Practical considerations of surveillance of *Salmonella* serovars other than Enteritidis and Typhimurium

J.A. Wagenaar (1, 2)*, R.S. Hendriksen (3) & J. Carrique-Mas (4)

(1) Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.165, 3508 TD Utrecht, the Netherlands
(2) Central Veterinary Institute of Wageningen UR, Lelystad, the Netherlands
(3) World Health Organization (WHO) Collaborating Centre for Antimicrobial Resistance in Food-Borne Pathogens and European Union Reference Laboratory for Antimicrobial Resistance, National Food Institute, Technical University of Denmark
(4) Centre for Tropical Medicine, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

*Corresponding author: j.wagenaar@uu.nl

Summary

Non-typhoid *Salmonella* serovars other than *Salmonella enterica* serovars *S.* Enteritidis (SE) and *S.* Typhimurium (ST) are isolated throughout the world with huge variations in prevalence. Besides the more generally occurring serovars, such as *S.* Infantis and *S.* Hadar, there are many examples of serovars that are principally reported from the regions and are most probably associated with local reservoirs. In most countries of the world, no formal surveillance systems for human salmonellosis are in place and data are limited to *ad hoc* studies. Data on animals, food and animal feed are even more scarce. The identification of non-SE/ST serovars may be hampered by a lack of experience in serotyping and the availability of quality-assured antisera. Subtyping *Salmonella* remains important to identify sources of human infections and to target interventions and control measurements. However, in the future, there will be an increasing use of culture-independent diagnostic assays, with the consequence that epidemiological subtyping and antimicrobial susceptibility data will no longer be generated. The validation of these assays for all serovars, particularly the rare ones, needs attention. Although current subtyping based on the Kauffmann-White scheme is well established, and has been shown to be robust, a new generation of subtyping methods will replace it in the near future.

Keywords


Introduction

Non-typhoid *Salmonella* is the most frequently recognised foodborne bacterial gastrointestinal illness in the United States (USA) (59) and, after *Campylobacter*, the second most frequently reported zoonotic bacterial agent in humans in Europe (41). Integrated surveillance programmes, with the systematic collection, analysis and reporting of national data from animals, food and humans, are expensive and therefore only implemented in developed countries, such as the USA, Canada, Australia, New Zealand and European countries. Examples are the fully integrated monitoring and reporting programmes in the European Union (EU) and Canada (4, 54). As a result of their high cost, surveillance programmes on non-typhoidal salmonellosis are lacking in most developing countries, with a few exceptions (68). In some developing countries, data are collected on *Salmonella enterica* serovars Typhi and Paratyphi; however, these efforts are primarily aimed at diagnostic confirmation and
determination of antimicrobial susceptibility for patient treatment (5). Most developing countries do not collect data on the prevalence and distribution of non-S. Enteritidis (SE) and S. Typhimurium (ST) serovars in food, animals and animal feed.

Although there are serious limitations on the availability of data, the total number of cases of non-typhoidal salmonellosis globally is estimated to be approximately 93.8 million (ranging from 61.8 to 131.6 million), of which an estimated 85.6% are foodborne (45).

This review focuses on the presence and relevance of serovars other than SE and ST in animal production, and the influence of isolation methods, serotyping and reporting of non-SE/ST serovars in comparison with those of SE/ST. The paper also discusses the emergence of five specific non-SE/ST serovars in humans in the EU, with importance for public health.

**Sampling and isolation: will one method fit them all?**

Sampling along the food chain highlights a great variation in serovars, depending on the geographical location, type and source of the sample. In addition, since the presence of *Salmonella* is highly dynamic, there may be important seasonal and spatial variations in the frequency of the serovars. Certain serovars capable of causing disease in animals (i.e. S. Gallinarum, S. Dublin) are rarely isolated from food and human samples. *Salmonella* in food of animal origin typically reflects the presence of the microorganism in healthy, colonised animals (e.g. S. Paratyphi B var. Java). As the susceptibility of humans to developing clinical disease is serovar dependent (39), and exposure levels to food items may differ, serovars in human blood or stools do not necessarily mirror their prevalence in a particular type of animal or food. An example is the prevalence of S. Paratyphi B var. Java in the Netherlands. In 2009, 56% and 70% of the *Salmonella* isolates from broilers and broiler meat at retail, respectively, belonged to this serotype. However, in humans, it represents only 0.7% of all *Salmonella* cases (56).

There are international standards for the isolation of non-typhoid *Salmonella* from food, animal feedstuffs, animal faeces and environmental samples, such as the ISO standards (ISO 6579:2002/Am1:2007 [Annex D] and ISO 6887-6, ISO 13307) (34, 35, 36). Protocols provided by such organisations as the World Organisation for Animal Health (OIE) and the World Health Organization (WHO) are based on the ISO standards (21, 67). These protocols are evaluated for the isolation of the most frequently isolated serovars from the matrices mentioned above but it is impossible to perform the evaluation for all serovars. As indicated in these protocols, some isolation techniques are not suitable for the isolation of non-motile *Salmonella* serovars (e.g. S. Gallinarum). However, there might be differences in their performance for other serovars as well. For that reason, a certain degree of flexibility is allowed when choosing the selective media in order to target the most prevalent serovars in the source/reservoir. In the absence of data about the sensitivity of various isolation techniques for specific serovars, the ISO method is considered as the best ‘fit’ for all. When isolating *Salmonella* from human faeces samples, protocols that have been specifically developed for isolation from clinical samples are used (21). Once a suspect *Salmonella* organism has been isolated, it should be confirmed by phenotypic or genotypic methods.

**Sero**typing for epidemiology

Studies on the epidemiology of *Salmonella* are carried out worldwide, using the internationally accepted Kauffmann-White serotyping scheme (24). Since its first publication in 1934, when 44 serovars were identified (57), it has become the standard procedure for *Salmonella* serotyping. Based on agglutinating reaction with (absorbed) antiserum specific for O (somatic) and H (flagella) antigens, about 2,500 serovars have been identified, belonging to two species, *S. enterica* and *S. bongori* (24). Reporting of the different *Salmonella* serovars differs greatly from country to country and region to region (26, 30). *Salmonella* Enteritidis and *S. Typhimurium* are globally distributed and are responsible for the largest fraction of human cases of salmonellosis by far (30). Other serovars also have a global occurrence but are less prevalent compared to SE and ST (e.g. S.Infantis), and there are some serovars that are extremely rare. About 30 serovars may account for more than 90% of *Salmonella* isolates in a given country (24).

Some serovars have a very strong host preference, and typically cause disease in a limited number of host species (63). Examples of these host-restricted serovars are *S. Typhi* and *S. Paratyphi* (humans), *S. Gallinarum* (poultry), *S. Abortusequi* (horses), *S. Typhimurium* (swine), and *S. Abortusovis* (sheep). A few host-adapted serovars which are normally found in one animal species are capable of occasionally infecting a limited number of other hosts, e.g. *S. Choleraesuis* (from swine to humans) and *S. Dublin* (from cattle to humans). Most serovars are able to cross the species barrier and have zoonotic potential. The capacity of a serovar to cause detectable clinical illness is dependent on the exposure dose and immune status of the host. Even the host-specific *S. Gallinarum* was able to cause illness in humans in experimental infections when using a high dose (44). The importance of the immune status of the host has been shown in Africa, where the epidemiology
of salmonellosis in humans is strongly influenced by the presence of malaria, human immunodeficiency virus (HIV) and malnutrition in the population (9).

The animal population serves as a large reservoir of Salmonella for humans, with many animal species being asymptomatic carriers of Salmonella. Salmonella can be transferred to humans through direct contact, through contaminated food of animal origin (meat, eggs, milk) or through contaminated vegetables or water. Companion animals or pets, including mammals, birds, amphibians and reptiles, can also serve as the source for human Salmonella infections (32). Reptiles are a particularly common source of human infections (19, 51). Microbiological source-attribution models for human Salmonella infections require subtyping, using methods such as serotyping, antimicrobial susceptibility testing, phage typing, pulsed-field gel electrophoresis and/or multiple loci variable number of tandem repeats (VNTR) analysis. Based on the subtyping data, the distribution of Salmonella subtypes in potential sources (animals, food) is compared with the presence of subtypes in humans, linking human infections to the potentially responsible source of infection (52).

The harmonised serotyping system has clearly been crucial in establishing the epidemiology of Salmonella at all levels. Ideally, a large proportion, if not all, of Salmonella isolates from humans, animals and food should be subtyped, to support epidemiological insight and to target interventions to prevent infections from identified sources.

Limitations in serotyping Salmonella

To perform serotyping, antisera should be available. Commercial (quality-assured) antisera are expensive. Thus, countries with limited resources have severe constraints in performing serotyping. In some countries, even the national reference laboratories do not have reference sera available, or are only able to serotype to group level (e.g. group O:4 or O:9). The quality of the serotyping data strongly depends on the quality of the antisera. Cross-reactions of antisera leading to errors are a common problem when the sera are not well absorbed. The WHO Collaborating Centre for Salmonella (Pasteur Institute, Paris) has made a protocol available for producing antisera and performing quality assurance. An external quality assurance system (EQAS) for serotyping Salmonella has been conducted by the WHO Global Foodborne Network (WHO–GFN, see Box 1) since 2000. This system shows that, although most laboratories throughout the world are capable of correctly serotyping Salmonella species, there is a continuing need to train people and inform them about the availability of high-quality antisera (28).

Box 1
An integrated approach to food safety and zoonoses: the Global Foodborne Infections Network

A 1997 survey of national reference laboratories showed that only 69 (66%) of 104 responding countries conducted routine Salmonella serotyping for public health surveillance (31). This study, which showed the lack of basic infrastructure for laboratory-based Salmonella surveillance, prompted the establishment of the WHO Global Salm-Surv (GSS), now called the Global Foodborne Infections Network (GFN). This is a network of institutions and individuals committed to enhancing the capacity of countries to detect, respond to and prevent foodborne and other enteric infections. In brief, WHO-GFN is a capacity-building programme that promotes integrated, laboratory-based surveillance and fosters intersectoral collaboration among human health, veterinary and food-related disciplines through training courses and activities around the world. Key activities include training courses, country-specific projects, the Country Databank and an external quality assurance system for serotyping Salmonella (EQAS). Membership is free (www.who.int/gfn/activities/en/).

Distribution of Salmonella serovars around the world

Due to limitations in clinical detection, diagnostic capacity and reporting, data from developing countries are generally scarce and difficult to find. Most data on serovar distribution are available from industrialised countries.

Convenient sampling and ad hoc data collections should be looked at with caution because of sampling bias. However, even limited data from some countries may contribute towards highlighting problems with certain serovars. Examples of serovars with a predominantly local distribution are S. Hiduddify and S. Kedougou in poultry in Nigeria and Thailand, respectively (53, 55), and S. Concord among humans in Ethiopia (29). Local serovars may also pose a risk to other areas due to travel and trade. Examples of this are the serovars S. Stanley and S. Schwarzengrund in Thailand and S.Kentucky in northern Africa. All of these have been linked to cases among European travellers (2, 27, 42).

As part of the WHO Global Foodborne Infections Network (WHO-GFN), a worldwide databank has been in operation since 2000 (see Box 1). The GFN Member Institutions (national or regional Salmonella reference laboratories) are asked to provide, each year:

- the number of Salmonella isolates identified
- the number of Salmonella isolates serotyped
the top 15 Salmonella serotypes identified
the sources of Salmonella isolates (e.g. human, non-human).

As of 10 March 2013, 1,181 data sets from 84 countries had been supplied to the database. These data are publicly available through the GFN website (www.who.int/gfn/activities/en/). Two scientific papers have summarised these global data (20, 30). The data show that SE and ST are the most prevalent serovars worldwide (43.5% and 17.1%, respectively). Except for S.Infantis, which was dominant in all regions, the other non-SE/ST serovars showed geographical differences. Salmonella Newport was mainly observed in the Americas, as well as in European countries; S.Virchow was found in Asian, European and Oceanic countries, S.Hadar was seen in European countries, and S.Agona in Latin and North American and European countries. These serovars were isolated with an overall proportion of 3.5%, 1.5%, 1.5% and 0.8%, respectively.

Data on the prevalence of Salmonella serovars from some industrialised countries are published annually on-line (Box 2).

Salmonella serovars in the European Union from 2006 to 2010

In 2006, the European Food Safety Authority (EFSA) reported 160,649 human cases of salmonellosis among the then 25 EU Member States (17). Non-typhoid serovars SE and ST were responsible for 75.4% of Salmonella cases with a known serotype. Other serovars contributed <1% to the burden of human salmonellosis; the main serovars being (17):

- S. Infantis (0.9%)
- S. Virchow (0.7%)
- S. Hadar (0.5%)
- S. Newport (0.5%).

All top five serovars (SE, ST, S.Infantis, S.Virchow and S.Hadar) were considered by the EU as ‘serovars of public health importance’. Over the years 2004 to 2008, baseline studies using standardised methodology were carried out on primary production sites of chickens, turkeys and pigs across EU Member States. Although SE and ST were common, a high fraction of serovars (36%–90%, depending on the target species) were non-SE/ST.

Box 2
Links to data on Salmonella surveillance systems in a selected group of countries

Australia
Humans: OzfoodNet
Animals: National Animal Health Information System

Canada
Humans and animals: C–EnterNet

European Union
Humans, food and animals: European Food Safety Authority

New Zealand
Humans: New Zealand Public Health Surveillance
www.surv.esr.cri.nz/surveillance/NZPHSR.php
Animals: Biosecurity in New Zealand
www.biosecurity.govt.nz/

United States
Humans: Laboratory-based Enteric Disease Surveillance (LEDS) system (replacing the former Public Health Laboratory Information System or PHILIS)
www.cdc.gov/nationalsurveillance/salmonella_surveillance.html
Animals: Animal and Plant Health Inspection Service/National Animal Health Reporting System
www.aphis.usda.gov/animal_health/nahrs/reports.shtml

The most common non-SE/ST serovars were:
- S. Infantis, S.Mbandaka and S.Livingstone (laying chickens)
- S. Infantis, S.Mbandaka and S.Hadar (broiler chickens)
- S. Derby, S.London and S.Infantis (pig production holdings)
- S. Derby, S.Infantis and S.Rissen (pig-breeding holdings)
- S. Bredeney, S.Hadar and S.Saintpaul (fattening turkeys)
- S. Saintpaul, S.Kottbus and S.Heidelberg (breeding turkeys).

Reduction targets of Salmonella were set, which focused largely on the five serovars of public health significance (in breeding and commercial poultry production) and SE/ST (in commercial poultry production). Over the 2006 to 2010 period, cases of human salmonellosis in the EU decreased by 40.6% (99,020 cases were reported in 2010). This was driven mainly by a 51.8% reduction in SE cases, even though there was a 16% increase in ST. Of the three additional serovars of public health importance, S.Infantis increased markedly (+42.5%), whereas both S.Virchow and S.Hadar decreased (~35.1% and ~28.9%, respectively) (17, 18).
Examples of emerging serovars in the European Union

Reports indicate increases in a number of serovars in the EU, and these now form part of the top ten serovars in humans. They are:
- S. Newport
- S. Kentucky
- S. Derby, and
- a variant of ST (called monophasic ST).

**Salmonella Infantis**

In 2010, there were 1,776 S.Infantis cases reported in the EU (1.8% of cases), making it the third most common serovar in 2010. *Salmonella* Infantis has a worldwide distribution (30). During 2008 to 2010, S.Infantis was by far the most frequently isolated serovar from broiler meat, due mainly to a large number of isolations from a few countries. In Hungary, widespread contamination with S. Infantis has been demonstrated in the poultry production chain (48), and a number of these clones are multi-drug-resistant strains (49). To a lesser extent, S. Infantis is also present in pig, bovine and turkey meat, and the serovar has been shown to be present in several other countries, in chicken breeding and table egg production. Beyond the EU, this serovar has been increasingly reported from a number of countries, such as Iceland (62), Israel (6) and Japan (47). There is little evidence of this serovar being implicated in egg-borne transmission. In Japan, molecular analyses, using amplified fragment length polymorphism, have linked human cases to broiler meat, but not to chicken eggs (47).

**Salmonella Derby**

Data compiled by EFSA indicate a moderate increase (+39.4%) in cases of salmonellosis due to S.Derby over the period from 2006 to 2010. This serovar is prevalent in a number of countries in Europe, Asia and Latin America (30). Data from the 2006/2007 EU baseline survey of slaughter pigs indicated that S. Derby was the second most common serovar after ST. In 2010, S.Derby still ranked as the second most common serovar in pig production. The baseline survey showed that this serovar was the third most common in fattening turkey flocks (11.3% of all *Salmonella* serovars), and was also present in a small number of countries in layer and broiler chicken production. In some EU countries, S.Derby has been increasingly detected in turkey production. Outside the EU, a high prevalence of S.Derby has been reported from Uruguay in chicken egg production, despite the fact that this serovar has not been detected in people in Uruguay, indicating that eggs are not likely to be a source for human S.Derby infection (7). Molecular analyses have shown a great similarity between isolates from pigs and humans, suggesting that pigs are the main source of this serovar (25).

**Salmonella Newport**

*Salmonella* Newport is mainly present in Europe and the Americas (30). In 2010, S.Newport ranked as the fifth most common serovar infecting humans in the EU. In that year it was the third most common serovar isolated from turkey flocks and turkey meat, but was practically absent from pigs, broilers and bovine meat. Over the last few years, a number of outbreaks of S.Newport in the EU have been traced back to the consumption of salads (37, 43, 65), imported horse meat (15), imported nuts (40) and watermelon. Phylogenetic studies have identified three main lineages in Europe (I, II and II), one of which (I) was isolated only at very low frequencies from non-animal hosts, suggesting human-to-human transmission (58). In the USA, highly resistant variants of this serovar have become established in cattle production over recent years (13, 33). Globally, outbreaks of S.Newport have been reported as being associated with the consumption of ground beef (60), tomatoes irrigated with pond water (23), unpasteurised cheese (10) and imported mangoes treated with hot water (61). It has been suggested that humans infected with S. Newport have a longer duration of shedding than with other serovars (46).

**Salmonella Kentucky**

A 69% annual increase of cases of S. Kentucky was reported in the EU in 2010. In that year, this serovar was the second most common serovar in broiler meat (driven by its high prevalence in Ireland) and the fourth most common in turkey meat. Two distinct lineages have been described; one in the USA, which has an increased ability to colonise poultry and cause extra-intestinal disease (38), and a ciprofloxacin-resistant lineage present in Africa and EU countries (42). In Switzerland, a high number of elderly female patients with S. Kentucky infection in the urinary tract has been reported (8).

**Monophasic Salmonella Typhimurium**

Monophasic variants of ST (1,4,[5],12:i:-) (i.e. lacking the first or the second phase of the H antigen, due to deletions) have been emerging over the last few years in many EU countries. By 2010, these variants were among the fourth most commonly reported serovars in the EU (1.5% of all human cases) (18). Results from EU baseline surveys in slaughter pigs (2006/2007) indicated that monophasic ST was the fourth most common serovar in slaughter pigs. In contrast, it was virtually absent in all baseline surveys in poultry (chickens and turkeys). Surveillance data from throughout the EU indicate that, in 2010, monophasic ST became the second most common serovar in pigs and the third in pig meat, suggesting an increase since the baseline
surveys. Monophasic ST was also common in cattle and bovine meat (the third most common serovar). An outbreak linked to the consumption of dry pork sausages has recently been reported in France (22).

Control of non-typhoid serovars in animal production

The large degree of variation exhibited by Salmonella serovars in their biological properties, host preferences and environmental survival presents a particular challenge for controlling the presence of Salmonella in animal production. In practice, this means that there is no ‘one size fits all’ solution, and different production systems may require different approaches to control the various serovars. The main factors to consider include:

- the current prevalence status (i.e. whether the serovar is already established in farms, hatcheries, feedmills or the environment)
- the public health implications of the serovar (i.e. the numbers of human cases, its antimicrobial-resistant profile), and
- the overall costs of monitoring and control.

Defining priorities for Salmonella control, based on the specific serovars found in particular types of production, is especially challenging, given the differences between countries, host species, year-to-year variations, and policy decisions that may be driven by economic considerations.

For serovars established in particular production niches, such as SE in egg production in Europe (16), farm-targeted control measures (improved cleaning and disinfection, vaccination, pest control) have proved quite effective in reducing or eliminating infection in the animal reservoir, resulting in notable reductions in human SE cases (18, 50). This has been made easier by the fact that SE is relatively rare in animal production supply systems (hatcheries, feedmills, the breeding pyramid). Therefore, once elimination from the farms has been achieved, reintroduction of SE is relatively uncommon. For situations where serovars are well established in animal production (i.e. ST, S.Derby in pigs), measures aimed at eliminating contamination from the farms are clearly essential. However, these efforts need to start at the top of the breeding pyramid to be of any value, since costly efforts to eliminate contamination from farms can be overrun by the introduction of infected stock. For serovars that are well established in animal production, more research is needed to define the effectiveness of on-farm control measures in providing cost-effective solutions.

Farming systems with animals that have considerable exposure to the environment (i.e. free-range poultry, grazing cattle) may theoretically be more exposed to serovars present in environmental sources (i.e. wildlife, water). However, in practice, the infection of farmed animals with Salmonella through contact with wildlife sources is relatively rare.

In modern animal production systems, where animals are confined with relatively good biosecurity, feed is likely to play a major role in the introduction of new serovars which may eventually impose a burden on human health (12). Ensuring an 100% success rate in preventing the introduction of exotic Salmonella serovars is virtually impossible, given the wide range of ingredients used in modern feed production, many of them linked to global trade. This poses unforeseen risks that should be minimised by adopting effective monitoring systems along the feed production chain.

Future developments influencing the surveillance of non-typhoid serovars

Clinical laboratories are gradually introducing culture-independent diagnostic assays to replace the conventional culture systems. For Salmonella, this will result in a loss of information on the various subtypes (11).

Over the last decade, alternatives to conventional serotyping have been developed but these only target the most common serovars. The advantage of molecular serotyping is that it is ‘backwards compatible’; i.e. newly collected data can be compared with the historic data. Other molecular alternatives have been developed that only partially correspond with the serotyping system (3, 66). With the availability of high throughput and relatively cheap options for whole-genome sequencing, it is to be expected that this approach will be used in surveillance programmes in the near future (1, 14, 64).

Acknowledgements

J. Carrique-Mas is supported through the VIBRE Project, funded by the Netherlands Organization for Scientific Research (WOTRO) and the Netherlands Organization for Health Research and Development (ZonMw) (Project Number 205100012).
Consideraciones prácticas en torno a la vigilancia de serovares de *Salmonella* distintos de Enteritidis y Typhimurium

J.A. Wagenaar, R.S. Hendriksen & J. Carrique-Mas

**Resumen**

En todo el mundo se aislan, con enormes diferencias de prevalencia, serovares no tifoideos de *Salmonella* distintos de *Salmonella enterica* sérovar Enteritidis (SE) y *S*.Typhimurium (ST). Además de los serovares más frecuentes, como *S*.Infantis o *S*.Hadar, hay muchos ejemplos de serovares descritos principalmente en una determinada región y asociados con toda probabilidad a reservorios locales. Dado
que pocos países del mundo tienen instaurado un sistema oficial de vigilancia de la salmonelosis humana, los contados datos al respecto provienen de estudios especiales sobre el tema. Aún más escasos son los datos relativos a animales, alimentos o piensos para animales. La identificación de serovares distintos de SE y ST puede verse dificultada por la falta de experiencia en tipificación sérica y el problema que supone obtener antisueros (de calidad). Tipificar las variantes séricas (subtipificación) de Salmonella sigue siendo importante para determinar el origen de las infecciones humanas y realizar intervenciones selectivas. En el futuro, sin embargo, habrá un uso creciente de ensayos de diagnóstico que no exigen el paso por un cultivo, lo que tendrá por consecuencia que dejen de generarse datos de subtipificación epidemiológica y de sensibilidad a los antimicrobianos. Conviene procurar que esos ensayos estén validados para todos los serovares, en especial los que son poco comunes. Aunque los actuales procedimientos de subtipificación, basados en el esquema de Kauffmann-White, están bien implantados y revisten probada solidez, en un futuro próximo serán sustituidos por una nueva generación de métodos de subtipificación.

**Palabras clave**

---

**References**


