escherichia coli*, *Yersinia enterocolitica*, *Campylobacter* species and *Helicobacter pylori*, which cause...
similar gastrointestinal symptoms to those of non-typhoid Salmonella, was investigated by Isomäki et al. (1989), Dalby et al. (2005) and Strid et al. (2007) and 51%, 59% and 15–17% of samples, respectively, were found to cross-react.

A combination of the literature search and a broad internet search (www.google.com) using the words ‘salmonella ELISA antibody assay’ identified only one commercially available antibody assay for detection of non-typhoid Salmonella in human sera. The ‘IMTEC-Salmonella-Antibodies Screen (IgG/IgA/IgM)’ and the ‘IMTEC-Salmonella-Antibodies IgA’ (Human-IMTEC) are two versions of an ELISA based on pooled antigens from S. Enteritidis and S. Typhimurium, primarily using high IgA titres as a marker for the diagnosis of reactive arthritis. The test is based on similar principles as those employed by Isomäki et al. (1989) and Strid et al. (2007) and, apart from IgA, also allows detection of IgG and IgM. The sensitivity of the test is reported in human sera. Encouragingly, four articles use the same commercially available LPS antigens of S. Typhimurium and S. Enteritidis, two of which were undertaken in larger study populations and with good results (Isomäki et al., 1989; Strid et al., 2007). Following the results presented above, we suggest the future use of a pooled ELISA containing a mixture of LPS from S. Enteritidis (serogroup D) and S. Typhimurium (serogroup B). The choice of LPS, however, depends entirely on the circulation of serovars in the human population. An ELISA based on the LPS listed above would cover 70–90% of all reported Salmonella isolates in the European Union Member States. A test designed for use in the United States would also need to contain a mixture of S. Enteritidis and S. Typhimurium in addition to LPS from S. Newport (group C), which is responsible for almost 10% of reported Salmonella cases (Hendriksen et al., 2011). S. Heidelberg (group B), the fourth most common type in the USA, will be captured by the S. Typhimurium (also group B) antigens. In order to be used commercially, the ELISA must be validated on a suitable number of known positive sera and a group of controls, both negative and with other known infections, such as Y. enterocolitica and H. pylori, to test for cross-reactivity. We suggest at least 100 Salmonella patients, 100 negative controls and 100 patients with other infections. With respect to performance, the required sensitivity and specificity will depend on the chosen cut-off as well as the purpose of the test. For diagnosis of reactive arthritis, we recommend a high specificity (at least 95%), while for general diagnostic and research purposes (such as seroepidemiology), a sensitivity and specificity of 85% will be sufficient. The cut-off value for a positive result can be determined by various methods from a fixed percentage of the OD and simple histograms to more elaborate statistical methods (Baum et al., 2005). In two commercially available ELISAs for detection of S. Typhi antibodies (BioQuant and the Genway Salmonella Typhi IgM and IgG ELISAs), the cut-off is the product of a ‘calibrator factor’ and the calibrator OD while the cut-off for the IMTEC ELISA is 10% standard OD. A preliminary suggested cut-off for a standardized non-typhoid Salmonella ELISA could also be 10% OD, given that this cut-off is suitable for the standards and samples used.

Developing a standardized assay for serodiagnosis of non-typhoid Salmonella in humans

The literature search undertaken in this study identified relatively few articles describing the use of ELISAs to detect non-typhoid Salmonella antibodies in human serum samples. Out of 206 abstracts manually screened for relevance, only eight described such assays.

All eight studies reviewed for this purpose used S. Enteritidis and/or S. Typhimurium LPS antigens for in-house ELISAs, four of which were based on the same commercially available LPS antigens (Isomäki et al., 1989; Dalby et al., 2005; Seuri et al., 2005; Strid et al., 2007). The two largest studies (Isomäki et al., 1989; Strid et al., 2007) showed similar results with respect to sensitivity and specificity, and in both studies, cut-off for seropositivity was defined as the geometric mean optical density (OD) value +2 standard deviations in a control group of healthy blood donors. Cross-reactivity with antibodies against other enteric pathogens, especially Y. enterocolitica and H. pylori (Dalby et al., 2005; Strid et al., 2007), was observed. Comparison of these results with those from the other studies identified in the literature search was difficult due to a variety of reasons such as lack of details for ELISA results, no information about cut-off values, no reporting of sensitivity and/or specificity (e.g. Seuri et al., 2005) and different definitions of seropositivity (e.g. Svenungsson et al., 1979).

Based on the eight articles in this review, it seems likely that an LPS-based ELISA can be a suitable candidate for a standardized, commercially available method of detecting Salmonella antibodies in human sera. Encouragingly, four articles use the same commercially available LPS antigens of S. Typhimurium and S. Enteritidis, two of which were

Present and future status for standardized Salmonella ELISAs

Standardized ELISAs are today not widely used in Europe for diagnosis of non-typhoid human salmonellosis and identification of its sequelae. In our search of both general internet sources as well as the worldwide scientific literature, we found evidence of only one commercially available kit; namely the IMTEC anti-Salmonella ELISA as described above. Contrastingly, at least four standardized antibody assays exist for the diagnosis of typhoid salmonellosis. Although not as prevalent as other forms of salmonellosis, typhoid fever is a serious problem in many areas of the developing world (Narayanappa et al., 2010; Naheed et al., 2008) and its relatively high mortality rate makes rapid and accurate diagnosis crucial. Particularly for the developing world (as well as in veterinary and food testing settings), the
use of lateral flow rapid tests (LFRTs) or dipsticks has been proposed. These LFRTs are very rapid, simple to use and performance results are similar to those of ELISAs; however, they are expensive to purchase and distribute and still require sample handling. In clinical settings, methods such as PCR, microarrays and loop-mediated isothermal amplification (LAMP) are increasingly being used for diagnosis of *Salmonella* infections. Both PCR and LAMP are rapid as well as highly specific and sensitive. However, several pre-assay steps (DNA extraction) are often needed, the sample purity requirements are considerable and amplification and analysis are undertaken using expensive, specialized equipment, making the cost of diagnosis high. Additionally, these analyses are not quantitative and they are only reliable during the acute phase of disease, meaning that they cannot be used to identify the cause of post-infection complications. The bacterium must be present in order for these tests to have any diagnostic value. In comparison, although less rapid and slightly less sensitive, ELISAs are quantitative, samples do not need pre-assay handling, sample purity is not required and the overall running costs are lower. Furthermore, the results of an ELISA can also be easily interpreted using simple relation to a standard curve. ELISAs can confirm infection in all phases of the disease and will additionally identify carriage of the bacterium in asymptomatic humans, particularly relevant for research purposes. In settings with focus on reliability and affordability, it is therefore unlikely that the newly developed molecular methods can be as applicable as ELISAs.

In the veterinary sector, a range of commercial ELISAs are available for monitoring the infection status of pig herds and poultry flocks as well as testing meat and foods for the presence of *Salmonella* species (e.g. Vico *et al.*, 2010; Szabó *et al.*, 2008). Standard methods such as the International Organization for Standardization Method 6579 (ISO, 2002) in Europe and the United States Food and Drug Administration’s method (FDA, 2007), both culture-based, are expensive and time-consuming and are now gradually being replaced by ELISA techniques and other relevant methods (Perelle *et al.*, 2004). Not surprisingly, this has created a large demand for the development of commercially available tests for the veterinary and food sectors.

On a worldwide basis, ELISAs are probably at present among some of the most reliable, accurate and cheapest methods of serodiagnosis for infectious diseases. Then why has a standardized test for identifying *Salmonella* antibodies in human sera not yet been developed? In our opinion, the development of such a test has been primarily hindered by practical, rather than technical, problems. As already mentioned, it is technically possible to create standardized ELISAs for animal sera, tissues and food as well as human sera. However, most laboratories are currently using (and have been for some time) their own in-house tests with acceptable success rates and therefore do not see a need to purchase a standardized one. Additionally, some institutions may even consider the ELISA an old-fashioned test, in comparison to PCR. The development of a standardized ELISA will require significant ground-work and an extensive production of new standards and controls that is logistically challenging and most likely not a priority for many countries which consider *Salmonella* a minor problem. However, given the fact that ELISAs have obvious advantages over

Fig. 1. (a) Burden of illness pyramid illustrating the chain of events in foodborne disease reporting and the imbalance between actual cases and number of cases reported. (b) Example for gastrointestinal illness in England in 1999 (number of persons); numbers are from Wheeler *et al.* (1999).
other serological methods and that standardized tests have already been successfully developed, these practical issues should be considered of minor importance.

By reaching an international consensus to develop a commercial ELISA kit for non-typhoid human *Salmonella* detection and using this test in routine diagnostics, we will allow confirmation of infection in slightly symptomatic and asymptomatic persons as well as in those with a non-culturable faecal sample. From a broader perspective, widespread use of ELISAs for diagnosing bacterial infections can also create a more realistic estimate of disease burden as they can detect infection in patients who would otherwise not have been diagnosed. Current figures of *Salmonella* incidence represent a very small fraction of total cases as they rely heavily on the health-seeking behaviour of patients as well as the tendency of physicians to request a stool culture (Fig. 1). Simonsen et al. (2008) presented a population-based method to estimate a more accurate incidence, using *Salmonella* antibody titres in 4691 sera from Danish general population surveys. Their model accurately predicted the increasing trend of culture-confirmed cases in the 1980s and showed that the true incidence in 1 year could have been almost 600 times higher than reported. Such an approach demonstrates the promise for using serological results to make disease burden estimates and ultimately compare the sensitivity of different national surveillance systems as well as evaluate the impact of food safety programmes on the incidence of human infections.

**Concluding remarks**

As shown in this review, the trend for using antibody assays to detect *Salmonella* in the veterinary and food sectors has not extended to diagnostics of non-typhoid human salmonellosis. Four studies reviewed for this article used commercially available antigens but developed their own in-house ELISA for the study purpose. The remaining studies also used in-house ELISAs. It is evident from this literature search that no international consensus for an established ELISA method, e.g. guidelines on the choice of antigens, cut-off values for antibody titres and overall definition of seropositivity, exists, which may explain the lack of a ‘universal’ non-typhoid *Salmonella* ELISA. In 2008, a total of 131 468 cases of human salmonellosis were reported from the European Union, making it the second most commonly reported zoonotic disease in humans, only surpassed by *Campylobacter* (EFSA, 2010). Considering the burden of this disease, and the likelihood that many patient samples may not be culturable, the development of a commercial antibody assay to detect *Salmonella* in human sera is now very timely.

Evidence from typhoid fever diagnosis and the veterinary sector has shown that it is feasible to develop a rapid, cheap and potentially accurate commercial antibody assay for *Salmonella*. We suggest that now is the time to take the next step and reach an international consensus for the development of an ELISA kit to diagnose non-typhoid human *Salmonella* infections.

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