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The Salmonella Network, a tool for monitoring Salmonella "from farm to fork"

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The ANSES Maisons-Alfort Laboratory for Food Safety is associated with the National Reference Laboratory for Salmonella (NRL-Salmonella) for the serotyping of Salmonella (Associate NRL). It coordinates the network for epidemiological surveillance of Salmonella in the food chain in France. This Salmonella Network is made up of about 140 French laboratories that send their serotyping results or strains for confirmation to the Associate NRL on a voluntary basis. By centralising all these results, it has been possible for more than 10 years to monitor trends in the isolation of Salmonella serovars in the food chain and detect the emergence of particular serovars or strains with characteristics critical to human health.

Background

Salmonella is one of the main microbiological contaminants responsible for foodborne illnesses in Europe. In 2010, EFSA reported 99,020 cases of human salmonellosis in Europe, although the decline in the number of annual cases observed for several years seems to be continuing (EFSA, 2012). In France, the number of foodborne illness outbreaks due to Salmonella, which has steadily declined since 2002, remained stable between 2009 and 2010 (InVS, 2012). In 2010, this bacterium was (or was suspected of being) responsible for 141 outbreaks of foodborne illness (20% of outbreaks with a confirmed or suspected agent), corresponding to 1357 human foodborne cases. Food items mainly involved are eggs and egg products, as well as meat.

Identification and characterisation of *Salmonella* remain essential for the epidemiological surveillance of contamination throughout the food chain and for the control of this pathogen.

The system for monitoring Salmonella and salmonellosis in France

Several organisations are involved:

- the National Reference Centre (NRC) for Salmonella at the Institut Pasteur performs serotyping of strains of human origin, sent by medical biology testing laboratories and hospital laboratories, and collects information on strains whose serovar has already been determined. These data are analysed in order to monitor changes in the number of Salmonella strains isolated from humans and detect outbreaks. The antimicrobial resistance of Salmonella is also studied;
- the French Institute for Public Health Surveillance (InVS), whose main task is to monitor the population's health status, analyses the signals sent by the NRC (clustered cases, outbreaks, etc.) and where necessary initiates investigations to identify any common source for the human cases. The aim is to take measures to limit the number of human cases (withdrawal and recall of a product, for example). The InVS also centralises and analyses data from the mandatory reporting of any foodborne illnesses notified to the Departmental Directorates for the Protection of Populations and Regional Health Agencies;
- the ANSES National Reference Laboratory (NRL) for *Salmonella* and its Associate NRL, the Maisons-Alfort Laboratory for Food Safety, deal with *Salmonella* strains of non-human origin. The Maisons-Alfort Laboratory for Food



Safety characterises strains and coordinates a network of 140 food and veterinary testing laboratories, both public and private, known as the *Salmonella* Network, which collects strains from a variety of isolation contexts (self-inspections conducted by food-processing industries, official monitoring and control plans, investigations, food scares) and the epidemiological information associated with these isolates (David *et al.*, 2011).

Each year, in addition to the serotyping performed systematically, some strains are tested for their sensitivity to antimicrobials. Resistance mechanisms associated with phenotypes of interest to public health are studied. As a result of this, in 2009 the *Salmonella* Network identified a bacteria for the first time in food (*Salmonella* serovar S.I 4,12:i:- isolated from chicken meat) that carried the *armA* gene conferring high-level resistance to aminoglycosides of clinical interest (Granier *et al.*, 2011).

Centralising data on the phenotypic and genotypic characterisation of *Salmonella* collected by the *Salmonella* Network enables emerging clones to be detected and reveals epidemiologically related strains during investigations of episodes of clustered human cases.

Between 2005 and 2010, the *Salmonella* Network was called on 47 times by the Directorate General for Food and the InVS to identify potential sources of contamination and assist with epidemiological investigations.

The regular collection of serotyping information and results combined with a statistical time-series analysis of isolation of



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Salmonella enables the detection of signals corresponding to a new or emerging situation of concern. The Salmonella Network has already shown its value to risk managers through its former alert function (Danan *et al.*, 2011).

Salmonella Network operation

The network has two objectives: (1) To provide food and veterinary testing laboratories with technical support for serotyping of *Salmonella* isolates, (2) To develop vigilance with respect to monitoring *Salmonella* isolated from the food chain ("from farm to fork") and detect signs indicating any unusual increase in a serovar.

Each year since 1997, a subscription charter has been signed by each partner laboratory (approximately 140 per year). Information in three areas is collected: (i) animal health and production (sick animals, healthy carriers or the farming environment); (ii) food hygiene (intended for human or animal consumption, slaughterhouse environment, cutting and processing units); (iii) the natural ecosystem.

Salmonella are isolated from samples taken throughout the food chain by numerous laboratories that currently provide good national coverage of first-line analyses. Almost all (97%) of France's public departmental laboratories are network members. The Salmonella serotyping method (Danan et al., 2009) used by the Associate NRL on strains received for confirmation is implemented according to the NF EN ISO/IEC 17025 standard, under COFRAC accreditation (www.cofrac.fr, accreditation no. 1-2246).

The *Salmonella* Network partner laboratories send pure strain cultures or summary tables of serotyping results. For each result, information is collected about the sample's context, type and origin (Figure 1).

The data collected cannot be treated as prevalence data because the Salmonella Network receives no indication about



Figure 2. Number of *Salmonella* serotyping results recorded within the ANSES *Salmonella* Network between 2005 and 2010.

the total number of tests performed. European regulations on zoonoses, which target certain farming sectors and serovars, impose a selective pressure that may have an impact on the feedback of information.

However, the relative stability of the network data and the similarities observed in the past regarding trends in certain serovars isolated in both humans (NRC) and food (NRL) underline the network's importance in the national *Salmonella* monitoring system. Its annual reports are available from http:// www.ansespro.fr/reseausalmonella.

Main trends observed in recent years

The Salmonella Network collects about 15,000 Salmonella serotyping results each year (Table 1). Between 2005 and 2010, depending on the year, 55% to 65% of these serotyping results were obtained by the laboratories and sent to the Salmonella Network. In the remaining cases (35% to 45%), the strains were serotyped by the Associate NRL, either because the originator laboratory does not perform complete serotyping, the serotyping was more complex, or confirmation was needed in the context of official controls.

Each year, two thirds of the serotyping results ultimately obtained come from the "animal health and production" sector (of which 80% are obtained from partner laboratories and 20% by the Associate NRL) and one third from the "food hygiene" sector (40% and 60% respectively).

Figure 2 shows the overall decline in the relative annual share of the serovars S. Enteritidis and S. Typhimurium observed by the *Salmonella* Network. A similar observation was reported by the NRC for strains of human origin isolated between 2002 and 2010 (Jourdan-Da Silva and Le Hello, 2012). This decrease is probably due to the impact of control and management measures applied in the poultry sector in recent years.

The frequency of isolation of strains S.I 1,4,[5],12:i:-, known as



Figure 3. Trends between 2005 and 2010 in the relative proportions of serovars Typhimurium and Enteritidis in the *Salmonella* Network, emergence of the monophasic variant S.I 1,4,[5],12:i:-.

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"monophasic variants" of S. Typhimurium, has been increasing for several years in humans (NRC data) and since 2008 in all the animal and production sectors monitored by the Salmonella Network (Figure 2; Table 2). These trends are consistent with the increase observed since 2007 in the number of outbreaks of clustered cases involving these strains in France (Danan et al., 2012; Gossner et al., 2012) and Europe (Bone et al., 2010; Hopkins et al., 2010).

The annual monitoring data presented in the inventories of Salmonella of non-human origin (2005 to 2010) available on the network's website, highlight a specific association of certain serovars with certain animal sectors or food types (Table 2), such as Dublin in dairy products, Indiana in poultry or Enteritidis in egg products.

In food hygiene

Among Salmonella isolated from pork meat, the relative proportion of serovar Typhimurium has been stable since 2005 (30 to 35% of the sample panel), while that of serovars Derby and S.I 4, [5], 12: i:- increased from 20% to about 40%, and from 0 to 5.5% respectively. For delicatessen meats, Typhimurium and Derby remain the most frequently identified serovars, but the growing relative importance of serovar S.I 4,[5],12:i:between 2008 (3.4%) and 2010 (10%) is noteworthy.

The relative proportion of S. Typhimurium in dairy products is decreasing (12% in 2005 compared with 6% in 2010). Concerning egg products, the few isolates identified in this food category only emphasise the relative stability of serovars Typhimurium and Enteritidis between 2005 and 2010.

With regard to the hygiene of duck carcasses, meat and offal, the serovars Indiana, Typhimurium and Kottbus are the most frequently isolated and have been relatively stable since 2005. The distribution of serovars is much more variable for the "turkey" and "Gallus gallus" sectors, although since 2005 the

main serovars have remained Agona, Bredeney, Derby, Hadar, Indiana and Typhimurium in turkeys, and Enteritidis, Indiana, Typhimurium and most recently Paratyphi B in Gallus gallus. The serotype S.I 4,[5],12:i:- has also emerged in the "turkey" and "Gallus gallus" sectors since 2009.

In animal health and production

Since 2005, Senftenberg has been the serovar most frequently isolated from the Gallus gallus and turkey farming environments whereas in the duck sector it is the serovar Indiana. In the cattle sector, Typhimurium, Montevideo and Dublin are predominant, with relative stability since 2005, isolated both from farming environment samples and in the context of animal disease. In the pork sector, each year since 2005, the two main serovars (Typhimurium and Derby) have accounted for between 60% and 80% of all Salmonella isolates.

Conclusion

Despite not providing consolidated data on prevalence, the Salmonella Network provides an appreciation of the diversity and spatiotemporal evolution of isolated serovars, for the entire food chain. In particular, it is a source of information on rare serovars or those not covered by the regulations, and can act as an alert mechanism for the health authorities.

The voluntary mobilisation of the Salmonella Network's partner laboratories and the close collaboration between the reference laboratories (NRC and NRL) are essential prerequisites to the efficient running of the national Salmonella monitoring system. Coordination and regular assessment of the Salmonella Network's operation, harmonisation of analytical methods and data repositories to be shared, and the resources and communication tools implemented are critical to achieving monitoring objectives.

Table 1. Relative frequency (%) of the main serovars detected within the Salmonella Network, by food category, in 2010 (N = total number of isolates)

SEROVARS	Poultry (N = 629)	Egg products (N = 35)	Pork (N = 1155)	Delicatessen meats (N = 523)	Beef (N = 154)	Dairy products (N = 815)	Animal feed (N = 1113)
TYPHIMURIUM	14.2	5.7	30.6	32.5	37.7	5.8	2.9
DERBY	3.2	0	37.4	17.9	5.8	5.4	1
HADAR	1.9	0	0	0.2	0	0	0.1
MONTEVIDEO	1.4	0	0	0.2	2.6	2.9	19.9
INDIANA	25.6	0	0	1.3	0.6	0	0.4
AGONA	2.7	0	0.5	3	1.3	4	0.9
DUBLIN	0	0	0	0.2	10.4	57.6	0
ENTERITIDIS	4.7	22.9	0.3	0	0.7	0.3	0.3
MBANDAKA	1.7	22.9	0.3	0.2	13	2.5	6.3
RISSEN	0.5	0	2.2	9.8	0	0.4	3
S.I 1,4,[5],12:i:-	3.5	0	5.5	10	11	1.3	0.5
S.IIIb 61:[k]:1,5,7	0	0	0	0.2	0.7	5.6	0
TOTAL %	59.4	51.5	76.8	75.5	83.8	85.8	35.3
Total number of serovars identified (100%/category)	51	11	44	50	30	37	109





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We would like to thank all the partner laboratories that regularly send strains and epidemiological information to the Salmonella Network.

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Figure 1. Example of the form used by the Salmonella Network to collect information associated with an isolate from a food intended for humans or from the ecosystem.