Bulletin pidémiologique

Animal health and nutrition

January 2017/Issue 77

Special Edition on Food Safety Monitoring

Detailed contents on page 2

EDITORIAL

This special edition is the first issue of the *Bulletin Épidémiologique (BE) - Animal Health - Food* dedicated to an annual review of food safety monitoring. This new *BE* issue presents the organisation and the results of the main surveillance programmes for chemical and biological contaminants that may be found in food.

The contaminants of interest are associated with adverse effects in humans, whether proven or potential, and for which there are surveillance programmes at the national level. Three categories of contaminants are being monitored: contaminants of environmental origin, those related to pesticides and fertilisers used in livestock rearing and agriculture, and those related to a food safety deficiency in food product processing methods. The articles present the basic concepts of epidemiological surveillance in the area of food safety and show the position of all the stakeholders in the process.

For each article, an inset summarises how surveillance is organised and gives the regulatory context. The programmes are described along with a review of the most recent year for which consolidated data are available at the national level; where appropriate, these results are compared with those of previous years.

This special issue of the *BE* is a supplement to the annual reviews published in parallel – specifically the summary sheets issued by the technical units of the French General Directorate for Food, published on the Ministry of Agriculture's website. In fact, the specific task of co-authoring these articles, carried out by the various contributing entities, including government administrations, ANSES, National Reference Laboratories, and agro-industrial technical centres, helps to structure exchanges. This ultimately contributes to optimisation of surveillance activities by developing common technical terminology, sharing and harmonising of objectives, and by promoting exchanges regarding data quality, and the interpretation of results and putting them into perspective in order to assess or manage risks.

This is already a sign of the benefits expected from synergies between the various stakeholders involved in surveillance, some of whom have been identified as potential members of the Food Chain Surveillance Platform, provided for by the new French law on the Future of Agriculture, Food and Forestry.

This document also serves as a tool for promotion and communication at the national and international levels. It provides detailed feedback on surveillance programmes and epidemiological data to all the stakeholders in the surveillance process, thus promoting direct involvement in this activity at each stage, from collection through to analysis and interpretation of results (operators, laboratories, and agents in charge of official controls at the local and national levels). These reviews can also be considered as reference data for use at the international level, for example in the context of cross-border trade. In addition, the reviews and analyses presented serve as a basis for risk assessment and management.

Overall, the general level of food quality in France is considered to be very good and is characterised by very low levels of non-compliance. Moreover, the results of monitoring of foodborne illnesses in humans provide indicators of the level of overall control of biological contamination.

The reviews underscore the joint responsibility of the various stakeholders, from the livestock farmer to the final consumer, in characterising and controlling sanitary risks. For all contaminants, a critical analysis of the safety situation, and an assessment of the surveillance and control activities implemented – and their relevance – provide the foundation for safety management, from the local to the national level.

The Editorial Board - BE special Food Safety

TABLE OF CONTENTS *SOMMAIRE DÉTAILLÉ*

Special Edition on Food Safety Monitoring

The *Bulletin Épidémiologique* is a joint publication of the French Agency for Food, Environmental and Occupational Health & Safety, and the Directorate General for Food of the Ministry of Agriculture, Food and Forestry.

Reflections on food chain surveillance

Corinne Danan (corinne.danan@agriculture.gouv.fr) (1), Didier Calavas (2)

(1) Directorate General for Food, Sub-directorate for Food Safety, Support Office for Food Chain Surveillance, Paris, France
(2) ANSES, Lyon Laboratory, France

Abstract

Food surveillance activities produce valuable safety-related data under the responsibility of many stakeholders in the food chain but are often under-exploited. The optimisation of surveillance systems at national level is expected in the framework of the French law for the future of Agriculture, implementing an epidemiological surveillance Platform. In the food safety sector, this project is being built step by step through consultations with the different stakeholders. This paper summarises the results of these consultations organised by the Directorate General for Food since the end of 2015; it describes the fundamental elements of an epidemiological surveillance approach on which future work can be based.

Keywords
Food safety, Epidemiological surveillance

Résumé

Réflexions autour de la surveillance épidémiologique des aliments

Les activités de surveillance des aliments, sous la responsabilité de nombreux acteurs, représentent une source précieuse de données sanitaires souvent sous- exploitées. Une optimisation des dispositifs de surveillance des aliments au niveau national est envisagée avec la mise en place d'une plateforme d'épidémiosurveillance, telle que prévue par la loi d'avenir pour l'agriculture. Dans le secteur de la sécurité sanitaire des aliments, cette perspective se construit progressivement par une concertation avec les différents partenaires. Cet article fait la synthèse des résultats des consultations organisées par la DGAL depuis fin 2015 et décrit les éléments fondamentaux d'une approche d'épidémiosurveillance sur lesquels pourront se fonder les travaux futurs.

Mots-clés
Sécurité sanitaire des aliments, surveillance épidémiologique

Epidemiological surveillance is essential to any public health policy, because it helps provide accurate and reliable information and analyses on the status and development of biological and chemical safety hazards. It does not directly "act" on the spread of a safety hazard but provides information about its status and development.

The relevance and quality of a surveillance programme are therefore factors that directly influence the relevance of the measures taken by risk managers, the quality of expert appraisals undertaken for risk assessment purposes, and the quality and feasibility of research work to be carried out[1]. It should also be noted that epidemiological surveillance covers both surveillance and vigilance activities, devoted respectively to current safety hazards in France and to exotic or emerging hazards (i.e. hazards not identified in France at a given time).

In the areas of human and animal health, epidemiological surveillance benefits from more experience than epidemiological surveillance in the field of food safety. The concepts, definitions and tools developed in this framework should thus be adapted to the characteristics and particularities of food safety. The Epidemiological Surveillance Platform for Animal Health (ESA Platform), established in October 2011, is an example to be taken into account, but cannot be transposed unless it is adapted, in view of developing such a Platform for food safety.

Epidemiological surveillance relies on multidisciplinary and multi-partner activities. In a sector as broad and varied as food production, there are many stakeholders, who are often focused on one type of product or one stage in the food chain for which they are responsible. The reflections under way to develop epidemiological surveillance actions in the area of food safety should therefore optimise relations between stakeholders in the food chain. They should also ensure that stakeholders take ownership of the guidelines and tools offered by epidemiologists, to help them implement effective programmes and interpret their results.

In this context, an essential prerequisite consists in agreeing on a common vocabulary, in a sector that is generally unfamiliar with this type of approach. Moreover, a distinction should be made between epidemio-surveillance and risk management or assessment, even though the stakeholders are sometimes the same.

The reflections presented in this article draw on the experience of the ESA Platform and collective brainstorming sessions organised by the DGAL since the end of 2015 with several representatives of inter-professional associations involved in the food chain, ANSES scientists, agricultural and agro-industrial technical centres, and analytical laboratories. During these sessions, the use of the terms "epidemiological surveillance" and "epidemio-surveillance" did not seem natural, since surveillance applies to categories of foods that cannot be associated with a "state of health" in the strict sense. In addition, epidemiological surveillance was instinctively associated with the surveillance of "epidemics" in most cases. And yet this association does not fit with the definitions used by epidemiologists in the fields of human and animal health who are more familiar with epidemio-surveillance approaches.

The three sectors of animal health, human health and food safety ultimately use common definitions, referring to a population of individuals (foods, animals, plants or humans) with a state of safety to be monitored for which it is necessary to adopt monitoring, control measures, etc. (Box 1).

In the rest of this document, preference will be given to the expression "Food chain surveillance" (FCS) instead of "Epidemio-surveillance of foods"; this term seems more suitable and avoids the use of "epidemio-surveillance" which has too many "health" connotations.

Food chain surveillance: objectives and methods

Objectives

The objectives of surveillance activities are different from those of control activities which involve, when a non-conformity or safety status of concern is identified, implementing measures to eliminate the source or reduce the risk of consumers being exposed to the detected contaminant.

1. According to a working document on the future of the ESA Platform (2016).

FCS can have various objectives:

- estimate the level of contamination in a "population" (i.e. a category of food in a stage of the food chain) and analyse its trends. This objective can contribute to verifying the level of safety control in upstream stages, assessing the impact of a management measure, or disseminating/communicating representative data for a "population" to users of this information (risk assessors and managers),
- detect unusual contamination early on, as part of a risk prevention approach, before pathological cases emerge in humans.

Stakeholders in food chain surveillance

Managers of FCS programmes can be:

- public risk managers (national control authorities: DGAL, DGCCRF, DGS) managing official surveillance plan and official control programmes,
- private risk managers (operators in all stages of the food chain) managing their own-checks programmes on an individual or collective basis,
- managers of integrated thematic surveillance programmes, most often in National Reference Laboratories (e.g. the *Salmonella* network managed by ANSES).

Within these programmes, there can be many stakeholders taking place at national and local level (Box 3). The sustainability of surveillance actions relies on the ongoing coordination of the stakeholders involved and feedback for producers and users of data (private or public risk managers, risk assessors, and consumers as needed).

Box 1. Definitions

In the area of animal health, epidemio-surveillance is an observation method based on continuous recording to monitor the state of health or risk factors in a defined population, particularly to detect the emergence of pathological processes and to study their development over time and space with a view to adopting appropriate prevention measures (Toma *et al.*, 1991).

In the area of human health, epidemiological surveillance means the systematic ongoing collection, collation and analysis of data for public health purposes and the timely dissemination of public health information for assessment and public health response as necessary (International Health Regulations[1]).

In the area of food safety, the "epidemio-surveillance of foods" is a set of activities aiming to: i) continuously collect data on levels of one or more contaminants (Box 2) in a category of food in a stage of the food chain (the "population"), ii) interpret them, and iii) communicate the resulting information to organisations and structures responsible for food safety. In all cases, the "epidemio-surveillance of foods" encompasses long-term activities and is ultimately focused on human health issues for which risk assessment, risk management or other prevention or surveillance measures need to be taken.

1. http://www.who.int/ihr/publications/9789241596664/en/.

Box 2. What is a contaminant?

A contaminant is any chemical element, chemical substance or biological agent not intentionally added to food which is present in such food as a result of the production (including operations carried out in crop and animal husbandry), processing, preparation, treatment, packing, packaging, transport or holding of such food, or as a result of environmental contamination. Radionuclides are considered physical contaminants in the context of official surveillance. Extraneous matter (such as, for example, insect fragments, animal hair, etc.) is not covered by this definition.

In relation to Regulation (EEC) No 315/93, we include biological agents (viruses, bacteria, parasites) in the definition of contaminant.

"Contaminant/product" pairs to be monitored

The choice of contaminants to be included in FCS activities should take into account diseases and adverse health effects in humans.

The scope covers all contaminants likely to be found in foods of plant or animal origin (Box 2). The surveillance stage can differ depending on the contaminant, as a function of its development across stages in the food chain (some contaminants appear or disappear as a result of production processes) and as a function of the surveillance programme's objective. This choice should be risk-based, using an integrated approach, and corresponds to the most suitable stage of the food chain for taking effective action. Due to the risk of the possible transfer of contaminants from animal feed to food, the surveillance of animal feed should be included in the scope of food chain surveillance.

Unlike in the areas of animal and plant health, food-related safety hazards have not yet been officially classified. The discussions held as part of the action plan of the Interministerial Committee for the Modernisation of Public Action (CIMAP) are expected to lead to such a classification (see above).

Regulatory context of food surveillance

Principles of the European legislation

General principles

The objective of the European legislation on food safety is to guarantee a high level of safety for consumers. No foods are to be placed on the market if they are considered hazardous under Regulation (EC) No 178/2002. In order to achieve this objective, the European regulations have laid down general principles relying on risk analysis, the primary responsibility of operators, and traceability and information requirements for the control authorities (Hygiene package). Risk assessment and management are clearly defined.

In addition, Member States are to implement surveillance programmes whose results (regarding agents responsible for zoonoses and chemical contaminants in foods) are reported annually to the European Food Safety Authority (EFSA).

Role of own-checks

Food chain operators have performance obligations and rely on an analysis of hazards and critical points for their control (HACCP) to define their own-checks schemes. This own-checks enables them to confirm the effectiveness of safety control measures. It is to be undertaken in all stages of the food chain (production, processing, distribution) from feed to food, except for primary production. For microbiological agents found in foods, Regulation (EC) No 2073/2005 establishes a minimum list of criteria to be included in the health control plans of operators. This list is not exhaustive and should be tailored to the hazard analysis of each company. For chemical contaminants, the choice of contaminants to be included in safety control plans is based only on the hazard analysis undertaken by each company.

The Hygiene package thus gives priority to own-checks to demonstrate the effectiveness of the programmes put into place by operators in the food sector in controlling contamination. These own-checks therefore represent a massive quantity of data on food contaminants, spread out among companies.

Official controls

Official controls contribute to the overall assessment of the safety control plans implemented in companies and to the verification of compliance with the legislation. They are organised according to a harmonised European approach to their design and implementation (Regulation (EC) No 882/2004). This verification partly relies on annual food sampling campaigns for the detection of contaminants, whether or not there are regulatory maximum values (system/programme of surveillance & control plans, SCPs).

Box 3. Stakeholders in food chain surveillance[1]

- The administrative authority (General Directorate and decentralised services) takes all measures intended to collect, process and disseminate epidemiological data and information regarding Category 1 health hazards as well as, when necessary, Category 2 health hazards
- (Articles L. 201-3 and -4 of the French Rural Code); these measures currently apply only to the sectors of animal health and plant health, for which health hazards have been classified; discussions are being held in the area of food safety as part of the action plan of the Interministerial Committee for the Modernisation of Public Action (CIMAP).
- Sanitary networks: a sanitary network is a group of stakeholders recognised by the State, representing 60% of the monitored population; the authority can recognise these sanitary networks in order to promote the prevention of sanitary hazards, the surveillance of animal and plant health, and the pooling of related costs (Article L. 201-10 of the French Rural Code; Order No 2015-1242 of 7 October 2015); specific reflections are necessary in the area of food safety for which no sanitary networks are currently recognised.
- Regional sanitary associations: a federation of sanitary organisations in the form of an association governed by the French Act of 1901 can be recognised by the State for the prevention, surveillance and control of sanitary hazards (Article L. 201-11 of the French Rural Code); specific reflections are necessary in the area of food safety for which no regional sanitary associations are currently recognised.
- Accredited analytical laboratories contribute to epidemiological surveillance and the early detection of outbreaks and at-risk sanitary situations, through their analytical knowledge and involvement in the local epidemiological context. They can participate in the epidemio-surveillance Platforms mentioned in Article L. 201-14 of the French Rural Code. (Decree No 2015-1902 of 30 December 2015). French *départements* are involved in sanitary monitoring through departmental analytical laboratories (Order No 2015-1242 of 7 October 2015).
- National Reference Laboratories (NRLs) contribute to the epidemio-surveillance missions undertaken by the State, primarily through the confirmation of first-line analysis results, the development and deployment of analytical methods, and the coordination of official laboratory networks.
- ANSES provides its supervisory ministries with scientific and technical support for surveillance and reference activities. It also carries out monitoring, alert, surveillance and vigilance missions; as part of its reference missions, ANSES is responsible for issuing alerts in the areas of veterinary medicinal products, plant protection substances, food safety (including drinking water) and animal and plant health. ANSES relies on data collection systems, primarily those of networks of laboratories run by NRLs, by definition giving it surveillance missions.
- Agro-industrial technical institutes (ITAIs) can provide scientific and technical support to operators in the implementation of their safety control plans; they perform general interest missions and are recognised by the authorities (Articles D823-1 and 2 of the French Rural Code).
- Joint technology networks (RMTs), recognised by the State pursuant to Article 91 of the French Act on agriculture No 2006-11 of 5 January 2006, are dedicated to the pooling of human resources by network members for carrying out collaborative work on priority topics for the development of the agricultural and agri-food sectors. Some RMTs have activities dealing with food safety (e.g. the Qualima and Quasaprove RMTs).

(1) To date, no regional health associations or health networks have been recognised in the target sectors of animal health and plant health.

Moreover, Regulation (EC) No 854/2004 defines specific rules for the organisation of official controls for products of animal origin intended for human consumption. Among other things, official controls are routinely organised at the slaughterhouse to reduce the risk of transmitting food-borne zoonoses (in particular testing for bovine cysticercosis and trichinellosis). These "controls" are part of programmed surveillance in reality.

National regulations

The State is responsible for organising food safety throughout France. As such, it has to implement conditions for the detection and control of health hazards, together with all stakeholders.

General provisions on epidemio-surveillance in the areas of plant health, animal health and food safety were specified in Order No 2015-1242 of 7 October 2015 on the organisation of surveillance related to animal health, plant health and food. This order provides for "epidemio-surveillance platforms" in order to provide (public and private) risk managers with support.

Definition and expected missions of the FCS platform

Definition and objectives

A platform can be defined as a multidisciplinary and multi-partner consultation space whose objective is to optimise surveillance actions to achieve a high level of food safety. It should provide support to risk managers for the "design, deployment, coordination, promotion and assessment of surveillance programmes" (Order No 2015-1242) as well as validated information to risk assessors. Consultations between partners also aim to identify research actions in the area of surveillance.

Nonetheless, every manager remains responsible for his/her programme. Such a platform can only be put into place if private and public partners from different fields agree to share resources, expertise and tools to the benefit of all.

Missions

For information, in the area of animal health, the general objective of the ESA Platform is to "facilitate the coordination, operational implementation and monitoring of the animal health surveillance policies adopted and implemented by its members. It should in particular ensure that the measures taken to monitor threats to animal health are adequate for dealing with current health hazards or hazards which threaten French territory"[2]. From an operational standpoint, it also leads and coordinates the surveillance systems that make up its work programme and is a centre of epidemiological expertise for these various systems.

For the establishment of the FCS Platform, it is essential to clarify the boundaries of surveillance support with missions involving:

- surveillance strictly speaking, whose management and organisation remain the responsibility of surveillance programme managers (see above),
- risk assessment, which is the responsibility of ANSES at national level,
- risk and alert management, under the supervision of private and public risk managers.

Note that the primary objective of a Platform is not to access or *a fortiori* to hold data but rather to strengthen systems enabling high-quality data to be acquired.

Thus, the actions taken in the framework of an epidemio-surveillance Platform provide two types of support: scientific and technical

2. Calavas *et al.* (2015). Bulletin Épidémiologique on animal health & nutrition, No 48. http://bulletinepidemiologique.mag.anses.fr/sites/default/files/BEP-mg-BE48-art1.pdf.

support (which can be described as "surveillance engineering") as well as strategic support:

- Scientific and technical support upstream of data collection.
  - Methodologies for the development of surveillance programmes.
  - Sampling protocols (sampling plan, identification of stakeholders, analytical methods, sampling tools, etc.).
  - Recommendations for data collection, information systems, programme coordination.
  - Charter for the use of data.
- Scientific and technical support downstream of data collection.
  - Statistical analysis and result reporting methods.
  - Expert appraisal and multidisciplinary interpretation of the health situation.
- Strategic support for surveillance.
  - Assessment of the effectiveness and efficiency of programmes (Oasis, RiskSur, etc.).
  - Monitoring of emerging hazards (in particular related to technological developments or new consumer practices).
  - International monitoring (e.g. risk of importing contaminated raw materials or finished products).
  - Identification of requirements for research into surveillance methodologies.

The DGAL is currently holding discussions to produce a proposal for the organisation and governance of the FCS Platform at national level. Based on the commitments of the various private and public partners, the FCS Platform is expected to start its work by the first quarter of 2017.

The surveillance system for **contaminants in the food chain** managed by the DGAL: report on the 2014 plan campaign

Marion Bordier (marion.bordier@agriculture.gouv.fr)
Directorate General for Food, Ministry of Agriculture, Agri-food and Forestry, Paris, France

Abstract
The Directorate General for Food (DGAL) of the French Ministry of Agriculture, Agri-food and Forestry manages a surveillance system for contaminants in food and feed. The system is complex and involves many stakeholders interacting with one another. Its main objectives are to verify if products are safe and to monitor trends in contamination over time.
In 2014, 25 surveillance programmes were implemented, across the different food sectors all along the food chain. No less than 58,179 samples were collected and approximately 800,000 analytical results were produced. As in previous years, contamination levels in food and feed were low. Data were processed on the one hand by the authorities to implement immediate risk-mitigation measures and to communicate about official actions, and on the other hand by the scientific community to conduct research work.
In 2014 again, when we look at the results, the surveillance system in place has shown evidence of effectiveness, despite many regulatory and methodological constraints, thanks to the strong commitment of the different stakeholders and the significant allocation of human and financial resources. However, a number of points could be improved to optimise the system and thus improve data quality and communication on the results.

Keywords
Surveillance, Food chain, Contaminant, Targeted surveillance, Random surveillance

Résumé
Le système de surveillance des contaminants dans la chaîne alimentaire piloté par la DGAL : bilan de la campagne des plans de surveillance et de contrôle en 2014
La direction générale de l'Alimentation (DGAL) du ministère de l'Agriculture, de l'Agroalimentaire et de la Forêt pilote un système de surveillance de la contamination des productions alimentaires. Le système fait intervenir et interagir de nombreux acteurs. Son objectif principal est de vérifier la conformité sanitaire des productions et de suivre les niveaux de contamination susceptible de se retrouver dans les denrées alimentaires.
En 2014, 25 plans de surveillance ou de contrôle ont été mis en œuvre, répartis dans toutes les filières et aux différentes étapes de la chaîne alimentaire. Un total de 58 179 prélèvements ont été effectués et environ 800 000 résultats d'analyses ont été produits. Comme les années précédentes, les niveaux de contamination des denrées et des aliments pour animaux, et les taux de non-conformités évalués au regard des seuils réglementaires, sont faibles. Les données sont exploitées d'une part par les autorités pour la mise en place des mesures de gestion immédiates du risque et d'autre part par la communauté scientifique pour la réalisation de travaux de recherche. Elles permettent par ailleurs aux autorités de communiquer sur leurs actions. Au vu des résultats de 2014, le système de surveillance mis en place a montré son efficacité, malgré les contraintes réglementaires et méthodologiques, grâce à une implication forte des différents acteurs et aux importants efforts humains et financiers consentis. Cependant, un certain nombre de points pourraient être améliorés pour optimiser le système, et ainsi améliorer la qualité et la valorisation des données produites.

Mots-clés
Surveillance, chaîne alimentaire, contaminant, plan de surveillance, plan de contrôle

As part of the official controls implemented by the French authorities to ensure food safety, the Directorate General for Food (DGAL) of the French Ministry of Agriculture, Agri-food and Forestry (MAAF) manages a surveillance system for contaminants in primary animal and crop production, food of animal origin and feed. Within this system, various programmes are implemented targeting detection of a contaminant or specific class of contaminants in a given production sector (contaminant/product combination), in a specific stage of the food chain. These programmes are called surveillance plans (SPs) or control plans (CPs), depending on the objective and the sampling strategy. For SPs, sampling is random, so that the calculated level of contamination provides an estimate of that in the monitored production sector. For CPs, sampling is targeted and involves products for which health control is deemed inadequate or poor (products from areas potentially contaminated by organic pollutants) or for which the misuse of pharmacologically active substances is suspected.

The tested contaminants have a suspected or confirmed harmful effect on health, either in the short or long term, and may be: i) chemical substances (residues of veterinary drugs, hormones, plant protection products), ii) environmental or industrial chemical contaminants, iii) physical contaminants (radionuclides), iv) pathogens (bacteria, viruses, parasites), or v) antimicrobial-resistant bacteria. All food-production sectors are concerned, and the chosen sampling stage depends on the contaminant, the surveillance objective, levels of control of the related risk in the various stages of the food chain, and whether there are other surveillance systems or programmes.

Objectives of the surveillance system

The system of surveillance & control plans (SCPs) is part of the general organisation of the assessment and control of food safety. It meets several objectives. Firstly, it contributes to the verification of food safety and enables control pressure to be placed on operators in the agricultural and agri-food sectors (when the tested contaminant has a regulatory threshold for the monitored product). It also enables the monitoring of contamination in domestic and imported products, and the identification of trends and emerging contamination events. In addition, since some of the tested contaminants are inputs used

in agriculture (veterinary medicinal products, plant protection products), SCPs are able to detect the misuse of substances in agricultural practices (non-compliance with withdrawal periods for veterinary drugs, use of unauthorised plant protection products for a treated crop) or even fraudulent practices (use of unauthorised products). The system also contributes to the collection of data for estimating consumer exposure to food hazards and proposing risk-mitigation measures. Lastly, the system represents a health guarantee, for products imported from third countries and monitored at European border points, as well as for French products exported to foreign markets.

A number of contaminant/product combinations are monitored to fulfil specific European regulatory requirements. These SCPs thus contribute to the harmonisation of the sanitary quality of European products vis-à-vis certain health hazards.

How the system works

The official surveillance system for food-chain contaminants involves many stakeholders interacting with one another. The institutional organisation of the system is shown in Figure 1.

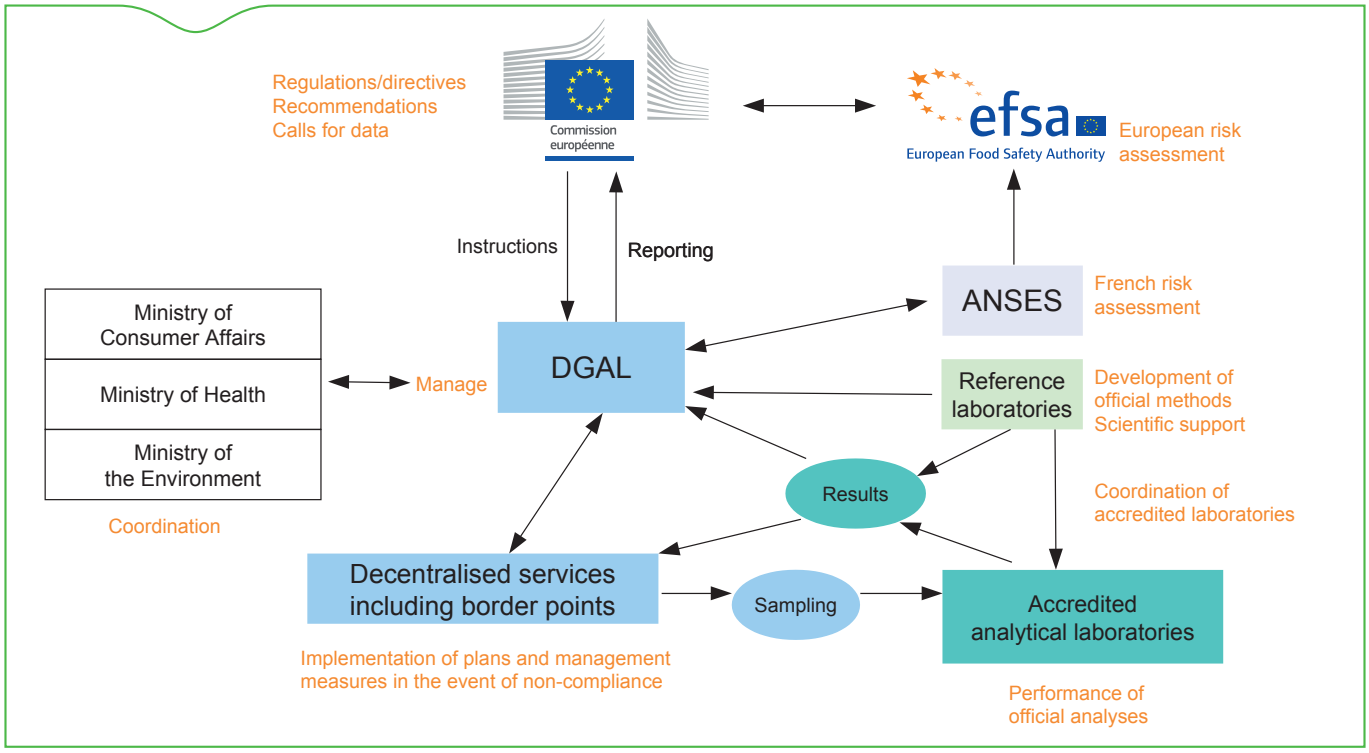

Figure 1. Institutional organisation of the official food-chain surveillance system

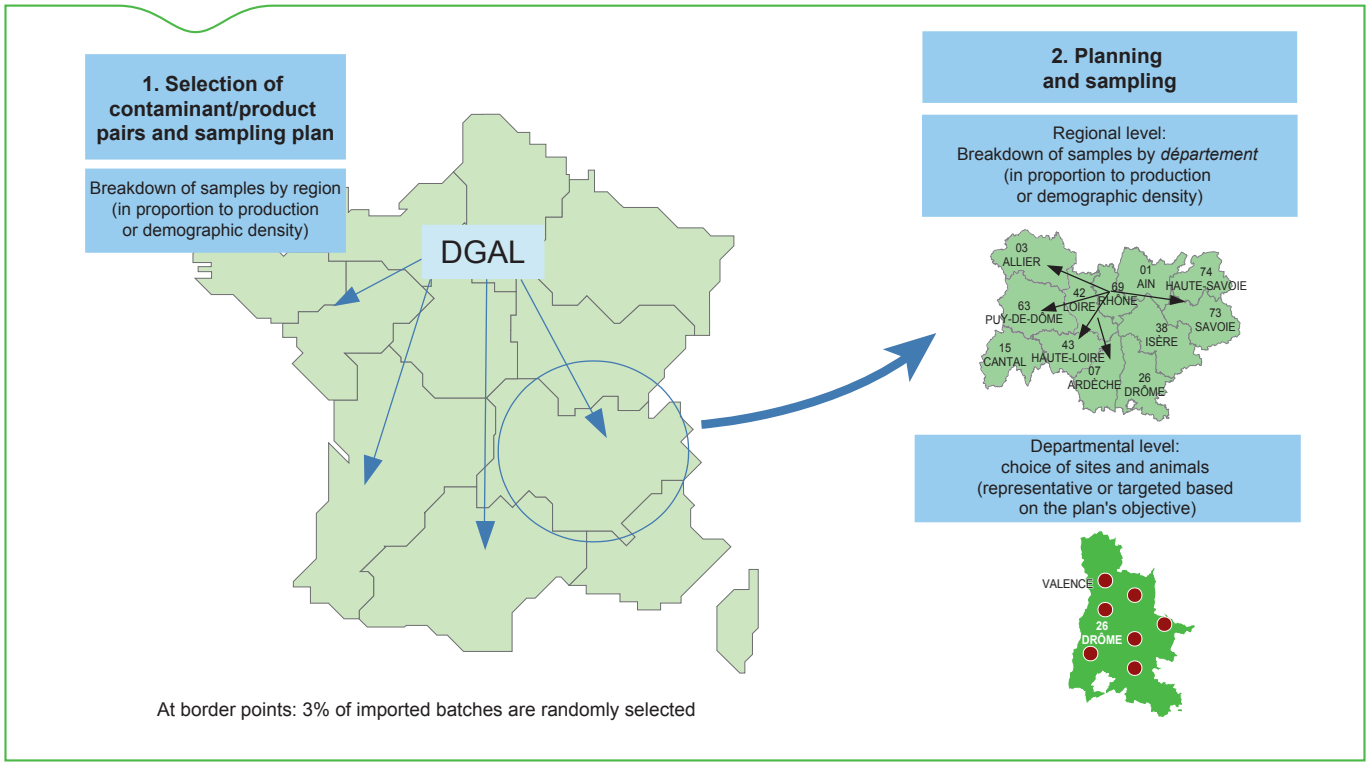

Figure 2. Functional organisation of the official food-chain surveillance system

The DGAL is in charge of the coordination of the system. Its role is to develop surveillance protocols and oversee their implementation. Every year, based on the available regulatory texts, European calls for data, risk assessment work and analytical capacities, it defines the national campaign. To do so, it selects the contaminant/product combinations that will be monitored, defines the sampling plan, and formulates the definition of a case (compliant, suspicious or non-compliant samples). This stage takes place in collaboration with other managers of surveillance systems and programmes and with the support of ANSES and the National Reference Laboratories (NRLs). At the same time, it ensures that networks of accredited laboratories, the sole recipients of samples taken in the context of SCPs, are operational to receive and analyse samples in accordance with official methods (international and national standards or methods developed and approved by NRLs). Once planning has been carried out at national level, samples are allocated to regions and *départements*, in proportion to their production for plans occurring upstream in the food chain, and to the human population size for plans taking place during distribution. Decentralised services are then responsible for selecting sampling sites and dates based on the characteristics of each plan, taking samples, and sending them to a laboratory (accredited laboratory or NRL). They manage the monitoring of results as they come in; in the event of non-compliant results, they have to implement suitable mitigation measures to reduce the risk of consumer exposure and impose sanctions on operators when necessary. Figure 2 illustrates the functional organisation of the system.

Results of the 2014 SCP campaign

In 2014, 25 programmes were implemented, across the different food sectors all along the food chain, from production to marketing, in the field of DGAL's mandate (Table 1). No less than 58,179 samples were collected and approximately 800,000 analytical results were produced. The budget allocated by the DGAL to the implementation of these SCPs totalled approximately €12 million for analytical, sampling and logistical costs alone. The number of inspectors assigned to take samples and monitor planning corresponded to approximately 110 full-time equivalent days worked (FTEW).

In the animal production sector, the large majority of samples were taken on farms and at the slaughterhouse (91%), *versus* 4.5% at the retail stage and 2% at the processing stage. The productions with the highest sampling rates were the livestock and poultry production with 57.1% and 21.9% of samples respectively. The fishery products were in third position with 7.2% of samples. The tested contaminants were primarily anabolic substances, banned or undesirable substances (38.8% of samples) such as chloramphenicol and hormones, and residues of veterinary drugs (28.4% of samples) such as antibiotics and anti-inflammatory drugs. Testing for environmental and industrial contaminants accounted for 12.5% of samples, and testing for biological contaminants (including toxins) accounted for approximately 11.6% of samples.

In the crop production sector, 1525 samples were taken to screen for plant protection product residues. They were taken in the primary production stage, at harvest, primarily from fruits and vegetables, in support of controls among users of these products or otherwise.

Figure 3 shows the breakdown of the samples by class of contaminants and by production.

This breakdown reflects the fact that, in the division of competences between the various administrations in charge of food safety, the DGAL is in charge of primary animal and crop production, and that foods from the "slaughter animals', "poultry" and "fishery products" sectors are the most commonly consumed foods. In this stage of production and in these sectors, unauthorized substances, veterinary drug residues, environmental contaminants and residues of plant protection products are the hazards that require the highest level of vigilance. In 2014, there was an increase in samples for the detection of industrial and environmenta contaminants, which pose a chronic health risk and are of major concern to consumers.

Table 1. The DGAL's surveillance and control plans for the 2014 campaign

Surveillance of the chemical and physical contamination of animal products
Control plan for chemical residues (anabolic substances, banned substances, veterinary medicinal products, pesticides, polychlorinated biphenyls (PCBs), dioxins, trace metals (TMs)) in slaughter animals, poultry, rabbits, game, farmed fish, milk, eggs, honey
Surveillance plan for the contamination of foods of animal origin derived from land animals by certain brominated flame retardants (BFRs)
Surveillance plan for the contamination of foods of animal origin by radionuclides
Surveillance plan for the antimicrobial resistance of certain sentinel and zoonotic bacteria in poultry and swine
Surveillance of the biological contamination of terrestrial animal products
Surveillance plan for the contamination of marinated poultry and pork meat by *Salmonella* spp. in the production stage
Surveillance plan for the contamination of fresh poultry meat by *Salmonella* spp. at the slaughterhouse
Surveillance plan for the contamination of raw-milk cheeses by Shiga toxin-producing *Escherichia coli* (STEC) in the production stage
Surveillance of seafood and freshwater products (excluding aquaculture)
Surveillance plan for phycotoxins and chemical contaminants (TMs, dioxins, PCBs, pesticides, polycyclic aromatic hydrocarbons (PAHs), BFRs) in bivalve molluscs
Surveillance plan for chemical contaminants (TMs, dioxins, PCBs, pesticides, PAHs, BFRs) from the aquatic environment in fishery products
Surveillance plan for veterinary medicinal products in farmed fishery products placed on the market
Exploratory plan for the detection of methylmercury in fish placed on the market
Surveillance plan for histamine in fishery products
Surveillance plan for *Escherichia coli* contamination in live bivalve molluscs
Surveillance of animal feed
Surveillance plan and control plan for undesirable substances and products in raw materials and compound animal feed
Surveillance of primary crop production
Control plan for residues of plant protection products in primary plant products
Surveillance plan for residues of plant protection products in primary plant products
Surveillance of imported products at border points
Surveillance plan for food and feed of animal origin originating from third countries
Surveillance plan for the contamination of animal feed of non-animal origin originating from third countries

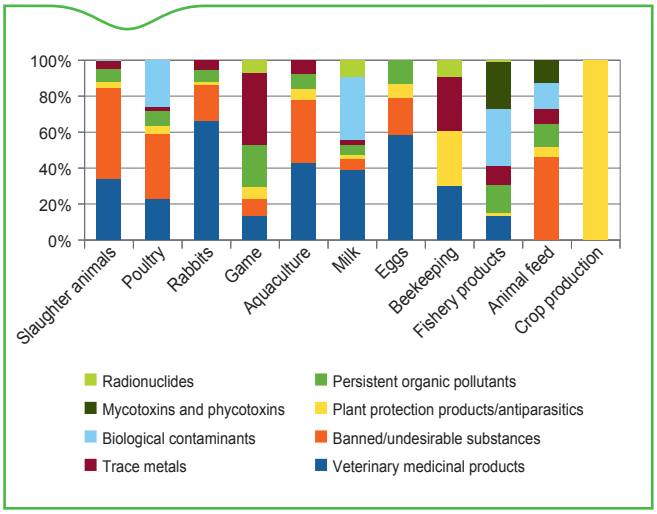

Figure 3. Breakdown of samples by class of contaminants and by sector in 2014

Table 2. SCP non-compliance rates for the 2014 campaign

Description of the plan: contaminant/product	S	C	Monitored contaminant or class of contaminants	Monitored products	Non-compliance rate (95CI)*
Chemical residues/slaughter animals		X	Anabolic substances, banned substances, veterinary medicinal products, environmental contaminants	Cattle, sheep/goats, swine, equines	0.1% (0.1-0.2)
Chemical residues/poultry		X		Spent hens/roosters, broiler chickens/ cockerels, turkeys, other	0.0% (0.0-0.1)
Chemical residues/rabbits		X		Meat rabbits	0.0% (0.-0.8)
Chemical residues/game		X		Small game birds, large game animals	0.3% (0.1-1.6)
Chemical residues/milk		X		Whole raw cow's, sheep's, goat's milk	0.1% (0.0-0.4)
Chemical residues/eggs		X		Chicken eggs, quail eggs	0.4% (0.1-1.0)
Chemical residues/farmed fish		X		Sea and freshwater (lakes, ponds) fish	0.2% (0.0-1.1)
Chemical residues/honey		X		Local honey	0.7% (0.1-3.8)
Animal feed	X		Chemical and microbiological contaminants (excluding PAPs)	Animal feed of animal and plant origin	0.1% (0.0-0.5)
		X	PAPs		0.3% (0.1-1.0))
Histamine/fishery products	X		Histamine (+ 3 biogenic amines)	Histaminogenic fish	0.4% (0.1-1.3)
Phycotoxins/bivalve molluscs	X		Lipophilic toxins, PSP and ASP	Mussels, oysters, scallops	0.4% (0.2-1.1)
Escherichia coli/live bivalve molluscs	X		*Escherichia coli*	French and imported bivalve molluscs	3.8% (2.4-5.8)
Persistent organic pollutants/fishery products (excluding aquaculture)	X		Dioxins, DL-PCBs, NDL-PCBs, BFRs, PAHs	Sea and freshwater fish, shellfish, cephalopods, molluscs	1.0% (0.4-2.4)
Trace metals/fishery products (excluding aquaculture)	X		Cadmium, lead, mercury	Sea and freshwater fish, shellfish, cephalopods, molluscs	1.8% (0.9-3.5)
Escherichia coli STEC/raw-milk cheeses	X		*E. coli* STEC	Raw-milk cheese from cows and small ruminants	0.2% (0.1-0.7)
Salmonella spp/marinated meat	X		*Salmonella* spp	Marinated poultry and pork meat	3.9% (1.8-8.2)
Residues of plant protection products/primary crop production		X	Plant protection products	Fruits and vegetables	5.7% (4.2-7.6)
Residues of plant protection products/primary crop production	X		Plant protection products	Cereals, leafy vegetables, storage cereals	2.8% (1.9-4.2)
Products of animal origin presented at border inspection points	X		Chemical and biological contaminants	Products of animal origin (food and feed)	0.4% (0.2-0.8)
Animal feed of non-animal origin, presented at designated entry points	X		Chemical and biological contaminants	Plants, minerals, additives, pre-mixes	0.0% (0.0-3.4)

S = surveillance plan; C = control plan; 95CI = 95% confidence interval
* calculated with OpenEpi software (http://www.openepi.com/Proportion/Proportion.htm)

As in previous years, contamination levels and non-compliance rates in food and feed, assessed against the regulatory thresholds, were low. Table 2 shows SCP non-compliance rates for the 2014 campaign.

In animal production, non-compliance rates ranged from 0.0% to 3.8%. The surveillance of fresh poultry meat at the slaughterhouse showed 14% prevalence for *Salmonella*. Contamination thus remained very high, in particular in the "fattening turkeys" production, but with a very pronounced slaughterhouse effect (most of the strains were isolated in a limited number of slaughterhouses). CP non-compliance rates were generally higher than those for SPs since they targeted at-risk products. Their value thus depended on the level of contamination and the definition and fulfilment of targeting criteria.

In crop production, non-compliance rates were 2.8% for the SP and 5.6% for the CP regarding residues of plant protection products. Once again, the difference might be due to the different sampling strategies, which is risk-based for the CP. The results of this CP were lower than those from 2013 (8.8%) but this decrease is not statistically significant.

Analysis of the surveillance system

In 2014, the surveillance system demonstrated its effectiveness, with the coordinated management of approximately 60,000 samples in a framework limited by regulatory and methodological constraints, thanks to harmonised procedures shared by the various stakeholders. While the system's main objective was the surveillance of food-borne human health hazards, it also served as an operational and functional framework for the deployment of plans outside this scope (testing for contaminants in pet food, exploratory plan for the detection of methylmercury in fish), in order to optimise resources.

The allocated budgets and the very high sampling rate demonstrated the significance of this mission for the DGAL and its decentralised services. Central government officials were heavily involved in the development of relevant surveillance protocols, likewise, officials from decentralised services were committed to ensuring compliance with the planning. Data were evaluated at various levels. They were used by the authorities to implement immediate risk-mitigation measures in the event of non-compliant results, to

communicate about their actions to professionals and consumers[1] and to promote French products to commercial partners. They were added to contamination databases, which were used by the scientific community to undertake research work and in particular by risk assessors for consumer exposure studies.

However, a number of points could be improved to optimise the system.

For example, the monitored contaminant/product combinations are currently chosen on the basis of sectoral prioritisation, by production or by class of contaminants. While there are a number of collaborative actions with other public and private surveillance programme managers, there is no coordinated overall prioritisation for refining the scope of surveillance covered by the SCP system and ensuring optimal coverage of the food chain in terms of surveillance.

Furthermore, the development and implementation of plans are subject to regulatory provisions that are more or less binding depending on the programme and are often not harmonised from one production or class of contaminants to the next. This complicates the coordination of the system and the implementation of surveillance protocols (difficulties accessing certain matrices, complying with the sampling strategy, etc.) and is not always in line with national concerns (requirement to monitor certain non-priority contaminant/product combinations in France). Some surveillance protocols lack a scientific basis for the definition of the sampling plan (sample size, sampling methodology, etc.), which can cause biases in the interpretation of results; implementation constraints are sometimes poorly anticipated, which leads to implementation problems in the field and therefore failures in terms of compliance with the sampling and data collection requirements. Ongoing and end-of-campaign feedback provided to the system's various stakeholders (especially decentralised services and accredited laboratories) is not specific to each group of stakeholders and does not secure their full support for the system's procedures. Lastly, the quality of data related to samples and analytical results still needs to be improved, in order to optimise their analyse and use, by the DGAL to implement suitable mitigation measures and coordinate the system, and also by the scientific community at French and European levels for contamination and exposure studies.

Expected improvements to the system

Despite the solid "quantitative" performance of the SCP system, the results of the 2014 campaign have identified areas for improvement to optimise the role of SCPs in the overall food safety scheme, in particular in terms of data quality. Various actions, in all stages of the surveillance system, could contribute to this improvement in data:

i) more robust prioritisation of the contaminant/product combinations to be monitored and improvement of the epidemiological and operational quality of surveillance protocols, ii) enhanced coordination of the system to increase the participation of field stakeholders and thus improve compliance with requirements relating to planning, sampling strategies and data collection, iii) provision to the authorities and NRLs of a tool for analysing the quality of data entry (automated indicators) and facilitating the activities the networks they supervise. Moreover, the system should undergo robust and ongoing evaluations, in order to assess its performance and cost-effectiveness.

Some areas for improvement have already been translated into concrete actions. In the framework of the action plan that followed the report on the mission for the assessment of the food safety policy in France, conducted at the request of CIMAP (Interministerial Committee for the Modernisation of Public Action), ANSES was asked to investigate two issues: the optimisation of the official surveillance of chemical contaminants in foods, and the prioritisation of microbiological and chemical hazards to be targeted for official controls to be targeted. As part of the establishment of the Food Chain Surveillance Platform[2], a project is under way to improve the quality of the data produced by SCPs, founded on the coordination of the surveillance system and the monitoring of automated indicators.

1. Annual surveillance campaign results are available on the Ministry of Agriculture's website at the following address: http://agriculture.gouv.fr/plans-de-surveillance-et-de-contrôle.

2. Under Order 2015-1242 of 7 October 2015 on the organisation of surveillance in the fields of animal health, plant health and food, pursuant to the French Act on the future of agriculture, food and forests (LAAAF).

Short item. Guide to the definition of epidemiological surveillance requirements in the food safety sector

Brève. Guide d'aide à la définition des besoins en matière de surveillance épidémiologique dans le secteur de la sécurité sanitaire des aliments

Corinne Danan (1) (corinne.danan@agriculture.gouv.fr), Isabelle Berta-Vanrullen (2), Anne Bronner (3), Didier Calavas (4), Pascal Hendrikx (5)

(1) Directorate General for Food, Food Department, Support Office for Food Chain Surveillance, Paris, France
(2) ANSES, Laboratory Affairs Department, Unit for the Coordination and Support of Surveillance, Maisons-Alfort, France
(3) Directorate General for Food, Department of Health Actions for Primary Production, Paris, France
(4) ANSES, Lyon Laboratory, Epidemiology Unit, Lyon, France
(5) ANSES, Laboratory Affairs Department, Unit for the Coordination and Support of Surveillance, Lyon, France

This short item supplements the framework article by Danan and Calavas (Reflections on food chain surveillance) published in this issue. It specifies the scope of surveillance in terms of risk assessment and management activities in the area of food safety and intends to help all stakeholders in the surveillance system precisely position themselves in the process.

General objectives of food chain surveillance

Epidemiological surveillance refers to a set of activities that provide confirmed reliable information about the status of and changes in contamination in a stage of the food chain. This information is intended to help risk managers and assessors scale their actions.

The activities cover the ongoing collection of data, their analysis and interpretation, the coordination of surveillance schemes with various stakeholders (see below), and the transmission of information to the authorities in charge of implementing prevention and monitoring actions. Data collection can be organised at regular intervals using a methodology enabling its comparison. Data interpretation consists in assessing levels of contamination, which includes detecting emerging contamination, making assumptions about risk factors in contamination, and/or assessing the impact of implemented control or prevention measures.

Depending on expectations, surveillance schemes adhere to specific protocols (choice of matrix, type of sampling, sampling plan, sampling frequency, analytical method, information system, etc.).

To ensure the proper functioning of the entire process, the stakeholders involved in surveillance activities should be informed of the purpose of the missions of risk managers and assessors. The table opposite illustrates the synergies between these various activities.

Surveillance objectives	Risk management (M) or assessment (A) actions that may be taken on the basis of surveillance results
Define the level of contaminant or pathogen X in matrix Y (the definition of the matrix is related to the stage of the food chain) and its change over time. This objective applies to known and detected contaminants and pathogens in standard production situations, most often at low levels of contamination. Note: this objective is in particular associated with a process for assessing the impact of one or more control measures and verifying their effectiveness	M: Adapt current control measures if necessary M: Put into place new control measures by identifying the most relevant stage of the food chain, including recommendations for consumers or preventive measures M: Define or revise a regulatory criterion (number of units of a sample (n), tolerance (c), limit of detection, stage and nature of sampling, method) M: Scale a sampling plan (procedures, sampling frequency) tailored to the surveillance objective
Detect the emergence of a rare or exotic contaminant or pathogen	A: Assess related health risks, in a new context M: Define ad hoc monitoring, prevention or communication measures
Characterise contamination	A: Evaluate flows of contaminants and thus better understand attributable sources of human cases M: Identify sources of contamination as quickly as possible to take action and reduce consumer exposure

Food chain surveillance process

SURVEILLANCE STAKEHOLDERS*	Operators	DDecPP[1] inspectors, Veterinarians, Technicians, Operators	Private or public analytical laboratories	DDecPP, DRAAF[2], Veterinarians, Local interprofessional associations	NRL[3], NRC[4]	DGAL, DGCCRF, ANSES, Interprofessional associations	NRL, Actia[5], Acta[6], Veterinarians, Interprofessional associations	Heads of surveillance schemes
MISSIONS	Provision of data	Sampling	1st-line analyses	Local coordination	Reference analyses	Leadership National coordination	Technical support	Management
ACTIONS	Collect, report (own-checks)	Sample	Detect	Coordinate locally	Confirm	Plan	Develop protocols	Recommend
			Quantify	Undertake preventive actions	Characterise contamination	Harmonise	Develop analytical methods	Guide
				Approve	Coordinate networks of laboratories	Coordinate	Analyse data	Classify
				Transmit	Contribute to improving data quality	Raise awareness		Approve
				Monitor		Train		Communicate to all organisations involved in the health issue, in particular those in charge of prevention and monitoring actions
						Interpret		
						Communicate		
						Check data quality		

* This table lists only actions and stages relating to epidemiological surveillance (some stakeholders are both surveillance stakeholders and risk managers or assessors).

1. Departmental Directorate for Protection of the Population
2. Departmental Directorate for Food, Agriculture and Forestry
3. National Reference Laboratory
4. National Reference Centre
5. French Technical Coordination Association for the Food Industry
6. French Technical Coordination Association for Agriculture

Surveillance of **persistent organic pollutants** in foodstuffs of animal origin in 2014

Jean-Cédric Reninger (1) (jean-cedric.reninger@anses.fr), Sandra Favret (1), Laurent Noël (2)
(1) ANSES, Risk Assessment Department, Food Observatory Unit, Maisons-Alfort, France
(2) French Directorate General for Food, Coordination Office for Chemical and Physical Contaminants, Paris, France

Abstract
In France, foodstuffs are regularly monitored in order to track contamination levels in French and imported products. This monitoring makes it possible to study trends and ensures that the maximum limits defined in the regulations are not exceeded. This article deals with the surveillance system managed by the Directorate General for Food (DGAL) in 2014 concerning persistent organic pollutants (POPs) (dioxins and PCBs, brominated flame retardants (BFRs) and polycyclic aromatic hydrocarbons (PAHs)) in foodstuffs of animal origin. A comparison with data from 2013 is also proposed.

In 2014, various programmes were implemented to monitor levels of POPs in animal foodstuffs (mainly set by Commission Regulation (EC) No 1881/2006), for a total of 4,932 samples taken, the vast majority of which involved DL-PCBs (1,954 samples) and NDL-PCBs (2,666 samples). This number of samples was higher than in 2013 (2,697 samples), but for these two years, conclusions were similar: observed contamination levels were low and the maximum limits were seldom exceeded (at a rate of less than 1%). Exceeded limits involved only dioxins and PCBs (DL and NDL) in fish meat. The alert thresholds (defined at national level) were also exceeded for the same compounds in game meat.

However, the conclusion should be confirmed in light of future sampling, due to small sample numbers and/or changes in the sampled matrices (foodstuffs of different natures, with different places of origin, etc. from one year to another).

Keywords
Persistent organic pollutants, Surveillance programmes, Monitoring programmes, Polychlorinated biphenyls, Dioxins, Polycyclic aromatic hydrocarbons, Brominated flame retardants

Résumé
Surveillance des polluants organiques persistants dans les denrées alimentaires d'origine animale en 2014
En France, les denrées alimentaires sont régulièrement contrôlées dans le but de suivre les niveaux de contamination dans les productions nationales et importées. Cette surveillance permet de suivre des tendances et de s'assurer du respect des teneurs maximales imposées par la réglementation. Cet article s'intéresse au dispositif de surveillance, piloté par la direction générale de l'Alimentation en 2014, relatif aux polluants organiques persistants (POP): dioxines et polychlorobiphényles (PCB), retardateurs de flammes bromés (RFB) et hydrocarbures aromatiques poly- cycliques (HAP) dans les denrées animales. Une mise en perspective par rapport aux résultats obtenus en 2013 est également proposée.
En 2014, plusieurs plans ont été mis en œuvre pour le suivi des teneurs en POP dans les denrées animales (principalement fixées par le règlement CE n°1881/2006), soit 4932 prélèvements dont une grande majorité concernant les PCB dioxin-like (DL) et dioxines (1 954 prélèvements), ainsi que les PCB non-dioxine like (NDL) (2 666 prélèvements). Ce nombre de prélèvements est supérieur à celui de 2013 (2 697 prélèvements) mais pour ces deux années le constat est identique: les niveaux observés de contamination restent faibles et les non-conformités sont peu fréquentes (moins de 1 %). Ces non-conformités concernent exclusivement des dioxines et PCB (DL ou NDL) dans la chair de poissons. On peut également observer des niveaux de contamination supérieurs aux seuils d'alerte fixés au niveau national pour ces mêmes composés dans la viande de gibier. Toutefois, certaines des conclusions devront être précisées à la faveur des prélèvements qui seront effectués à l'avenir, du fait des faibles nombres de prélèvements et/ou des changements dans les matrices prélevées (denrées de natures, de lieux d'origine, etc., différents d'une année à l'autre).

Mots-clés
Polluants organiques persistants, plans de surveillance, plans de contrôle, polychlorobiphényles, dioxines, hydrocarbures aromatiques polycycliques, retardateurs de flamme bromés

Every year, government administrations, including the Directorate General for Food (DGAL), the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF), and the Directorate General for Health (DGS), implement surveillance and control programmes (PSPC) in order to monitor the levels of chemical contaminants in food.

These PSPC involve a wide range of different substances such as inorganic and organic contaminants, veterinary medicinal products, pesticide residues and mycotoxins, and concern all the food products available on the market in France[1].

This review focuses more specifically on the PSPC implemented by the DGAL for the year 2014 that aim to monitor contamination levels of persistent organic pollutants (POPs) in food matrixes of animal origin. These compounds, which can be found in the environment and result mainly from human activity, whether industrial or domestic, are persistent, bioaccumulative and mobile. The scientific community has defined toxicity reference values for the compounds that have known toxic effects in humans.

1. See the article by Marion Bordier, "The surveillance system for food-chain contaminants managed by the DGAL: report on the 2014 surveillance and control plan campaign", in this edition

Persistent organic pollutants under monitoring

The POPs examined in this review are the following:

- brominated flame retardants (BFRs),
- dioxins and furans (PCDDs/PCDFs),
- polychlorinated biphenyls (PCBs), including dioxin-like PCBs (DL-PCBs), which have a toxic effect related to the same mechanism as PCDDs and PCDFs, and non-dioxin-like PCBs (or NDL-PCBs), which have a toxic effect that is different from dioxins,
- polycyclic aromatic hydrocarbons (PAHs).

Brominated flame retardants

BFRs are chemical substances included in a wide range of products and materials to reduce their flammability, from plastics and textiles to electronic equipment. The most commonly used are polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), tetrabromobisphenol-A (TBBPA), and polybrominated biphenyls (PBBs). Since some of these BFRs have been identified as having toxic properties, use of several of these compounds has been prohibited for a number of years. This is the case specifically for PBBs and almost all PBDEs, with the exception of decabromodiphenyl ether (BDE-209), which is still authorised for use. Due to their persistence in the environment, these BFRs are still to be found in the environment, even if they are no longer used. This is why the programmes implemented in 2014 covered detection of eight PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209) as well as three PBBs (BB-52, BB-101 and BB-153) in every sample, in addition to three forms of HBCD (alpha, beta and gamma), along with TBBPA.

Box. Surveillance of persistent organic pollutants in foods of animal origin carried out by the DGAL in 2014

Objectives

Monitoring of contamination levels for dioxins, dioxin-like polychlorinated biphenyls (DL-PCBs) and non-dioxin-like polychlorinated biphenyls (NDL-PCBs), and brominated flame retardants (BFRs) in foods of animal origin.

Programming framework

Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products.

Decision 97/747/EC fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products.

Decision 98/179/EC laying down detailed rules on official sampling for the monitoring of certain substances and residues thereof in live animals and animal products.

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

Commission Regulation (EU) No 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs.

Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.

The methods of sampling for monitoring BFRs are not defined in regulations but were established at the national level with the National Reference Laboratory responsible for these substances.

Protocol

POP contamination levels in foods of animal origin were assessed on the basis of various programmes:

- chemical residue monitoring programmes (including dioxins and PCBs) in animals for slaughter, poultry, rabbits, game,
- farmed fish, as well as milk and eggs,
- a surveillance programmes for certain BFRs in foodstuffs from terrestrial animals,
- a surveillance programmes for dioxins, PCBs, PAHs, and BFRs in
- bivalve molluscs,
- a surveillance programmes for chemical contaminants, including dioxins, PCBs, PAHs, and BFRs, in fishery products.

Production areas of interest: animals for slaughter (cattle, sheep, goats, and horses), poultry, rabbits, game, farmed fish, eggs, milk, and fishery products (fish, shellfish, cephalopods, and bivalve molluscs).

Food chain stage: primary production or first processing. All distribution channels for fishery products (hyper- and supermarkets, fishmongers, itinerant markets, etc.).

Analytical methods: official methods by gas chromatography coupled with high-resolution mass spectrometry or liquid chromatography coupled with tandem mass spectrometry.

Non-compliant sample: a sample is considered non-compliant when the level of a contaminant quantified in the sample exceeds the regulatory threshold given the expanded measurement uncertainty (k = 2) associated with the analytical result.

Dioxins and furans

The dioxin (polychlorinated dibenzo-p-dioxins (PCDDs)) and furan (polychlorinated dibenzofurans (PCDF groups)) include 75 and 135 different molecules, respectively. Among these multiple congeners, only those that are considered the most toxic are regulated, i.e. 7 PCDDs and 10 PCDFs.

Polychlorinated biphenyls

In addition to the dioxins, twelve congeners of the polychlorinated biphenyl (PCB) group are characterised by toxic properties similar to those of dioxins (DL-PCBs) and are also tested for during analyses.

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a group of more than one hundred organic compounds with at least two aromatic rings. European regulations were initially based solely on benzo(a)pyrene levels, but an update in force from 2012 (Commission Regulation (EC) No 1881/2006) also established maximum levels for the sum of four PAHs: benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene, and chrysene.

Surveillance and control programmes implemented in 2014

The transfer of the various compounds in these POP groups from the environment (soil, sediment, suspended matter) to living organisms leads to their accumulation in animal fats. This lipophilic property underlies their accumulation in foods of animal origin. The PSPC implemented in 2014 by the DGAL involved the following foods:

- for terrestrial animals: meats, offal, fats, milk, and eggs,
- for seafood and freshwater products: fish meat, shellfish, cephalopods, and bivalve molluscs.

Samples are taken from farmed animals (animals for slaughter, farmed fish stocks, etc.) and from wild animals (game, fishery products, etc.).

The data collected for 2014 are also compared with equivalent data from the previous year to highlight any differences in contamination levels between these two years.

Materials and methods

Sampling and analyses

Samples are taken randomly for the surveillance programmes, i.e. there are no defined targeting criteria, while samples for monitoring programmes target foods from production sites in areas that are likely to be contaminated (based on the IREP[2] and BASOL[3] databases, among others). However, it is possible that targeting cannot be defined at the time of sampling. The observed contamination levels are therefore based on both randomly obtained samples and targeted samples, including on occasion within a single plan.

Implementation of the programme requires input from various different stakeholders. The DGAL determines a number of samples to be collected by region, generally on the basis of production levels. Each region then divides this number up among its various *départements*, which carry out sampling *via* decentralised services. The distribution among *départements* can follow various criteria on the basis of production volumes or number of production sites for instance, or be organised numerically by simply dividing the number of samples among the *départements*.

2. IREP: French register of pollutant emissions.
3. BASOL: database on polluted sites or contaminated land.

The analyses are carried out by laboratories accredited[4] by the Ministry of Agriculture, Food and Forestry to perform analyses, and by the Laboratory for the Study of Residues and Contaminants in Food (Laberca), National Reference Laboratory (NRL) for certain specific plans.

Censored data management

The results presented in this review are based on the upper bound hypothesis defined by the World Health Organization (WHO, 1995). This hypothesis results in processing of censored data[5] as follows: when the amount of substance is lower than the limit of detection (LOD), the amount is considered equal to the LOD. Likewise, when the amount of substance is lower than the limit of quantification (LOQ), the amount is considered equal to the LOQ. Quantified values are however retained as is.

Calculation of the sums in toxic equivalents (TEQ) for dioxins, furans, and PCBs

The overall concentration of dioxins and DL-PCBs in a sample is characterised by the sum of the mixture of the various congeners. Since dioxins and DL-PCBs each have a specific degree of toxicity, toxic equivalency factors (TEFs) have been defined in relation to the most toxic congener: 2,3,7,8-TCDD, also called the Seveso dioxin (Martin van den Berg *et al.*, 2006). This weighting coefficient indicates the degree of toxicity in relation to this reference compound, which was attributed the value 1. The product of "TEF x congener concentration" is used to calculate a toxic equivalent (TEQ) for each compound. The toxic equivalents of all the constituents of the sample mixture are then added together and define, in TEQ, the relative toxicity of the mixture of this sample.

Regulatory compliance

For control purposes, the results of the analyses carried out in the programmes of interest are compared with the maximum limits (MLs) established in the regulations or with nationally determined thresholds that apply to certain analyte/matrix pairs for which MLs have not been defined. In the second case, we can use the term alert threshold because these thresholds have no regulatory value.

The regulatory limits for dioxins, PCBs, and PAHs in food of animal origin are defined in Commission Regulation (EC) No 1881/2006. From a regulatory perspective, PCB and dioxin levels have not been defined for game. In this area, the DGAL has defined national alert thresholds using, as a reference, MLs for slaughter animals or poultry that are the closest to the game species in question. In this way, an alert threshold equal to the ML for swine was for example used for wild boars, and an alert threshold equal to the ML for poultry was retained for game birds.

There is no regulatory limit for BFRs. There is, nonetheless, a European surveillance recommendation (Commission Recommendation 2014/118/EU of 3 March 2014 on the monitoring of traces of brominated flame retardants in food). This recommendation calls for the Member States to monitor the presence of BFRs in different foodstuffs in order to reflect consumption habits and thus better characterise consumer exposure.

Concerning PAHs, it is important to be aware that new levels for benzo(a)pyrene and the sum of the four PAHs established in Regulation (EC) No 1881/2006 for smoked fish apply as of 1 September 2014.

4. List available at: http://agriculture.gouv.fr/laboratoires-agrees-et-reconnus-methodes-officielles-en-alimentation.
5. Tests with results below the analytical limit.

Results and Discussion

The surveillance programme implemented for 2014 concerned 4932 samples, including 1954 that were intended for the detection of DL-PCBs and dioxins, 2666 for NDL-PCBs, 121 for PAHs, and 191 for the quantification of BFRs.

Every year, the results of these analyses are communicated to ANSES by the control authorities as part of a data exchange agreement signed by the administrations and the Agency.

Brominated flame retardants

Concerning BFRs, it is not appropriate to define non-compliance levels given that there is no regulatory threshold applicable to these compounds.

The completion rate, i.e. the ratio of the number of samples planned to the number of samples effectively collected, was 97.4% for the 2014 BFR programme (5 planned samples were not collected). This rate is very similar to that achieved in 2013 (100%).

Table 1 presents the results for BFRs according to the various matrix groups. The contamination means are expressed in ng/g of fat, except for fishery products for which the results are expressed in ng/g of fresh product.

It is important to note that these results are not comparable between products produced on land and seafood because of a different denominator (g of fat *vs* g of wet product).

Table 1. Number of samples and contamination means (as per the upper bound hypothesis), in ng/g of fat or wet weight for fishery products, for the 2013 and 2014 PSPC carried out by the DGAL regarding brominated flame retardants (PBDE, PBB, HBCD, and TBBPA)

Matrix	Number of samples		Mean of sums of 8 PBDEs (ng/g)		Mean of sums of 3 PBBs (ng/g)		Mean of sums of 3 HBCDs (ng/g)		Mean for TBBPA (ng/g)	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Beef meat	10	10	0.61	5.63	0.02	0.04	0.36	0.42	0.04	0.02
Pork meat	10	9	0.28	0.93	0.01	0.01	0.13	1.56	0.03	0.03
Mutton/lamb meat	9	9	2.05	1.04	0.01	0.01	0.12	0.36	0.04	0.02
Sheep liver	10	9	0.74	1.03	0.03	0.04	0.12	0.27	0.07	0.03
Rabbit meat	4	6	1.06	5.75	0.03	0.02	0.10	0.12	0.04	0.02
Poultry meat	10	10	1.73	1.05	0.02	0.01	1.57	0.70	0.21	0.07
Eggs	20	19	0.80	0.35	0.01	0.01	0.38	0.10	0.01	0.01
Milk	25	25	0.18	0.50	0.01	0.01	0.06	0.08	0.03	0.01
Shellfish	6	5	0.02	0.01	0.00	0.00	0.00	0.01	0.00	0.00
Molluscs	52	47	0.08	0.07	0.00	0.00	0.09	0.07	0.00	0.00
Cephalopods	1	2	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00
Fish	37	40	0.26	0.17	0.01	0.00	0.08	0.10	0.03	0.00
Game meat	10	0	1.04	-	0.02	-	0.17	-	0.09	-
Total	**204**	**191**								

For the products from land animals, the maximum values observed for beef and rabbit meat led to a significant increase in the mean for the eight PBDEs *versus* 2013. However, the low number of samples in the land animal sectors (less than or equal to 10) implies low accuracy of these results. This low number of samples, associated with a high maximum value, is also the cause of the increased mean of the sums of the three HBCDs in pork between 2013 and 2014.

Conversely, between 2013 and 2014, a decrease was observed in mean contamination regarding the sum of the three HBCDs in poultry meat. Unfortunately, since this decrease was again based on a low number of food samples (only 10 analyses carried out each year), it is difficult to draw conclusions.

Table 2. Number of samples, contamination means (as per the upper bound hypothesis), in pg TEQ/g of fat or wet weight for fishery products, and number of non-compliant samples for the 2013 and 2014 PSPC carried out by the DGAL regarding dioxins and DL-PCBs

Matrix	Number of samples		Mean of sums of dioxins and DL-PCBs (pg TEQ/g)		Number of non-compliant samples	
	2013	2014	2013	2014	2013	2014
Poultry meat	53	478	0.43	0.21	0	0
Rabbit meat	7	10	0.47	0.35	0	0
Eggs	36	20	0.50	0.57	0	0
Milk	43	54	0.91	1.00	0	0
Beef fat	61	195	0.91	0.78	0	0
Pork fat	50	575	0.15	0.12	0	1
Sheep/goat fat	24	99	0.71	0.60	0	0
Sheep/goat liver	0	99	-	0.28	-	0
Game meat	12	45	2.38	1.35	1*	8*
Farmed fish	8	10	0.33	0.29	0	0
Wild fish	205	184	1.04	0.77	4	3
Shellfish	29	31	0.27	0.11	0	0
Cephalopods	7	4	0.04	0.21	0	0
Molluscs	150	150	0.71	0.59	0	0
Total	**685**	**1,954**				

* above national alert threshold (non-regulatory value).

Table 3. Number of samples, contamination means (as per the upper bound hypothesis), in ng TEQ/g of fat or wet weight for fishery products, and number of non-compliant samples for the 2013 and 2014 PSPC carried out by the DGAL regarding NDL-PCBs

Matrix	Number of samples		Mean of sums of NDL-PCBs (ng TEQ/g)		Number of non-compliant samples	
	2013	2014	2013	2014	2013	2014
Poultry meat	227	477	3.84	2.69	0	0
Rabbit meat	13	10	4.76	4.68	-	-
Eggs	97	90	3.02	3.56	0	0
Milk	80	81	3.91	4.25	0	0
Beef fat	364	594	4.09	3.17	0	0
Pork fat	329	576	2.20	2.20	0	0
Sheep/goat fat	95	298	4.41	2.55	0	0
Sheep/goat liver (fat weight)	0	99	-	9.31	-	0
Sheep/goat liver (wet weight)			-	0.51	-	0
Game meat	32	42	20.83	12.59	2*	1*
Farmed fish	32	30	3.35	4.53	0	0
Wild fish	207	184	9.96	5.87	2	2
Shellfish	29	31	0.91	0.20	0	0
Cephalopods	7	4	0.44	1.11	0	0
Molluscs	150	150	3.22	2.58	0	0
Total	**1,662**	**2,666**				

* above national alert threshold (non-regulatory value).

Dioxins (PCDDs/PCDFs) and PCBs

Completion rates were very similar for 2013 and 2014, with respectively 98.9% and 96.8% of planned samples effectively collected.

Table 2 presents the results for the sum TEQ for dioxins and DL-PCBs according to the various matrix groups. The observed levels are expressed in pg TEQ/g of fat for all the matrices, except for fishery products with results expressed per wet weight.

Given the specific regulatory context requiring a monitoring rate representative of the national production level (Council Directive 96/23/EC), the number of samples was essentially stable from one year to the next for most of the matrices. However, the number of samples increased in 2014 for fats (beef, pork, and sheep/goat meat), and for poultry and game muscle. The sheep liver matrix was also added. These changes led to a total of 1954 samples in 2014, *versus* only 685 in 2013.

The contamination levels observed in 2014 remain low and were overall about the same as those recorded in 2013. Non-compliance rates were below 1%, with 0.60% for 2013 and 0.22% for 2014, respectively.

For both years, game meat was the matrix with the highest dioxin and DL-PCB levels. The fall in the mean level between these two years should, however, be interpreted with caution given that the number of samples was multiplied by three and that the species sampled within the game group may have changed. Moreover, the alert thresholds were exceeded on more occasions in 2014 compared to 2013. In 2013 and 2014, these alerts concerned respectively one ostrich sample, and eight wild boar samples.

For wild fish, the three non-compliant samples in 2014 were two mackerels (Atlantic Ocean for one and non-specified area for the other), and one tuna (Mediterranean). In 2013, the alert thresholds were exceeded four times. They involved two salmon samples (Baltic Sea), one tuna (Mediterranean), and one eel (from the Netherlands).

Concerning NDL-PCBs, of all the congeners, six represented about half of the total amount of PCBs contained in food (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180). Since 2011, the sum of these six PCBs has been regulated because it is considered a good indicator of NDL-PCB contamination. As these congeners do not have the same toxicity characteristics as dioxins, the calculated sum is not weighted using a toxicity equivalence coefficient.

Between 2013 and 2014, the completion rate was stable at 97.2% despite a much higher number of samples in 2014 (2666 samples *versus* 1662 in 2013).

Table 3 presents the results for NDL-PCBs according to the various matrix groups. The observed levels are expressed in ng TEQ/g of fat for all the matrices, except for fishery products where results are expressed per fresh weight. Two separate lines are indicated for sheep and goat livers: one with the result expressed in ng TEQ/g of fat and the other with the result expressed in ng TEQ/g of fresh weight. This is explained by the change in the definition of maximum levels that occurred in 2014 *via* Regulation (EC) No 1881/2006 (change from maximum level of 40 ng TEQ/g of fat to 3.0 ng TEQ/g of wet weight).

The contamination levels observed in 2014 remain low and were comparable to the levels reported in 2013. The non-compliant rate calculated for 2014 was very close to that for 2013 (0.08% and 0.12%, respectively).

Table 4. Number of samples, contamination means (as per the upper bound hypothesis), in µg/g of wet weight, and number of non-compliant samples for the 2013 and 2014 PSPC carried out by the DGAL regarding PAHs

Matrix	Number of samples		Mean of benzo(a) pyrene (µg/g)		Mean of sums of four PAHs (µg/g)		Number of non-compliant samples for benzo(a) pyrene		Number of non-compliant samples for the sum of four PAHs	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Smoked fish	75	53	0.21	0.22	0.70	0.55	0	0	0	0
Bivalve molluscs	71	68	0.30	0.33	2.95	2.41	0	0	0	0
Total	**146**	**121**								

In 2014, a sample of muscle from a wild boar exceeded the alert threshold above which an investigation is initiated to identify the source of contamination in the environment. In 2013, the two cases in which the alert threshold was exceeded were related to farmed ostrich and wild boar muscle samples. Like in the case of dioxins and DL-PCBs, the decrease in the NDL-PCB level observed in game meat between 2013 and 2014 could be related to sampling (different sampled species from one year to the next) and not to an actual decrease in contamination. This trend would therefore need to be confirmed.

The two non-compliant fish samples in 2014 were the same samples of mackerel (Atlantic Ocean and non-specified area) as the non-compliant samples for dioxins and DL-PCBs. For 2013, one tuna (Mediterranean) and one eel (from the Netherlands) were non-compliant. These were the same samples that were already non-compliant for dioxin and DL-PCB levels.

Polycyclic aromatic hydrocarbons

For 2013 and 2014, the completion rates were 100% and 99%, with only one sample not being collected in 2014. We can nonetheless observe that the number of samples decreased between these two years, particularly concerning smoked fish (about 20 fewer samples).

Table 4 presents the results for PAHs according to the various matrix groups. The levels observed are expressed in µg/kg of wet weight for all the matrixes.

The contamination levels were essentially the same between 2013 and 2014. For both these years, no samples exceeded the compliance thresholds for PAHs. During 2014, the regulatory thresholds for smoked fish were lowered for benzo(a)pyrene (2 µg/kg instead of 5 µg/kg) and for the sum of the four PAHs (12 µg/kg instead of 30 µg/kg), without this leading to any non-compliance.

Conclusions and outlook

For all the POPs monitored in the PSPC, the observed contamination levels remain low overall and are below the thresholds established either by European regulations (maximum limits), or nationally by the DGAL (alert

thresholds). In the case of maximum limits, the observed cases of non-compliance exclusively involved exceeded levels for dioxin and PCBs (DL or NDL) in fish meat. These same compounds were also implicated in the exceeded alert thresholds established nationally for game meat. Nonetheless, joint efforts on sources of contamination, particularly incinerators, and food controls have enabled a significant reduction in consumer exposure to dioxins and PCBs (ANSES, 2011). The new regulations concerning NDL-PCBs have helped reinforce this programme.

In 2015, the sampling plan regarding dioxins, PCBs, and PAHs was renewed practically in line with 2014, except for fishery products. This is due to an analysis of all the contamination data available for fishery products over the last five PSPC campaigns that was used to develop a new sampling plan. Although it has the same number of samples collected nationally as in previous years, this new programme focuses on the most relevant species, i.e. those with the highest levels of contamination (e.g. predator fish) and/or those with the highest consumption levels. A portion of the sampling remains targeted at low contamination and low consumption species to maintain minimal surveillance of these species.

Concerning BFR, there are currently no European regulations setting maximum limits for these compounds in foodstuffs. In 2015, detection of BFRs continued, to comply with Recommendation 2014/118/EU regarding their monitoring. It would be interesting to review monitoring of BFRs between 2012 and 2015 to improve the precision of results for each matrix.

Lastly, some of the conclusions will need to be confirmed through future sampling given the occasionally limited number of samples for a food group/contaminant group pair. For 2014, only a dozen analyses of BFRs were performed for each type of butchery meat (beef, mutton/lamb, and pork). As a result, it is essential to maintain high sampling levels because in addition to the main objective of monitoring contamination levels, the current programme also generates contamination data that are used by risk assessment experts (ANSES, EFSA). These data help to regularly update this assessment. This assessment is more accurate when the analytical limits retained by laboratories are low, and the programmes implemented by the DGAL meet the objectives defined by all the stakeholders.

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Surveillance of trace metals in foods of animal origin - focus on the exploratory plan to test for methylmercury in fish

Rachida Chekri (1) (rachida.chekri@anses.fr), Jean-Cédric Reninger (2), Thierry Guérin (1), Laurent Noël (3)

(1) ANSES, Laboratory for Food Safety, Maisons-Alfort , France.
(2) ANSES, Risk Assessment Department, Maisons-Alfort, France
(3) French Directorate General for Food, Coordination Office for Chemical and Physical Contaminants, Paris, France

Abstract

The surveillance of trace metals such as arsenic, lead, cadmium, nickel and mercury in foodstuffs of animal origin is ensured by an operational plan aiming at risk identification and the quantification and characterisation of the hazards related to trace metals found in foods.

In 2014, several surveillance and control plans (targeted sampling) as well as an exploratory plan were implemented to monitor trace metals (lead, cadmium, mercury and methylmercury) in foodstuffs. These plans generated 6,908 analyses in various matrices (fish products, livestock products, milk, game, poultry, rabbits and honey). Processing of the results showed a completion rate of 99.3% and a rate of non-compliance (with the regulatory maximum levels or national alert thresholds) ranging from 0.7% to 16% across all sectors, excluding the equine industry. The identified non-compliances were managed based on the identified risk. They also helped to maintain or strengthen the surveillance of certain analyte/matrix pairs, such as lead in game meat and cadmium in equine liver.

In general, the surveillance system in place has contributed to estimating consumer exposure to trace metals as well as to populating databases (methylmercury exploratory plan) for enhanced risk assessment. The analysis of the monitoring system was an opportunity to present prospects for improvement including the need to define more suitable sample targeting criteria that are easier to implement. Another area for improvement would be the implementation of a tool for improving the quality of data generated by monitoring and control plans.

Keywords

Surveillance, Trace metals, Lead, Cadmium, Mercury, Methylmercury

Résumé

Surveillance des éléments traces métalliques dans les denrées alimentaires d'origine animale - focus sur le plan exploratoire de la recherche du méthylmercure dans les poissons

La surveillance officielle des éléments traces métalliques (ETM) tels que l'Arsenic, le Plomb, le Cadmium, le Nickel ou le Mercure dans les denrées alimentaires d'origine animale est assurée grâce à un dispositif qui permet de maîtriser le risque alimentaire par l'identification, la quantification et la caractérisation du danger lié à la présence de ces éléments dans les aliments.

En 2014, divers plans de surveillance et de contrôle (échantillonnage ciblé) ainsi qu'un plan exploratoire ont été mis en œuvre pour la surveillance des ETM (Plomb, Cadmium, Mercure et Méthylmercure) dans les denrées alimentaires. Ces plans ont engendré 6 908 analyses dans diverses matrices (produits de la pêche, animaux de boucherie, laits, gibiers, volailles, lapins et miels). L'exploitation des résultats obtenus, a indiqué un taux de réalisation de 99,3 % et un taux de non-conformités (au regard des teneurs maximales réglementaires ou des seuils d'alerte nationaux) variant de 0,7 à 16 %, toutes filières confondues, hors filière équidés. Les non-conformités mises en évidence ont fait l'objet de mesures de gestion adaptées en fonction du risque identifié. Elles ont également permis de maintenir ou de renforcer la surveillance de certains couples analyte/matrice tels que le Plomb dans le muscle de gibier ou le Cadmium dans le foie d'équidés. De manière générale, le système de surveillance mis en place a contribué à l'évaluation du niveau d'exposition du consommateur aux ETM ainsi qu'à l'alimentation des bases de données de contamination (plan exploratoire Méthylmercure), pour une meilleure évaluation du risque. L'analyse du dispositif a permis de présenter des perspectives d'amélioration, notamment la nécessité de définir des critères de ciblage des prélèvements, plus adaptés et plus simple à mettre en œuvre; ainsi que la mise en place d'un outil pour l'amélioration de la qualité des données générées par les plans de surveillance et de contrôle.

Mots-clés

Dispositif de surveillance, éléments traces métalliques, Plomb, Cadmium, Mercure, Méthylmercure

Every year, various surveillance and control plans (SCPs) are implemented to monitor contamination of primary plant and animal production, foodstuffs of animal origin, and animal feed. These plans are also a way of collecting data on contamination with a view to assessing the risks related to food.

Trace metals (TMs) in foodstuffs of animal origin are a group of contaminants that are monitored through this programme. The main elements under monitoring are lead (Pb), cadmium (Cd), and mercury (Hg). The sources of TMs are either natural or anthropic, i.e. related to human activities such as industry and agriculture. Through the various transformation processes that they undergo (physico-chemical, oxidation-reduction, biological activity, absorption-desorption, etc.), TMs are found in different chemical forms, whether organic or inorganic, with a variable lifespan, and are more or less toxic depending on the element of interest. They are adsorbed in soil, sediments, and in aquatic environments, and can also be found in the air. This is how these substances enter the food chain (water-phytoplankton/plant-fish/animal) where they undergo biomagnification and/or bioaccumulation. Ingestion of these TMs *via* food is associated with disruptions of essential metabolic functions in humans. Toxicity related to lead and mercury cause kidney, neurotoxic, and cardiovascular lesions, and cadmium is classified

as "carcinogenic in humans"; it affects renal function and causes reproductive disorders.

In this article, we will present the objectives of the surveillance programme for TMs implemented in 2014, its operational aspects as highlighted by surveillance scheduling and the surveillance protocol (choice of analyte/matrix pairs to monitor, sampling strategy and plan, analytical methods, etc.), as well as the results and areas for improvement. We will specifically focus on the methylmercury (MeHg) plan concerning fish. Mercury, a regulated substance, accumulates in fish mainly in the form of methylmercury, which is not regulated, and this form carries a toxic risk for the consumer. However, the surveillance plans implemented concern mainly mercury. As a result, in 2014, an exploratory plan was initiated to collect contamination data specifically on the most toxic species (MeHg) in fish consumed in France.

Objectives of the surveillance programme

The objectives are to: i) monitor the compliance of animal foodstuffs placed on the market in France on a pro rata basis of the quantities produced, and ii) provide data for assessing the risk to consumers related to contamination of animal foodstuffs by TMs. In addition, possible European alerts (RASFF[1]) are taken into account for implementation of the programme to enable further vigilance or to set up targeted plans regarding specific analyte/matrix pairs.

The SCPs implemented in 2014 concerned foodstuffs of animal origin at the stage of primary production or primary processing: meat, offal, milk and honey (for land animals), fish meat for farmed fish, as well as seafood and freshwater products. The plans were organised as follows:

- control plan for lead and cadmium in animals for slaughter, poultry, rabbits, game, farmed fish, and honey,
- control plan for lead in milk, beef, mutton/lamb, and goat meat,
- surveillance plan for lead, cadmium, and mercury in fishery products (fish, shellfish, cephalopods and bivalve molluscs),
- exploratory plan for methylmercury in fish.

Most of the programmed plans meet the regulatory objectives fixed by the European Union to monitor contamination levels by various contaminants in food, and to harmonise food safety monitoring for European production regarding certain health hazards. This is the case for TMs in primary animal production.

Other specific plans are aimed at national monitoring and involve analyte/matrix pairs that are not regulated but that are of interest. Examples include lead and cadmium in honey and in rabbits, and cadmium in game meat (farmed and wild) to which detection of lead was added in 2014.

Aside from these plans, implemented to monitor compliance of products, an exploratory plan for the detection of MeHg in fish was organised. Its aim was to collect data on MeHg and total mercury (HgT) concentrations observed in fish placed on the market. Currently, European regulations establish only the HgT concentration in foodstuffs, with a regulatory maximum limit (ML) of 1 mg/kg for predator fish and 0.5 mg/kg for other fish. However, mercury toxicity depends on its speciation (different chemical species of an element, i.e. chemical entity: atom or group of related atoms that can be an ion, a molecule, or a radical) and on the amount of these different species ingested, which can be different from the HgT concentration. Organomercury species are far more toxic than inorganic species. This is the case for MeHg, the most hazardous form to humans, which is neurotoxic and teratogenic.

The main source of exposure of humans to MeHg is consumption of fishery products. Moreover, the calculation of population exposure to MeHg is generally carried out on the basis of a hypothesis in which the mean proportion of mercury present in the form of MeHg in fish meat varies from 80% to 100% of HgT. To assess this exposure as precisely as possible, knowledge of the MeHg levels, in addition to the HgT levels, would enable the European authorities to issue new toxicity reference values and more specific food recommendations (committee of the *Codex Alimentarius*).

1. RASFF: rapid alert system for food and feed – European Commission.

Box.

Objectives

Surveillance plan: monitor contamination levels of regulated trace metals in foodstuffs of animal origin: lead (Pb), cadmium (Cd), and mercury (Hg) in primary production.

Exploratory plan for the detection of methylmercury (MeHg) in fish: provide additional data for assessing the risk related to consumption of fish.

Programming framework

Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products.

Decision 97/747/EC fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products.

Decision 98/179/EC laying down detailed rules on official sampling for the monitoring of certain substances and residues thereof in live animals and animal products.

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.

Protocol

The plans for the detection of TMs in foodstuffs of animal origin implemented in 2014 include: a control plan for lead and cadmium in animals for slaughter, poultry, rabbits, honey and game; a control plan for lead in milk; a surveillance plan for lead, cadmium, and mercury in fishery products; and an exploratory plan for MeHg in fish.

Production areas of interest: animals for slaughter (cattle, sheep, goats, and horses), poultry, rabbits, game, farmed fish, eggs, honey, milk, beef, mutton/lamb, goat meat, and fishery products (fish, shellfish, cephalopods, and bivalve molluscs).

Food chain stage: primary production or first processing. All distribution channels for fishery products (hyper- and supermarkets, fishmongers, itinerant markets, etc.).

Non-compliant samples: as a general rule, a result is considered non-compliant when the maximum levels of a contaminant present in a product are exceeded, taking into account the expanded measurement uncertainty (k = 2) associated with the result.

The surveillance programme for TMs implemented in 2014 involved 3140 samples, and 59 samples were analysed as part of the MeHg exploratory plan.

Sampling strategy: for control plans, sampling targeted at foodstuffs from areas that are likely to be contaminated, and for the surveillance and exploratory plans, random samples at the distribution stage.

Analytical methods: official methods for the determination of TME levels (Pb, Cd, Hg) in foodstuffs of animal origin by atomic absorption (AA) spectrometry or by inductively coupled plasma mass spectrometry (ICP-MS) and the method of determination of the MeHg content in fishery products by isotopic dilution.

How the surveillance programme operates

The programme calls on various players to perform different activities in specific areas of competence: management and scheduling (sampling strategy, choice of suitable analyte/matrix pairs, etc.), implementation (sampling, development of appropriate analytical methods, analysis, demonstration of non-compliance, etc.), and exploitation of results from the programme (measures implemented following cases of non-compliance or identified emerging risks, conclusions, and proposals for improving the programme)[2].

Management and scheduling framework

Management and scheduling are taken care of by the Directorate General for Food (DGAL). Control plans are developed and implemented in accordance with the provisions of Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products, and Commission Decisions 97/747/EC (fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC for the monitoring of certain substances and residues thereof in certain animal products) and 98/179/EC (laying down detailed rules on official sampling for the monitoring of certain substances and residues thereof in live animals and animal products).

The regulatory limits for TMs in animal foodstuffs are defined in Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels (MLs) for certain contaminants in foodstuffs.

Sampling methods and the performance criteria of analytical methods are defined in Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs.

Sampling strategy

The sampling strategy is managed by the DGAL in conjunction with other managing bodies of the programme with support from ANSES and the National Reference Laboratory (NRL) for trace metals in foodstuffs of animal origin.

The sampling methods (number of samples, representativeness of the sample lots and sublots, or units, packages, labelling, and transmission) are those specified in Commission Regulation (EC) No 333/2007.

The sampling strategy depends on the type of plan and is carried out as follows.

Surveillance plans

Sampling is carried out randomly. The choice of lots to sample is made at random, irrespective of the date, place, origin (farmed or wild), or species concerned, depending on the human population of each region. The samples are taken at the distribution level. Concerning the surveillance plan in fishery products, regardless of the species, samples are taken at the point of transfer to the final consumer in all distribution channels (hyper- and supermarkets, fishmongers, itinerant markets, etc.).

Control plans

All of the samples are collected in a targeted manner. The criteria used are for example the location of agricultural production sites near polluted or potentially polluted areas. Databases that provide information on risk areas – IREP[3] and BASOL[4] – are used to distribute the samples at the departmental level.

All livestock rearing or production methods, whether intensive, organic, or certified, are included. Samples to detect lead and cadmium in the equine sector are taken from muscle and liver in the same animal (addition of this matrix in 2014). Since there is a lack of recent data on contamination of offal from horses over two years of age, reinforced monitoring of lead and cadmium in the liver was planned for 2014 in order to assess the level of contamination in this matrix, and if necessary, revise current management methods (systematic collection of livers from animals over two years). For milk, samples are taken from production sites where the animals have access to the outside, with priority given to polluted or potentially polluted areas.

Exploratory plan for MeHg in fish

Samples are taken at the distribution stage, in a randomised manner. A total of 54 samples were planned and attributed to all regions in mainland France, as well as the five Overseas *départements*.

Since predatory species are highly bioaccumulative compared to other species, it was decided to take one sample of predator fish and one non-predator by region in mainland France, and two samples of different fish (predator or not) in the five Overseas *départements*.

Implementation methods

The programme is implemented jointly by the decentralised services that carry out sampling, accredited laboratories, and the National Reference Laboratory (NRL) responsible for analyses, and by all the stakeholders for the management of non-compliance.

Analytical methods

The analyses are carried out by laboratories accredited by the Ministry of Agriculture, Food and Forestry to perform analyses, and by the NRL for certain specific plans. All of the laboratories are accredited by the French Accreditation Committee (Cofrac) to carry out analyses in accordance with the provisions of Standard NF EN ISO/CEI 17025 "General requirements for the competence of testing and calibration laboratories", and according to the 99-3 accreditation programme "Analysis of chemical contaminants in animals and products thereof and in foodstuffs intended for humans or animals: metals". The methods used for the regulatory analyses are the official methods (DGAL guidance note No. DGAL/SDPPST/N2011-8081 "Official methods for the determination of TME levels (Pb, Cd, and Hg) in foodstuffs of animal origin").

Two main techniques are used depending on the availability of equipment in the laboratories, i.e. atomic absorption (AA) spectrometry, and inductively coupled plasma mass spectrometry (ICP-MS). These methods are developed and validated by the NRL, in accordance with applicable standards, in order to evaluate performance parameters such as limits of detection (LODs) and quantification (LOQs), trueness and intermediate precision. They are then transferred to accredited laboratories. In line with European regulations, these performance criteria must meet the predefined requirements, especially in terms of LOQ and measurement uncertainty of the result. The method must be sufficiently sensitive to quantify low concentrations at and below (1/5 to 2/5) maximum levels, and must have a measurement uncertainty in agreement with a maximum regulatory value (calculated based on the concentration of interest). As the statement of conformity of a sample is based on the analytical result, minus its uncertainty, a maximum limit for the uncertainty value has been established to prevent any overestimation of uncertainty that would affect the conclusion.

2. See the article by Marion Bordier, The surveillance system for food-chain contaminants managed by the DGAL: report on the 2014 surveillance and control plan campaign, in this edition.

3. IREP: French register of pollutant emissions. *http://www.irep. ecologie.gouv.fr/IREP/index.php*

4. Basol: Database on polluted sites or contaminated land. *http://basol.developpement- durable.gouv.fr/*

For non-regulated analyses in the MeHg exploratory plan in fish, the method used was the determination of MeHg levels in fishery products by isotope dilution: solid/liquid extraction and quantification by isotope dilution - gas chromatography coupled with inductively coupled plasma mass spectrometry (ID-GC/ICP-MS).

Regulatory compliance

For monitoring purposes, the analytical results are compared to the maximum levels or to the nationally determined thresholds that apply to the analyte/matrix pair under consideration. The sample is compliant if the result subtracted from expanded measurement uncertainty (coverage factor fixed at 2, for a confidence level of 95%) is less than or equal to this maximum level.

Since there are no regulatory thresholds for some analyte/matrix pairs under monitoring, alert thresholds determined nationally have been defined by the DGAL on the basis of previous data from SCPs and/or bibliographic data, or on the basis of MLs for similar matrices or species (e.g. game birds associated with poultry).

This is, in particular, the case for lead and cadmium in game, rabbits, and honey, and lead in horses. For example, concerning honey, the retained thresholds are 0.10 mg/kg for lead and 0.05 mg/kg for cadmium, with these thresholds representing compliance thresholds above which an investigation is triggered to identify a possible source of contamination in the environment. Since 2015, an ML has been set for lead in honey at 0.10 mg/kg (Commission Regulation (EC) No 2015/1005 of 25 June 2015).

When a non-compliant result is found, the laboratories inform the decentralised service that obtained the sample, and this service then informs the Public Health Emergency Unit (MUS) at the DGAL. The MUS provides technical support to decentralised services in conjunction with the relevant sector office to assess reports. It ensures execution of a possible batch withdrawal or recall procedure and, if there is no immediate risk, where necessary transmits case management to the DGAL sector office and the directorate generals that may be concerned.

Results and Discussion

The surveillance programme for TMs implemented in 2014 involved 3,140 samples, with a completion rate of 99.3%. This rate ranged from 73% to 114% depending on the analyte/matrix pair, except for lead in sheep's milk (40%) and lead and cadmium in small farmed game (32%). These low rates are related either to samples not being collected due to difficulties in the field or to a lack of communication of results in Sigal (non-transmission of results or unusable transmitted results). The surveillance programme involved 23 different analyte/matrix pairs, which is a high number given the quantity of samples. Nonetheless, the number of samples meets the minimum requirements in European regulations and forms part of overall surveillance of health risks on the basis of priority setting by sector or by contaminant group, taking account of budget restrictions.

The total number of analyses was 6790. Generally speaking, the quantified results (24.9%) are far below authorised maximum levels. The identified non-compliances concerned HgT in fish (2.6%), cadmium in shellfish (6.5%, 31 samples), lead in horse liver (0.6%), cadmium in horse muscle (0.6%), cadmium in poultry liver and game muscle (0.7%), and cadmium in cattle liver (9%, 22 samples). These cases of non-compliance triggered an epidemiological investigation that led to stock seizure or batch withdrawal when contamination was confirmed. For example, following a non-compliant result for cadmium in cattle liver from a production site located in an area where the ground is contaminated with lead and cadmium, systematic seizure of offal from animals in this area was implemented.

Moreover, a high, though expected, level of non-compliance (78%) was found for analyses of cadmium in horse liver. This plan was implemented to confirm continued systematic seizure of liver from horses over two years of age at the slaughterhouse, in line with specific French legislation concerning offal of animals that are "slaughtered late" and may bioaccumulate cadmium in their livers in amounts higher than the maximum level of 0.5 mg/kg, and that would therefore be unfit for human consumption.

With regard to game, the detection of lead was added to the detection of cadmium in 2014 to assess possible lead contamination that consumers may be exposed to. In all, 16.6% non-compliance for lead in muscle and 5.5% in liver was found in game meat, as well as 12% for cadmium in liver and 0.7% in muscle.

The liver is usually more highly contaminated than muscle and this was confirmed for cadmium but not for lead. This increased level of non-compliance for lead in muscle is probably due to the use of lead-containing bullets used in hunting, with resulting contamination of samples, despite recommendations concerning sampling away from the bullet trajectory.

Since game meat is consumed only occasionally, it does not constitute a major source of exposure to cadmium or to lead. However, the possibility that consumers with a specific diet (for example those consuming large quantities of game meat) may be exposed to a greater extent, cannot be ruled out. It would be interesting to better characterise this situation in order to issue consumption recommendations, if necessary. Sources of contamination can be natural or possibly artificial (human activities such as industry) with logical accumulation in game given the diet of certain animals and their age.

Moreover, the number of samples collected in 2013 was slightly lower in comparison with the SCPs implemented in 2014 (2,273 *versus* 3,140), with an equivalent completion rate. We also observed small quantities of TMs in the analysed matrices and several non-compliances that involved two samples of horse and cattle muscle, and one sample of swordfish (Indian Ocean) for cadmium, and two samples of ling (NE Atlantic) for mercury. Alert thresholds were also exceeded for 22 game liver and muscle samples for cadmium and lead, and one sample of sea urchin (NE Atlantic), and two samples of honey for lead (including one case related to the presence of lead in the material).

Focus on the exploratory plan for MeHg in fish

A total of 59 samples were analysed and the results for mean levels of HgT and MeHg according to the various species (23 predator fish and 36 non-predator fish) are shown in Figure 1.

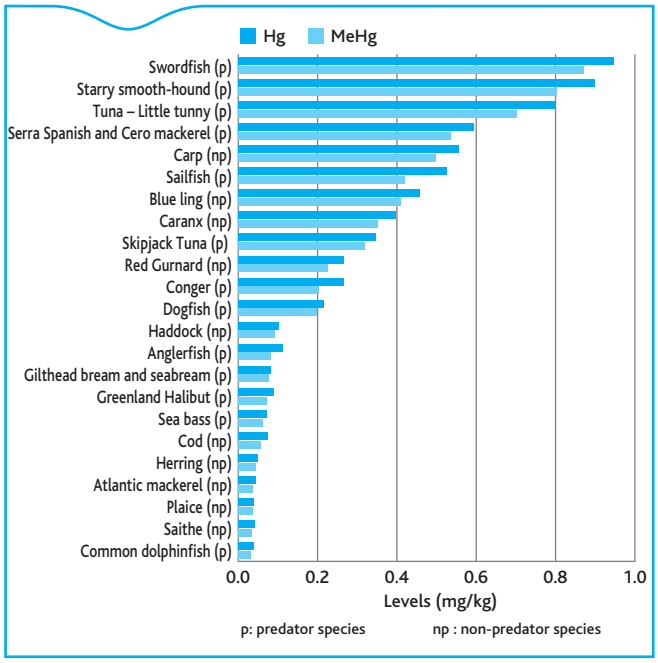

Figure 1. Distribution of HgT and MeHg levels according to fish species

Table 1. Mean data for HgT and MeHg contamination in fish

	Mean contamination values (mg/kg)			
	All fish (n = 59)	Non-predator fish (n = 23)	Predator fish (n = 36)	Predator fish, excluding swordfish and sharks (n = 19)
MeHg (mg/kg)	0.33	0.12	0.46	0.21
HgT (mg/kg)	0.37	0.14	0.51	0.24

The completion rate was 109%, with five additional samples of swordfish (*Xiphias gladius*) from Réunion Island analysed in addition with the scheduled sampling. All samples were quantified in HgT and in MeHg.

We can note six cases in which the maximum levels were exceeded for HgT given the individual levels measured in the species fished in unknown areas, except for Serra Spanish mackerel (*Scomberomorus brasiliensis*) caught in the Atlantic Ocean (1.6 mg/kg > 1.0 mg/kg (maximum level for predator fish)), carp (*Cyprinus carpio)* (0.82 mg/kg > 0.50 mg/kg (maximum level for non-predator fish)), swordfish (*Xiphias gladius*) (respectively 1.3 – 1.8 – 3.1 mg/kg > 1.0 mg/kg (maximum level for predator fish)), and the Little tunny (*Euthynnus alletteratus*) (1.3 mg/kg > 1.0 mg/kg (maximum level for predator fish)). This corresponds to a proportion of non-compliant samples of 10%, but this should be interpreted with caution given the sampling level in this plan (59 samples for 25 different species from various fishing locations). Targeted sampling and a higher number of samples would provide a better assessment of MeHg contamination in fish. Furthermore, MeHg content represents on average 87% of HgT (74% to 97% depending on the species), which is consistent with data from the literature (AFSSA request 2003, EFSA Opinion 2012).

As expected, HgT and MeHg levels are higher in predator species, which accumulate HgT and MeHg more readily (Table 1).

This exploratory plan provided data concerning MeHg and HgT in fish placed on the market in France. High consumers of predator species are likely to be more exposed to HgT and MeHg.

In addition, the European committee of experts on environmental contaminants is currently discussing the proposed revision of maximum levels for mercury in fishery products, on the basis of Commission Regulation (EC) No 1881/2006 of 19 December 2006. In this area, a new classification of MLs is under consideration: four levels are proposed: 0.30 – 0.50 – 1.0 and 2.0 mg/kg, established on the basis of a review of available contamination data showing that, depending on the species, mean contamination levels for mercury are either far below or far above current MLs. As an example, the fish with the highest levels of mercury are the oldest predator fish at the end of the food chain (tuna, swordfish, etc.), but also smaller predators that have slow growth. The current ML fixed for swordfish and sharks does not reflect commonly recorded contamination levels. Therefore, the MLs for these species should be fixed in Hg, applying the principle generally used to set maximum levels for contaminants (ALARA[5] principle resulting from the comparison of theoretical exposure deduced from available contamination data and the toxicity reference value (TRV) of the contaminant).

5. ALARA: As low as reasonably achievable

Analysis and areas for improvement of the surveillance programme

Overall, the 2014 surveillance programme of TMs in foodstuffs of animal origin contributed to consumer protection by managing contaminations considered non-compliant.

Specific national plans such as the detection of cadmium in horse liver and lead, and cadmium in honey enabled continued surveillance and suitable provisions according to the associated characteristic risks (confirmation of the need for systematic seizure of liver from horses over two years of age, contamination surveillance for lead and cadmium in honey and game, outside European requirements).

Moreover, data from the exploratory plan for the detection of MeHg/HgT were used to populate contamination databases, which are used by the scientific community to better assess risk.

In view of the high level of non-compliance for lead in game muscle, a joint project was launched by the DGAL, decentralised services, analysis laboratories, and the NRL. This project should be continued to identify whether the source of contamination is environmental or essentially related to hunting practices (lead bullets) and to implement measures that are in line with the identified risks. The aim is to ensure higher traceability of the programme, in accordance with applicable rules and recommendations (sampling outside the bullet trajectory by decentralised services and then by the laboratories. Inconsistent analytical results, related to dispersed lead bullets invisible to the naked eye in the matrix, should be reported by the laboratories), and reporting of any anomalies by all of the stakeholders involved. In addition, the DGAL has sent a request to ANSES concerning the health risks related to consumption of game meat due to environmental chemical contaminants (dioxins, polychlorinated biphenyls (PCBs), cadmium and lead).

Other actions could be implemented to optimise the system more generally, in particular by examining the sample targeting criteria for control plans. Those criteria are difficult to take into consideration due to the lack of instructions, precise indications, and difficulties associated with implementation in the field.

The quality of data is also an area for improvement in order to optimise the use of data from SCPs. The development of a computer-based tool for the qualitative follow-up of SCP data using indicators has also been initiated by ANSES through an agreement between the DGAL and the ANSES.

Surveillance of phycotoxins in shellfish

Marina Nicolas (1) (marina.nicolas@anses.fr), Catherine Belin (2), Pauline Favre (3), Laurence Rudloff (3)

(1) ANSES, French National Reference Laboratory for Marine Biotoxins, Maisons-Alfort, France
(2) IFREMER (French Research Institute for Exploitation of the Sea), Nantes, France
(3) Directorate General for Food, Sub-directorate for Food Safety, Office for Seafood and Freshwater Fish, Paris, France

Abstract

This paper presents the French national system for monitoring three groups of marine biotoxins regulated in shellfish, implemented firstly in marine production areas by the REPHY REPHYTOX network of IFREMER and secondly at the distribution level through the network of laboratories approved by the Directorate General for Food within the framework of official controls. The European regulations, the nature of the shellfish toxins, and analytical methods used are presented. The sampling procedures and strategy, as well as the results obtained by each of the two systems mentioned, are presented and discussed.

Keywords

Phycotoxins, Shellfish, Lipophilic toxins, ASP, PSP, Surveillance

Résumé

Surveillance des phycotoxines dans les coquillages

Cet article présente le dispositif national de surveillance de trois groupes de biotoxines marines réglementées dans les coquillages mis en œuvre, d'une part au niveau de zones marines de production par le réseau Rephy-Rephytox de l'Ifremer et, d'autre part au stade de la distribution par le réseau des laboratoires agréés de la direction générale de l'Alimentation dans le cadre des plans de surveillance et des plans de contrôle mis en place chaque année. La réglementation européenne, la nature des phycotoxines recherchées et les méthodes analytiques mises en œuvre sont présentées. Les modalités et la stratégie d'échantillonnage pour chacun des deux dispositifs sont décrites. Les résultats obtenus en 2015 sont exposés et discutés.

Mots-clés

Phycotoxines, coquillages, toxines lipophiles, ASP, PSP, surveillance

Shellfish are in direct contact with the marine environment and, due to their filtration activity (in the case of filter-feeding shellfish), concentrate contaminants found in the environment, particularly phycotoxins (algal toxins produced by toxic phytoplankton).

The following phycotoxins are regulated in shellfish under the EU hygiene package (Regulation (EC) No 854/2004 of 29 April 2004):

- lipophilic toxins including diarrhetic shellfish poison (DSP) (okadaic acid, dinophysistoxins, pectenotoxins, yessotoxins and azaspiracids), produced in particular by *Dinophysis*. These toxins are likely to cause rapid-onset gastrointestinal disorders in the consumer (30 minutes to 12 h after ingestion), mostly without severity except in people with a fragile state of health;
- amnesic shellfish poison (ASP) (domoic acid), produced in France by *Pseudo-nitzschia*. These toxins are likely to cause generally rapid-onset neurological disorders in the consumer (15 minutes to 38 h after ingestion) that can be serious, as seizures and coma may result in a fatal outcome;
- paralytic shellfish poison (PSP) (saxitoxin), produced in France by *Alexandrium*. These toxins are likely to cause rapid-onset neurological disorders in the consumer (30 minutes to 12 h after ingestion) that can be serious, as paralysis of the respiratory muscles may result in a fatal outcome.

Table 1. Regulatory thresholds for phycotoxins in shellfish

Name of toxin group		Regulatory threshold
Saxitoxins (PSP-type toxins)		800 µg/kg of meat
Domoic acid (ASP-type toxins)		20 mg/kg of meat
Lipophilic toxins	Okadaic acid (OA) group	160 µg of okadaic acid equivalents/kg of meat (for okadaic acid, dinophysistoxins and pectenotoxins together)
	Azaspiracids	160 µg of azaspiracid equivalents/kg of meat
	Yessotoxins	3.75 mg of yessotoxin equivalents/kg of meat

The maximum regulatory levels in shellfish are established in Regulation (EC) No 854/2004 of 29 April 2004 (Annex III, Section VII, Chapter V) (Table 1).

These phycotoxins are monitored in shellfish through two complementary programmes:

- firstly, in marine production areas *via* the REPHY-REPHYTOX networks of Ifremer, respectively the Phytoplankton and hydrology observation and monitoring network, and the Phycotoxin monitoring network,
- and secondly at the distribution level *via* the surveillance and control plans (SCPs) implemented by the DGAL.

Monitoring of phycotoxins in shellfish in marine production areas (REPHY-REPHYTOX networks)

Materials and methods

Shellfish production areas are regularly monitored to ensure the quality of the products. The surveillance method for phycotoxins in shellfish production areas is described in Ifremer's REPHYTOX procedures dossier (Neaud-Masson & Belin)[1].

The surveillance of phycotoxins is closely related to surveillance of toxic phytoplankton, which is managed within the framework of the REPHY network. Its procedures are currently being revised[2].

If necessary, local REPHY-REPHYTOX procedures provide more specific information with reference to the national provisions.

The objective of REPHYTOX is the detection and monitoring of toxins that may accumulate in commercial marine products, particularly bivalve molluscs found in production areas or in natural environments farmed professionally. To meet these objectives, REPHYTOX collects shellfish samples through a network of sampling sites located

1. http://envlit.ifremer.fr/content/download/83181/601705/version/9/file/Cahier-Procedures-REPHYTOX_v1.pdf
2. http://envlit.ifremer.fr/surveillance/phytoplancton_phycotoxines/mise_en_oeuvre

Box.

Objectives
The REPHYTOX network aims to detect and monitor regulated phycotoxins in shellfish located in marine production areas. This network is closely associated with the REPHY network, which includes in its missions the detection and monitoring of phytoplankton species producing toxins that may accumulate in shellfish.

The DGAL surveillance plans (SCP system) regarding phycotoxins in shellfish complement the REPHY-REPHYTOX monitoring programme on shellfish in the marine environment. The objective of these plans is to assess phycotoxin contamination levels of marketed shellfish and thereby, consumer exposure.

Programming framework
Regulations

- Regulation (EC) No 853/2004 of 29 April 2004 (Annex III, Section VII, Chapter V)
- Regulation (EC) No 854/2004 (Annex II, Chapter II, Point B)
- Regulation (EC) No 854/2004 (Annex II, Chapter II, Point D.2)

Protocol

- Type of contaminants detected: the three groups of regulated toxins, i.e. lipophilic toxins (okadaic acid, dinophysistoxins, pectenotoxins, yessotoxins, and azaspiracids).
- amnesic toxins in the domoic acid group, paralytic toxins of the saxitoxin group.
- Production of interest ("population"): shellfish.
- Food chain stage: shellfish sampled directly in the natural production environment (marine areas) for REPHYTOX monitoring, and shellfish placed on the market for surveillance plans.
- "Case" definition: sample contaminated by regulated phycotoxins above the thresholds determined by European regulations.
- Number of samples and sampling method: between 2500 and 3000 samples analysed each year for REPHYTOX monitoring, with at least half concerning lipophilic toxins. About 1000 samples per year for the surveillance plan.
- Sampling strategy: targeted for REPHYTOX monitoring, and random for surveillance plans, with the number of samples to collect per region being proportional to the human population.
- Analytical methods, types of samples:
  - > Testing (detection and quantification) for lipophilic toxins[1] by chemical analysis (liquid chromatography coupled with tandem mass spectrometry).
  - > Testing for saxitoxin group toxins by bioassay, with quantification based on the survival time of mice injected with shellfish extracts.
- Testing for domoic acid group toxins (domoic acid and its epimer epi-domoic acid) by chemical analysis (liquid chromatography coupled with ultraviolet detection).

1. Testing is performed for regulated toxins (okadaic acid, dinophysistoxins and pectenotoxins - OA+DTXs+PTXs, yessotoxins - YTXs and azaspiracids - AZAs) and also certain non-regulated toxins (spirolids - SPXs, gymnodimines - GYMs, and pectenotoxin-2-seco acid - PTX2sa).

along the entire coast, with spatial coverage that must fulfil two requirements: scientific relevance and optimisation of the cost/effectiveness ratio. There may be overlap between the REPHYTOX sampling sites and those of the REPHY network. In any event, there is a close relationship between REPHYTOX and a certain number of REPHY sites since the phytoplankton results at REPHY sites in a given area determine when detection of toxins at the REPHYTOX sites in the area is triggered. If toxic phytoplankton are found[3] (above the thresholds defined for each toxic species in the REPHYTOX procedures), toxin analyses are triggered in shellfish with a weekly interval.

In some cases, monitoring of toxic phytoplankton is not sufficiently reliable to guarantee the food safety of shellfish in an area, and analyses of toxins are then systematically carried out in shellfish. This is the case:

- in areas at risk for lipophilic toxins during predefined risk periods. These areas are considered more sensitive on the basis of historical toxin contamination data and may be subject to shellfish contamination even if there are only very low quantities of toxic phytoplankton that are difficult to detect, which justifies systematic analysis in shellfish,
- in offshore sources, which are systematically monitored for the three types of toxins every fifteen days (1 month before and then during the farming period). The depth of the water column in this case makes it impossible to clearly determine all the phytoplankton species present.

In the case of lipophilic toxins, mussels are considered a sentinel species because historical data have shown that they always become contaminated more quickly than all other shellfish. When there are mussels available for a production zone, they are therefore analysed on a first-line basis, while other shellfish are analysed as soon as mussels are found to contain toxins. There is no sentinel species for ASP or PSP.

There are about 250 potential sampling points for shellfish all along the coast of mainland France. The samples can be for various types of shellfish, from offshore sources or farmed using different methods (stake, rope, tray culture, etc.).

Changes to the system for monitoring shellfish production areas (specifically sampling conditions) are defined within the framework of a national steering committee (COPIL) that brings together the various government bodies concerned: the Directorate General for Food, the Directorate for Marine Fisheries and Aquaculture, the Directorate General for Health, Ifremer, ANSES, and the French Public Health Agency. The committee meets at least once a year.

The analytical methods used in the REPHY and REPHYTOX systems are as follows:

> Quantitative analysis of domoic acid (ASP toxin) in shellfish by high-performance liquid chromatography with ultraviolet detection (HPLC-UV): ANSES method PBM BM LSA-INS-0140.

Principle: domoic acid and its epimer epi-domoic acid (if present) are extracted from a homogenised tissue sample using 50% aqueous methanol. The extract is then filtered and analysed by isocratic high-performance liquid chromatography (HPLC) with ultraviolet detection.

> Determination of lipophilic marine biotoxins in molluscs by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS): ANSES method PBM BM LSA-INS-0147.

Principle: toxins in groups OA, PTX, AZA and YTX are extracted using methanol from a homogenised tissue. An aliquot of the methanol extract is treated by alkaline hydrolysis to convert possible acyl esters of OA and/or DTX into free toxins. The extracts are then purified by SPE (optional step) and analysed by gradient elution liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Non-hydrolysed extracts are used to test for the presence of free OA, free DTX1 and free DTX2, PTX1, PTX2, AZA1, AZA2, AZA3, YTX, homo YTX, 45 OH YTX, and 45 OH homo YTX. Hydrolysed extracts are used to determine the total quantity of toxins of the OA group.

> Bioassay in mice for the determination of saxitoxin group toxins (paralytic phycotoxins - PSP) in shellfish - ANSES method PBM BM LSA-INS-0143.

Principle: the bioassay method in mice includes a toxin extraction step from meat by hot acid hydrolysis, followed by intraperitoneal (IP) injection of 1 ml of extract in at least three mice. The survival

3. The following toxic species are tested for: *Alexandrium, Dinophysis, Pseudo-nitzschia, Ostreopsis, Gonyaulax spinifera, Lingulodinium polyedrum, Protoceratium reticulatum,* and *Prorocentrum lima.*

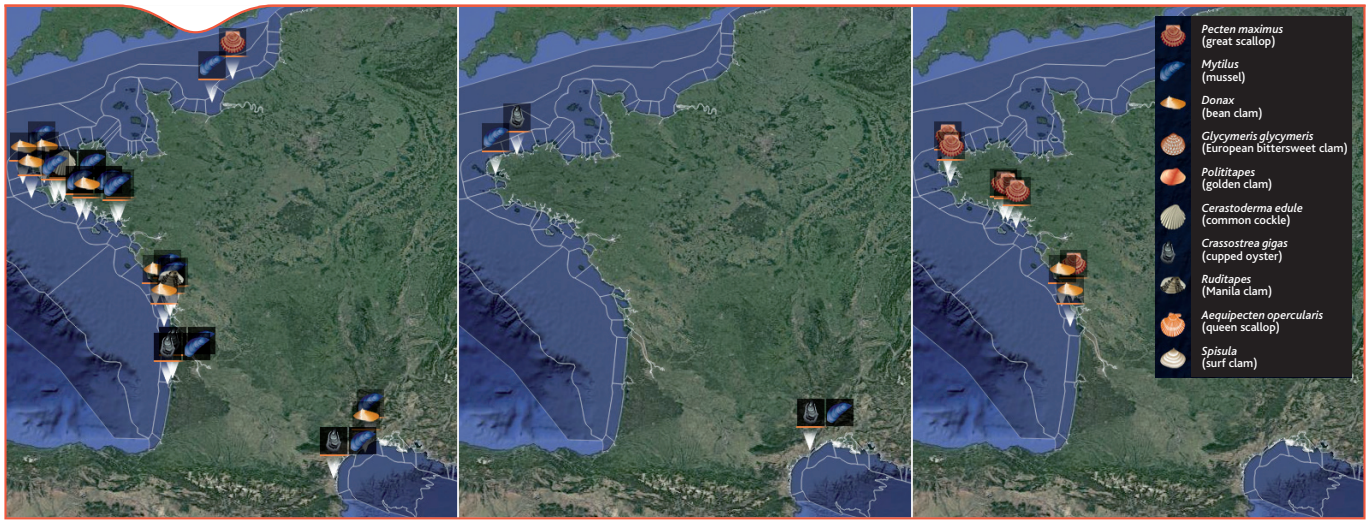

Figure 1. Geographic distribution of episodes of documented toxicity in shellfish on the coast: lipophilic toxins (left), paralytic toxins – PSP (centre), and amnesic toxins – ASP (right)

time (interval between injection and death) is recorded and the toxicity determined in mouse units (MU) from Sommer's table.

The bioassay is quantitative when the mice die between five and seven minutes after injection. Several dilutions may be needed to obtain a survival time between five and seven minutes. The MU measurement is then converted into µg STX diHCl equivalents (eq)/kg.

The National Reference Laboratory (NRL) for marine biotoxins coordinates three networks of accredited laboratories, one for each type of phycotoxin analysed: ASP network, PSP network, and lipophilic toxin network. These networks include Ifremer laboratories for the monitoring of production areas.

Results for 2015

Lipophilic toxins

Of the 1300 analyses performed for these toxins, 140 results (about 11%) were non-compliant (i.e. above the regulatory threshold of 160 µg/kg) for the okadaic acid + dinophysistoxins + pectenotoxin group. This percentage is higher if only mussels are considered (15%).

The maximum concentrations detected at the national level for the various shellfish species were as follows: 3003 µg/kg in mussels from Etang de Salses-Leucate (western Mediterranean) in January, 615 µg/kg in oysters from the Bay of Arcachon in May, 322 µg/kg in great scallops from Pays de Caux in January and 1315 µg/kg in *Donax* from the coast of Gironde in May (Figure 1). For the azaspiracid and yessotoxin groups, no non-compliant results were observed in 2015.

Paralytic toxins (PSP)

Of the 529 bioassays performed for these toxins, 19 results (i.e. 4% [95CI: 2-5]) were non-compliant (i.e. above the

regulatory threshold of 800 µg/kg). This percentage is much higher if only mussels are considered (19%), bearing in mind that only mussels and oysters were contaminated in 2015.

The maximum concentrations detected at the national level for these two shellfish species were as follows: 3136 µg/kg in mussels from Etang de Thau (western Mediterranean) in October and 1622 µg/kg in oysters from the Penzé river (north-west Brittany) in July (Figure 1).

Amnesic toxins (ASP)

Of the 661 analyses performed for these toxins, 40 results (about 6%) were non-compliant (i.e. above the regulatory threshold of 20 mg/kg). This percentage is higher if only great scallops are considered (10%), with these shellfish showing the highest contamination. The maximum concentrations detected at the national level for the two most affected shellfish species were as follows: 284 mg/kg in great scallops from the Roadstead of Brest in January and 33 mg/kg in *Donax* from the coast of Gironde in May (Figure 1).

Discussion

Lipophilic toxins

Concerning lipophilic toxins, the configuration of toxic episodes in 2015 is quite similar to what is observed each year. Firstly, from a geographical point of view: i) rare episodes in the Channel, primarily around the Seine estuary, ii) multiple episodes on the Atlantic coast, in particular in western and southern Brittany and in the Bay of Arcachon, areas where lipophilic toxins have been observed repeatedly for over 30 years, and iii) mostly localised episodes in lagoons in the Mediterranean. Secondly, in terms of distribution through the year: i) for coastal shellfish, toxicity was observed from the spring in the Atlantic areas, more commonly in summer in the Channel, and more during the winter in Mediterranean lagoons, ii) for pectinids (primarily great scallops), contamination can be observed during fishing periods, i.e. in the winter. In line with other years, mussels are the most highly affected shellfish, bearing in mind that many other shellfish species can be affected if the episodes continue for an extended period. Considering the results obtained since 2010 (first year when chemical analyses were used to detect these toxins), the 2015 results are rather high for certain types of shellfish in view of the national median value (340 µg/kg) calculated based on values above the food safety thresholds. These results are, however, well below the maximum levels reached in certain years, in particular for specific shellfish: for example, 37,296 µg/kg and 11,755 µg/kg in mussels and cockles, respectively, from the Bay of Arcachon in April 2012. Concerning the azaspiracid and yessotoxin groups, the lack of non-compliant results in 2015 confirms the results obtained for these toxin groups since their detection was implemented along the coastline of France.

Paralytic toxins (PSP)

For paralytic toxins, the three areas most affected in 2015 (Abers in Brittany, the Roadstead of Brest, and the Etang de Thau in the Mediterranean) were among the four zones most commonly affected by episodes of contamination by PSP phycotoxins (adding to these the Penzé River in north-west Brittany) since 1988[4]. These episodes, which thus remain limited from a geographic point of view, are still of concern given how dangerous these toxins are. In terms of occurrence through the year, the results for 2015 confirm trends observed to date: contamination is always observed between June and September in the Channel-Atlantic zone and always between September and December for the Etang de Thau. Until now, non-compliances have only been observed in mussels, oysters, cockles, or clams. Shellfish from offshore sources (including great scallops) have never been affected by a PSP episode. Taking into account the results obtained since 1990, the results for 2015 are rather high for mussels in view

4. Year of first detection of PSP toxins in France.

of the national median (1622 µg/kg) calculated on the basis of values higher than the food safety threshold for all shellfish. Importantly, however, results are well below the maximum values reached in some years, in particular for certain shellfish, such as: 11,664 µg/kg in mussels from the Roadstead of Brest in July 2012, and 7,360 µg/kg in oysters from the Abers (north-west Brittany) in August 2001.

Amnesic toxins (ASP)

For amnesic toxins, the areas affected in 2015 (western and southern Brittany, Pertuis Charentais) are among the zones that have regularly been affected by ASP episodes since the year 2000, when the first ASP toxins were identified in France. The Seine estuary, and less often the western Mediterranean, are also zones that have been affected since 2000. In terms of occurrence through the year, the results for 2015 confirm trends observed to date: all year for great scallops, and generally between March and June for the other shellfish, irrespective of the region. As a general rule, episodes of ASP affect mainly, if not exclusively in certain years, great scallops. This type of shellfish also shows the highest concentrations with a particularly protracted decontamination period that can reach several months. Other shellfish may also be affected, including mussels, oysters, *Donax*, and clams, but at concentrations rarely exceeding 100 mg/kg, and above all with decontamination periods that are often very short. Taking into account the results for the period 2000-2015, the results for 2015 for great scallops are rather high in view of the national median (41 mg/kg) calculated on the basis of values higher than the food safety threshold for these shellfish. The values are nonetheless lower than the maximum levels reached in certain years, the record being 861 mg/kg in the Roadstead of Brest in April 2014.

Contamination records for the three toxin groups are available at: http://envlit.ifremer.fr/var/envlit/storage/documents/ parammaps/toxines/index.html#

Monitoring of phycotoxins in shellfish at the time they are placed on the market (SCP system)

Materials and methods

Surveillance plans for contamination of shellfish by phycotoxins at the distribution level, implemented by the DGAL, complement the REPHY-REPHYTOX monitoring programme.

These plans are part of the general framework for assessing compliance of foodstuffs, which falls under the responsibility of the competent authorities. The regulatory criteria for phycotoxins in shellfish at the distribution level are described in Annex II, Chapter II, Point D.2 of Regulation (EC) No 854/2004.

The objective of these plans is also to assess the level of phycotoxin contamination of marketed shellfish. As a result, the data help to estimate consumer exposure. In 2015, 918 samples were planned by the DGAL for the full year, with regional distribution determined proportionally to the human population, i.e. 306 samples for detection of ASP, PSP and lipophilic toxins, respectively.

Samples were taken randomly at the distribution level in hyper- and supermarkets or in retail stores (fishmongers): this involved samples of live farmed (shellfish aquaculture) or fished bivalve molluscs, preferably sourced in France or in another Member State of the European Union.

The collected samples were forwarded to the accredited laboratory networks according to the types of phycotoxins to detect. The analytical methods used were the same as those implemented for the REPHY-REPHYTOX system.

Results

Of the 918 collected shellfish samples, 897 yielded an analytical result. The analysis completion rate was 97%. Among the 897 analytical results, three values exceeding the regulatory thresholds were observed, i.e. a non-compliance rate of 0.33% (95CI-[0.11-0.98])[5] for the three groups of regulated toxins. Table 2 presents the overall results.

Amnesic toxins (ASP)

Of the 301 samples collected, 297 were analysed. No values exceeding the threshold for domoic acid were found, corresponding to a compliance rate of 100% (95CI-[98.7-100]) for samples in this toxin group.

Paralytic toxins (PSP)

Of the 303 samples collected, 300 were analysed. No values exceeding the threshold for saxitoxin were found, corresponding to a compliance rate of 100% (95CI-[98.7- 100]) for samples in this toxin group.

Lipophilic toxins

Of the 309 samples, 300 were analysed. Three values exceeding the threshold for lipophilic toxins of the okadaic acid group (OA+DTXs+PTXs) were detected, corresponding to a non-compliance rate of 1% (95CI-[0.34-2.90]) for samples in this toxin group.

The first case involved live bulk mussels sourced from Spain that showed levels above the regulatory threshold (170.3 µg of okadaic acid equivalents/kg). Following this non-compliance, the affected mussels were withdrawn and recalled, with information provided to consumers.

The second case involved live mussels sourced from Spain that showed levels above the regulatory threshold (204.1 µg of okadaic acid equivalents/kg). Following this non-compliance, the affected mussels were withdrawn and recalled, with information provided to consumers. In view of this non-compliant result and the closure of the corresponding production area a short time after the harvest, an alert report was forwarded to the Spanish authorities *via* the RASFF[6].

The third case involved living mussels sourced from Ireland that showed levels above the regulatory threshold (230.1 µg of okadaic acid equivalents/kg). It was not possible to implement management measures directly in France on the product batches affected by this non-compliance. The mussels had been distributed and consumed in full. An alert report was forwarded to the Irish authorities *via* the RASFF.

Furthermore, on the basis of the full results, it can be observed that 87.6% (263/300) of the samples did not have quantifiable lipophilic toxin levels.

5. 95CI: 95% confidence interval
6. Rapid alert system for Food and Feed

Table 2. Breakdown of the samples and results by type of matrix and by analyte

	Number of samples					Number of samples analysed	Number of non-compliant samples	Compliance rate (%)
	Mussels	Oysters	Great scallops	Others*	Total			
ASP toxins	162	56	7	76	301	297	0	100
PSP toxins	179	66	3	55	303	300	0	100
Lipophilic toxins	199	55	7	48	309	300	3 (mussels)	99
Total	**540**	**177**	**17**	**179**	**913**	**897**	**3**	**99.6**

* European bittersweet clam, queen scallop, cockles, clams, or no species indicated.

For okadaic acid, dinophysistoxins and pectenotoxins taken together, 28 samples showed a quantifiable toxin level below the regulatory threshold of 160 µg of okadaic acid equivalents/kg of meat:

- 15 samples had toxin levels between the quantification limit and 45 µg of okadaic acid equivalents/kg of meat,
- 13 samples had toxin levels between 45 µg and 160 µg of okadaic acid equivalents/kg of meat.

For the azaspiracids, only one sample had a quantifiable toxin level lower than the regulatory threshold of 160 µg of azaspiracid equivalents/kg of meat. This was a sample of mussels sourced from the Netherlands with a level of 80 µg of azaspiracid equivalents/kg.

For the yessotoxins, five samples had toxin levels between the quantification limit and 1711 µg of yessotoxin equivalents/kg of meat. This involved three samples of mussels from Italy, one sample of mussels from Denmark, and one sample of mussels from France (Etang de Diana in Corsica).

Discussion

The results of the 2015 surveillance plan for contamination of shellfish by phycotoxins at the distribution level indicate that, like in previous years, the contamination rate for bivalve molluscs by phycotoxins is low, with an overall non-compliance rate of 0.33% (95CI-[0.11-0.98]). The findings from this surveillance plan indicate that monitoring of marine production areas by Ifremer, associated with management measures, ensures a good food safety status for national products placed on the market. The three cases of non-compliance detected as part of the surveillance plan involved shellfish from other Member States of the European Union, which were therefore not produced and monitored in marine areas of France.

In addition, the surveillance plan ensures verification of compliance for products placed on the market in France, whether they are produced locally or imported. The combination of the two surveillance programmes makes it possible to ensure a high level of consumer protection.

Only one sample of French shellfish (mussels from Île de Groix) was involved in a case of collective foodborne illness in 2015, confirming the effectiveness of the national surveillance programme, and in particular upstream surveillance in the production areas.

In 2016, the DGAL decided to monitor only contamination of mussels by lipophilic phycotoxins at the distribution stage. This decision is based on the results of the REPHYTOX monitoring programmes implemented, which show that mussels are the bivalve molluscs that are most frequently contaminated by phycotoxins, and in particular lipophilic phycotoxins. The objective of this plan is to assess contamination levels of mussels on the market by lipophilic phycotoxins and thereby, consumer exposure.

Acknowledgements

The environment resources laboratories of Ifremer for implementation of the REPHYTOX network and for collection of data.

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Report on *Trichinella* spp. monitoring in meat

Isabelle Vallée (1) (isabelle.vallee@anses.fr), Gina Zanella (2), Pascal Boireau (1)

(1) ANSES, Maisons-Alfort Laboratory for Animal Health, Joint research unit (BIPAR) (ANSES-ENVA-Inra), National Reference Laboratory for Foodborne Parasites, Maisons-Alfort, France
(2) ANSES, Maisons-Alfort Laboratory for Animal Health, Epidemiology Unit, Maisons-Alfort, France

Abstract

Trichinella is a foodborne zoonotic parasitic nematode. The infective muscle larvae of the parasite enter the muscle cells of the host. Infection of humans or animals occurs through the consumption of raw or undercooked meat. *Trichinella* spp. is a major parasite of pigs, carnivores and omnivores. The parasite circulates in wildlife and can thus infect domestic animals in contact mainly with contaminated animals' carcasses. Meat inspection at the slaughterhouse is mandatory under international and European regulations, as is the inspection of all game animals intended for human consumption. In cases of private consumption, testing for larvae in meat is recommended. During the 1975-1999 period, human trichinellosis outbreaks occurred in France and led to the implementation of a monitoring system including the training of technicians from routine laboratories, standardisation and harmonisation of the network with the gold standard reference method of artificial digestion, as well as the establishment of a quality assurance programme with ring trials, the certification of routine laboratories by the Ministry of Agriculture, and laboratory accreditation. As a consequence, since 1999 the autochthonous cases of human contamination have been linked to consumption of meat that is not controlled by the veterinary services. The implemented system can thus be considered as effective in protecting consumers from *Trichinella* infections.

Keywords

Foodborne parasite, *Trichinella*, Zoonosis, Detection

Résumé

Bilan de surveillance de* Trichinella *spp. chez les animaux de boucherie

***Les trichines sont des nématodes parasites zoonotiques des viandes. Les larves musculaires de* Trichinella *spp. sont présentes dans les fibres musculaires et infectent l'Hôte définitif lorsque la viande est consommée crue ou peu cuite.* Trichinella *spp. est un parasite majeur des porcins, des carnivores et des omnivores. Il circule dans la faune sauvage et peut ainsi contaminer les animaux domestiques qui seraient en contact avec des cadavres d'animaux. La réglementation impose le contrôle des viandes à l'abattoir et des venaisons destinées à la consommation humaine en dehors du cercle privé familial. Dans ce cas (consommation familiale), le contrôle est recommandé mais non obligatoire. Les foyers de trichinellose humaine survenus en France pendant la période 1975-1999 ont conduit à la mise en place d'un dispositif de surveillance, alliant la formation des techniciens des laboratoires vétérinaires départementaux (LVD), la standardisation et l'harmonisation de la technique de détection directe et la mise en place d'un système d'assurance qualité comprenant l'organisation d'essais inter- laboratoires d'aptitude, la délivrance d'un agrément par le ministère en charge de l'Agriculture et l'accréditation des LVD. Grâce à ce dispositif, les carcasses positives sont identifiées dès l'abattoir et n'entrent pas dans la chaîne alimentaire. Ainsi, les seuls cas de contamination humaine autochtone déclarés depuis 1999 sont liés à la consommation de viande n'ayant pas été contrôlée par les services vétérinaires. Le contrôle officiel des viandes est donc aujourd'hui efficace pour protéger les consommateurs vis-à-vis du risque de contamination par* Trichinella.

Mots-clés

Parasites transmis par les aliments,* Trichinella*, zoonoses, détection

Trichinella spp. is a zoonotic parasitic nematode transmitted by the consumption of raw or undercooked meat. The *Trichinella* parasite is cosmopolitan and its various species have adapted to different climatic zones worldwide. It has a broad spectrum of hosts including all mono-gastric mammals. *Trichinella* spp. circulates across the globe and carries a health risk for humans (Box 1). This species regularly causes outbreaks that may affect a variable number of people depending on the infected slaughter animal.

Trichinella spp. remains a public health concern in some parts of the world, such as Latin America, Asia, Eastern Europe and the Balkans, and some Mediterranean regions (central Spain, Corsica and Sardinia). In other areas, the species poses an economic problem related to the cost of mandatory controls for the marketing of meat (Western Europe, North America). This parasite is in fact the only one covered by European and international regulations for meat intended for human consumption.

Official control of meat intended for human consumption

The surveillance programme in France is based on European regulations (EU 2015/1375) reinforced by guidance notes from the Directorate General for Food (DGAL) that are used to adapt these regulations (Box 2) to the epidemiological situation in the country and to livestock rearing conditions.

The exposure of indoor-raised pigs to *Trichinella* is considered negligible in Europe provided that the production sites are controlled (Commission Implementing Regulation EU 2015/1375). However, the lack of validated serological tests to ensure surveillance of these production facilities makes it impossible, at this time, to consider discontinuing control of these animals. As a result, the countries of the European Union are continuing monitoring. In France, one animal per thousand is therefore screened using direct survey testing to ensure surveillance of indoor production facilities. Outdoor or family-scale livestock production facilities are, however, a risk factor for contamination. This is why animals from these sources are controlled systematically, with higher test sensitivity through an increase in the analysed muscle mass (Table 1).

Direct detection of *Trichinella* spp. L1M larvae is required for horse meat and game meat from animals susceptible to this parasite, such as wild boars. Concerning [non-farmed] wild boars, analysis is mandatory for game meat marketed *via* short distribution channels (direct supply to retail distributors, restaurant owners, and hunting or association-related meals (Guidance note DGAL/SDSSA/N2008-8250)). Analysis of meat is strongly recommended for non-farmed wild boars intended for consumption within the family context.

Box 1. Trichinellosis

Trichinella spp. is a nematode parasite that causes trichinellosis, a major zoonosis resulting from consumption of raw or undercooked meat (ANSES, 2011). Humans (or animals, the definitive hosts) acquire the infection by eating meat that contains L1 muscle larvae (L1M) of *Trichinella* spp. These larvae are released in the stomach and then migrate to the epithelium of the small intestine where they moult to reach the sexually differentiated adult stage. Fertilised females produce newborn L1 larvae (NBL) in the intestinal epithelium. These larvae then migrate *via* the blood and lymphatic vessels to their definitive niche, skeletal striated muscle fibres. The NBL divert fibre muscle function for the benefit of a feeder cell and remain dormant for years at the L1M stage.

In animals, trichinellosis is asymptomatic, except in very rare cases. In humans, contamination by *Trichinella* remains silent at low ingested doses of parasites (fewer than 100 larvae). However, if there is significant or massive contamination (1000 L1M or more), more pronounced characteristic clinical signs develop after a short episode of diarrhoea accompanied by abdominal pain of variable intensity. The incubation period is proportional to the ingested parasite load and can range from one to four weeks. The clinical triad of myalgia, facial oedema and hyperthermia leads to suspicion of trichinellosis, which is confirmed by marked eosinophilia and specific serological results. Symptoms resolve within a few weeks but in 10% to 20% of cases, "chronic" trichinellosis may develop with recurrent muscle pain and/or persistent visual accommodation disturbances. Complications including encephalitis, myocarditis, pericarditis and acute heart failure may occur in the event of very high-level contamination (Dupouy-Camet *et al.*, 2015). The cost of treatment is high, estimated at 2000 euros on average per treated patient. There is no effective treatment to eliminate L1M settled in the muscle tissue (from about 15 days post-infection). This is why veterinary monitoring of carcasses is the only effective control method to prevent human cases.

Trichinella is the only foodborne parasite subject to European (EU 2015/1375) and international (OIE, *Codex alimentarius*) regulations.

Epidemiology

Affected species: trichinae are major parasites of swine and these animals are the main source of human contamination worldwide. Wild carnivores and omnivores are also a direct source of contamination for humans or an indirect source *via* contamination of outdoor-reared pigs exposed to parasitised meat/carcasses. Most wild and domestic monogastric mammals are at risk of being infected naturally. All Equidae are susceptible to this type of parasitosis: horses, ponies, donkeys, mules, etc. Nine species and three genotypes make up the *Trichinella* genus and have different geographical distributions. *T. spiralis* is a cosmopolitan parasite more commonly found in Europe and North America. There are three other species of trichinae in Europe (*T. britovi*, *T. nativa* and *T. pseudospiralis*). The prevalence of parasitic infection is higher in Eastern Europe, in Scandinavian countries and in Finland, in central Spain and in France in protected regions (natural parks).

Table 1. Mass to be analysed depending on the animal species, the type of rearing, and/or the animal status

Animal species	Type of rearing or status	Sampling site	Minimum mass to be analysed (in g)	Reference
Domestic swine	Indoor	Pillars of the diaphragm	1	Annex I, Chapter I of Regulation (EU) 2015/1375
		If no pillars of the diaphragm • Mastication muscles • Tongue • Abdominal muscles • Diaphragm	2	
	Outdoor or Breeding stock	Pillars of the diaphragm	2	Guidance notes: DGAL N2007-8054 of 27 Feb 2007 and N2007-8161 of 3 July 2007
		If no pillars of the diaphragm • Masseter muscles • Tongue	4	
	Special case	If meat is • from an unknown sampling site • intended for undercooked consumption	5	Annex I of Regulation (EU) 2015/1375, 2b
Wild boars	/	Tongue or pillars of the diaphragm	5	• Guidance note DGAL N2007-8003 of 02/01/2007 • Guidance note DGAL N2008-8250 of 24/09/2008 • Annex III of Regulation (EU) 2015/1375
Horses	/	Tongue or masseter muscles	10	• Guidance note DGAL N2006-8063 of 01/ 03/2006 • Annex III of Regulation (EU) 2015/1375
Other species	/	See Annex III of Regulation (EU) 2015/1375		

Despite this, the proportion of non-farmed wild boars actually tested is difficult to determine since numerical data on slaughtered wild animals managed directly by hunters or hunting federations are not systematically recorded by the Departmental Directorates for Protection of the Population (DDecPPs).

Muscle samples for analysis are taken at the slaughterhouse for pigs and horses, or at the processing facility for farmed wild boars. The regulatory analysis of carcasses involves an artificial digestion test of muscle samples taken at the slaughterhouse. These samples can be pooled into one test, making it possible to screen several animals at the same time provided that the minimum mass to analyse is in line with that required by the competent authority. This test is a direct method that leads to isolation of the parasite (L1M) in an acid-pepsin digestion liquid. The official method is described in Chapter I of Annex I of Regulation (EU) 2015/1375; the method was also recently standardised at the international level (ISO 18743-2015). Muscle sampling sites and masses for analysis are stipulated by European regulations. At the national level, applicable regulations reinforce European requirements, specifically for horse meat by doubling the mass to be analysed (Table 1).

The epidemiological situation in France

In horses

Between 1975 and 1999, twelve outbreaks of human trichinellosis occurred in France and in Italy as a result of consumption of infected horse meat originating from Eastern Europe or North America (Boireau *et al.*, 2000). Epidemiological case-control investigations

implemented for the major outbreaks (more than 10 clinical cases) led to horse meat being identified as the source of contamination. In 25 years, 3326 cases of human trichinellosis out of a total of 6250 for the entire EU were related to horse meat. Some 2296 people were affected in France over this period, with the other cases occurring in Italy. The emergence of this disease in these two countries can be explained by dietary habits, since only the French and Italians consume undercooked horse meat. Although the consumption of meat of equine origin is higher in Belgium (more than twice the level recorded for France), the custom of cooking horse meat thoroughly (well done) prevents any risk of parasite transmission. In each instance, the outbreaks were related to single infected horses with different geographic origins, although there was a slight predominance of Eastern European countries. The first horse naturally infected with *Trichinella* was seized at a slaughterhouse in Brescia, Italy in 1988. Contaminated horses were identified occasionally in France until the implementation of the quality assurance plan in 1999 (Box 3). Over this same period, eight other anademics (contamination from the same source) occurred and were the result of an insufficient sample volume or difficulty in standardising test readings, but these problems have been resolved since 1999.

In pigs

Data on official analyses for swine trichinellosis detection in France have been collected each year by the NRL *via* the DDecPPs for pigs depending on the rearing category (indoor, outdoor, breeding), and for wild boars marketed through short distribution channels since 1997. The health measures for management of indoor pig production holdings (control of feed, no contact with wildlife, rat control, etc.) enable the animals to be protected from contamination with *Trichinella* spp. Thanks to this system, there have been no detected cases of swine trichinellosis in indoor production holdings on the continent, with the exception of one pig found to be positive for *T. spiralis* in Brittany in 2007. This case, which

was detected through self-monitoring of meat intended for export as part of bilateral trade exchanges, was exceptional and unusual for a pig reared in this type of holding. Moreover, the resulting epidemiological investigation did not identify any other contaminated animals in the holding, nor among the wildlife (small rodents) living around the holding. One-off contamination of this pig by a small rodent may explain this case.

In 2004, two outdoor-reared pigs were found to be positive for *T. britovi* in the Haut-Taravo valley, Corsica, which was until then considered to be *Trichinella* spp. free. Since 2004, 25 domestic swine have been detected positive for *T. britovi* in this region or in neighbouring valleys. Serological monitoring surveys carried out in Corsica over the period 2006-2008 confirmed the low-grade circulation of the parasite among wild boar (*Sus scrofa*) populations, with a prevalence of 2.01% (95%CI 1.36-2.86) (Richomme *et al.*, 2010).

In wildlife

The parasite is also known to circulate in wildlife, and wild boars have been found positive for *T. britovi* mainly in the south of France (Occitanie and Provence-Alpes-Côte d'Azur regions). A positive wild boar was found in the Ariège *département* (*T. britovi*) in 2007, then another in 2011 in Gard (*T. britovi*), and a third in 2012 in Alpes-Maritimes (*T. britovi*). In addition, foxes were found to be infected in 2008 (3 in Var) and in 2013 (1 in Haute-Savoie), but also wolves in 2007 (4 in Savoie), in 2012 (1 in Isère), in 2013 (1 in Haute-Savoie), and in Alpes-Maritimes, with one in 2014 and one in 2015.

Box 3. Coordination of a network of accredited laboratories

Accredited departmental veterinary laboratories (LVD) carry out first-line screening of carcasses on a routine basis. If a suspect case is found, the larva or larvae are transferred to the National Reference Laboratory (NRL) for confirmation of the presence of *Trichinella* spp. larvae and identification of the species. Since 1999, the NRL has set up a quality assurance system in several stages regarding training, harmonisation of the official test and organisation of inter-laboratory proficiency tests (ILPTs), and lastly accreditation of official laboratories.

Training of technicians

At least once a year, the NRL organises a theoretical and practical training session on the official diagnosis of animal trichinellosis. This specialised two-day session covers: the biological and epidemiological cycle of *Trichinella* spp., the anatomy of the parasite, human trichinellosis, the official artificial digestion method, management of quality assurance as part of these analyses, applicable regulations, and the procedure for managing non-negative results. The session also looks at the limitations and critical points of the diagnostic technique. The training also covers other parasites that may be identified during trichinae analysis, such as the trematode *Alaria alata,* which circulates mainly in eastern France among wild boar populations (Portier *et al.*, 2011). Since 1999, about 400 technicians from the departmental veterinary laboratories have taken part in these training sessions.

Harmonisation of the detection technique and organisation of ILPTs

European regulations recognise several methods but the technique considered to be the reference is the "Magnetic stirrer method for pooled sample digestion" (Annex I, Chapter I, Commission Implementing Regulation (EU) 2015/1375). The network of laboratories in France was therefore harmonised for the use of this technique, which replaced trichinelloscopy (far less sensitive) and the Trichomatic 35® system.

In 2004, the NRL organised the first ILPT nationally with the aim of evaluating implementation of the official method in the participating laboratories. Participation in the ILPT is mandatory for departmental veterinary laboratories because the compliance of results is a prerequisite for obtaining and maintaining the accreditation granted by the DGAL (Official Journal 2008). Participation in the ILPT is also essential for accreditation of departmental veterinary laboratories and maintains the skills of accredited personnel. To organise these ILPTs, the NRL has developed an original method to prepare reference meat samples containing a determined number of capsules of L1M of *Trichinella spiralis* (Vallée *et al.,* 2007). Through implementation of this method, France was the first European country to organise ILPTs for the detection method of *Trichinella* larvae in the meat matrix. The proficiency of the accredited departmental veterinary laboratories improved rapidly, since it was found, as of the second ILPT (2nd half of 2004), that all laboratories were able to detect the larvae present in the meat sample. Changes in the network over eleven years clearly show that the proficiency of laboratories has stabilised with more than 80% of accredited laboratories achieving an average above 75% for the identification of larvae in the reference sample. This reflects a good level of proficiency in line with what is expected of laboratories routinely, given the sensitivity of the test (ICT guidelines). The ILPTs were organised every six months up to 2011 and became annual from 2012 because the network was shown to be stable for several years. In 2016, a total of 59 accredited departmental veterinary laboratories participated in the ILPT and obtained compliant results. These laboratories thus form an effective national network for the detection of *Trichinella* spp. muscle larvae in meat from pigs, wild boars and horses.

Laboratory accreditation

Regulations require that these laboratories be accredited to ensure traceability and proper performance of analyses. Since 2011, the 59 laboratories participating in the ILPTs therefore launched an accreditation procedure with the French Accreditation Committee (Cofrac) and the entire network will be recognised by the end of 2016. The accredited method is that described in Regulation (EU) 2015/1375, Annex I, Chapter I, which is recognised as the reference method (ICT guidelines).

Box 2.

Objectives of the surveillance programme

- Detect animals carrying larvae of *Trichinella* spp. at the slaughterhouse and remove them from the food chain.
- Ensure that animals presenting a risk for the consumer are controlled.

Surveillance framework

Regulation (EU) No 2015/1375

Wildlife is subject to outbreak surveillance with the reporting of confirmed cases of hunted wild boars for which detection of trichinae larvae was requested by the hunter or the hunting federation in question.

Organisation of the national programme

The surveillance network is made up of approved and accredited departmental veterinary laboratories (LVD), the National Reference Laboratory for Foodborne Parasites (ANSES, Maisons-Alfort), and the relevant departments of the DGAL. When a departmental veterinary laboratory detects a nematode larva in first-line screening, the specimen is forwarded to the NRL for identification and to confirm the presence of *Trichinella* spp. Molecular species typing is also performed to characterise the isolate and to identify the specific *Trichinella* species. If the case is confirmed, the incriminated carcasses are removed from the food chain in accordance with the regulations.

The NRL ensures coordination of the accredited LVD network by organising:

- theoretical and practical training sessions for LVD technicians (since 1999),
- inter-laboratory proficiency testing (since 2004; initially every six months until 2011, then annually),
- scientific and technical support.

The analytical method has been standardised at the national and international levels, and the LVD network was harmonised for use of this method. This is the regulatory magnetic stirrer method for pooled sample digestion described in Annex I of Regulation (EU) 2015/1375. The method involves direct detection of *Trichinella* larvae in muscle samples taken at the slaughterhouse or at the processing facility, depending on the masses and elective sites described in the European regulations, reinforced by DGAL guidance notes.

Discussion and conclusion

The trichinellosis monitoring programme implemented in France has proven to be effective and prevents many human cases. It can be estimated that one wild boar carcass is shared by about fifteen different consumers, one pig by thirty,

and one horse by 400 to 500 (on the basis of data from the most recent human outbreaks that occurred in France in 1997-1998). If we take into account the fact that two horse carcasses, 29 pig carcasses, and four wild boar carcasses were found to be infected between 1999 and June 2016, more than 1900 people have been spared from exposure since 1999. The result is that consumers in France can be regarded as protected from the risk of *Trichinella* provided that meat is controlled by official services.

However, when at-risk carcasses are not controlled by veterinary services, there is a potential hazard for the consumer, as was demonstrated by the recent contamination episode that led to three confirmed human cases due to *figatelli* sausage consumed raw and prepared from a non-controlled pig (Ruetsch *et al.*, 2016). Since 1999, the main autochthonous human cases have been associated with consumption of non-controlled wild boar meat. Although hunter training includes information on the risk of *Trichinella* contamination, the number of animals controlled in the context of private consumption remains low. Moreover, imported cases have also been recorded with the main source of contamination in recent years being consumption of polar bear meat following travel to the Arctic region (Canada, Greenland). Since 2004, 26 positive cases have been reported in this context, including three cases in 2016 related to consumption of polar bear meat in Greenland (Dupouy-Camet *et al.*, 2016).

Cases of human trichinellosis are recorded by the Parasitology Department of Cochin Hospital, a former national reference centre, which became a contracted laboratory of the French Public Health Agency (formerly the INVS) responsible for monitoring human trichinellosis (cnrdestrichinella.monsite-orange.fr). This laboratory, the NRL (ANSES), the DGAL, and the French Public Health Agency all work in close collaboration when there is a suspected case or a reported autochthonous human case in order to determine the incriminated parasite species (*T. spiralis, T. britovi, etc.*) as early as possible, along with the parasitic load of consumed products when possible, and to carry out an epidemiological investigation. Identifying the trichinae species and the parasitic load is important for the treatment of affected patients.

The NRL is charged with collecting data on trichinae health inspections for animals from the DDecPPs, as well as the total number of animals slaughtered by *département*. It now appears necessary to develop this collection system into a computerised tool in order to have data that can be rapidly quality controlled. This would also provide a more precise calculation of the total number of analyses performed with reference to recorded animals, either at the slaughterhouse or at the processing facilities. It would also be beneficial to integrate data concerning non-farmed wild boars managed directly by hunters or hunting federations, in order to estimate more precisely the number of animals that are in fact officially controlled for trichinae.

Trichinella spp. is a parasite that requires permanent control efforts because it cannot be eradicated, given its broad host range and its circulation in wildlife worldwide. Control of trichinellosis requires protection of indoor swine production holdings and monitoring of at-risk meat (from horses, wild boar and outdoor-raised pigs), as well as information to consumers on the risks related to dietary

habits that involve eating undercooked game meats. *Trichinella* spp. is at the centre of the "One Health" concept that includes animal health, food safety and public health.

Acknowledgements

The authors would like to thank the various players in the control scheme: accredited departmental veterinary laboratories, NRL personnel, DGAL departments, and colleagues from Cochin Hospital.

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Epidemiological monitoring of bovine cysticercosis in France: situation in 2015

Céline Dupuy (celine.dupuy@agriculture.gouv.fr) (1), Monique Fresnel (1), Pierre Guillet (2), Mylène Auge (3), Luc Serra (3), Claire Morlot (1), Emilie Gay (4)

(1) Ministry of Agriculture, Food and Forestry, French Directorate General for Food, Office for Slaughter and Cutting Plants, Paris, France
(2) Ministry of Agriculture, Food and Forestry, Office for project management of information systems concerning food, Paris, France.
(3) Ministry of Agriculture, Food and Forestry, Office for project management of information systems concerning food, Toulouse, France
(4) ANSES, Epidemiology Unit, Lyon Laboratory, France

Abstract

The French national meat inspection database (SI2A) was launched in all French cattle slaughterhouses on 1 January 2015. It has enabled the surveillance of annual bovine cysticercosis prevalence and incidence rates. In 2015, raw apparent prevalence was 0.123% [0.122-0.123] (95 CI) for both viable and degenerated cysts and 0.0096% [0.0095-0.0098] for viable cysts. True prevalence was estimated at 1.07% [0.72-1.67] and 0.08% [0.06-0.13] for both viable and degenerated cysts and for viable cysts respectively. The comparison of raw apparent prevalence in 2010 and adjusted prevalence for age-sex in 2015 showed a slight but statistically significant decrease during this period. This decrease could be attributed either to an improvement in the bovine cysticercosis situation or to lower meat inspection detection sensitivity in 2015 due to a difference in data collection methodologies. The implementation, in addition to the current surveillance system, of a method for identifying farms/areas at higher risk for infestation in France could enable the development of more appropriate prevention and control measures.

Keywords

Bovine cysticercosis, Surveillance, France

Résumé

Epidémiosurveillance de la cysticercose bovine en France : situation en 2015

Le déploiement, depuis le 1er janvier 2015, du Système d'information sur l'inspection en abattoir (SI2A) dans tous les abattoirs bovins français a permis la mise en place d'une surveillance annuelle de la prévalence et de l'incidence de la cysticercose bovine. En 2015, la prévalence apparente de la cysticercose bovine (tous types de cysticerques confondus, vivants et calcifiés) était de 0,123 % [0,122-0,123] (IC95) et de 0,0096 % [0,0095-0,0098] pour les cysticerques vivants. La prévalence réelle, prenant en compte la sensibilité estimée de la détection a été estimée à 1,07 % [0,72-1,67] pour les cysticerques quel que soit leur stade de développement et à 0,08 % [0,06-0,13] pour les cysticerques vivants. La comparaison de la prévalence apparente en 2010 et de la prévalence apparente ajustée sur l'âge et le sexe en 2015 a montré une diminution faible mais statistiquement significative sur cette période. Cette diminution pourrait être attribuée soit à une amélioration de la situation vis-à-vis de la cysticercose bovine, soit à une baisse de la sensibilité de détection liée à des modalités de collecte de données différentes. La mise en place, en complément de la surveillance actuelle, d'un dispositif d'identification des élevages dans les zones les plus à risque en France permettrait d'envisager des méthodes de prévention, de lutte et de détection plus adaptées.

Mots-clés

Cysticercose bovine, Surveillance, France

Cysticercosis (Box 1) has a considerable economic impact on the cattle rearing sector because of the associated condemnations and depreciation of carcasses following freezing treatment. The economic significance and zoonotic nature of this infestation warrant the implementation of an epidemiological surveillance system to contribute to better risk management (Box 2). To this end, it is necessary to use data collected at the slaughterhouse that were until recently very difficult to obtain. In 2010, a one-time survey was conducted to collect all necessary information regarding bovine cysticercosis in slaughterhouses in France *via* a questionnaire, but this process requires considerable energy and is not comparable to a permanent surveillance system.

On 1 January 2015, the Ministry of Agriculture launched the French national meat inspection database (SI2A) for use in all slaughterhouses in mainland France and overseas *départements* and territories. This tool is used to record and centralise the results of *ante-* and *post-mortem* inspections of animals that presented an anomaly (*ante-mortem* clinical signs/*post-mortem* lesions). The SI2A must be used in all cattle slaughterhouses. The database was designed to facilitate inspections by veterinary service officers at the slaughterhouse by enabling them to immediately issue registers, notifications (e.g. condemnation certificates) and letters. An application called Dedal (Decision-making system for food) was also developed to enable officers to access the results of pre-set queries. The direct use of recorded data to issue official documents on the one hand, and the

Box 1. Bovine cysticercosis

Cysticercus bovis-related cysticercosis is a parasitic zoonosis affecting cattle as the intermediate host and humans as the definitive host (Figure 1). Cattle are infected primarily by feeding on pastures infested with C. bovis eggs originating from excretion of the parasite by humans, particularly following landfarming with insufficiently treated water from water treatment plants (Cabaret *et al*., 2002). C. bovis larvae then migrate from the gastrointestinal tract to the muscles where they become encysted into cysticerci. These cysticerci remain viable for a few months and then degenerate and calcify at the latest nine months after ingestion. Humans are then infected by ingesting living cysticerci when they eat parasitised meat that is raw or undercooked. An adult taenia (tapeworm) then develops in two or three months resulting in the release of proglottids in the faeces, which is a source of discomfort (Scientific Committee on Veterinary Measures relating to Public Health, 2000).

Since infestation is asymptomatic in animals, detection is only possible at the slaughterhouse on *post-mortem* inspection. All slaughtered cattle are inspected visually looking at the heart, tongue, masseters, oesophagus and diaphragm, as well as mandatory muscle incisions (European Parliament, 2004). Infested carcasses are seized integrally if there is massive infestation. In the event of local infestation, partial seizure or freezing treatments are applied. Infested meat may be placed on the market because of the low sensitivity of inspection at the slaughterhouse, leading to the sale of infested carcasses. Humans are then contaminated *via* consumption of undercooked beef.

possibility of accessing reports on aggregated data on the other, are important components that help to guarantee both the sustainability of information recording and data quality.

This review presents the epidemiological situation concerning bovine cysticercosis in 2015 based on SI2A data compared with data from the study carried out in 2010. It makes use of epidemiological indicators adjusted for age and sex, two important factors to consider when calculating the prevalence of this disorder so as to limit interpretation bias (Dupuy *et al.*, 2014b).

Material

The data on prevalence and distribution of the cattle population by age and sex in 2010 originate from the article by Dupuy *et al.*, (2014a). For 2015, data were extracted from the SI2A database for cattle subject to a *post-mortem* inspection decision for one of the following reasons: muscular cysticercosis, localised viable form; muscular cysticercosis, localised degenerated form; and muscular cysticercosis, generalised. Data from the national cattle identification database (BDNI) were used to obtain information on the date of birth, date of slaughter, and sex of all the cattle slaughtered over this period.

Method

Apparent prevalence, true prevalence and apparent prevalence adjusted for age and sex were calculated for 2015. Apparent prevalence is defined as the number of cattle detected at the slaughterhouse with at least one cysticercosis lesion divided by the total number of slaughtered cattle. True prevalence was calculated by dividing apparent prevalence by the probability of detection of cysticercosis, estimated by EFSA at 11.5% [7.4-17.1] (Dupuy *et al.*, 2012).

Age and sex were identified as the main individual factors associated with variability in terms of cysticercosis lesions at the slaughterhouse. Since there may be significant fluctuations over time in the proportions of cattle slaughtered regarding age and sex, prevalence data must be adjusted for these variables to enable comparisons of prevalence levels between years, without any bias related to changes in the typology of the slaughtered population.

Prevalence adjusted for a combined age-sex variable was therefore calculated by direct standardisation. The cattle population slaughtered in the year 2010 was defined as the reference population, and the data on the cattle population slaughtered in 2015 were adjusted by weighting of the distribution of cattle slaughtered in 2010 using the age-sex variable.

For this standardisation, the following age-sex classes were used: 0-8 months-female; 0-8 months-male; 8-24 months female; 8-24 months-male; 2-3.5 years-female; 2-3.5 years-male; 3.5-5 years-female; 3.5-5 years-male; 5-10 years-female; 5-10 years-male; >10 years-female; >10 years-male.

The standardised cysticercosis rate (SCR) can be used to quantify the difference observed between two adjusted prevalence levels. It is determined by indirect standardisation (Bouyer *et al.*, 2009; Breslow and Day, 1987). The cattle population slaughtered in 2010 was defined as the reference population and the distribution of the population in terms of age-sex in 2015 was used to determine the expected number of cattle presenting cysticercosis lesions in 2015, if the age-sex–adjusted prevalence was similar to that in 2010. This number was obtained by multiplying the number of cattle observed presenting cysticercosis lesions in 2010 by the ratio between the number of cattle slaughtered in 2015 and 2010, for each age-sex variable group. The SCR was then defined as the ratio between the observed number of cattle with a cysticercosis lesion in 2015 divided by the expected number of cattle presenting cysticercosis lesions in 2015.

Box 2.

Objectives
The objective of the epidemiological surveillance system for cysticercosis is firstly to monitor incidence and annual prevalence of this disorder in France. Subsequently, it aims to identify the holdings/zones that are most at risk in France.

Programming framework
Regulation (EC) No 854/2004 provides for systematic inspection of all cattle carcasses aimed at detecting bovine cysticercosis through incisions and palpations.

Protocol
Officers from the veterinary inspection services screen for the presence of viable or degenerated/calcified cysticercosis lesions at the slaughterhouse in all the cattle slaughtered in France. A decision is made for each carcass (partial seizure, full seizure, freezing treatment) and is recorded in the national SI2A database that must be used in all cattle slaughterhouses across the country.

A case of cysticercosis with a viable cysticercus is defined as any animal recorded in the SI2A with a *post-mortem* inspection including the reason "muscular cysticercosis, localised viable form".

A case of cysticercosis for all types of cysticerci is defined as any animal recorded in the SI2A with a *post-mortem* inspection of the second degree including one of the following reasons: "muscular cysticercosis, localised viable form"; "muscular cysticercosis, localised degenerated form"; "muscular cysticercosis, generalised".

Table 1. Apparent prevalence and prevalence adjusted for a combined age-sex variable and standardised cysticercosis rate for all types of cysticerci and for viable cysticerci, with a 95% confidence interval for cattle slaughtered in France in 2010 and 2015 (reference = cattle slaughtered in 2010)

	2010	2015
All types of cysticercosis		
Apparent prevalence (%)	0.142 [0.142-0.143]	0.123 [0.122-0.123]
Adjusted prevalence (%)		0.121 [0.121-0.121]
Standardised cysticercosis rate	1	0.84 [0.84-0.84]
Only viable cysticerci		
Apparent prevalence (%) = incidence	0.013 [0.013-0.014]	0.0096 [0.0095-0.0098]
Adjusted prevalence (%) = adjusted incidence		0.0095 [0.0095-0.0095]
Standardised cysticercosis rate	1	0.71 [0.71-0.71]

Results

The survey carried out in 2010 included 4,564,065 cattle (91.3% of cattle slaughtered in 2010) in the prevalence analysis, after exclusion of cattle without data on age and sex. The estimated apparent prevalence levels are presented in Table 1 (Dupuy *et al.*, 2014a, Dupuy *et al.*, 2014b).

In 2015, 4,692,454 cattle were slaughtered in the 209 cattle slaughterhouses in France. Of these 209 slaughterhouses, 202 used the SI2A to record slaughterhouse seizures. The available data for the analysis of prevalence in 2015 concerned 4,689,095 cattle slaughtered in these 202 slaughterhouses (99.9% of the cattle slaughtered in France over this period), including 5736 that were condemned integrally or partially due to cysticercosis lesions. After exclusion of cattle without data on age and sex (n=28,214, 0.6%), the study population contained 4,660,881 cattle. In this study population, *post-mortem* inspection enabled the detection of at least one cysticercosis lesion (irrespective of the development stage) for 5729 cattle, i.e. an apparent prevalence of 0.123% [0.122-0.123].

Among the infested animals, 450 (7.9%) presented lesions with viable cysticerci, i.e. an apparent prevalence of 0.0096% [0.0095-0.0098]. Also among the infested animals, 148 cattle presented a generalised form (2.6%).

The true prevalence, irrespective of the cysticercus development stage, was estimated to be 1.07% [0.72-1.67]. The true prevalence of bovine cysticercosis due to viable cysticerci was estimated to be 0.08% [0.06-0.13].

The adjusted prevalence values and SCRs are shown in Table 1. The difference between two prevalence values was considered statistically significant when their confidence intervals did not overlap.

Discussion

The prevalence of bovine cysticercosis due to viable cysticerci can be regarded as the incidence from an epidemiological perspective, as the presence of this type of lesion is indicative of recent infestation (at the most a few months before slaughter). Simultaneous monitoring of the prevalence of bovine cysticercosis due to viable cysticerci and the prevalence of bovine cysticercosis due to all types of cysticerci therefore provides complementary information.

The differences between the apparent prevalence values and the adjusted apparent prevalence values in 2015 were low, though statistically significant. This is related to small differences in the distribution of the slaughtered cattle population in terms of age and sex between 2010 and 2015 (variation ranging from 0.1 to 1.8% depending on the age-sex class). This does not, however, detract from the usefulness of comparing adjusted prevalence values rather than apparent prevalence values, because considerable differences may be observed in the future. The decision to slaughter an animal is a complex multi-factorial process (Dupuy, 2014c).

The comparison of the apparent prevalence in 2010 with the adjusted apparent prevalence in 2015 shows a statistically significant but small decrease between these two years. There were, in fact, 1.2 (1/0.84) and 1.4 (1/0.71) times fewer cases observed in 2015 compared to the values that should have been observed if the prevalence had been identical to 2010, respectively for all forms of cysticercosis and for cases of viable cysticerci only (Table 1). The decrease is statistically greater for incidence than for prevalence.

This could lead to the conclusion that there has been an improvement in the situation regarding bovine cysticercosis in France with a lower prevalence, but above all a lower incidence. However, caution should be exercised when comparing these results. The 2010 data originate from a survey specifically asking slaughterhouses through a guidance note to report information on cysticercosis lesions detected at the slaughterhouse *via* questionnaires. It is possible that this had an effect on the sensitivity of detection of cysticercosis lesions through increased awareness of this disorder among inspection services. Hadorn and Stärk *et al.* (2008) have demonstrated the substantial impact that increased awareness of inspection officers could have on detection sensitivity. For tuberculosis, sensitivity was shown to increase from 50.6% to 80.4%. This study is based on the hypothesis that sensitivity was identical in 2010 and 2015, even though the data collection process was different. In addition, the 2010 survey involved double recording of information: use of the local tool by officers to issue the condemnation certificate and, in parallel, recording in the survey questionnaire. There is a concern that this double recording may not have been systematic, leading to an under-reporting bias.

The information available *via* the SI2A is less precise than that from the 2010 survey. For generalised lesions, the development stage of cysticerci was not specified systematically because the distinction between generalised lesions with or without the presence of viable cysticerci was not provided for. In some cases, the officers recorded both generalised cysticercosis and localised cysticercosis due to viable cysticerci, enabling us to conclude that viable cysticerci were present, and similarly with localised cysticercosis due to degenerated cysticerci. The 143 cattle that presented generalised lesions without

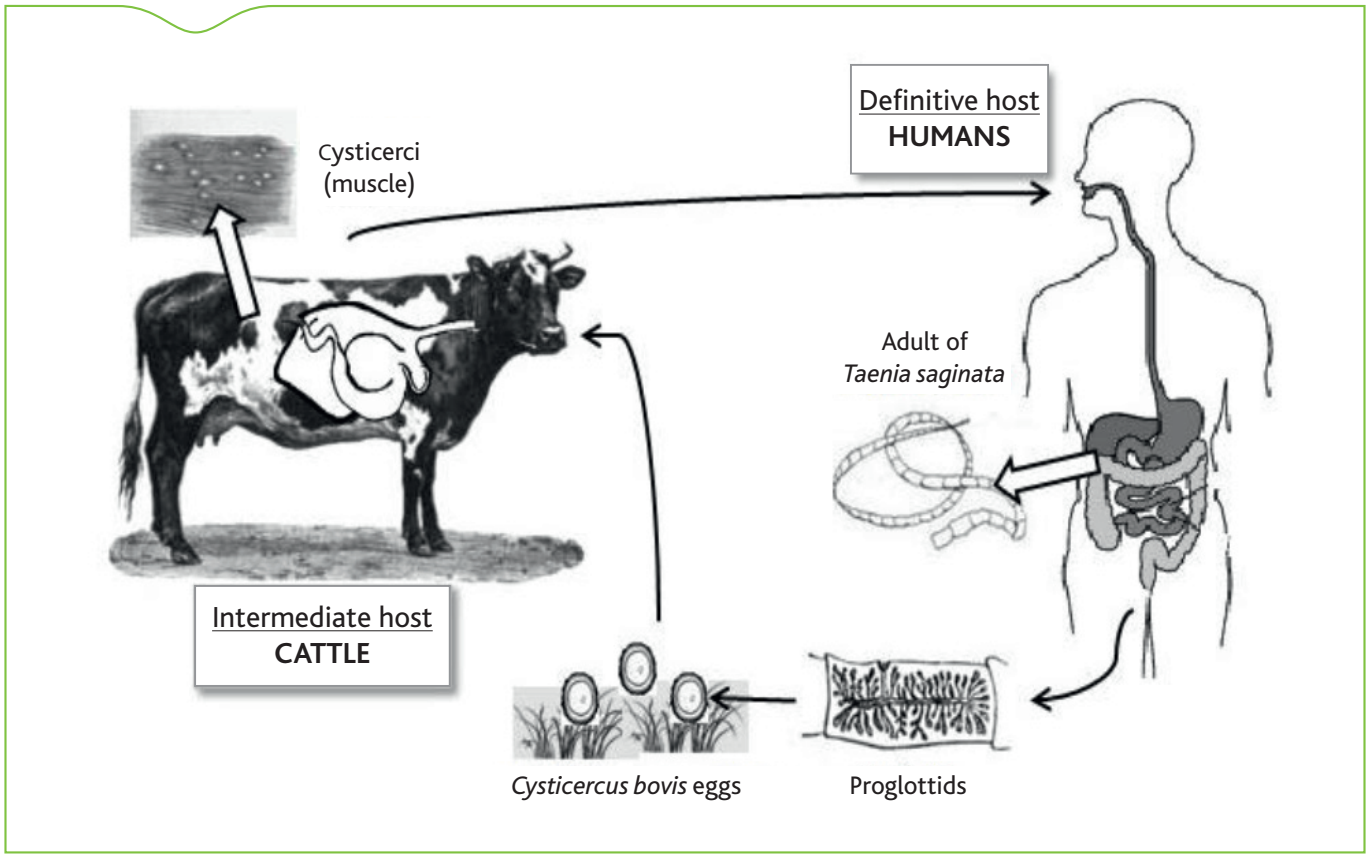

Figure 1. Cycle of *Taenia saginata* (Morlot, 2011)

other information were considered to have degenerated cysticerci lesions, which could have an effect on the incidence results if some of these animals had viable cysticerci lesions. It appears necessary to consider upgrading the lesion references in the SI2A to include this distinction for generalised lesions so as to increase the reliability of monitoring of bovine cysticercosis incidence and prevalence.

2015 was the first year of operation of the SI2A. Analysis of annual data on bovine cysticercosis from this system will be used to monitor changes in the incidence and prevalence of this disorder, on the basis of data collected in a similar way from one year to the next, thus limiting measurement bias.

Conclusion

The SI2A database has made it possible to set up an epidemiological surveillance system for bovine cysticercosis in France through routine collection of information regarding this disorder. We are now able to monitor the prevalence and incidence of bovine cysticercosis on an annual basis through practically exhaustive data for the whole country. This also facilitates feedback to farmers *via* standardised condemnation certificates and a possible move to digitise this information.

Inspections based on risk are already implemented using the Food Chain Information (FCI) forwarded by farmers. FCI covers all the relevant information that the breeder provides to the slaughterhouse on the animals intended for slaughter. A list of this information is defined by ministerial order, and includes information regarding bovine cysticercosis. However, FCI regarding bovine cysticercosis is based solely on cases recorded for the animal's most recent holding, which results in a considerable bias specifically for calcified cysticerci lesions (long interval between infestation and detection of the lesion at the slaughterhouse). This information could be improved by implementing a surveillance system that can identify farms or zones that are at the highest risk (high prevalence of cysticerci), taking into account the uncertainty about the place where the animal was infested (Dupuy *et al.,* 2015). Suitable prevention and control measures could also be implemented more easily. This would, however, require the use of data on cattle movements from birth to slaughter, and routine access to and analysis of these data are more complex.

Acknowledgements

The authors would like to thank all the officers of the inspection services at slaughterhouses for their inspection activities and recording of inspection data in the SI2A.

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Surveillance of **veterinary drug residues** in poultry meat and eggs

Brigitte Roudaut (brigitte.roudaut@anses.fr) (1), Isabelle Fournet (isabelle.fournet@agriculture.gouv.fr) (2)
(1) ANSES, Fougères Laboratory, Veterinary drug residues reference laboratory, Fougères, France
(2) Directorate General for Food, Office for agricultural inputs and public health in livestock rearing, Paris, France

Abstract
Some chemicals introduced intentionally (veterinary drugs, additives) or illegally (banned substances) in the diet (drinking water, feed) of poultry are likely to be transferred to the muscles and also to the eggs in laying females (hens, quails, etc.). In the EU, while some antibiotics are registered as veterinary drugs (Regulations (EC) No 470/2009 and (EU) No 37/2010), most coccidiostats are registered as additives in accordance with Regulation (EC) No 1831/2003 on additives for animal feed. This paper aims to present the results of French control plans for antibiotics, anthelmintics, coccidiostats and non-steroidal anti-inflammatory drugs (NSAIDs) in poultry meat (broilers, turkeys, other poultry) and eggs (hens, quails) for 2015. The results show that most poultry and eggs are marketed free of veterinary drug residues. The implementation of the Hygiene Package should further reduce the non-compliance rate for some of these substances and guarantee products against these risks.

Keywords
Control programme, Monitoring programme, Poultry, Meat, Eggs, Antibiotics

Résumé
Le dispositif de surveillance des résidus de médicaments vétérinaires dans les volailles et les œufs
Certaines substances chimiques introduites de manière volontaire (médicaments vétérinaires, additifs) ou frauduleuse (substances interdites) dans l'alimentation (eau de boisson, aliment) des volailles sont susceptibles d'être transférées vers les muscles chez les volailles et aussi vers l'œuf chez les femelles pondeuses (poule, caille...). Dans l'Union européenne, alors que certains antibiotiques sont enregistrés en tant médicaments vétérinaires (règlements 470/2009/CE et 37/2010/UE), la plupart des anticoccidiens sont enregistrés comme additifs selon le règlement 1831/2003/CE, relatif aux additifs destinés à l'alimentation des animaux. Le présent article a pour objectif de présenter un bilan des résultats des plans de contrôle français pour les antibiotiques, anthelmintiques, anticoccidiens et antiinflammatoires non stéroïdiens (AINS) dans les muscles de volailles (poulet de chair, dinde, autres volailles) et dans les œufs (poule, caille) pour l'année 2015. Les résultats montrent que les volailles et les œufs commercialisés sont en grande majorité exempts de résidus de médicaments vétérinaires. La mise en place du Paquet hygiène devrait permettre de diminuer encore le taux de non-conformité pour certaines de ces substances et de garantir les produits vis-à-vis de ces risques.

Mots-clés
Plan de contrôle, plan de surveillance, volailles, viande, œufs, antibiotiques

Veterinary medicinal products and additives used in animal nutrition are prescribed and used intentionally according to strictly controlled procedures (dosage, time of administration, and withdrawal before slaughter) to guarantee their safety and efficacy. All of these substances are evaluated in terms of risks before being authorised and placed on the market. In particular, the use of veterinary medicinal products must not lead to residue concentrations that exceed the maximum residue limits (MRLs) in foodstuffs from animals exposed to these substances. Moreover, certain substances are prohibited in animal production.

The assessment of veterinary medicinal products is carried out by the European Medicines Agency (EMA). This assessment is used to determine MRLs in foodstuffs of animal origin on the basis of the concept of withdrawal periods, and results in the establishment of the list of authorised active substances (Directive 2001/82/EC, Regulation (EC) No 470/2009). Several groups of veterinary medicinal products are authorised for use in poultry: antibiotics, antiparasitics, anthelmintics and coccidiostats. Coccidiostats are the most commonly used class of active substance in poultry in France, after antibiotics. However, the number of compounds available is limited in laying hens due to continual production of eggs and the risk of transfer of residues to eggs. Treatment is generally given orally in drinking water or feed for five days on average. The parenteral route accounts for less than 1% of treatments. Compliance with the withdrawal period is particularly sensitive in laying hens since the producer is required to withhold egg production from the market during treatment, and in some cases, for several days after treatment. Compounds that have obtained an MRL for eggs mostly do not exceed this MRL during treatments recommended by a veterinarian.

The assessment of food additives is carried out in accordance with Regulation (EC) 1831/2003 by the European Food Safety Authority (EFSA). Some coccidiostat additives, authorised for broilers and turkeys, are only allowed in pullets for egg production until the age of 12 to 16 weeks. All antibiotic additives have been banned in the European Union since 1 January 2006.

Objectives of the surveillance programme - Regulatory references

Official control plans aim to identify possible traces of drug residues in meat (muscle) and eggs, for which the public health risk has previously been evaluated and led to MRLs being defined in these foodstuffs for the authorised substances (Commission Regulation (EU) No 37/2010). The medicinal products containing the authorised substances undergo assessment with a view to granting of a marketing authorisation (MA), leading to the determination of withdrawal periods to comply with between the last administration of the medicinal product and marketing of the products originating from the animals (meat, eggs and offal). Compliance with the conditions of use (route of administration, dosage) and with the withdrawal period guarantee with very high probability that residue levels are below the MRLs and there is no toxicological risk for the consumer. Alongside these plans targeting substances that are authorised or not authorised for the poultry sector, other official control plans focus on substances that are currently banned from use in animal production, such as chloramphenicol, nitrofurans and nitroimidazoles. Sampling is either random or targeted. Samples are collected in accordance with the procedures laid down in Commission Decision 98/179/EC.

Non-compliance is reported either due to the simple presence of residues when the substance yielding the residues is banned, or due to the presence of residues at concentrations above those authorised (MRLs), taking into account the measurement uncertainty (decision limit).

Non-compliance thresholds are established:

- for veterinary medicinal products according to Commission Regulation (EC) No 470/2009 and Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin,
- for coccidiostats according to the various EU regulations concerning the authorisation of these substances as animal feed additives, and Commission Regulation (EC) No 124/2009 of 10 February 2009 (setting maximum levels for the presence of coccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed). Some coccidiostats are also used as veterinary medicinal products and therefore have an MRL (Commission Regulation (EU) No 37/2010).

Surveillance and control plans

Various control plans

Since 1989, control plans for the detection of antibiotic residues have been implemented in primary poultry production in order to meet EU requirements, in particular Council Directive 96/23/EC of 29 April 1996 *on measures to monitor certain substances and residues thereof in live animals and animal products,* supplemented by Commission Decision 97/747/EC *fixing the levels and frequencies of sampling provided for by Council Directive 96/23/EC*. Since 1996, detection has been expanded to cover other classes of veterinary medicinal products.

These plans must be targeted and, following this process, a total of 2459 meat samples were collected in 2015 from slaughterhouses using targeting criteria ranging from basic signs concerning the carcass to information from the food chain (official documentation), and 2984 samples were taken from production facilities or slaughterhouses to test for prohibited substances. In all, 557 egg samples were collected from farms or at packaging plants to test for authorised substances, and 69 samples for the detection of prohibited substances.

Other controls for antibiotic residues (self-monitoring) are also performed by the professional sector *via* in-house laboratories or certified laboratories. In this case, commercial screening kits (using biological, ELISA or immunochromatographic methods) can be used.

Sampling plan

The number of samples to collect per sampling site (farm or slaughterhouse) was calculated to meet the minimum requirements of Council Directive 96/23/EC, on a pro rata basis:

- of slaughtered tonnage for poultry (1,668,447 t in 2014). The minimum number of samples to collect for each category of poultry must be one sample for every 200 t of annual production, with a minimum of 100 samples per group of substances (for annual production greater than 5000 t). As such, in 2015, the breakdown of samples according to species was as follows for antibiotics: 59.5% for chickens, 25% for turkeys, 12.5% for other poultry, and 3% for cull chickens,
- of production volumes for eggs (772,213 t in 2014). The minimum number of samples to collect must be one sample for every 1000 t of annual production, with a minimum of 200 samples. The breakdown of samples according to species was as follows: 95% for chicken eggs and 5% for quail eggs.

The breakdown of these samples by group and class of contaminants is then determined according to the minimum requirements set out in the relevant regulations and according to a risk assessment related specifically to the number of non-compliant samples identified in previous years.

Veterinary medicinal product classes tested for in muscle and eggs

The choice of substances to be tested by class of contaminants is established jointly with the national reference laboratories based on known usage, the analytical methods used, and their performance.

Box.

Objectives
These control plans are aimed at assessing compliance with the conditions of use of veterinary medicinal products or coccidiostat additives (route of administration, dosage), and the withdrawal period between administration of the medicinal product (or additive) and consumption of the foodstuffs originating from the treated animals. The plans also aim to detect any use of prohibited substances that could present a toxicological risk for the consumer, and to identify and examine the reasons for the presence of residues in foodstuffs of animal origin.

Programming framework
Council Directive 96/23/EC, Commission Decision 97/747/EC.

Commission Regulation (EC) No 470/2009, Commission Regulation (EU) No 37/2010, and Commission Regulation (EC) No 124/2009.

Protocol

- **Contaminants of interest: veterinary drug residues**
  > Prohibited substances
  > Authorised substances: antibiotics, anthelmintics, coccidiostats and non-steroidal anti-inflammatories.
- **Targeted production types: poultry meat (chicken, turkey, other poultry), eggs (chicken, quail).**
- **Stage of the food chain: production facilities, slaughterhouses, packaging plants (for eggs).**
- **Definition of a "case"**

Non-compliance involving a concentration greater than the decision limit and triggering a management measure (investigation of the source of contamination).

- **Number of samples and sampling method**

Meat: 2459 samples were collected at the slaughterhouse for the detection of authorised substances, and 2984 samples at production facilities or the slaughterhouse for the detection of prohibited substances between January and December 2015.

Eggs: 557 samples were collected at production facilities or at packaging centres for the detection of authorised substances, and 69 samples for the detection of prohibited substances between January and December 2015.

- **Sampling strategy:** targeted controls, carried out in accordance with the procedures laid down in Commission Decision 98/179/EC, using targeting criteria. The sampling effort is distributed by region on a pro rata basis of the previous year's production.
- **Analytical methods, types of samples**

To test for prohibited substances, only techniques based on tandem mass spectrometry are used for screening and confirmation.

To test for authorised substances, broad-spectrum methods such as microbiological or immunological (biochip) techniques are used to screen for antibiotics. Over the past few years, more expensive very broad spectrum multi-residue chemical methods based on tandem mass spectrometry have also been used for the detection of antibiotics and other classes of veterinary medicinal products: anthelmintics, coccidiostats and non-steroidal anti-inflammatories. More conventional methods, including liquid and planar chromatography, are also used for certain classes of antibiotics.

Table 1. Non-compliance rate in meat and eggs by class of veterinary medicinal products in 2015

	Meat (slaughterhouse samples)			Eggs (farm or packaging site samples)		
	Number of recorded results	Number of non-compliant results	Non-compliance rate (%)	Number of recorded results	Number of non-compliant results	Non-compliance rate (%)
Prohibited substances	2,984	0	0	69	0	0
Chloramphenicol LC-MS/MS	1,387	0	0	29	0	0
Nitrofurans LC-MS/MS	271	0	0	20	0	0
Nitroimidazoles LC-MS/MS	1,326	0	0	20	0	0

No cases of non-compliance were detected for 2015 concerning these prohibited substances, whether for meat or eggs.

Table 2. Non-compliance rate for other substances in meat and eggs by class of veterinary medicinal products in 2015

		Meat (slaughterhouse samples)			Eggs (farm or packaging site samples)		
		Number of recorded results	Number of non-compliant results	Non-compliance rate (%)	Number of recorded results	Number of non-compliant results	Non-compliance rate (%)
Authorised substances		**2,459**			**557**		
Antibiotics		1,236	3	0.24	235	1	0.43
Multi-residue testing	4-plate test (muscle) or Evidence biochips (eggs) + LC-MS/MS	315	1	0.32	62	0	0
	LC-MS/MS + LC-MS/MS	340	1	0.29	/		
Testing by class	Sulfonamides: HPTLC + HPLC-UV	331	1	0.30	173	1	0.57
	Tetracyclines: HPLC-UV	230	0	0	/		
	Quinolones: HPLC-fluorimetry	20	0	0	/		
Anthelmintics HPTLC + LC-MS/MS		708	0	0	/		
Coccidiostats LC-MS/MS		510	0	0	322	1	0.31
NSAIDs LC-MS/MS		5	0	0	/		

4-plate test: microbiological screening method for bacterial inhibitors – LC-MS/MS: liquid chromatography coupled with tandem mass spectrometry – HPTLC: high-performance thin-layer chromatography – HPLC: high-performance liquid chromatography coupled with UV or fluorimetric detection.

The classes of medicinal products tested are listed in Tables 1 and 2. They derive from the regulatory requirements of Council Directive 96/23/EC.

Screening and confirmation methods

In the poultry sector, conventional broad-spectrum microbiological methods or more innovative immunological methods (Evidence biochips) are used to screen for antibiotics. Over the past few years, more expensive very broad spectrum multi-residue chemical methods based on tandem mass spectrometry have also been used for the detection of antibiotics and other classes of veterinary medicinal products: anthelmintics, coccidiostats and non-steroidal anti-inflammatories. More conventional methods, including liquid and planar chromatography, are also used for certain classes of antibiotics.

The analytical methods used for these official controls are listed in Tables 1 and 2 based on the targeted medicinal product classes. These methods are regularly reviewed and validated by the National Reference Laboratory for veterinary drug residues to include the new compounds placed on the market and thereby to follow changes in practices. To test for prohibited substances, only techniques based on tandem mass spectrometry are used for screening and confirmation.

Results

The contamination levels found *via* SCPs for prohibited substances in muscle and eggs are shown in Table 1, while those for authorised substances in muscle and eggs are shown in Table 2.

For antibiotics, the muscle samples were divided up and analysed according to two analytical strategies combining different analytical methods: two for multi-residue detection (testing for residues of several classes of antibiotics), and three for targeted testing of one antibiotic class (sulfonamides, tetracyclines and quinolones that are not well detected using microbiological methods). In 2015, the cases of non-compliance detected in poultry muscle concerned oxytetracycline (duck, cull hen) and sulphadimethoxine. Traces of doxycycline were also found in turkey muscle at a concentration below the MRL. For the other classes of veterinary medicinal products, no cases of non-compliance were detected in poultry muscle.

Concerning eggs, no cases of non-compliance were found for antibiotics *via* biochip screening. However, residues of oxytetracycline were detected (63 and 100 µg/kg), but at concentrations below the MRL in eggs (200 µg/kg), indicating good adherence to dosages in poultry farming. One case of non-compliance was found for sulphonamides with the detection of 5.8 µg/kg in a quail egg. This compound is not authorised for use in egg-laying species. Another case of non-compliance was identified with residues of monensin (a coccidiostat) at 2.3 µg/kg in a chicken's egg, while the maximum residue level is 2 µg/kg.

Interpretation

In 2015, the cases of non-compliance detected for antibiotics in poultry muscle involved oxytetracycline and sulfadimethoxine in birds other than broilers (ducks, cull hen). Production site inspections were carried out and showed that these non-compliant cases were associated with treatments using medicated feedingstuffs. Moreover, the results (Table 2) showed a greater ability to detect non-compliance for multi-residue strategies, in particular screening using the LC-MS/MS method, in comparison to approaches with methods targeting a single antibiotic class.

The targeting criteria at the slaughterhouse were based on the state of the poultry or on other information from the food chain (medicinal product treatment before slaughter). The overall rate of

non-compliance of 0.12% for poultry meat can be considered low in view of the targeting criteria used (*Review of the surveillance and control plans implemented by the DGAL in 2014*). However, the 0.24% rate of non-compliance for antibiotics should be monitored, particularly in minor species (duck, quail, etc.).

Concerning eggs, the cases of non-compliance mainly involved sulphonamides and coccidiostats, medicinal products or additives that are not authorised for use in egg-laying chickens. The concentrations found are very low (< 6 µg/kg). The assumption made by the DGAL for the non-compliance was exposure *via* feed that should not contain these substances (cross-contamination of non-supplemented feed by medicated feedingstuffs at the feed manufacturing stage, during transport, or at the farming site). An exploratory plan on the presence of antibiotics and coccidiostats in animal feed at the farming site is to be implemented in 2017 to document this assumption.

Discussion

Although sales of antibiotics for poultry have decreased by 30.3% over the past seven years (Chevance and Moulin, 2015) and exposure of poultry in terms of the Animal Level of Exposure to Antimicrobials (ALEA) decreased by 12.3% between 2009 and 2013, exposure remains higher in comparison to other animal sectors, such as sheep and goats, cattle, and fish. Despite the fact that poultry are among the three most exposed species to antibiotics (along with rabbits and swine), the rate of non-compliance for antibiotic residues is lower in poultry when compared to ruminants in particular.

Non-compliance identified through surveillance and control plans remains rare. Overall, the antibiotic classes most commonly implicated in non-compliance were tetracyclines and sulphonamides (Roudaut *et al.*, 2013). These classes are among the most frequently used antibiotics in poultry, after polypeptides which are very poorly absorbed in the intestines and which do not lead to non-compliance. Production site inspections have, nonetheless, identified other cases of non-compliance related to inappropriate farming practices (absence of a livestock register, an incomplete livestock register, poor management of the farm's medicinal stocks). Warnings and/or reminders regarding the regulations were issued to the breeders in question.

Concerning veterinary medicinal products, the main causes of non-compliance in other European countries are intentional or accidental use of medicinal products that are not authorised for use in egg-laying chickens, failure to observe the maximum age for administration, infringement of withdrawal periods or dosages, and cross-contamination by supplemented feed during preparation of these feedingstuffs at the manufacturing site or farm (Cannavan *et al.*, 2000). Another source of contamination, though less frequent, is recycling of medicinal products by ingestion of litter by chickens (Kan, 2005).

Regarding coccidiostat additives, non-compliance results mainly from cross-contamination at different stages of the process, between non-supplemented feed and feed containing additives (Cannavan *et al.*, 2000, Mortier *et al.*, 2005). Certification of production facilities associated with the management of available information on medicinal product use (livestock register) ensures traceability of products and treated animals, reduces the risk of residues being present, and guarantees the products in terms of this risk.

Conclusion and outlook

The results of control plans (EFSA report, 2014) and surveys show that muscle and eggs marketed in Europe are for the most part free of regulated chemical contaminants. This high level of safety is achieved through strict regulations on animal feed and on use of veterinary medicinal products and additives, and application by those involved in the production sector. The implemented approach with good practice guidelines in line with the Hygiene Package should help further reduce the non-compliance rate and guarantee products regarding these risks. Given the current context in France of promoting careful use of antibiotics to reduce the risks of antibiotic resistance in veterinary medicine (EcoAntibio 2017 plan), the focus at the farm level is on appropriate use of these drugs in the various animal sectors. Breeders are also responsible for ensuring that the products they market comply with residue concentrations set in corresponding regulations. However, provision of information to breeders and satisfactory quality control of feed transferred to the farm by self-monitoring and by the regulatory authorities remain essential. Changes have already been made in the use of antibiotics in the poultry sector, with a reduction in the use of the "medicated premix" form, which currently represents only 4% of body weight treated.

Furthermore, the ability to monitor a wider range of residues related to the use of different classes of antibiotics is fundamental with the aim of detecting new practices. This is why it was decided for the 2015 and 2016 programmes to increase the number of samples to be analysed directly by the LC-MS/MS method (liquid chromatography coupled with tandem mass spectrometry) for multi-residue testing. For 2016, the sampling schedule for the detection of antibiotic residues in the poultry sector provides for more than 1300 meat samples and 235 egg samples. In parallel, the NRL has developed and validated a new multi-residue method including new compounds, that can screen for more than 80 antibiotics, along with another method targeting different antiparasitic classes with an LC-MS/MS method. These methods will be operational in 2017 in accredited laboratories. An exploratory plan is also scheduled for 2017 to measure the exposure of poultry to antibiotics in feed, resulting from cross-contamination. As part of its research activities, the Fougères Laboratory is also working on the development of new analytical methodologies based on non-targeted analysis in muscles, offal and droppings using an LC-MS/MS method to examine the use of certain antibiotics (cephalosporins) in poultry.

Acknowledgements

The authors would like to thank all the teams at the accredited laboratories and the NRL for their efforts in obtaining the data in these control plans, as well as the DDecPPs.

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The French system for surveillance of contamination by plant protection products in foodstuffs of animal origin

Chanthadary Inthavong (1) (chanthadary.inthavong@anses.fr), Anne-Claire Martel (2), Isabelle Fournet (3)
(1) ANSES, Laboratory for Food Safety, Pesticides and Marine Biotoxins Unit, Maisons-Alfort, France.
(2) ANSES, Sophia-Antipolis Laboratory, Honeybee Pathology Unit, Sophia Antipolis, France
(3) French Directorate General for Food, Department for health actions in primary production, Sub-Directorate for health and animal protection, Office for agricultural inputs and public health in livestock rearing, Paris, France

Abstract
Every year, programmes for the surveillance and control of contamination in foodstuffs of animal origin are organised by the Directorate General for Food (DGAL). These programmes constitute an important tool in the food safety system. In animal production, eleven surveillance programmes are carried out for the detection of pesticide residues. Samples are collected in the preliminary stage in farms. Multi-residue methods are used to test for pesticide residues in foodstuffs. Programmes organised in 2014 and 2015 generated nearly 161,000 analysis results. Detected contamination levels were very low (no non-compliant samples in 2014, two in 2015) in accordance with the results obtained by other Member States. The only two non-compliant samples detected concerned lindane. This contamination was probably due to the persistence of this substance in the environment.

Keywords
Pesticides, Residues, Foodstuffs of animal origin, Surveillance programmes, Control programmes

Résumé
Le dispositif français de surveillance des produits phytosanitaires dans les denrées alimentaires d'origine animale
Les plans de surveillance et de contrôle de la contamination des denrées alimentaires d'origine animale sont mis en place chaque année par la direction générale de l'Alimentation en application de la réglementation européenne. En production animale, onze plans de surveillance sont mis en œuvre pour la recherche des résidus de pesticides. Les prélèvements sont réalisés au stade de la production primaire chez les éleveurs français. Les résidus de pesticides sont recherchés dans ces denrées alimentaires par méthodes multi-résidus. Les plans de 2014 et 2015 ont engendrés plus de 161000 résultats d'analyses. Le taux de contamination détecté est très faible (deux prélèvements non conformes en 2015 et aucun en 2014), ce qui est cohérent avec ce qui est observé dans les autres États membres. Les deux seules non-conformités détectées concernaient le lindane. Cette contamination est probablement d'origine environnementale, due à la rémanence de cette substance.

Mots-clés
Pesticides, résidus, denrées alimentaires d'origine animale, plans de surveillance, plans de contrôle

The use of pesticides (or phytosanitary products or plant protection products) developed from the end of the Second World War. Pesticides are classified into four categories depending on their use: fungicides, herbicides, insecticides and a fourth category for miscellaneous substances. In terms of production, the breakdown in tonnage in 2014 was 45%, 40%, 2% and 13%, respectively (French Plant Protection Industry Association - UIPP, 2014). The first insecticides used were synthetic products belonging to the organochlorine compound class, which, as a result of their persistence, are still found in the environment today, several decades after their use was discontinued. These chemical products accumulate all along the food chain and, due to their high lipophilic affinity, may contaminate certain foodstuffs of animal origin with high fat content. Despite the gradual phasing-out of pesticides associated with known health risks (the most problematic substances), use of plant protection products has remained common practice in conventional agriculture since the 1980s. Manufacturers have progressively replaced these pesticides by organophosphate compounds, synthetic pyrethroids, carbamates, triazoles and neonicotinoids (Table 1).

Although foodstuffs of plant origin are the main food category likely to contain pesticide residues, foodstuffs of animal origin may also lead consumers to be exposed to these contaminants. This is because once a substance is applied to a crop, residues of this substance (parent compound and/or degradation products) may be found in plant products consumed by animals, and pesticide residues are known to accumulate in animal tissues.

Objectives of the surveillance programme - Regulatory references

The European system for monitoring plant protection products in foods of animal origin addresses one of the missions of the European Food Safety Authority (EFSA), established by Regulation (EC) No 178/2002, i.e. the collection of data with the purpose of measuring consumer exposure to these residues and identifying any emerging risks.

This system is governed by the following regulations:

- Council Directive 96/23/EC, which requires that Member States of the European Union implement surveillance and control plans for chemical residues (more specifically, residues of plant protection products) in foodstuffs of animal origin. Since 1997, France has organised control plans to meet this regulatory requirement and communicates the results to the European Commission on an annual basis. Similarly, the Commission forwards compiled results from the various Member States to EFSA, mandated for this purpose within the framework of Article 31 of Regulation (EC) No 178/2002, and
- the various EU implementing regulations (788/2012 - 400/2014 - 2015/595) concerning the coordinated multiannual control programme for the years 2013 to 2018. These regulations list the active substance/foodstuff pairs to be assessed over this period. These provisions aim to evaluate compliance with maximum levels of pesticides in or on foodstuffs of plant and animal origin, and assess consumer exposure to these residues. These maximum residue levels (MRLs), applicable to pesticides found in or on food

and feed of plant or animal origin, are established in Regulation (EC) No 396/2005. These MRLs for foodstuffs, established for each substance, guarantee that the residues found in the food do not represent a risk for the consumer further to use of the active plant protection product in accordance with good agricultural practices for the treatment of crops.

Surveillance and control plans

To meet the requirements of the various regulations, the Directorate General for Food (DGAL) organises surveillance plans (SPs) and control plans (CPs). The Directorate sets up a national and then regional sampling schedule in line with the sampling plans it determines or those stipulated in European regulations. The regional players (Regional Food, Agriculture and Forestry Directorates - DRAAF and Regional Food Authorities - SRAL) are in charge of departmental scheduling, working jointly with the DDecPPs tasked with carrying out the sampling.

The difference between SPs and CPs lies in their objectives, resulting in a different sampling strategy. In the case of SPs, the aim is to evaluate the representative level of contamination of a food category (ultimately, these data help assess consumer exposure), by random sampling within a population or sub-population, and thus without

Table 1. Groups of pesticides used

Product groups over time			
	Herbicides	**Fungicides**	**Insecticides**
Before 1900	Copper sulphate Iron sulphate	Sulphur Copper salts	Nicotine
1900-1920	Sulphuric acid		Arsenic salts
1920-1940	Nitro derivatives		
1940-1950	Plant hormones		Organochlorines Organophosphates
1950-1960	Triazines, Substituted ureas, Carbamates	Dithiocarbamates Phthalimides	Carbamates
1960-1970	Bipyridyl, Toluidines, etc.	Benzimidazoles	
1970-1980	Amino phosphonates, Propionates, etc.	Triazoles, Dicarboximides, Amides, Phosphites, Morpholines	Pyrethroids, Benzoylureas (growth regulators)
1980-1990	Sulphonylureas		
1990-2000		Phenylpyrroles, Strobilurins	

Source: French Plant Protection Industry Association (UIPP) - Brochure on research in plant protection products

Box.

Objectives

Since 1998, control plans for the detection of pesticide residues for agricultural use have been implemented in primary production to meet the requirements of Council Directive 96/23/EC of 29 April 1996 *on measures to monitor certain substances and residues thereof in live animals and animal products*.

The objective of these control plans is to detect any illegal treatments and/or inappropriate practices in primary production that may adversely affect the safety of foodstuffs. They help to manage the risk of foodstuff contamination by chemical substances that are considered to have probable or proven chronic toxicity. They provide surveillance data regarding this contamination in order to contribute to national and European risk assessments. The implementation of Directive 96/23/EC is aimed at guaranteeing harmonisation of national controls in each Member State to maintain the same level of safety.

Programming framework

Regulation (EC) No 78/2002, i.e. the collection of data with the purpose of measuring consumer exposure to these residues and identifying any emerging risks.

Council Directive 96/23/EC of 29 April 1996 *on measures to monitor certain substances and residues thereof in live animals and animal products*.

Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 *on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC*.

Commission Implementing Regulations (EU) (88/2012 - 400/2014 - 2015/595) concerning the coordinated multiannual control programme for the years 2013 to 2018.

Protocol

- Type of contaminants of interest: Pesticides for agricultural use and veterinary medicinal products (acaricides).
- Targeted production sectors (populations): foodstuffs of animal origin.
- Stage of the food chain: slaughterhouses, beekeepers for honey.
- Definition of a "case": Non-compliance involves either the simple presence of pesticide residues when the substance yielding the residues is banned, or the presence of residues at concentrations above those authorised (> MRLs).
- Number of samples and sampling method: the number of samples to collect by sector and by sampling site (farm or slaughterhouse) was calculated to meet the minimum requirements of Council Directive 96/23/EC, on a pro rata basis of the number of animals slaughtered (meat and large game), slaughtered tonnages (poultry, small game and rabbits), and production volumes (farmed fish, milk, eggs and honey).
- Sampling strategy: exhaustive.
- Analytical method, type of samples: the Directorate General for Food (DGAL) organises surveillance and control plans (SCPs). The Directorate sets up a national and then regional sampling schedule in line with the chosen sampling plans or those stipulated in European regulations. The regional players (Regional Food, Agriculture and Forestry Directorates - DRAAF and Regional Food Authorities - SRAL) are in charge of departmental scheduling, working jointly with the DDecPPs tasked with carrying out the sampling.

Almost all analyses are carried out by accredited laboratories in accordance with Standard NF EN ISO/CEI 17025 and approved by the Ministry of Agriculture, Food and Forestry, and by National Reference Laboratories (NRLs).

taking into account the contamination risk level. In the case of CPs, the aim is to characterise abnormal situations and to detect suspected non-compliance or cases of fraud. Sampling in this case is targeted at a portion of the production that is assumed to have a higher risk of contamination (sampling undertaken on the basis of predetermined targeting criteria).

The planning of regional and then departmental sampling, the quality of sampling, and the precision of the collected data *versus* expected results are critical factors affecting the credibility of the generated safety information. The robustness of the system underpins satisfactory risk management and non-biased risk assessment.

The official analyses carried out on these samples are performed by laboratories approved by the Ministry of Agriculture, Food and Forestry on the basis of a clear statement of requirements, including accreditation by the French Accreditation Committee (Cofrac) in accordance with Standard NF EN ISO/CEI 17025. Only these laboratories are authorised to analyse samples taken within the framework of official controls. The network of laboratories is coordinated by the National Reference Laboratories (NRLs), which develop and validate official methods, provide technical support to laboratories, and ensure their technical proficiency to perform analyses. Some of these NRLs carry out official analyses themselves as part of the SCPs: in the event of development of a new method

Table 2. SCPs for plant protection products in foodstuffs of animal origin in 2014 and 2015 in France

	Meat (beef, pork, lamb and mutton, goat's meat, horse meat)			Poultry			Farmed fish	Rabbits	Game	Dairy products			
	Muscle	Fats	Liver	Muscle	Muscle and fats	Liver	Meat	Muscle	Muscle	Milk	Butter	Eggs	Honey
Carbamates	A			A									
Pyrethroids		A	T	A	T	T	A	A	A	A	T	A	A
Organochlorines		A	T	A	T	T	A	A	A	A	T	A	A
Organophosphates		A	T	A	T	T				A	T	A	A
Other pesticides			T		T	T	A				T	T	A
Neonicotinoids													A

A: annual; T: triennial

Table 3. Size of samples and number of analyses performed for the surveillance plans of plant protection products in foodstuffs of animal origin for 2014 and 2015 in France

	Target population annual mean	Size of the minimum annual national sample required by regulations for the testing of plant protection products		Size of the effective annual national sample		Number of results for pesticide residue concentration rates obtained 2014+2015
		N	Proportion (%)	2014	2015	
Cattle	4,775,000 (total number of cattle slaughtered over 12 months)	430	0.009	450	450	47,200
Pigs	23,933,000 (total number of pigs slaughtered over 12 months)	430	0.002	500	450	40,000
Small ruminants	4,472,000 (total number of sheep and goats slaughtered over 12 months)	90	0.002	100	60	10,000
Horses	19,000 (total number of horses slaughtered over 12 months)	Absence	Absence	10	5	1,000
Poultry	1,703,000 tonnes produced over 12 months	255 (batches)	0.01	505	445	42,000
Rabbits	46,000 tonnes produced over 12 months	10 (batches)	0.02	5	5	300
Farmed fish	50,000 tonnes produced over 12 months	Absence	Absence	30	90	3,000
Farmed game	3000 large game (red deer, roe deer, fallow deer) 9000 tonnes of small game (pigeon, quail, partridge, pheasant) slaughtered over 12 months	Absence	Absence	5	5	1,800
Milk	24,703,000 tonnes collected over 12 months	Absence	Absence	70	40	8,000
Butter		66: every 3 years		0	66	
Eggs	772,000 tonnes produced over 12 months	Absence	Absence	70	90	8,000
Honey	11,800 tonnes produced over 12 months	0.3 %	35	50	50	
TOTAL				**1,795**	**1,756**	**161,300**

or testing on a new matrix (e.g. detection of pesticides in butter or analyses of pesticides in honey).

Surveillance and control plans implemented in 2014 and 2015

Council Directive 96/23/EC, supplemented by Commission Decision 97/747/EC, governs the strategy, the level and the frequency of sampling for the 11 surveillance plans to be implemented in primary production each year, in the following matrices:

- beef, pork, and poultry from the farm and slaughterhouse,
- sheep/goat's meat, horse meat, rabbit, and farmed game from the slaughterhouse,
- farmed fish and milk from the farm or first processing level,
- eggs from the collection site,
- honey from the beekeeper (or elsewhere if traceability to the beekeeper is guaranteed).

The samples are taken unannounced for CPs and preferably targeted at risk criteria. However, given the difficulty in targeting, a random system of sampling was retained for pesticides. They are collected in accordance with the procedures laid down in Commission Decision No 98/179/EC.

Most of the pesticides tested for annually as part of the SCPs belong to the organochlorine, organophosphate, synthetic pyrethroid and carbamate pesticide classes, in line with the requirements of Directive 96/23/EC. However, other groups such as the neonicotinoid or benzoylurea classes may also be tested for in animal matrices known to contain this type of pesticide, or on the strength of implementing regulations regarding the multiannual pesticide control programme (Table 2).

Sampling plan for the SCPs of 2014 and 2015

The number of samples to collect by sector and by sampling site (farm or slaughterhouse) was calculated (Table 3):

- to meet the minimum requirements of Council Directive 96/23/EC, i.e. pro rata of:
  - the number of animals slaughtered for meat and large game,
  - tonnages of animals slaughtered for poultry, small game and rabbits,
  - production volumes for farmed fish, milk, eggs and honey;
- to establish prioritisation based on the number of non-compliant samples detected in previous years.

The choice of substances to be tested by class of contaminants was established jointly with the National Reference Laboratories based on expected risks of use, regulatory obligations, analytical methods used, and their performance.

The sampling strategy implemented jointly by the DGAL and the NRL for pesticides, in line with regulatory obligations, aims to define a representative pesticide residue contamination level for a food group. Although the size of the sampling may appear small compared to the target populations, the power of the methods implemented provides a large number of concentration measurements for a broad range of plant protection substances. The confidence intervals for the results obtained range from 1 to more than 3% depending on the sectors, which is not very precise and difficult to analyse as is. However, the repeatability of this plan can enable possible emerging trends to be identified.

Analytical methods

The official methods can be used to cover about 70 pesticides belonging to different classes.

Official methods

There are currently several multi-residue methods that can determine pesticide levels in foodstuffs of animal origin. These methods are generally based on an extraction protocol of pesticide residues and fats, and are therefore mostly aimed at liposoluble pesticides (Ledoux *et al.,* 2011).

Assays of pesticides are carried out by gas chromatography (GC) coupled with electron capture detectors (ECDs) and nitrogen-phosphorus detectors (NPDs). Although these detectors are still used for searching certain pesticides, mass spectrometry (MS) is now used as a detector coupled with gas chromatography (GC-MS). Laboratories now increasingly use gas and liquid chromatography coupled with tandem mass spectrometry (GC-MS/MS and LC-MS/MS). Recent developments in mass analysers and data processing mean that more accurate and specific assays can be performed for pesticides using techniques such as liquid chromatography coupled with high-resolution mass spectrometry.

Now that mass spectrometry is used routinely in laboratories, broad-spectrum multi-residue methods can be developed.

Broad-spectrum multi-residue methods

The first Quick Easy Cheap Effective Rugged and Safe (QuEChERS) type method was developed in 2003 (Anastassiades *et al.,* 2003a, 2003b). It basically includes three steps: extraction, purification, and detection. Over the past 10 years, QuEChERS methods have evolved to address the specific problems related to foodstuffs of animal origin. The European Union Reference Laboratory (EURL) for pesticides in food of animal origin and commodities with high fat content, along with the NRLs are working on these types of methods known as broad-spectrum methods because they not only have the advantage of screening a large number of pesticides of low to high polarity, but are also rapid and effective. These methods, developed and then validated according to the SANCO 12571/2013 guidance document by NRLs, can be applied to samples in the context of SCPs.

Results

A non-compliant result indicates either the simple presence of pesticide residues, when the substance of interest has been banned from use, or the presence of residues at a level higher than the MRL for authorised products.

For 2014, the overall results of the surveillance and control plans carried out in France did not reveal any non-compliance. In 2015, in 1622 samples (3034 analyses performed), two cases of non-compliance were identified: one in a muscle sample of beef (0.031 mg/kg), and the other in an egg sample (0.03 mg/kg). This involved contamination by hexachlorocyclohexane (the chemical name of lindane), for which the MRLs are 0.02 mg/kg in muscle and 0.01 mg/kg in eggs. Lindane is an organochlorine insecticide that was marketed starting from 1938. This substance has a very broad spectrum of insecticidal activity against plant-eating insects, soil-dwelling insects, and human and animal parasites. Lindane was therefore used widely in agriculture, and in pharmaceutical products for the treatment of scabies and the elimination of lice.

In France, lindane has been discontinued in agriculture since 1 July 1998, and since 2009 in the rest of the world. There are no plant protection products containing lindane authorised for sale. However, concerning the use of pesticides that are not authorised in the EU, Regulation (EC) No 396/2005 sets MRLs at a sufficiently low level to protect consumers against ingestion of pesticide residues given the persistence of some of these substances in the soil.

The investigations carried out at the farm level were not able to identify the source of contamination.

For the non-compliant beef muscle, the investigation carried out at the farm (a small-scale site with about 20 animals) was not able to identify sources of contamination via:

- environmental pollution (the production site is in a mountain environment with no nearby industry or craft trades),
- food (only hay produced on-site and mineral supplements in the form of a mineral lick),
- veterinary medicinal products (only Closamectin Pour-on). The hypothesis of treatment of surrounding trees was considered but not confirmed.

Concerning the non-compliant chicken egg sample, the investigation carried out in an outdoor holding was also unable to identify the source of contamination *via* food or drinking water. The hypothesis of soil pollution was retained. The producer has subsequently stopped outdoor egg production.

Comparison of data with plans in other European countries

According to the 2015 EFSA annual report that summarised, among other data, the results of analyses for regulated pesticides in foodstuffs of animal origin for 2013 for all European countries, of the 8257 samples analysed, 25 (0.3%) showed values exceeding the MRL (Table 4). The most commonly identified or detected pesticides were hexachlorobenzene, DDT, thiacloprid, lindane, endosulfan, amitraz and pirimiphos-methyl. For the most part, these products, like the organochlorine substances, are no longer used in Europe but are frequently found given their persistence in the environment.

For 2013, there were no samples with levels exceeding the MRL among the 1021 cow milk samples analysed. In contrast, a few pesticides were found in trace amounts. This involved hexachlorobenzene and DDT, both of which have been banned since 1979. A similar result was found for the 753 pork muscle samples analysed.

Discussion - Outlook

None of the results for 2014 in France showed cases of non-compliance across all the plans implemented. The results for 2015 were also satisfactory with a non-compliance rate between 0.3% (beef plan) and 1.2% (egg plan). In both these cases, the pesticide residue found was lindane. Further to the investigations carried out at the production site, it appears that the presence of this substance in the samples was not due to its use, but rather its persistence in the environment in the case of eggs.

To ensure more in-depth future investigations and to confirm or rule out the hypothesis of soil contamination, a procedure is under consideration to sample and analyse soil for this type of persistent pollutant.

The results obtained for the various national surveillance and control plans in France are comparable to those reported in other Member States, i.e. a low level of contamination by pesticides in foodstuffs of animal origin. However, some cases of non-compliance were recorded and are probably more likely related to environmental contamination than use of the pesticides themselves. Currently, the official methods cover about 70 pesticides belonging to various classes. Over the last few years, new pesticides have been produced by manufacturers and are used by agricultural producers. The list of pesticides to test has therefore evolved in Europe. National Reference Laboratories are working on developing methods that are more rapid with a broad spectrum. These will be used to extract a larger number of pesticides to better evaluate contamination of foods of animal origin.

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Table 4. Results for detection of pesticides in foodstuffs of animal origin in Europe in 2013 exceeding the MRL

Foodstuff/pesticide	Origin of foodstuff	Number of non-compliant samples (/values > MRL)	Residue levels (mg/kg) min - max	MRL (mg/kg)
Honey		**6/2**		
Azoxystrobin	Denmark	5/2	0.011 - 0.086	0.01
Thiacloprid	Austria	1/0	0.233	0.2
Game		**4/0**		
DDT	Denmark	4/0	0.057 - 0.095	0.05*
Chicken eggs		**3/3**		
Lindane	Austria	2/2	0.254 - 0.295	0.01*
DDT	Denmark	1/1	0.209	0.05
Pork fat, beef muscle, poultry muscle		**5/4**		
Permethrin	Estonia	3/3	0.077 - 0.183	0.05*
Methoxychlor	Estonia, Belgium	2/1	0.018 - 0.021	0.01*

(*) Value corresponding to the limit of quantification (LOQ) of the analytical method

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Results of the surveillance and control plans on **pesticide residues in honey** for 2014 and 2015

Anne-Claire Martel (1) (anne-claire.martel@anses.fr), Adrien Bégaud (1), Patrick Mangoni (1), Isabelle Fournet (2)

(1) ANSES, Sophia Antipolis Laboratory, Honeybee Pathology Unit, Sophia Antipolis, France
(2) French Directorate General for Food, Department for health actions in primary production, Sub-Directorate for health and animal protection, Office for agricultural inputs and public health in livestock rearing, Paris, France

Abstract
Plans for the surveillance and control of the contamination of foodstuffs of animal origin are organised each year by the Directorate General for Food (DGAL) in accordance with European regulations. For the beekeeping sector, samples are collected at the preliminary stage from French beekeepers. Pesticide residues (veterinary drugs and plant protection products) are analysed in honey using gas chromatography (GC) and liquid chromatography coupled with electrospray tandem mass spectrometry (LC-MS/MS). The results of the 2014 and 2015 plans show low levels of contamination below the maximum residue limits (MRLs).

Keywords
Honey, Pesticides, Residues, Surveillance and control plans

Résumé
Les plans de surveillance et de contrôle de la contamination des denrées alimentaires d'origine animale sont mis en place chaque année par la Direction générale de l'Alimentation (DGAL) en application de la réglementation européenne. Pour la filière apicole, les prélèvements sont réalisés au stade de la production primaire chez les apiculteurs français. Les résidus de pesticides (médicaments vétérinaires et phytosanitaires) sont recherchés dans les miels par chromatographie en phase gazeuse (GC) et par chromatographie en phase liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS). Les résultats des plans de 2014 et 2015 ont montré des taux de contamination très faibles inférieurs aux limites maximales en résidus (LMR).

Mots-clés
Miel, pesticides, résidus, plan de surveillance, plan de contrôle

For many years now, France has implemented surveillance and control plans (PSPC) for chemical residues in foodstuffs of animal origin. Since 1997, these plans have been carried out in accordance with the requirements of Directive 96/23/EC, under which Member States of the European Union (EU) must test for chemical residues (more specifically pesticides) in their products of animal origin. The main objective of these plans is to assess the level of contamination of foodstuffs placed on the national market with a view to protecting public health and identifying and removing possible sources of pollution. In the beekeeping sector, a sampling programme for honey is carried out each year by the DDecPPs at beekeeper holdings in France. Analyses of pesticide residues are performed at the ANSES Sophia Antipolis Laboratory.

In France, the beekeeping sector is made up of professional beekeepers, who generate 63% of honey production, and multi-activity or recreational beekeepers. Concerning organic beekeeping, the estimated production of certified honey ranged between 1200 and 1500 tonnes in 2014, and accounted for about 10% of national honey production [1]. Three regions represent more than 40% of production: Provence-Alpes-Côte d'Azur (PACA), Midi-Pyrénées and Rhône-Alpes (Figure 1). In 2014, 13,200 tonnes of honey were produced, including about 10,000 by beekeepers with more than 50 hives (Table 1). Beekeepers collect honey in the supers in late spring to late summer depending on the region and transhumance routes. Production can be multi-flower honey (or polyfloral honey) or monofloral honey, when one type of flower is the main source of the product. The breakdown of honeys sampled by floral origin is shown in Figure 2 for 2014 and 2015.

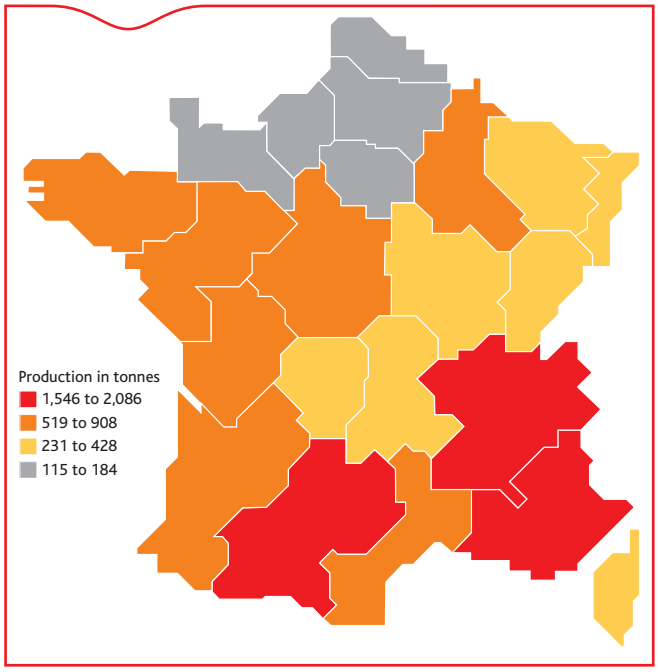

Figure 1. Regional distribution of estimated honey production in 2014 [1]

Table 1. Honey production in 2014 by class of hives [1]

Number of hives held	Number of beekeepers	Honey production (in tonnes)
0-10	25,304	1277
10-50	8,721	1,956
50-150	1,451	1,550
150-450	1,362	4,962
>450	355	3,461
Total	**37,193**	**13,206**
>50	3,168	9,973

Source: AND International 2014/2015 survey

Sampling

In accordance with the instructions in the guidance notes DGAL/SDSPA/SDPA/N2013-8214 [2] and DGAL/SDSPA/2014-999 [3], honey sampling is conducted in a targeted manner from beekeepers in France. In 2014 and 2015, the number of honeys sampled was established by product type on the basis of production size, using a distribution key fixed at the EU level (Directive 96/23/EC) and the results of plans carried out in previous years. Sampling was random

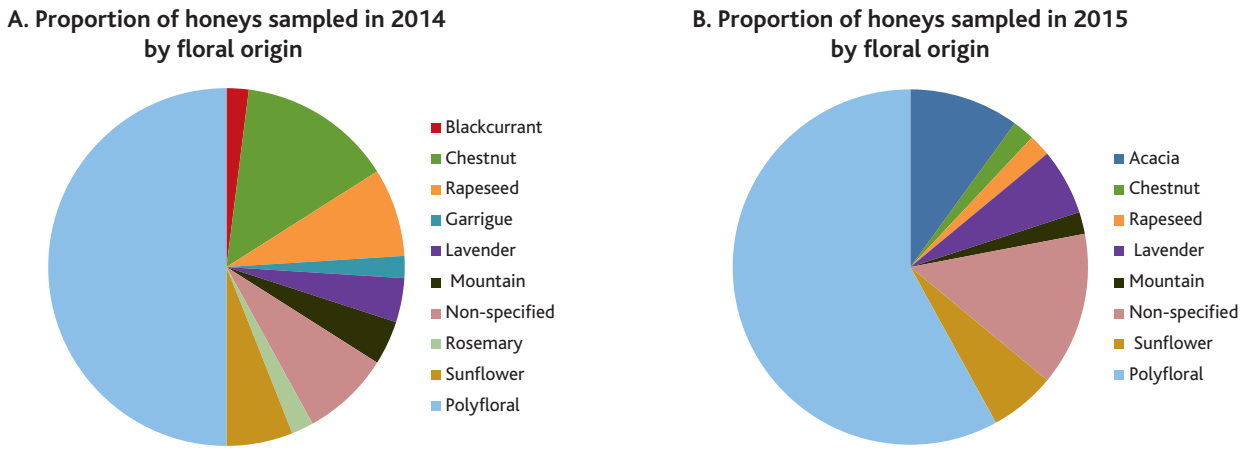

Figure 2. Proportion of honeys sampled by floral origin as part of the 2014 control plan (A) and the 2015 control plan (B)

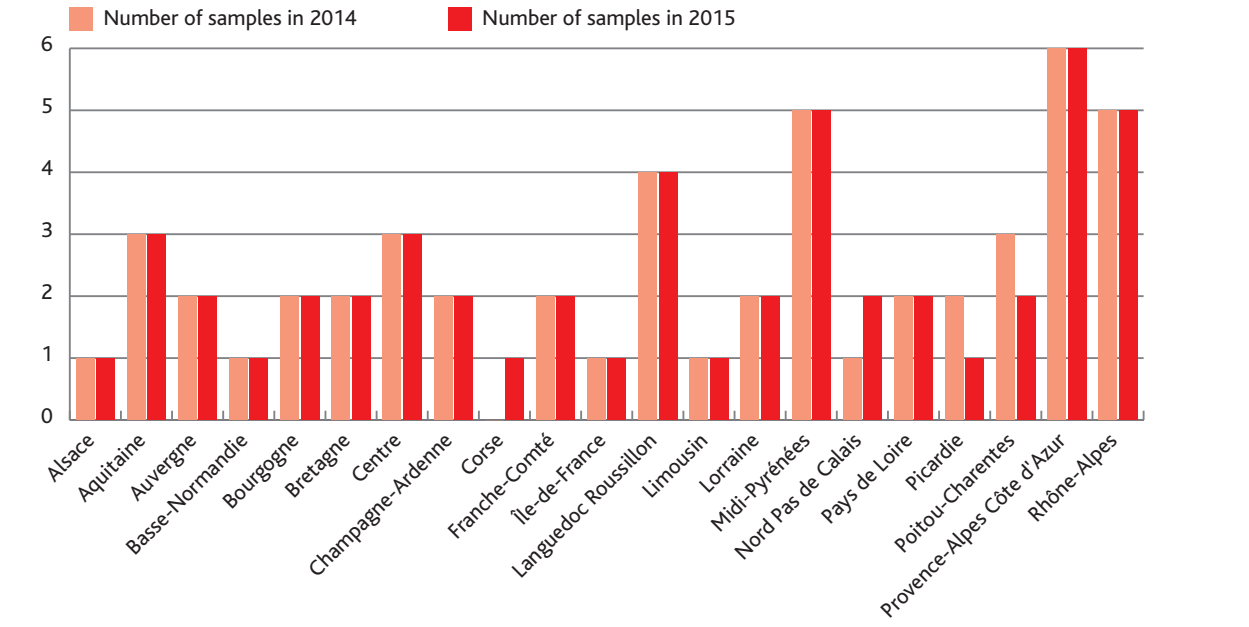

Figure 3. Distribution of samples by region as part of the 2014 and 2015 PSPC

(surveillance plan) for 54% of honeys in 2014, and 56% of honeys in 2015. For other honeys, sampling was targeted (control plan). The plans covered 20 regions (Figure 3). For 2014 and 2015, the control plan involved the collection of 50 samples of monofloral or polyfloral honey, excluding mixed honeys. Different flower varieties were represented among the 50 honeys sampled in 2014 and 2015 (Figure 2). Polyfloral honeys were the most commonly sampled variety (at least half of the samples over the two years).

Testing for pesticide residues

The main compounds tested for are acaricides used to control *Varroa destructor*, a mite parasite of bees. Residues of acaricides can be found in honey. Apivar® (amitraz) and Apistan® (tau-fluvalinate) are the main veterinary drugs used and they have been granted marketing authorisations (MAs) for the treatment of bee colonies. Other products are also authorised such as Thymovar®, Apilife Var® or Apiguard® (thymol), or more recently, MAQs (formic acid) or Api-Bioxal® (oxalic acid) and Apitraz® (amitraz). On the sampling forms, the DDecPPs indicate the beekeepers' use of these various products, which must be mentioned in the beekeeping registers maintained at the production holdings. Some beekeepers also use other veterinary products with MAs for other species, such as Taktic® for ruminants (amitraz). A few years ago, Asuntol® (coumaphos), which has an MA for species other than bees, was used by certain beekeepers. Residues of coumaphos accumulated in beeswax and could lead to contamination of honey. This product is no longer authorised.

Insecticides belonging to the neonicotinoid class (imidacloprid, clothianidin, acetamiprid, thiacloprid and thiamethoxam) are also tested for in honey. They are water-soluble compounds that may be found in honey. These insecticides are used in agriculture either for seed coating or foliar spray of crops. It should be noted that the EU suspended the use of imidacloprid, clothianidin and thiamethoxam in four field crops (maize, rapeseed, sunflower and cotton) as of late 2013.

All analyses for these pesticide residues are performed by the ANSES Sophia Antipolis Laboratory, which is accredited in accordance with Standard NF EN ISO/IEC 17025. The methods used have been validated in line with SANCO/12571/2013 [4] and are accredited by the French Accreditation Committee (COFRAC). Quantitative analyses are carried out using gas chromatography coupled with

electron capture detector (GC-ECD) and nitrogen-phosphorus detector (GC-NPD) to assay acaricides, and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) to assay neonicotinoids in honey.

Results

Because the laboratory received all the planned samples, the effective sampling rate for 2014 and 2015 was 100%. For certain compounds, maximum residue limits (MRLs) in honey have been defined [5, 6 and 7]: amitraz (200 µg/kg), coumaphos (100 µg/kg), acetamiprid (50 µg/kg) and thiacloprid (200 µg/kg). The analytical methods used can detect the presence of residues below these values. The limits of quantification (LOQs) are 1 µg/kg for imidacloprid, acetamiprid and thiacloprid, 4 µg/kg for bromopropylate, chlorfenvinphos, thiamethoxam and clothianidin, 5 µg/kg for tau-fluvalinate, 6 µg/kg for amitraz, and 8 µg/kg for coumaphos. The results of the 2014 and 2015 plans are shown in Figures 4 and 5. Results obtained for 2014 show traces of residues for acetamiprid, thiacloprid, coumaphos, tau-fluvalinate and chlorfenvinphos. Certain honey samples (8%) contained two pesticide residues. Chlorfenvinphos, a plant protection product, is tested for in honey because it may be used outside this scope as a treatment in hives against *Varroa*. Traces of chlorfenvinphos (<LOQ) were found in one polyfloral honey from the Aveyron *département* in 2014. As a reminder, its use has been prohibited in France and the European Union since 31 December 2007. Residues of acetamiprid and thiacloprid are mainly found in spring-time honeys (rapeseed), polyfloral honeys, and those from sunflowers, blackcurrants and lavender. Honeys containing coumaphos and tau-fluvalinate residues came from colonies for which the treatments were not indicated in the sampling forms. However, these pesticide residues came from either application of the corresponding veterinary drugs by the beekeeper, or contact of the honey with contaminated beeswax. This is because certain liposoluble pesticides (e.g. amitraz, tau-fluvalinate and coumaphos) tend to accumulate in beeswax and some of them are stable and persistent in this matrix.

Conclusion - Outlook

All of the pesticide residues found in honey as part of the 2014 and 2015 control plans were at very low levels, below the MRL for coumaphos, acetamiprid and thiacloprid. However, one prohibited plant protection product, chlorfenvinphos, was found in a honey sample from the 2014 control plan. It is important to monitor this pesticide in future control plans for honey in France. In such cases,

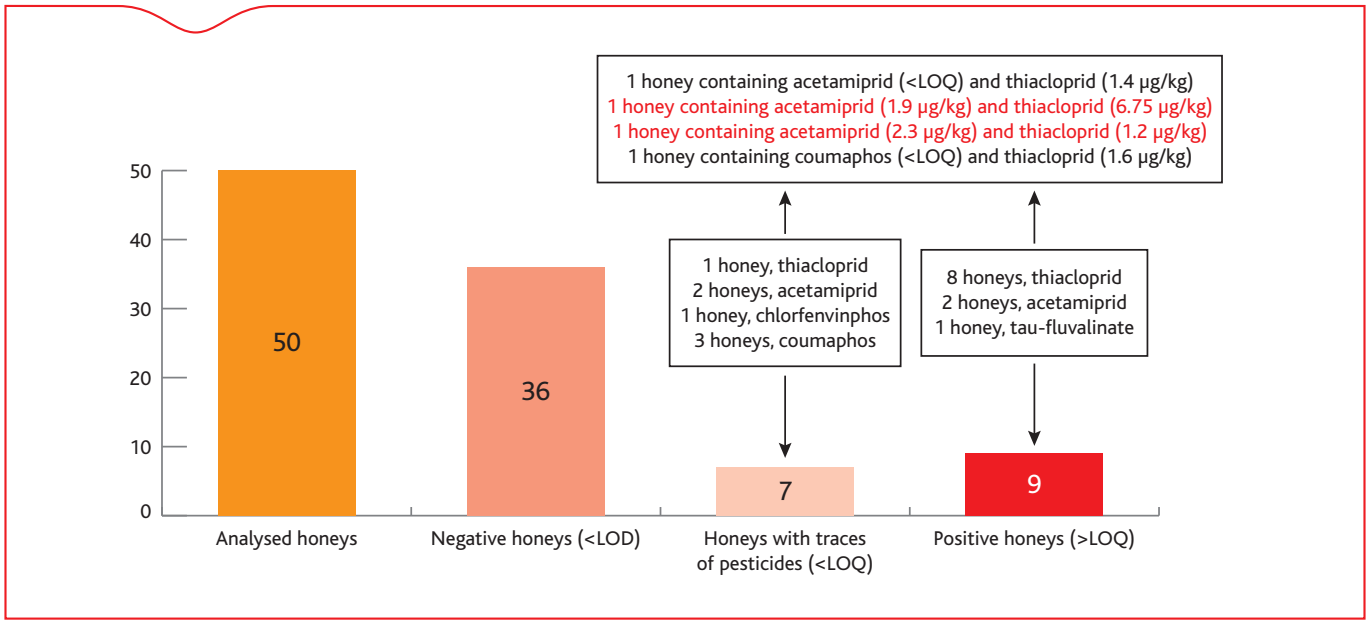

Figure 4. Results of pesticide residue analyses in honey in the 2014 plan

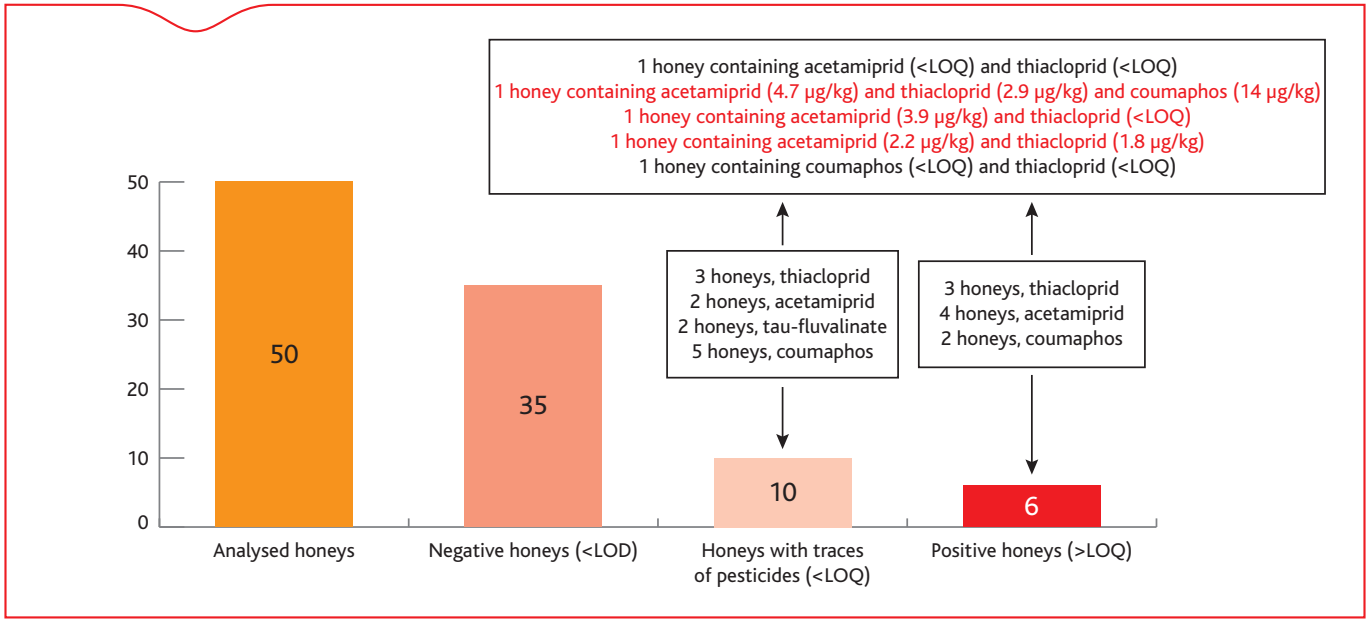

Figure 5. Results of pesticide residue analyses in honey in the 2015 plan

the veterinary services carry out investigations to determine the source of honey contamination. Another important point is that it is crucial to mention on the sampling forms the treatment schedules shown in the beekeepers' registers.

It would also be beneficial to have the harvesting date of the sampled honey, to better determine the possible source of contamination. Depending on the apiary's environment, plant protection products applied to neighbouring crops can be found in the nectar and pollen collected by the bees, and thus contaminate the honey.

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Surveillance of **growth promoters**

Stéphanie Prévost (1) (laberca@oniris-nantes.fr), Ludivine Sérée (1), Parina Sittisack (1), Gaud Dervilly-Pinel (1), Bruno Le Bizec (1), Isabelle Fournet (2)

(1) National Veterinary, Food and Nutrition School, Nantes-Atlantique (ONIRIS), Laboratory for the Study of Residues and Contaminants in Food (Laberca), National Reference Laboratory for growth promoters and environmental organic contaminants, L'UNAM, Nantes, France
(2) French Directorate General for Food, Department for health actions in primary production, Sub-Directorate for health and animal protection, Office for agricultural inputs and public health in livestock rearing, Paris, France

Abstract

The use of growth promoters in farm animals has been banned within the European Union since 1988. In order to guarantee to consumers that foodstuffs are free from residues of this type of substance, a European surveillance and control system supports this measure, which has been organised in France since 1988 within the framework of the surveillance and control programmes implemented by the Directorate General for Food. This paper aims to describe the regulatory framework and the terms of implementation regarding compounds of interest, animal species concerned, relevant biological matrices and appropriate analytical strategies. Data obtained from the 2014 plans illustrate the entire system.

Keywords

Growth promoters, Surveillance programme, Mass spectrometry

Résumé

Le dispositif de contrôle des promoteurs de croissance

L'utilisation des promoteurs de croissance est interdite en élevage au sein de l'Union européenne depuis 1988. Afin de garantir au consommateur des denrées exemptes de résidus de ce type de substances, un dispositif européen de surveillance et de contrôle accompagne cette mesure, qui en France est organisé depuis 1988 dans le cadre des plans de surveillance et de contrôle mis en place par la direction générale de l'Alimentation. Le présent article décrit le cadre réglementaire, les modalités de mise en œuvre en termes de composés d'intérêt, d'espèces animales concernées, de matrices biologiques pertinentes et de stratégies analytiques adaptées. Les données issues des plans 2014 illustrent l'ensemble du dispositif.

Mots-clés

Promoteurs de croissances, plan de surveillance, spectrométrie de masse

Growth factors or growth promoters are defined as anabolic substances that increase muscle mass with the aim of improving physical and/or economic performance. Throughout history, humans have attempted to improve their performance by artificial means. The first mentions of doping date back to Antiquity (the Iliad and Odyssey). As early as the 6th century B.C., Greek athletes consumed different meats depending on their sporting discipline: jumpers ate goat's meat, boxers and throwers ate bull meat, while wrestlers preferred fatty pork meat.

The concept of doping in livestock rearing is far more recent and the first scandals related to its use date from the 20th century. Growth stimulants or their synthetic derivatives were used at that time to improve feed conversion and thereby growth in animals. With this type of treatment, animals develop more quickly for the same amount of feed.

Initially, cheap synthetic hormones such as diethylstilbestrol (DES), used at the time in human medicine, were administered to animals. Following a number of scandals and the very strong consumer reactions due to the related health risks, natural hormones were used instead:

- sex hormones (testosterone, oestradiol, progesterone),
- synthetic steroid hormones (trenbolone acetate),
- synthetic antithyroids (thiouracil),
- adrenaline-analogue ß-agonists (β2 adrenergic agonists) (clenbuterol),
- pituitary growth hormone (somatotropin).

Since the concentrations used were very low and did not result in residue levels above those in non-treated animals in the case of natural hormones, the debate then focused on ethical issues. However, residues are still the subject of highly controversial reports, with supporters proving the safety of treatments and detractors arguing that the data are insufficient.

Producers in the United States, Canada and other countries use these stimulants for three main reasons: to improve the quality of meat (treated animals produce leaner meat at the expense of fat), to improve conversion rates (a higher weight is obtained with less feed), and to reduce production costs (the meat price is lower as a higher amount of meat is produced with lower production costs).

In the European Union, the use of growth promoters in livestock rearing is governed by a regulatory framework; its application is monitored through an EU-wide harmonised control system. The system involves the detection and identification of possible residues of these substances or their markers in animal matrices or food of animal origin.

Regulatory references

The use of steroids and thyrostatics has been prohibited in livestock rearing since 1988 (Directive 88/146/EEC). This legislation has been amended over the years and in 1996 led to the implementation of a regulatory system governing the use in livestock rearing of substances with hormonal effects (oestrogens, androgens, progestagens), or with thyrostatic effects, as well as ß-agonist substances (Council Directive 96/22/EC, amended by Directives 2003/74/EC and 2008/97/EC). Prohibited substances are listed in this regulation in Annex II. It is, however, possible for certain Member States to derogate from the ban on these substances for specific therapeutic or zootechnical indications, provided that the substances are used in veterinary medicinal products that have received a marketing authorisation (MA), and that the corresponding analytical tests for residue detection are available.

The use of growth hormone has been banned in Europe since 1990 (Decision 90/218/EEC), this was followed by a moratorium (Decision 94/936/EC), extended since 1999 by Decision 1999/879/EC.

The first controls of the illegal use of these substances were governed by Directive 85/358/EEC. This legislation has changed in parallel with that concerning the use of growth promoters and led to Directive 96/23/EC in 1996, which, in addition to controls on the illegal use of growth promoters, covers and harmonises the surveillance and control of all types of chemical residues in foodstuffs of animal origin that involve a proven or potential hazard for human health (residues of veterinary medicinal products and environmental contaminants).

The legislation emphasises the obligation to designate National Reference Laboratories and their fundamental role in the organisation of laboratory networks carrying out official analyses. The text is supplemented by:

- Decision 97/747/EC fixing the levels and frequencies of sampling for certain sectors,
- Decision 98/179/EC laying down detailed rules on official sampling,
- Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

European Directives are transposed into national law to become effective in each Member State. In France, Articles 234-1 to R. 234-14 of the Rural and Maritime Fishing Code (CRPM) partially cover the directives regarding growth promoters.

Control plans implemented in 2014 (Table 1)

Directive 96/23/EC, supplemented by Decision 97/747/EC, governs the strategy, level and frequency of sampling for the eight control plans to implement each year in primary production for the detection of growth promoters in the following sectors:

- cattle, swine, and poultry at the farm and slaughterhouse,
- sheep/goats, horses, rabbits, and farmed game at the slaughterhouse,
- farmed fish at the farm or first processing levels.

Samples are targeted and unannounced. The targeting criteria can be related to the production type or any other information that the DDecPPs have. The groups of growth promoters to be tested for annually as part of these control plans are in line with Directive 96/23/EC: stilbenes and stilbene derivatives (Group A1), antithyroid agents (Group A2), steroids (Group A3), resorcylic acid lactones (Group A4), and ß-agonists (Group A5). It is important to note that corticosteroid testing (Group B2f) is traditionally associated with growth promoter testing, because historically in Europe, they were found in the context of investigations related to misuse of ß-agonists and/or steroids.

Outside the scope of regulatory obligations, France has decided to also control for the presence of growth hormones (somatotropins) in cattle and fish.

The choice of matrices to be sampled was defined based on their relevance, either in terms of possible administration routes (feed) or matrices that best concentrate residues of administrated substance.

Sampling and breakdown of samples in 2014

The number of samples to collect by sector and by sampling site (farm or slaughterhouse) for the control plans on growth promoters was calculated (Table 2):

1. to meet the requirements of Council Directive 96/23/EC, i.e. pro rata of:
   - the number of animals slaughtered for meat and large game;
   - the tonnage produced for poultry, small game, and lagomorphs;
   - production volumes for farmed fish.

2. to establish prioritisation based on the number of non-compliant samples detected the previous years.

The regional distribution of these samples was based on pro rata volumes of livestock for production site samples and on pro rata volumes of slaughtered animals for slaughterhouse samples, as illustrated in Figure 1.

Growth promoters to be detected and analytical methods

Directive 96/23/EC requires that Member States develop reliable analytical methods for the control of the fraudulent use of growth factors at production sites, under the coordination of the European Union Reference Laboratories (EURLs) appointed by the European Commission. The RIKILT (Wageningen, Netherlands) is the EURL for growth promoters with hormonal effects, and the BVL (Berlin, Germany) is the EURL for ß-agonist type substances. The missions of these laboratories include contributing to the development and validation of analytical methods, and harmonising performance within the EU.

Box.

Objectives

Verify compliance with the regulatory ban on the use of growth promoters.

Verify the absence of growth promoter residues in animal matrices intended for human consumption.

Detection of fraudulent practices.

Programming framework

Directive 88/146/EEC of 7 March 1988 prohibiting the use in livestock farming of certain substances having a hormonal action.

Directive 96/22/EC amended by Directives 2003/74/EC and 2008/97/EC concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of beta-agonists.

Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products.

Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

French Public Health Code (CSP) and Rural and Maritime Fishing Code (CRPM).

Protocol

- **Type of compounds of interest:** substances with hormonal effects (oestrogens, androgens, progestagens), stilbenes, resocylic acid lactones, antithyroids, as well as β-agonist substances and corticosteroids.
- **Target production sectors:** cattle, swine, sheep, goat, horse, poultry, aquaculture, lagomorph, and game production sectors.
- **Stage of the food chain:** farms, slaughterhouses.
- **Definition of non-compliance:** a sample is considered non-compliant if the concentration of the analyte of interest measured exceeds the decision limit of the confirmation method (Article 6, Decision 2002/657/EC).
- **Number of samples and sampling method:**
- The number of samples to collect by sector and sampling site (farm or slaughterhouse) was calculated to meet the requirements of Directive 96/23/EC. The number of samples to collect depends on:
  - > the number of animals slaughtered for meat and large game,
  - > the tonnage produced for poultry, small game, and lagomorphs,
  - > production volumes for farmed fish.
- **Sampling strategy:** targeted (conformation of the animals, for example).
- **Analytical methods:** multi-dimensional mass spectrometry (MS/MS) for screening and confirmation analyses. Specific techniques such as high-resolution mass spectrometry (HRMS) and isotope-ratio mass spectrometry (IRMS) are also used in the context of confirmation analyses.
- **Types of samples:** biological matrices such as urine, appendages, tissues, retinas, faeces, and blood.

Table 1. Control plans for growth promoters in animal matrices for 2014

Sector	Promoter group	Animal feed	Blood	Urine	Hairs	Lungs	Eyes	Thyroid	Muscle or liver
Cattle	Stilbenes	X		X	X				X
	Antithyroids	X		X				X	
	Steroids	X		X	X				X
	Steroid esters				X				
	Resorcylic acid	X		X	X				X
	ß-agonists	X		X	X	X	X		
	Glucocorticosteroids				X				X
	Recombinant bovine somatotropin		X						
Pigs	Stilbenes	X		X					X
	Antithyroids	X		X					
	Steroids	X		X					X
	Steroid esters				X				
	Resorcylic acid	X		X					X
	ß-agonists	X				X	X		
	Glucocorticosteroids				X				X
Sheep, goats, horses	Stilbenes			X					
	Antithyroids			X					
	Steroids			X					
	Resorcylic acid			X					
	ß-agonists					X			
	Glucocorticosteroids				X				X
Poultry	Stilbenes	X							X
	Steroids	X							X
	Resorcylic acid	X							X
	ß-agonists	X				X			
Rabbits, Game	Stilbenes								X
	Steroids								X
	Resorcylic acid								X
	ß-agonists					X			
Fish	Stilbenes								X
	Steroids								X
	Resorcylic acid								X
	Somatotropin		X						

In addition to the classes listed in Annex I of Directive 96/23/EC, other non-regulated substances may also be monitored on the basis of information emanating from the National Veterinary and Plant Protection Squad (BNEVP) or the National Reference Laboratory. An example is selective androgen receptor modulators (SARMs). These substances are currently tested for as part of an exploratory plan.

The official methods used today can detect and identify about 70 different growth promoters. The first-line (screening) analysis of a sample must be rapid, easy to implement, cheap, sensitive and robust. These methods have a high processing capacity and are applied by the eleven laboratories in the growth promoters network, covering the whole country, to screen multiple samples in order to rapidly distinguish between "compliant" and "suspect" samples. A sample is considered suspect when the identity of the compound is confirmed following screening and, if relevant, when the compound has a maximum residue level, its concentration exceeds this threshold.

This first step is used to identify suspect samples that will then need to be assessed as compliant or not using a confirmation method. The sample is then re-extracted to ensure that the results are not false (contamination, sample switch, etc.). Non-compliance is reported when the concentration of the identified compound is higher than the decision limit or CCa. The performance of the methods developed must have a false-compliant (false-negative) rate below 5% for the screening step, and a false-non–compliant (false-positive) rate below 1% for the confirmation step. The requirements concerning method performance and interpretation of the results are described in Decision 2002/657/EC.

While screening methods can use various analytical techniques (immunoassays, mass spectrometry), confirmation methods require targeted analysis of the administered compound and/or its direct metabolites by chromatography coupled with detection by mass spectrometry for non-ambiguous identification and quantification of the analyte of interest.

Screening methods

Official screening analyses are performed by the network of first-line laboratories approved by the General Directorate for Food. These establishments have official multi-residue methods developed and validated by the NRL, in accordance with Decision 2002/657/EC. These methods are used to test for growth promoters in complex biological matrices such as urine, appendages (e.g. hairs), or other matrices retained for their relevance. For example, the retina is a useful biological matrix because it durably fixes residues of ß2-adrenergic agonists and can be used to demonstrate fraud a long time after administration of the substance. This matrix is preferred at the slaughterhouse. Hairs are also able to fix residues of steroids or ß-agonists, thus extending the detection window. This matrix is used both on the farm and at the slaughterhouse. There are in fact several matrix/compound pairs that increase the effectiveness of the control (e.g. ß-agonists/lung or retina, steroids/faeces, progestagens/fat tissue, thyrostatics/thyroid, etc.).

The nature of the biological samples, which are often complex, means that they generally require several extraction and purification steps before characterisation of their contents. The measurement

Table 2. Number of samples to collect by sector and sampling site

	2014 target population	Size of the minimum annual national sample required by regulations for the detection of growth promoters and other prohibited substances (Group A)		Minimum size of the national sample by sub-group				Remainder to distribute depending on prioritisation of the MS (reference year 2014)	DGAL scheduling 2014		
									Farm	Slaughter-house	Total
Cattle	4,775,000 (total number of cattle slaughtered over 12 months)	0.25% of production, including half on the farm	11,937 samples, including 6,000 on the farm	A1	Stilbenes	5%	597	8,356	2,100	2,100	**4,200**
				A3	Steroids (+esters)	5%	597				
				A4	Resorcylic acid	5%	597				
				A2	Antithyroids	5%	597		400	300	**700**
				A5	ß-agonists	5%	597		1,800	1,900	**3,700**
				A6	Substances included in 37/2010 - Table 2	5%	597		1,700	1,700	**3,400**
		Abs*	Abs*	B2f	Glucocorticosteroids	Abs	Abs			600	**600**
					Somatotropin	Abs	Abs		200		**200**
Total promoters, cattle											**9,400**
Pigs	23,933,000 (total number of pigs slaughtered over 12 months)	0.02% of the production with a minimum of 0.001% on the farm	4,787 samples (different animals), including 239 on the farm	A1	Stilbenes	5%	239	3,351	130	190	**320**
				A3	Steroids (+esters)	5%	239				
				A4	Resorcylic acid	5%	239				
				A2	Antithyroids	5%	239		40	200	**240**
				A5	ß-agonists	5%	239		40	200	**240**
				A6	Substances included in 37/2010 - Table 2	5%	239		90	3,910	**4,000**
		Abs*	Abs*	B2f	Glucocorticosteroids	Abs	Abs			200	**200**
Total promoters, pigs											**1,000**
Small ruminants	4,472,000 (total number of sheep-goats slaughtered over 12 months)	0.01% of production	447 samples	A1	Stilbenes	5%	22	313		100	**100**
				A3	Steroids	5%	22				
				A4	Resorcylic acid	5%	22				
				A2	Antithyroids	5%	22			30	**30**
				A5	ß-agonists	5%	22			100	**100**
				A6	Substances included in 37/2010 - Table 2	5%	22			220	**220**
		Abs*	Abs*	B2f	Glucocorticosteroids	Abs	Abs			140	**140**
Total promoters, small ruminants											**370**
Horses	19,000 (total number of horses slaughtered over 12 months)	No minimum requirement but obligation to test for substances in Group A		A1	Stilbenes	Abs	Abs		4	4	**4**
				A3	Steroids	Abs	Abs				
				A4	Resorcylic acid	Abs	Abs				
				A2	Antithyroids	Abs	Abs		4	4	**4**
				A5	ß-agonists	Abs	Abs		4	4	**4**
				A6	Substances included in 37/2010 - Table 2	Abs	Abs		4	4	**4**
				B2f	Glucocorticosteroids	Abs	Abs		4	4	**4**
Total promoters, horses											**16**
Poultry	1,703,000 tonnes produced over 12 months	0.25% of the tonnage produced with a minimum of 0.05% on farms	4,269 samples (different batches)	A1	Stilbenes	5%	213	3,204	68	247	**315**
				A3	Steroids	5%	213				
				A4	Resorcylic acid	5%	213				
				A5	ß-agonists	5%	213		187	695	**882**
				A6	Substances included in 37/2010 - Table 2	5%	213		616	2,444	**3,060**
Total promoters, poultry											**1,197**
Rabbits	46,000 tonnes produced over 12 months	30 samples + 0.1% of "tonnage produced -3000 t"	73 samples (different batches)	A1	Stilbenes	30%	22			5	**5**
				A3	Steroids						
				A4	Resorcylic acid						
				A5	ß-agonists					10	**10**
				A6	Substances included in 37/2010 - Table 2	70%	51			60	**60**
Total promoters, rabbits											**15**

Abs: No minimum sample imposed by regulation

Table 2. Number of samples to collect by sector and sampling site (cont'd)

	2014 target population	Size of the minimum annual national sample required by regulations for the detection of growth promoters and other prohibited substances (Group A)		Minimum size of the national sample by sub-group				Remainder to distribute depending on prioritisation of the MS (reference year 2014)	DGAL scheduling 2014		
									Farm	Slaughter-house	Total
Farmed game	3,000 heads of large game 9,000 tonnes of small game produced over 12 months	20 samples	20 samples (different batches)	A1	Stilbenes	Abs	Abs			4	**4**
				A3	Steroids	Abs	Abs				
				A4	Resorcylic acid	Abs	Abs				
				A5	ß-agonists	Abs	Abs			4	**4**
				A6	Substances included in 37/2010 - Table 2	Abs	Abs			28	**28**
Total promoters, game											**8**
Farmed fish	50,000 tonnes produced over 12 months	0.333%	165 samples (different batches)	A1	Stilbenes	Abs	Abs			50	**50**
				A3	Steroids (+esters)	Abs	Abs				
				A4	Resorcylic acid	Abs	Abs				
				A6	Substances incluses dans 37/2010 - Tableau 2	Abs	Abs			120	**120**
				B2f	Somatotropin	Abs	Abs			50	**50**
Total promoters, fish											**100**
Total promoters, all sectors											**12,106**

Abs: no minimum imposed specifically

methods must combine selectivity and sensitivity because the residues of these substances are mostly found at ultra-trace amounts ($ng.kg^{-1}$ to $pg.kg^{-1}$). One of the most commonly used methods today is chromatography coupled with mass spectrometry. The technique may use gas chromatography for small thermostable and volatile compounds (steroids, stilbenes, resorcylic acid lactones), or liquid chromatography for the others (ß-agonists, thyrostatics, somatotropin, corticosteroids). To increase the specificity of detection, mass spectrometry is systematically of the multi-dimensional type (MS/MS); high-resolution mass spectrometry (HRMS) may sometimes be used.

Confirmation methods

A confirmation analysis may be performed when the network laboratory suspects the presence of one of the target compounds after the screening analysis. The confirmation strategy and analytical technique used are defined specifically based on the type of suspect analyte and its concentration. In this context, there are two sub-groups of substances among the growth promoters: xenobiotic substances for which simple detection clearly demonstrates fraudulent use of chemical substances in animals, and endogenous substances, such as oestradiol or testosterone, for which detection does not necessarily imply non-compliance of the sample. This is because androgenic steroids (testosterone, nandrolone, boldenone) and oestrogenic steroids (oestradiol) can be detected at highly variable concentrations depending on the animal's age, sex and physiological state. In the case of testosterone and oestradiol, measurement of the carbon $^{13}C/^{12}C$ isotope composition by isotope-ratio mass spectrometry (GC-C-IRMS) is used to determine the endogenous or exogenous nature of the residues, in particular in the animal's urine. Hairs can also be used for this type of compound since residues of ester forms of steroids administered can bind to this matrix, demonstrating without a doubt that the substance of interest was used because the animal's body does not produce this type of derivative (e.g. boldenone undecylenate, nandrolone cypionate, etc.). The presence of some substances may also be attributed to the animal's diet. This is the case specifically for zeranol (Group A4) or thiouracil (Group A2), which can be related to feed contaminated with a mycotoxin (zearalenone) or feed fortified with *Brassicaceae*, respectively (Pinel *et al.*, 2006). For these sensitive situations, the NRL handles confirmation and interpretation of results.

Results - 2014 review

Following the screening step performed on all samples, the analyses performed for confirmation purposes mainly involved compounds considered potentially endogenous, such as boldenone, nandrolone, oestradiol, testosterone, zeranol and taleranol, classed as natural hormones, but also strictly xenobiotic compounds such as β-agonists and steroid esters (A1 and A3). The breakdown of confirmation analyses by substance group of interest for 2014 is shown in Figure 2.

The observed non-compliant cases included values exceeding the MRL for dexamethasone in the liver matrix for the B2f substance group, and the presence of thiouracil identified in urine at variable concentrations higher than 10 $\mu g.L^{-1}$ in two samples. These thiouracil concentrations are, however, not incompatible with feed enriched with *Brassicaceae* (Pinel *et al.*, 2006).

At the European level, we should point out that most of the Member States carry out the minimum number of samples required by Directive 96/23/EC and Decision 97/747/EC. The sampled matrices are essentially the same among the Member States. Samples of urine, tissue and appendages, and feed are the most common for the detection of growth promoters.

The trend since 2013 appears to indicate an increase in cases of non-compliance reported by the Member States. However, the summary report issued by the European Food Safety Authority (EFSA, 2016) points out that the detected substances are not systematically attributed to illegal use, but are rather the result of notifications for natural hormones, particularly the A3 substance group, i.e. steroids, and for which non-compliance represents 0.08% of the measurements associated with this group of compounds. This is because some of the detected compounds can be found in the relevant species endogenously, without any illegal treatment. This is the case for instance for boldenone (a and b forms), 17a-nandrolone and 17a-testosterone. These results can be explained by the fact that the Member States still do not have specific, adapted confirmation methods and/or techniques for the particular case of natural hormones. Concerning thyroid agents (Group A2), 0.59% of the analysed samples were reported to be non-compliant and concern exclusively thiouracil. The B2f group is also represented at the European level by 28 reported non-compliant samples.

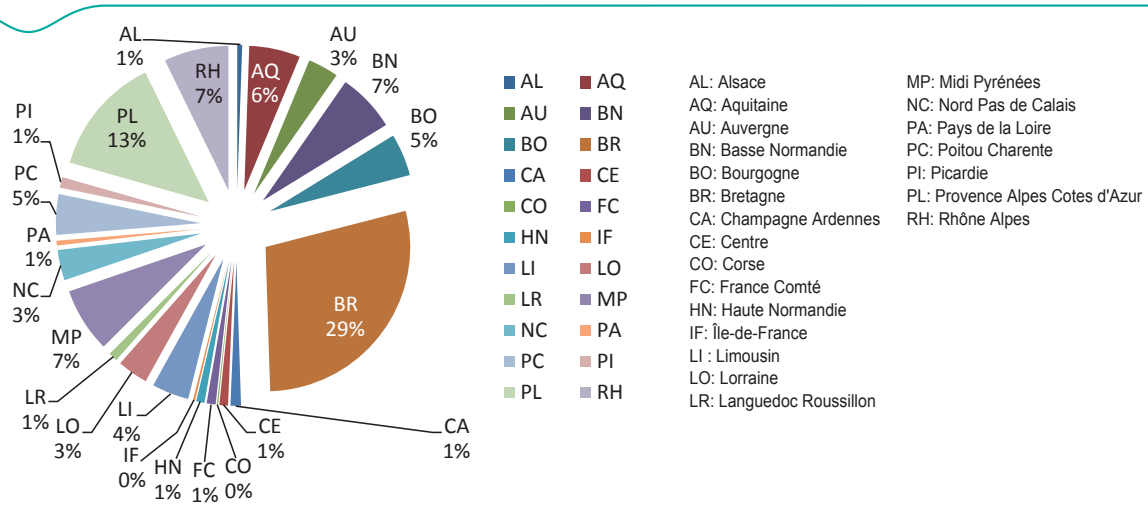

Figure 1. Regional distribution of samples taken for substance groups A1 to A5 and B2f (2014)

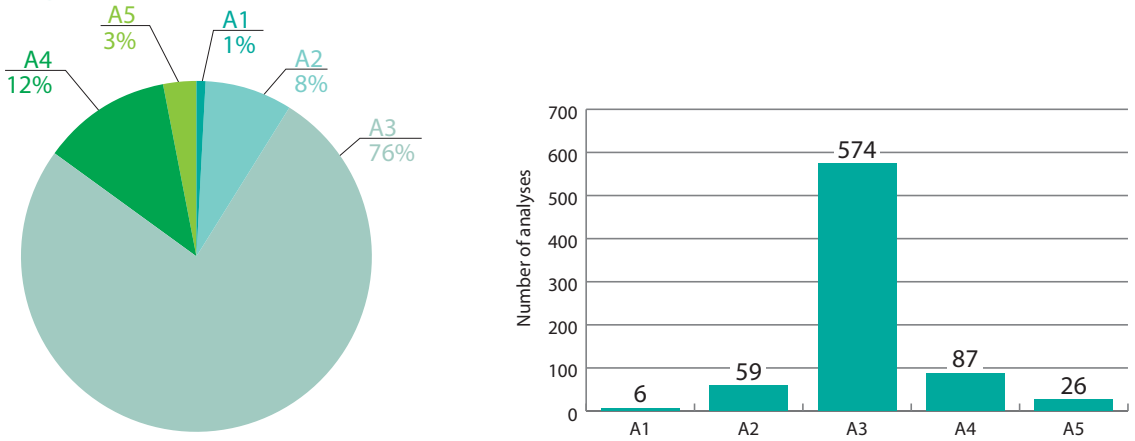

Figure 2. Distribution of the number of confirmation analyses performed by substance (2014)

Conclusion - Outlook

Control of the use of growth promoters is currently based on a range of competencies bringing together:

- the DDecPPs, which help to target the sampling;
- the network of official laboratories, which implement screening methods to test for about 70 different compounds belonging to various groups of growth promoters;
- the NRL, which develops and implements effective and specific confirmation methods.

There are three main types of obstacles that currently hinder even more effective control of the use of growth promoters:

- difficulties related to sampling certain matrices in target animals;
- detection of natural substances that have anabolic properties;
- identification of unknown compounds.

Concerning the first obstacle, the problem is considerable because if these sampling issues are not taken into account, it is highly unlikely that targeting is appropriate and thus that testing in fact identifies fraudulent practices, irrespective of the method used and its performance. An experimental plan involving the sampling of faeces has been implemented to evaluate the scientific usefulness of this matrix in testing for steroid substances, and to consider collecting faeces rather than urine, which is difficult to sample in target animals.

In the second case, and in line with an agreement between the DGAL and Laberca, specific analytical methods were developed recently. These methods rely on isotope-ratio mass spectrometry enabling high-precision measurement of the carbon 12/carbon 13 ratio in the compound, a proportion that differs depending on whether the compound is endogenous or synthetic (Buisson *et al.*, 2005; Janssens *et al.*, 2015). This strategy is, however, only present in a limited number of Member States (three laboratories). Alternative, more affordable strategies for all Member States are also under investigation and rely specifically on the combination of relevant matrix/residue pairs, for example blood/steroid esters, or hairs/steroid esters (Kaabia *et al.*, 2013).

Thirdly, concerning the detection of unknown compounds or more generally fraudulent physiological manipulation, overall exploratory approaches to the functioning of the species' bodies, implemented over the last decade, have already proven their worth. These strategies do not aim to detect the actual presence of suspect compounds or their direct metabolites, but rather to highlight a specific metabolic or physiological signal that could be associated with anabolic practices. These "indirect" or "non-targeted" approaches (Nebbia *et al.*, 2011; Pinel *et al.*, 2010) are based on methods such as transcriptomics (Riedmaier, 2015; Riedmaier *et al.*, 2009a; Riedmaier *et al.*, 2012; Riedmaier and Pfaffl, 2013; Riedmaier *et al.*, 2009b, c), proteomics (Cacciatore *et al.*, 2009; Cunningham *et al.*, 2009; Kinkead *et al.*,

2015), or metabolomics (Dervilly-Pinel *et al.*, 2015a; Dervilly-Pinel *et al.*, 2012; Gallart Ayala *et al.*, 2015; Jacob *et al.*, 2014; Kouassi Nzoughet *et al.*, 2015b), including derivative areas such as lipidomics (Kouassi Nzoughet *et al.*, 2015a) and steroidomics (Dervilly-Pinel *et al.*, 2011; Kaabia *et al.*, 2014). These new approaches are used to discover molecular markers of effects, which can then be monitored in a targeted way in a context of screening for anabolic practices. The first example of a monitoring method for biomarkers identified using a metabolomics approach (Dervilly- Pinel *et al.*, 2015a), and focusing on the screening of ß-agonist compound use in calves, has been implemented in France since 2013 for official controls (Dervilly-Pinel *et al.*, 2015b). The method is a world-first in this area.

These recent changes could prove to be effective in increasing the control pressure, and ultimately enable detection of a broader, realistic panel of anabolic practices.

In addition, concerning changes to the regulatory context, it is expected that the European regulations on the control of growth factor use will integrate new parameters that could be used to organise control plans even more effectively. This involves specifically the integration of technical progress regarding detection and new uses or substances with hormonal activity.

Against this backdrop, a review of Decision 2002/657/EC concerning the performance of analytical methods and the interpretation of results is currently under discussion at the European level to take into account new innovations and knowledge generated since its release.

It is also expected that changes will take into consideration possible harmonisation of procedures implemented in the various Member States in order to guarantee consistency of practices and decisions for greater quality of control. For example, Decision 2002/657/EC defined the concept of minimum required performance limits (MRPLs) which correspond to a fixed concentration that any control laboratory must be able to reach in a context of screening and confirmation, but only a few values have been published to date (e.g. MRPL for medroxyprogesterone acetate).

Regulation (EC) No 470/2009 indicates the possibility of determining reference points for action (RPAs) for non-authorised, pharmacologically active substances, when necessary, to ensure the control of imported or marketed foodstuffs of animal origin. RPAs are defined as action limits combining analytical possibilities that are both reasonable (i.e. that official laboratories can maintain) and compatible with residue levels which do not involve a risk for the consumer's health. Foodstuffs that contain residues of

substances at a concentration greater than or equal to the RPA are thus considered unfit for consumption. If the concentration is below this limit, the non-compliance is recorded but does not warrant management measures concerning the food. The outlook in this area therefore involves considering analytical and toxicological aspects to determine these values, but without replacing the full process of associated risk assessments (EFSA 2013).

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Surveillance of **shigatoxin-producing *E. coli*** (VTEC) in refrigerated fresh minced beef on the French market in 2015

Estelle Loukiadis (1) (estelle.loukiadis@vetagro-sup.fr), Christine Mazuy-Cruchaudet (1), Aurélie Granjon (1), Sophie Félix (1), Marie-Pierre Donguy (2), Sébastien Rémy (3), Sabine Itié-Hafez (3), Corinne Danan (3)

(1) Lyon University, VetAgro Sup, National Reference Laboratory for *E. coli* (including VTEC), Marcy l'Etoile, France
(2) Directorate General for Food, Mission for Health Emergencies, Paris, France
(3) Directorate General for Food, Support Office for Food Chain Surveillance, Paris, France

Abstract

Shigatoxin-producing *Escherichia coli* (VTEC) are considered as major pathogens causing severe and sometimes lethal infections in humans. Although more than 200 serotypes have been reported, only seven of them have been consistently associated with severe cases. Transmission of VTEC to humans occurs mainly through consumption of undercooked minced beef contaminated by animal faeces. Although there are no statutory criteria, meat containing one of these strains is considered as harmful to health. Thus, the surveillance plan conducted in 2015 aimed to assess, for fresh minced beef on the French market, the rate of contamination by VTEC identified as a higher risk in order to assess consumer exposure.

The results obtained confirm that the contamination rate for meat was low (0.3%; 95CI [0.01-1.9]) and similar to those obtained previously, suggesting that the risk of human exposure *via* the consumption of minced beef in France remains limited. The only strain isolated was an O103:H2 VTEC strain showing genetic markers of greater virulence.

The Directorate General for Food will continue to monitor VTEC contamination in beef collected on the market in 2016.

Keywords

VTEC, EHEC, Surveillance, Minced beef, 2015, France

Résumé

Surveillance des* E. coli *producteurs de shigatoxines (VTEC) dans les viandes hachées de bœuf réfrigérées mises sur le marché en 2015.

Les Escherichia coli *producteurs de shigatoxines (VTEC) sont des agents pathogènes majeurs, responsables d'affections parfois mortelles. Bien que plus de 200 sérotypes aient été rapportés, seuls sept sont responsables de la majorité des épidémies et affections sévères recensées. La viande hachée de bœuf contaminée par le contenu digestif des animaux porteurs et insuffisamment cuite reste une des principales sources de contamination de l'Homme. Bien qu'il n'existe aucun critère réglementaire, une viande contenant une de ces souches est considérée comme « dangereuse ». Aussi, le plan de surveillance 2015 visait à établir le taux de contamination des viandes hachées de bœuf réfrigérées mises sur le marché en France par les souches VTEC identifiées comme les plus à risque et, par conséquent à apprécier l'exposition du consommateur à ce danger.*

Les résultats obtenus confirment que le taux de contamination des viandes hachées de bœuf réfrigérées est faible (0,3% ; IC95 [0,01-1,9]) et du même ordre de grandeur que ceux obtenus précédemment, ce qui suggère que le risque d'exposition de l'Homme via la consommation de viande hachée de bœuf en France reste limité. L'unique souche isolée est une souche VTEC O103:H2 possédant des marqueurs génétiques de virulence accrue.

En 2016, la direction générale de l'Alimentation poursuivra la surveillance de la contamination des viandes hachées de bœuf par ces agents pathogènes au stade de la distribution.

Mots-clés

VTEC, EHEC, surveillance, viandes hachées de bœuf, 2015, France

Pathogenic shigatoxin-producing *Escherichia coli* (VTEC) are considered a major public health concern in several regions of the world due to the extreme severity of the symptoms they cause (AFSSA, 2003). Indeed, pathogenic VTEC are responsible for sporadic cases and outbreaks of haemorrhagic colitis as well as rare life-threatening infections affecting children in particular, such as haemolytic uremic syndrome (HUS). HUS is the main cause of acute renal failure in children under three years of age. The mortality rate varies from 3% to 5% and more than a third of patients suffer long-term kidney damage (AFSSA, 2003).

Although more than 200 serotypes of pathogenic VTEC strains have been involved in human infections, only a few have been consistently associated with severe outbreaks and infections. In France, VTEC strains belonging to one of the five O26:H11, O103:H2, O111:H8, O145:H28 and O157:H7 serotypes are associated with 70% to 80% of reported cases and have been defined as highly pathogenic (AFSSA, 2010; Brugère *et al.*, 2012). In the United States, VTEC strains of the same serotypes as well as the VTEC O45 and O121 strains are considered as presenting the greatest risk.

The natural reservoir for pathogenic VTEC is the digestive tract of ruminants. The consumption of raw or undercooked contaminated minced beef has been identified as one of the main routes of contamination during investigations of HUS cases identifying a responsible food (AFSSA, 2003).

In accordance with Directive 2003/99/EC, European Union Member States are required to set up a surveillance system for zoonoses and zoonotic agents. VTEC are included on the list of agents to be monitored, featured in Annex I (A) of this directive. In addition to the control pressure exerted on production sectors, the implementation of surveillance plans for VTEC contamination in at-risk matrices (mainly minced beef and raw-milk cheeses) is one of the actions taken for the protection of public health. These plans provide estimates of food contamination levels in various stages of the food chain. The data obtained also make it possible to make assumptions about the risk factors. Mitigation measures can be then established. Surveillance plan results are communicated to risk assessment agencies: i) ANSES in France, and ii) the European Food Safety Authority (EFSA) in Europe, for summarising with the data of other Member States.

There are currently no statutory microbiological criteria for VTEC in minced beef. However, the French authorities consider a beef burger containing a highly pathogenic VTEC strain to be "unsafe" as defined in Article 14 of Regulation (EC) No 178/2002, since it is injurious to health, given the severity of the related infections and the French habit of consuming this food undercooked[1].

The aim of the VTEC surveillance plan conducted in 2015 was to collect data for assessing the rate of contamination in refrigerated minced beef on the French market and therefore to evaluate the consumer exposure to this hazard.

Materials and methods

Sampling protocol

The surveillance plan should include 306 samples from different one-unit (n=1) batches of refrigerated minced beef in the distribution stage. All sample were taken in retail outlets such as supermarkets, hypermarkets and discount stores, which account for 95% of purchases of butcher's meat in France.

These 306 samples were planned in mainland France, in proportion to the number of inhabitants per region (Figure 1), in order to be as representative as possible of consumer exposure. The samples should be spread out throughout the year 2015.

Each sample should correspond to at least 100g of minced beef, prepacked in its original packaging (shrink-wrapped, vacuum-packed or packaged in a protective atmosphere) and labelled. The expiration date should be valid until the date of the analysis.

Nature of the tested contaminants

The following pathogenic bacteria were detected:

- VTEC strains considered in France as highly pathogenic to humans (AFSSA, 2010), i.e. strains owning the *stx* (*stx1* and/or *stx2*) and *eae* virulence genes and belonging to one of the five O157:H7, O26:H11, O145:H28, O103:H2 and O111:H8 serotypes,
- VTEC strains considered as pathogenic (AFSSA, 2010), harbouring the *stx* (*stx1* and/or *stx2*) and *eae* virulence genes, and belonging either to the O45 serogroup or to the O121 serogroup, targeted by the American regulations.

Analytical method used

In order to take into account potential differences in the contamination of minced beef, 100g of meat were collected in various places in minced beef in order to establish a sample. After homogenisation, the test portion per sample was 25g.

The detection of target VTECs was realised in accordance with the official authorised methods[2], adapted from the ISO TS 13136 method[3], recommended by EFSA (EFSA, 2009), and the official American method MLG 5B(4)(4) (Figure 2):

- a first step of enrichment of the investigated food allows the potential pathogenic strains to multiply and reach detectable levels,
- a second real-time PCR detection step uses nucleic acids extracted from this polymicrobial enrichment broth. The main markers of the target VTEC strains are detected: *stx* genes (Perelle *et al.*, 2004), *eae* genes (Nielsen *et al.*, 2003), and genes associated with the seven serogroups of interest (Perelle *et al.*, 2004 and MLG 5B method),
- a third bacterial isolation step implemented only if the results obtained previously are positive, i.e. if the *stx* gene AND *eae*

Box.

Objective
This surveillance plan aimed to assess contamination by VTEC strains in refrigerated minced beef on the French market and therefore evaluate consumer exposure.

Programming framework
- Directive 2003/99/EC.
- EFSA Opinion of 30 October 2009.
- AFSSA Opinion of 27 May 2010.

Protocol
- **Target bacteria**
  - VTEC strains highly pathogenic to humans. These are strains owning the stx and eae virulence genes and belonging to one of the five O157:H7, O26:H11, O145:H28, O103:H2 and O111:H8 serotypes.
  - Pathogenic VTEC strains, i.e. strains harbouring the *stx* and *eae* virulence genes and belonging to the O45 or O121 serogroup.
- **Affected products:** viandes de bœuf hachées réfrigérées (toutes origines).
- **Stage of the food chain:** distribution.
- **Definition of a "case':**
  Non-compliance if isolation of one of the targeted strains.
- **Number of samples and sampling method**
  Three-hundred and six samples were taken in mainland France between February and December 2015, broken down by region in proportion to the number of inhabitants.
  Each sample was collected in its original packaging in the refrigerated self-service section of supermarkets and hypermarkets.
- **Sampling strategy:** random.
- **Analytical method, nature of sampling**
  The test portion (25g) was analysed in accordance with the official methods adapted from Technical Specification ISO 13136: 2012.

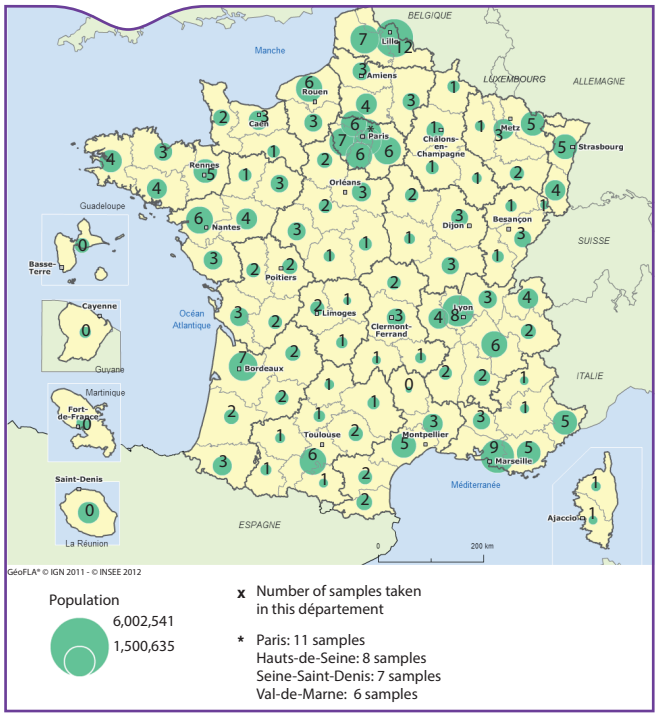


Figure 1. Departmental breakdown of the number of samples planned by number of inhabitants (http://www.statistiques-locales.insee.fr and 2012 INSEE data)

1. http://agriculture.gouv.fr/sites/minagri/files/documents/pdf/_Guide_Gestion_Alerte_Revision_2_jlt_2009_COMPLETEE_VDef__cle09fc34.pdf.
2. The official authorised methods are listed in Guidance Note DGAL/ SDSSA/ SDPRAT/N2013-8179 and are available online at the following address: http://agriculture.gouv.fr/laboratoires-agrees-methodes-officielles-alimentation-568.
3. Technical Specification ISO TS 13136:2012 "Microbiology of food and animal feed – Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens – Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (VTEC) and the determination of O157, O111, O26, O103 and O145 serogroups".
4. Official American method MLG 5B.05 "Detection and Isolation of non-O157 Shiga Toxin-Producing *Escherichia coli* (VTEC) from Meat Products and Carcass and Environmental Sponges" available at the following address: http://www.fsis.usda.gov/wps/wcm/connect/7ffc02b5-3d33-4a79-b50c-81f208893204/MLG-5B.pdf?MOD=AJPERES.

gene AND one of the specific genes of the targeted serogroups are detected concomitantly in the enrichment broth. This specific isolation step for bacteria belonging to the serogroup detected from the enrichment broth uses both immunomagnetic separation (IMS) techniques and direct isolation,

- a fourth step for the phenotypic (API20E) and genotypic characterisation of the *E. coli* strains isolated in the previous stage. More specifically, somatic and flagellar antigens are analysed by PCR to confirm the serotype of the *E. coli* strains isolated (Perelle *et al.*, 2004; Auvray *et al.*, 2008; Madic *et al.*, 2010). The *stx1, stx2*

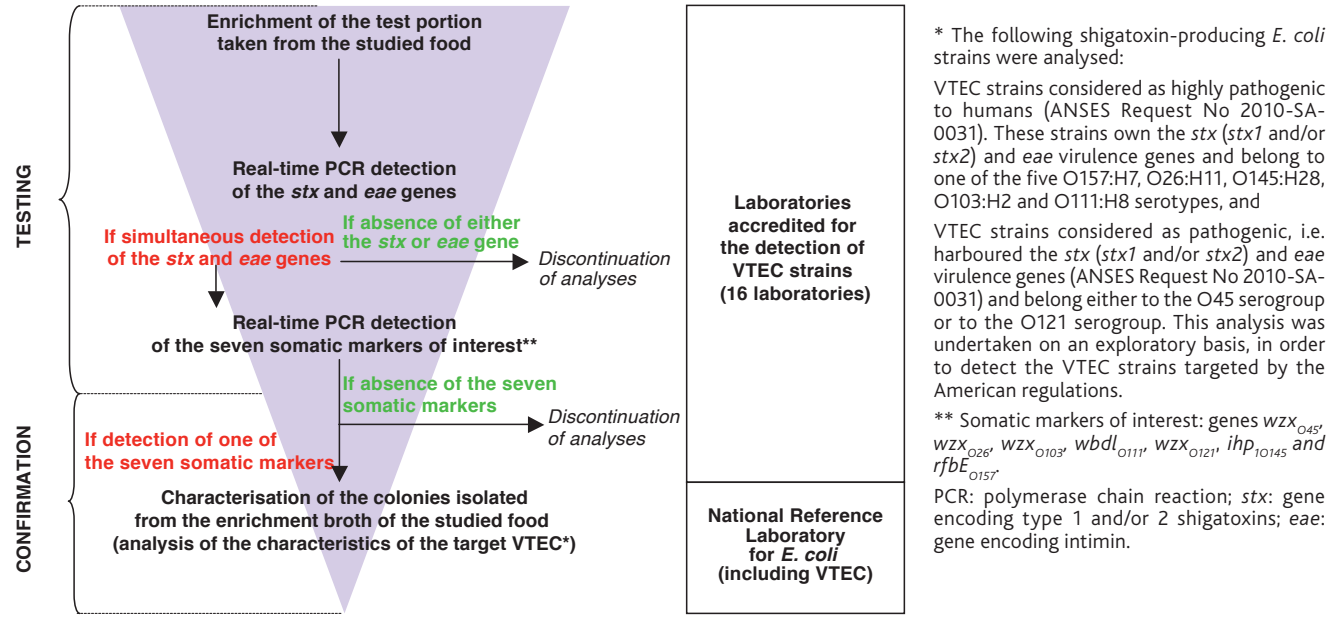
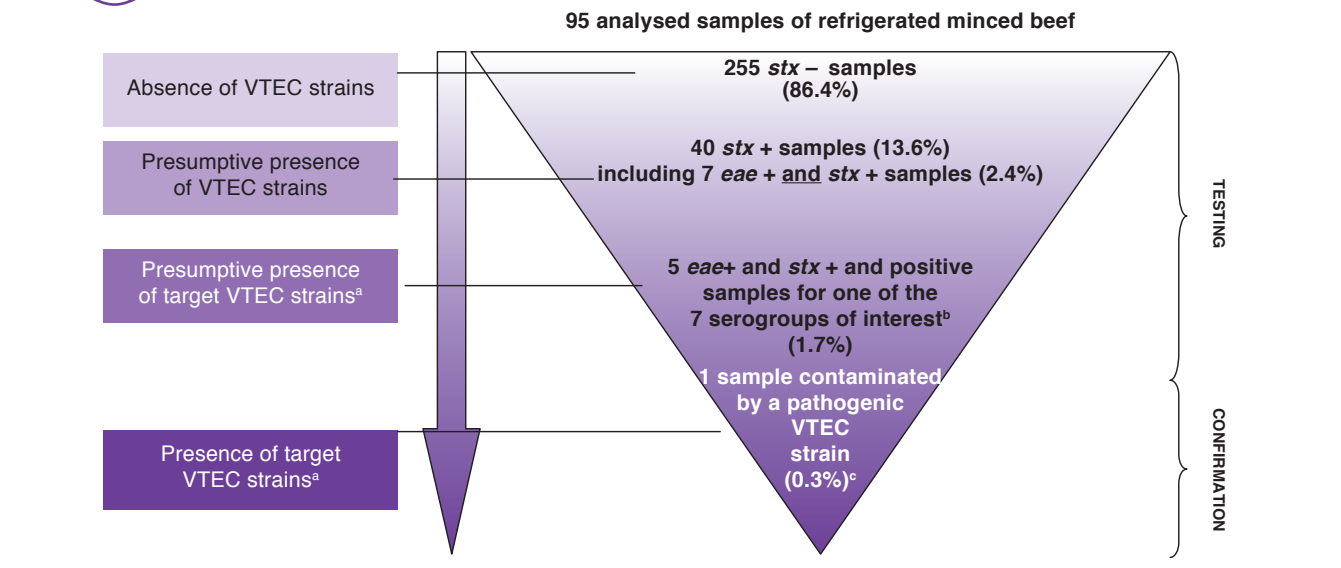


Figure 2. Diagram of the main stages of the method used for the analysis of VTEC strains, and stakeholders responsible for its implementation as part of the surveillance plan conducted in 2015 (adapted from Loukiadis *et al.*, 2012)



a. The following shigatoxin-producing *E. coli* strains were analysed:
(1) VTEC strains considered as highly pathogenic to humans (ANSES Request No 2010-SA-0031). Such strains own the *stx* (*stx1* and/or *stx2*) and *eae* virulence genes and belong to one of the five O157:H7, O26:H11, O145:H28, O103:H2 and O111:H8 serotypes, and
(2) VTEC strains considered as pathogenic, i.e. owning the *stx* (*stx1* and/or *stx2*) and *eae* virulence genes (ANSES Request No 2010-SA0031) and belonging either to the O45 serogroup or to the O121 serogroup. This analysis was undertaken on an exploratory basis, in order to detect the VTEC strains targeted by the American regulations.

b. Analysed somatic markers of interest: genes *wzxO45, wzxO26, wzxO103, wbdlO111, wzxO121, ihp1O145* and *rfbEO157.*

c. The isolated highly pathogenic VTEC strain was an O103:H2 strain having all the phenotypic and genotypic characteristics of the major typical EHEC strains (ANSES Request No 2010-SA-0031) (combination of O103:H2, *eae* e and presence of the gene). This strain also has the *ehx* gene (encoding enterohaemolysin) and the entire OI-122 island.

+: gene detected by PCR; -: gene not detected by PCR.

eae: gene encoding intimin; OI: O island; PCR: polymerase chain reaction; *stx*: gene encoding type 1 and/or 2 shigatoxins.

Figure 3. Summary of the results obtained from the surveillance plan for contamination by VTEC strains in refrigerated minced beef on the French market in 2015

and *eae* virulence factors in the collected isolates were analysed by PCR (Perelle *et al.*, 2004; Nielsen *et al.*, 2003). Genotypic characterisations in addition to those proposed by Technical Specification ISO/WD TS 13136:2012 were also undertaken: PCR analysis of the *ehx* gene (Tzschoppe *et al.*, 2012), *eae* gene variants (Nielsen *et al.*, 2003), *stx* gene variants (Scheutz *et al.*, 2012) and the presence of OI-122 (Karmali *et al.*, 2003).

The detection of the *stx* and *eae* genes and markers associated with any of the seven analysed serogroups was performed by the network of laboratories accredited for undertaking official analyses for the detection of VTEC, spread out across France[5][5]. Additional and confirmatory analyses were undertaken by the National Reference Laboratory (NRL) for *E. coli* including VTEC[6] (Figure 2).

Statistical analyses

In order to take into account uncertainties related to sampling fluctuations, the confidence interval with a 95% probability of containing the actual contamination rate was calculated with the R software (version 3.0.1, R Core Team., 2013) (error risk a set at 5%). The obtained rates were compared using Fisher's test (significance with a p-value £ 0.05) after verification of data normality.

Results

A total of 306 samples were collected: this corresponds to a 100% sampling rate in relation to the initial specification. However, only 295 of these 306 collected samples (96.4%) were analysed since eleven samples did not comply with the plan's instructions. The analysed minced beef was primarily of French origin (97.3%, 99% and 100% of the samples were collected from animals born, raised and slaughtered in France respectively). Such beef mainly intended to be consumed cooked (286/295, i.e. 96.9%) and usually had a fat content of 5% (135/295, i.e. 45%) or 15% (136/295, i.e. 45%).

Figure 3 summarises the sampling results. Of the 295 analysed samples, 290 were found to be negative. More precisely:

- 235 samples (79.7%) showed negative results for both the *eae* and *stx* genes,
- 20 samples (6.8%) had a positive PCR result for the *eae* gene only,
- 33 samples (11.2%) showed a positive PCR result for *stx* (*stx*1 and/or *stx*2) genes only,
- 2 samples (0.7%) had a positive PCR result for both the *stx* and *eae* genes but a negative result for all the seven analysed serogroup markers.

Only five samples (5/295, i.e. 1.7% of the analysed samples) had positive PCR results for the *stx* and *eae* genes and a positive signal for at least one of the seven tested serogroups. Such samples were considered as presumptive positive samples. None of the markers associated with serogroups O157, O111, O45 and O121 were detected. The detected serogroup markers corresponded to serogroups O103 (*wzxO103*, three samples), O145 (*ihp1O145*, two samples) and O26 (*wzxO26*, two samples). Two samples showed a positive signal for two serogroups simultaneously.

Only one presumptive positive sample out of 5 was confirmed as containing a VTEC strain considered as highly pathogenic (1/295, i.e. 0.3% of the analysed samples; 95CI [0.01- 1.9])) (Figure 3). This sample was a beef burger with a fat content of 15% that was intended to be consumed cooked. The meat was of French origin (animals born, raised and slaughtered in France).

The phenotypic and genotypic characteristics of the isolated strains are shown in Table 1. The highly pathogenic VTEC strain isolated belongs to the O103:H2 serotype and has all the phenotypic and genotypic characteristics of the major typical EHEC strains as defined in the AFSSA Opinion of 2010 (Request No 2010-SA-0031) (combination of serotypes, *eae* gene variants and presence of the genes encoding one of the types of shigatoxins). This strain also contains the *ehx* gene (encoding enterohaemolysin) and the entire OI-122 island, suggesting that it may have increased pathogenicity (AFSSA, 2008). Indeed, OI-122 contains genes encoding Nle effectors (non-LEE encoded effectors, whose role in the pathogenicity of strains remains unclear, even though they are not found in non-pathogenic strains). In general, the more complete this island (e.g. presence of one, two, three or four of the analysed OI-122 genes), the more the disease associated with these strains is severe (HUS) (AFSSA, 2008).

Discussion

The data from the VTEC surveillance plans show, irrespective of the surveillance stage and therefore irrespective of the biases inherent in the programme, low and similar contamination rates in minced beef over the past few years. The contamination rate observed in 2015 in refrigerated minced beef on the market was not significantly different from the results of previous surveillance plans (Loukiadis *et al.*, 2012). In fact, during the 2009 and 2010 surveillance plans on VTEC contamination in refrigerated minced beef collected during distribution, 0.1% (95CI [0.0-0.5]) and 0.2% (95CI [0.1-0.5]) of the analysed samples were respectively confirmed as being contaminated by a highly pathogenic VTEC strain. These results underline that consumer exposure to this hazard *via* the consumption of minced beef poses a low risk.

The highly pathogenic VTEC strain isolated in 2015 belongs to the O103:H2 serotype. It is thus potentially capable of causing characteristic attaching and effacing lesions of the intestinal mucosa in humans, responsible for diarrhoea symptoms, and of isproducing shigatoxin type 1, variant a. This toxin, involved in the destruction of the capillary endothelial cells of the colon, kidneys and brain, which can cause haemorrhagic colitis, HUS or even coma (AFSSA, 2003). This serotype of VTEC strains has been isolated in minced beef in previous surveillance plans. However, it is generally less prevalent than O26:H11 and O157:H7 VTEC in such foodstuff (Loukiadis *et al.*, 2012). VTEC O103:H2 strains were responsible for 2% of the 114 HUS cases identified in children under the age of fifteen in France in 2014 and 1.4% of the 698 cases identified over the 2010-2014 period (http://www.invs.sante.fr/Dossiers-thematiques/Maladies-infectieuses/Risques-infectieux-d-origine-alimentaire/Syndrome-

5. A total of sixteen laboratories were accredited for VTEC detection for the implementation of the 2015 plan (the list is available at the following address: http://agriculture.gouv.fr/sites/minagri/files/e._coli_VTEC_dans_le_cadre_des_pspc_-_liste_des_ laboratoires_agrees_v13.pdf).
6. National Reference Laboratory (NRL) for *E. coli* including VTEC – Research laboratory for pathogenic microorganisms in food (LMAP) – VetAgro Sup Veterinary Campus in Lyon (formerly ENV Lyon).

Table 1. Phenotypic and genotypic characteristics of the highly pathogenic VTEC strain isolated in refrigerated minced beef collected in the distribution stage in the framework of the 2015 surveillance plan

Strain	Phenotypic characteristics		Genotypic characteristics*							
	API 20E identification profile	Serotype**	*eae* (variant)	*stx1* (variant)	*stx2* (variant)	*ehx*	OI122			
							papC21	*sen 26*	*efa132*	*efa133*
85-93	5 144 572	O103:H2	+ (ε)	+ (1a)	-	+	+	+	+	+

* determined by PCR (ISO TS 13136:2012 and other references cited in the Materials and methods section).
** determined by PCR (the target genes for the determination of serotypes are given in AFSSA Opinion No 2008-SA-0122 and were analysed by PCR according to ISO TS 13136:2012 for somatic markers and according to Madic et al., 2010 for flagellar markers)

hemolytique-et-uremique) but have never been involved in episodes of clustered food-borne cases in France (Loukiadis *et al*., 2012).

Note that no VTEC O45 or O121 strains tested in beef in the United States have ever been found in France.

In all cases, when VTEC strains are detected, operators must withdraw the product from the market, search for possible sources of contamination, and take suitable control measures to reduce risks of contamination. These mitigation measures apply in accordance with the instructions of the DGAL[7].

All of the obtained results highlight the significance of measures taken upstream by professionals to control this hazard. Health control plans help reduce the risk of marketing contaminated products, at the slaughterhouse by taking into account the cleanliness of animals and controlling certain at-risk stages (oesophageal ligation, bagging the rectum, stripping the hide and evisceration in particular (ANSES, 2014)) and then during processing by complying with good hygiene practices and verifying the effectiveness of mitigation measures through self-inspections at critical points (including the inspection of raw materials in the production stage). Moreover, raising the awareness of consumers as to observance of the cooking instructions indicated on product labels, for minced beef in particular (see "Set of recommended good hygiene practices for consumers"[8]), is also a way to reduce the risk of human contamination downstream.

The obtained results were published in the "summary" note for the French authorities and were communicated to EFSA for publication in its "zoonoses" report (available at the following address: http://www.efsa.europa.eu).

In 2016, the DGAL continued to monitor contamination by VTEC strains in minced beef (refrigerated and frozen) by implementing a surveillance plan in the distribution stage.

Acknowledgements

The authors would like to thank all of the teams of the accredited laboratories and the *E. coli* NRL for their involvement in obtaining the surveillance plan data, as well as the services of the DDecPPs.

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Surveillance of *Salmonella* contamination of pig carcasses through own-check undertaken at the slaughterhouse

Sabine Itié-Hafez (1) (sabine.itie@agriculture.gouv.fr), Alain Le Roux (2), Françoise Chartier (3), Daniel Fort (3), Corinne Danan (1)
(1) Directorate General for Food, Sub-directorate for Food Safety, Support Office for Food Chain Surveillance, Paris, France
(2) French Pork and Pig Institute (IFIP), Le Rheu, France
(3) Directorate General for Food, Sub-directorate for Food Safety, Office for Slaughter and Cutting Plants, Paris, France

Abstract
Salmonellosis is the major cause of foodborne outbreaks caused by bacteria in Europe. In 2014, the European Commission reinforced the supervision of this contamination in the pig sector. In this context, the General Directorate for Food implemented a new system to centralise regulatory own-check for *Salmonella* in pig carcasses. The results provide an estimate of the level of contamination of carcasses, at national level and for each slaughterhouse. Variability in levels of contamination can be associated with risk factors, which could be the subject of dedicated studies. These results are intended to be transmitted each year to the European Food Safety Authority for comparison among Member States. They could also be used at national level to raise the awareness of stakeholders.

Keywords
Salmonella, Carcasses, Pigs, Slaughterhouses, Own-check

Résumé
Surveillance de la contamination des carcasses de porcs par* Salmonella *via le bilan des autocontrôles réalisés à l'abattoir
Les salmonelloses sont la première cause de toxi-infection alimentaire collective d'origine bactérienne en Europe. La viande de porc est une des sources associée aux cas humains. La Commission européenne a renforcé en 2014 la supervision de la maîtrise de cette contamination en filière porcine. Dans ce cadre, un nouveau système de centralisation des autocontrôles réglementaires vis-à-vis de Salmonella *dans les carcasses de porcs a été mis en place par la direction générale de l'Alimentation dans les abattoirs. Les résultats donnent une estimation du niveau moyen de la contamination par* Salmonella*, au niveau national et dans chaque abattoir. La variabilité des taux de contamination entre les abattoirs peutêtre associée à des facteurs de risque, qui pourraient faire l'objet d'études dédiées. Ces résultats sont destinés à être transmis à l'Autorité européenne de sécurité des aliments chaque année pour une comparaison entre États membres. Ils pourront être également utilisés au niveau national pour sensibiliser les opérateurs.*

Mots-clés
Salmonella*, carcasses, porcs, abattoir, autocontrôles*

The health control of food production systems is regulated by the European texts of the Hygiene package. In this context, operators in the food sector are responsible for the foodstuffs they place on the market and must ensure they are not hazardous . To do so, they must develop a health control plan in order to guarantee the control of identified hazards (including good hygiene practices, procedures founded on the HACCP principles, traceability, and the management of non-compliance) and verify that the defined control measures are effective. This verification relies on own-check, among other things. The competent authorities ensure that operators in the food sector comply with the regulatory requirements.

Although the number of salmonellosis cases has been decreasing since control programmes were implemented in the poultry sector, *Salmonella* remains the major cause of food-borne outbreaks of bacterial origin in Europe (EFSA & ECDC, 2015). Pork is one of the sources associated with human cases. In 2014 in France, 15% of food-borne outbreaks caused by *Salmonella* involved meat and 11% involved delicatessen meat (all species combined) (InVS, 2014). The lack of harmonised control programmes in the pig and pork sector in Europe led the European Commission to reinforce supervision by the competent authorities in this area in 2015. Of the various supervision methods proposed by the European Commission under Regulation (EU) 218/2014, the Directorate General for Food (DGAL) chose to implement a system for the collection and centralisation of the results of own-check undertaken in accordance with Regulation (EC) No 2073/2005 in all pig slaughterhouses. This innovative approach was defined in collaboration with representatives of professionals from slaughterhouses and the pork and pig sector.

Member States send the results collected annually to EFSA in accordance with Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents.

Materials and methods

Slaughterhouses concerned

Data are collected from all pig slaughterhouses, including both those slaughtering pigs only and those slaughtering several animal species including pigs.

Sample identification

The samples come from own-check for *Salmonella* undertaken in accordance with Regulation (EC) No 2073/2005 (Process hygiene criterion 2.1.4). These own-check are intended to verify control of the slaughter process. Slaughterhouses thus identify these own-check in order to distinguish them from other samples taken in the more specific framework of hygiene control process management or after an isolated loss of control.

Sampling procedure

Own-check are undertaken weekly in every slaughterhouse, randomly, with five carcasses from the same slaughter day, according to technical instruction DGAL/SDSSA/2015-619[1]. The sampling day must change every week. For slaughterhouses that do not operate

1. Technical instruction DGAL/SDSSA/2015-619 of 20 July 2015 on microbiological criteria for own-check of carcasses of slaughter animals.

Table 1. Characteristics of the slaughterhouses for which results are available

	Number of slaughterhouses in France	Annual pig slaughter volume in 2015 (in tonnes)	Main species slaughtered in 2015 (by volume)	Number of slaughter chains	Classification level/ compliance with EU regulations*
Multi-species slaughter-houses (including pigs)	133 (83.1%)	560,523 (28.5%)	Cattle: 63.9% Pigs: 34.5% Sheep: 0.8% Equines: 0.8%	chain: 25.6% chains: 25.6% chains: 47.3% chains: 1.5%	Level I: 6.0% Level II: 84.2% Level III: 9.8%
Pig slaughterhouses	27 (16.9%)	1,404,678 (71.5%)		1 chain: 100%	Level I: 7.4% Level II: 88.9% Level III: 3.7%
Total	**160**	**1,965,201**		**chain: 38.1% chains: 21.25% chains: 39.4% chains: 1.25%**	**Level I: 6.25% Level II: 85.0% Level III: 8.75%**

* The classification level of a slaughterhouse is established by the DDecPP/DAAF during official controls and corresponds to the slaughterhouse's risk control level: Level I = adequate risk control – Level 2 = acceptable risk control – Level III = inadequate risk control. It corresponds to the slaughterhouse's level of compliance.

Table 2. Categorisation of slaughterhouses by number of samples taken in 2015 as part of regulatory own-check

Category	Number of slaughterhouses	Annual number of samples (N)
1	55 (35%)	N < 50 (less than one 5-carcass sample per month)
2	72 (46%)	50 ≤ N < 240 (from one 5-carcass sample per month to one sample every two weeks)
3	29 (19%)	N ≥ 240 (at least one 5-carcass sample per week)

five days a week, samples can be taken every five days of actual slaughter. For plants with several slaughter chains, a own-check plan is established for each chain. This sampling frequency can be reduced to every fortnight (or every 10 days of actual slaughter) if the interpretation of the results is satisfactory for 30 consecutive weeks or for slaughterhouses for which the slaughter volume is less than 1000 heads per year.

Samples are collected using a non-destructive method, with a sponge used for the sampling of four different sites per carcass. The sampling area is at least 100 cm² per site. Samples are commonly taken from the leg, loin, belly and neck[2].

Salmonella testing is performed using reference method NF EN ISO 6579 "Microbiology of foods – Horizontal method for the detection of *Salmonella* spp.", or any equivalent alternative method certified by AFNOR Validation.

Centralisation of results

In 2015, the official control authorities entered, in a Sphinx form created by the DGAL, the results of the regulatory own-check undertaken by each slaughterhouse, specifying the following information : corresponding period, number of samples taken, number of positive results.

These data were centralised and analysed by the DGAL to estimate the average contamination rate for pig carcasses in France and in each slaughterhouse.

It is important to clearly distinguish between this supervision activity and the verification of process control by operators *via* the regulatory process hygiene criterion 2.1.4 of Regulation (EC) No 2073/2005:

- for this supervision undertaken by the competent authority, a positive result corresponds to the presence of *Salmonella* in a carcass; there is no interpretation of compliance for these results,
- for the implementation of the regulatory criterion, own-check results are routinely interpreted by the operator for 10 consecutive sampling times and corrective measures must be taken immediately in the event of non-compliance (more than three contaminated carcasses out of 50 tested for the time period in question).

2. Standard NF EN ISO 17604 recommends thirteen sampling sites.

Results and discussion

Characteristics of pig slaughterhouses

In 2015, 167 pig slaughterhouses were identified in France.

However, own-check results are available for only 160 slaughterhouses; data are missing or incomplete for seven slaughterhouses (three in Brittany, two in Corsica, one in Languedoc-Roussillon-Midi-Pyrénées and one in Auvergne-Rhône-Alpes). These 160 slaughterhouses are spread out across 74 *départements* in mainland France and four overseas *départements* and regions.

Of the 160 slaughterhouses for which results are available, 27 (16.9%) slaughter pigs only and 133 (83.1%) slaughter several animal species including pigs (Table 1).

Pig slaughter volumes range from two to 208,579 tonnes per year depending on the slaughterhouse, with an average volume of 12,362 tonnes per year. The largest slaughter volumes are observed in plants slaughtering pigs only.

Overall own-check results

In France, in 2015, 16,223 samples were collected by pig slaughterhouses as part of their regulatory own-check. In total, 1108 samples showed a positive result, corresponding to an average contamination rate of 6.8% (min. = 0.0%, max. = 28.1%, median = 1.4%).

The annual number of samples taken in the framework of regulatory own-check varies depending on the slaughterhouse. The number of own-check undertaken in the context of the regulations is related to the slaughter volume for most slaughterhouses. In fact, the smaller the volume, the more the number of samples can be reduced, since the number of samples taken can be modulated based on the number of actual slaughter days (for slaughterhouses not operating every day of the week) or in proportion to the tonnage for plants slaughtering several species on the same chain (see above).

For certain slaughterhouses however, it appears that the number of analyses performed was lower than expected; this may have been due to reduced sampling frequencies authorised in some specific cases (see above) or, in other cases, to the misinterpretation of or non-compliance with the regulatory provisions.

Table 3. Characteristics of slaughterhouses that collected between 1 and 50 samples in 2015

	Number of slaughterhouses in France	Annual pig slaughter volume in 2015 (in tonnes)	Main species slaughtered in 2015 (by volume)	Number of slaughter chains	Classification level/ compliance with EU regulations
Multi-species slaughter-houses (including pigs)	53 (96.4%)	25,231 (58.8%)	Cattle: 71.7% Pigs: 24.5% Sheep: 1.9% Equines: 1.9%	1 chain: 34.0% 2 chains: 26.4% 3 chains: 37.7% 4 chains: 1.9%	Level I: 5.7% Level II: 81.1% Level III: 13.2%
Pig slaughterhouses	2 (3.6%)	17,706 (41.2%)		1 chain: 100%	Level II: 100%
Total	**55**	**42,937**		**1 chain: 36.4% 2 chains: 25.4% 3 chains: 36.4% 4 chains: 1.8%**	**Level I: 5.5% Level II: 81.8% Level III: 12.7%**

Table 4. Characteristics of slaughterhouses that collected between 50 and 240 samples in 2015

	Number of slaughterhouses in France	Annual pig slaughter volume in 2015 (in tonnes)	Main species slaughtered in 2015 (by volume)	Number of slaughter chains	Classification level/ compliance with EU regulations
Multi-species slaughterhouses (including pigs)	65 (90.3%)	132,059 (42.3v%)	Cattle: 63.1% Pigs: 36.9%	1 chain: 20.0% 2 chains: 20.0% 3 chains: 58.5% 4 chains: 1.5%	Level I: 4.6% Level II: 86.2% Level III: 9.2%
Pig slaughterhouses	7 (9.7%)	180,380 (57.7%)		1 chain: 100%	Level II: 85.7% Level III: 14.3%
Total	**72**	**312,439**		**1 chain: 27.8% 2 chains: 18.0% 3 chains: 52.8% 4 chains: 1.4%**	**Level I: 4.2% Level II: 86.1% Level III: 9.7%**

Table 5. Characteristics of slaughterhouses that collected more than 240 samples in 2015

	Number of slaughterhouses in France	Annual pig slaughter volume in 2015 (in tonnes)	Main species slaughtered in 2015 (by volume)	Number of slaughter chains	Classification level/ compliance with EU regulations
Multi-species slaughterhouses (including pigs)	11 (37.9%)	403,233 (25.0%)	- Cattle: 18.2% - Pigs: 81.8%	1 chain: 18.2% 2 chains: 45.4% 3 chains: 36.4%	Level I: 18.2% Level II: 81.8%
Pig slaughterhouses	18 (62.1%)	1,206,592 (75.0%)		1 chain: 100%	Level I: 11.1% Level II: 88.9%
Total	**29**	**1,609,825**		**1 chain: 69.0% 2 chains: 17.2% 3 chains: 13.8%**	**Level I: 13.8% Level II: 86.2%**

Four slaughterhouses did not undertake any own-check. These were slaughterhouses for which the pig slaughter volume was extremely low and/or minor.

For the 156 slaughterhouses that conducted analyses in 2015, results are given for three categories of slaughterhouses established based on the number of samples collected (Table 2).

For the overall processing of data, the results for the 156 slaughterhouses that conducted analyses in 2015 (categories 1 to 3) are described below.

Category 1: slaughterhouses that collected between one and 50 samples

For the 55 slaughterhouses that collected between one and 50 samples in 2015, the average contamination rate was 1.8% (min. = 0.0%, max. = 21.4%, median = 0.0%). These were almost exclusively plants slaughtering several animal species (mainly cattle) (Table 3).

Category 2: slaughterhouses that collected between 50 and 240 samples

For the 72 slaughterhouses that collected between 50 and 240 samples in 2015, the average contamination rate was 4.3% (min. = 0.0%, max. = 28.1% and median = 1.6%). These were almost exclusively plants slaughtering several animal species (mainly cattle) and having several slaughter chains (Table 4).

Category 3: slaughterhouses that collected more than 240 samples

For the 29 slaughterhouses that collected more than 240 samples, the average contamination rate was 9.5% (min. = 0.0%, max. = 21.5% and median = 6.8%). These were mainly plants slaughtering pigs only or multi-species plants mainly slaughtering pigs. No slaughterhouses in this category had a Level III classification (Table 5).

Considering all of the results, the average *Salmonella* contamination rate in pig carcasses increased with the number of samples taken in the framework of regulatory own-check (Table 6).

Nonetheless, these results varied considerably between slaughterhouses (Figure 1).

Moreover, contamination rates did not seem related to the classification levels of slaughterhouses (Table 7).

Conclusions and outlook

The implementation of this new system provides an estimate of the

Table 6. Average *Salmonella* contamination rate in pig carcasses by number of samples taken in the framework of regulatory own-check in 2015

Number of samples collected in 2015 per slaughterhouse	Average *Salmonella* contamination rate
Between 1 and 50 (category 1)	1.8% (min. = 0.0%, max. = 21.4%, median = 0.0%)
Between 50 and 240 (category 2)	4.3% (min. = 0.0v%, max. = 28.1%, median = 1.6v%)
More than 240 (category 3)	9.5% (min. = 0.0%, max. = 21.5%, median = 6.8%)

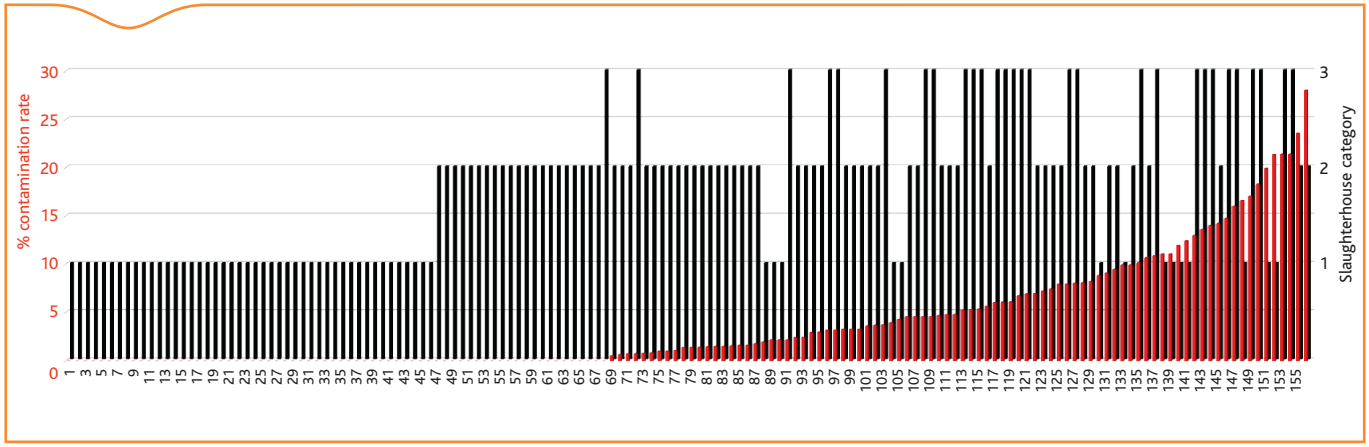

Figure 1. Breakdown of slaughterhouses that conducted analyses in 2015 by category and contamination rate

average level of contamination in pig carcasses from slaughterhouses and supplements national operator awareness-raising campaigns.

The observed results show significant variability in contamination rates between slaughterhouses. This variability may be related to various factors such as the slaughter volume, the characteristics of the slaughtered species, process control, the choice of carcass sampling sites, etc. The impact of these factors on hygiene control could be examined through specific studies. These studies could also include other factors likely to modify contamination rates observed in pig slaughterhouses: animal procurement radius, animal waiting time at the slaughterhouse before slaughter, cleaning/disinfection procedure, process used (singeing/double singeing), slaughter rate, etc. In addition, the individual results of each slaughterhouse should be processed by decentralised services in the context of official controls, especially in the event of deviation from the regulations.

In parallel, since December 2015, the French Pork and Pig Institute (IFIP), with funding from the French Pig and Pork Producers' Association (INAPORC), has developed a Web interface to collect own-check results from pig slaughterhouses, summarise them and interpret them for operators and the industry. So as to not maintain two parallel and redundant collection systems at national level, the DGAL would like to use the data from this database in the coming years. This transition will be gradual, with a stage for comparing the equivalence of the two systems (national coverage, collected results) in 2016.

At European level, vigilance should be maintained as to the interpretation by EFSA of all of the Member States' results, especially since the Commission let the Member States choose between three options for this supervision (organisation of official controls, use of validated control programme results, collection of own-check). The multi-partner group made up of members of the DGAL, the IFIP, French meat companies (Culture-viande), the French Federation of Slaughterhouse Operators (FNEAP), the French Federation of the Wholesale Meat Industry (FNICGV) and the INAPORC association, set up to monitor the French system, will be mobilised as needed to ensure good communication around these data.

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Table 7. Average *Salmonella* contamination rate in pig carcasses by slaughterhouse classification level

Classification level	Number of slaughterhouses that conducted analyses in 2015	Number of analyses conducted in 2015	Average *Salmonella* contamination rate
I	10 (6.4%)	1,325 (8.2%)	6.7% (min. = 0.0%, max. = 13.0% and median = 1.9%)
II	132 (84.6%)	13,951 (86.0v%)	7.1% (min. = 0.0%, max. = 28.1%, median = 1.4%)
III	14 (9.0%)	947 (5.8%)	3.0% (min. = 0.0%, max. = 8.3%, median = 0.7%)

Box.

Objectives
The objective of this surveillance is to collect and centralise the results of *Salmonella* self-inspections undertaken in pig carcasses at the slaughterhouse, in accordance with Regulation (EC) No 2073/2005. This system was implemented for the first time in France in 2015.

Programming framework
Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents.

Regulation (EU) No 218/2014 amending Regulation (EC) No 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption.

Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

Protocol
- **Nature of the tested contaminants:** *Salmonella*.
- **Affected products ("population"):** carcasses of pigs slaughtered in France.
- **Stage of the food chain:** slaughterhouse.
- **Definition of a "case":** sample contaminated by *Salmonella* spp.
- **Number of samples and sampling method:** the protocol enables the collection of all the results of the *Salmonella* self-inspections identified in the health control plans of operators, in accordance with Regulation (EC) No 2073/2005.
- **Sampling strategy:** random inspection of carcasses at regulatory frequencies for all pig slaughterhouses.
- **Analytical method, nature of sampling:** samples are collected at the end of the chain before the chilling of carcasses, with a sponge rubbed onto an area of at least 400cm^2. *Salmonella* testing is performed for each sample with the ISO/CEN 6579 method or any equivalent alternative method certified by AFNOR Validation.

Programmed surveillance of *Salmonella* spp. contamination of fresh poultry meat at slaughterhouse and the antimicrobial resistance of strains isolated in 2014

Muriel Marault (1), Sabine Itié-Hafez (2), Viviane Morel (1), Isabelle Berta-Vanrullen (3), Sophie A. Granier (1) (sophie.granier@anses.fr), Claire Born (4), Corinne Danan (2)

(1) ANSES, Laboratory for Food Safety, Laboratory associated with the National Reference Laboratory for Antimicrobial resistance, Maisons-Alfort, France
(2) Directorate General for Food, Sub-directorate for Food Safety, Support Office for Food Chain Surveillance, Paris, France
(3) ANSES, Laboratory Affairs Department, Unit for the Coordination and Support of Surveillance, Maisons-Alfort, France
(4) Directorate General for Food, Sub-directorate for Food Safety, Office for Slaughter and Cutting Plants, Paris, France

Abstract

Programmed surveillance of *Salmonella* spp. contamination of fresh poultry meat at slaughterhouse and the antimicrobial resistance of strains isolated in 2014. In 2014, implementing Decision 2013/652/EU on the surveillance and reporting of antimicrobial resistance in zoonotic and commensal bacteria, the Directorate General for Food (DGAL) organised a surveillance programme on poultry carcass contamination by *Salmonella* spp. at slaughterhouse. The antimicrobial resistance of these *Salmonella* isolates was also assessed. In order to produce data representative of the slaughtered volume nationwide, only certified poultry slaughterhouses were targeted in mainland and overseas France. Contamination by *Salmonella* spp. was on average greater than 10%. Turkey carcasses displayed higher contamination rates than chicken carcasses. The most commonly observed serovars were not those regulated in fresh poultry meat. Therefore, non-compliance rates remained very low, at around 1%. The resistance profiles observed rarely involved critically important antibiotics for human health. Multi-drug resistance appeared to be quite rare in chickens, while it was more frequent in turkeys. This programme is designed to be reproduced every other year in order to provide temporal trends as well as comparable data at European level.

Keywords

Monitoring program, *Salmonella*, Poultry, Carcasses, Antimicrobial resistance

Résumé

Surveillance programmée de la contamination par* Salmonella *spp. des viandes fraîches de volaille au stade de l'abattoir et de la résistance aux antibiotiques des souches isolées en 2014

En application de la décision 2013/652/UE concernant la surveillance de la résistance aux antimicrobiens chez les bactéries zoonotiques et commensales, la direction générale de l'Alimentation a organisé en 2014 un plan de surveillance de la contamination par Salmonella *spp. des carcasses de volailles au stade de l'abattage et de la résistance aux antibiotiques des souches isolées. Seuls les abattoirs de volailles agréés dans l'ensemble des régions de France métropolitaine et d'Outre-mer étaient concernés afin de produire une information représentative des volumes d'abattage au niveau national. Le taux de contamination moyen des carcasses de volailles par* Salmonella *est supérieur à 10 %. Les carcasses de dindes présentent un taux de contamination plus élevé que celles de poulets. Les sérovars majoritairement isolés ne sont pas ceux qui sont concernés par le critère réglementaire de sécurité défini pour les viandes fraîches de volailles dans le règlement (CE) n°2073/2005 ; les taux de non-conformité sont donc faibles, proches de 1 %. Les profils d'antibiorésistance obtenus concernent peu les antibiotiques critiques pour la santé humaine. Par ailleurs, si les souches multi-résistantes sont peu nombreuses chez le poulet, leur nombre est plus élevé chez la dinde. Ce plan est destiné à être reconduit les années paires afin de comparer l'évolution du niveau de résistance des souches de* Salmonella *isolées au sein de ces filières, au niveau européen.*

Mots-clés

Plan de surveillance, Salmonella, *volaille, carcasses, antibiorésistance*

Salmonella is the second-leading cause of food-borne infections in humans and remains the most common cause of food-borne outbreaks of bacterial origin in Europe. The principal reservoir of *Salmonella* is the gastro-intestinal tract of mammals (pigs and cattle) and birds (domestic poultry). Transmission to humans mostly occurs through the consumption of raw or undercooked contaminated foods. For the most susceptible individuals, antimicrobials are administered to treat salmonellosis. However, the bacteria can acquire patterns of antimicrobial resistance and therefore resist treatments. This phenomenon is a public health threat.

In accordance with Directive 2003/99/EC, European Union Member States are required to set up a surveillance system for zoonoses, zoonotic agents and related antimicrobial resistance. *Salmonella* are included on the list of agents to be monitored featured in Annex I (A) of this directive. For food-borne *Salmonella*, official surveillance consists in: i) supervision of the implementation of Regulation (EC) No 2073/2005 by operators, and ii) implementation of Decision 2013/652/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria.

The main objective of this surveillance programme was to characterise the antimicrobial susceptibility profiles of the *Salmonella* strains isolated from poultry carcasses at the slaughterhouse, in accordance with Decision 2013/652/EU. The programme also provided for verification of the compliance of poultry carcasses with microbiological safety criterion 1.28 of Regulation (EC) No 2073/2005, introduced in 2011, for *Salmonella* Typhimurium (including its monophasic variant 1,4,[5],12:i:-) and *Salmonella* Enteritidis.

Box.

Objectives

Descriptive study on the antimicrobial susceptibility of *Salmonella* strains isolated from poultry carcasses at the slaughterhouse.

Verification of the compliance of poultry carcasses with the regulatory safety criterion.

Framework

Decision 2013/652/EU, Regulation (EC) No 2073/2005 (safety criterion 1.28).

Protocol

The sampling plan was designed to obtain 170 *Salmonella* isolates in the chicken sector and 170 *Salmonella* isolates in the turkey sector.

- **Nature of the tested contaminants:** *Salmonella*, susceptibility to 14 antimicrobials representing 12 antimicrobial classes.
- **Affected products ("population"):** turkey and chicken carcasses at the slaughterhouse
- **Definition of a "case":** a sample was considered non-compliant if it was contaminated by *Salmonella* Enteritidis or Typhimurium (including its monophasic variant 1,4,[5],12:i:-).

Number of samples and sampling method: 3000 (1200 samples from fattening turkeys and 1800 samples from broiler chickens) in proportion to slaughter volumes.

- **Sampling strategy:** random in each slaughterhouse.
- **Analytical method, nature of sampling:** *Salmonella* testing on neck skin according to reference method NF EN ISO 6579 "Microbiology of foods – Horizontal method for the detection of *Salmonella*" or equivalent alternative methods validated by AFNOR Certification.

Materials and methods

Sampling protocol

In accordance with Decision 2013/652/EU, the sampling plan was designed so as to obtain 170 *Salmonella* isolates in the chicken sector and 170 in the turkey sector to test their antimicrobial susceptibility.

The number of samples was calculated based on the results of a similar surveillance programme, implemented by the Directorate General for Food (DGAL) in 2010 (average contamination rate in chicken carcasses: 10.4% and in turkey carcasses: 16.7%)[1].

Thus, taking a margin of safety into account, assuming a decrease in *Salmonella* prevalence in poultry carcasses related to the introduction of microbiological safety criterion 1.28, the total number of samples was set at 3000 (1200 samples from fattening turkeys and 1800 samples from broiler chickens).

The samples were spread out across eighteen regions and three overseas territories, in proportion to the slaughter volumes of accredited poultry slaughterhouses. The samples were then divided up between the various slaughterhouses by the regions, in accordance with the protocol on the organisation of surveillance and control plans defined by the DGAL, which specifies, among other things, requirements for the geographic and temporal distribution of samples (distribution in proportion to slaughter volumes, smoothing of samples throughout the year).

Sampling and sending to laboratories

The batches to be sampled were to be randomly selected. In accordance with Regulation (EC) No 2073/2005 (Annex I, Chapter 3), the samples were made up of five units of poultry neck skin (n=5), prepared as follows (Figure 1):

- an approximately 10g piece of neck skin was collected from fifteen randomly selected poultry carcasses from the same original holding, after chilling,

1. http://agriculture.gouv.fr/sites/minagri/files/documents/pdf/recueil_tt_publicPSPC_2010_v4.pdf.

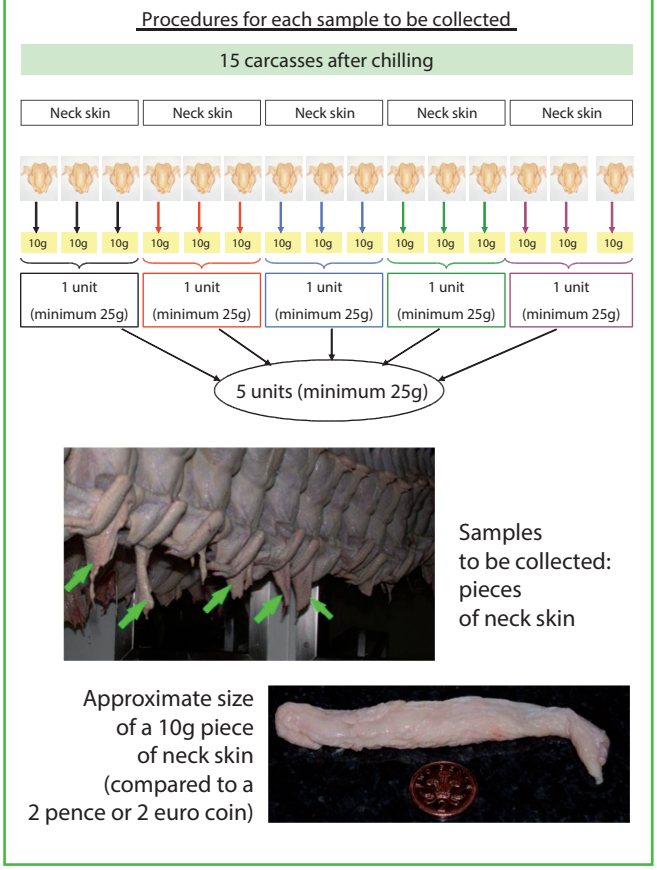

Figure 1. Sampling procedure (extracted from technical instruction DGAL/SDSSA/2013-9926 of 24/12/2013)

Table 1. List of tested antimicrobials and interpretative thresholds according to EUCAST (www.eucast.org)

Antimicrobial class	Tested antimicrobial (abbreviation)	Epidemiological cut-off values (ECOFFs) (mg/L)
Penicillins	Ampicillin (AMP)	> 8
3GC	Cefotaxime (CTX)	> 0.5
	Ceftazidime (CAZ)	> 2
Carbapenems	Meropenem (MEM)	> 0.125
Macrolides	Azithromycin (AZM)	> 16*
(Fluoro)quinolones	Nalidixic acid (NAL)	> 16
	Ciprofloxacin (CIP)	> 0.064
Aminoglycosides	Gentamicin (GEN)	> 2
Phenicols	Chloramphenicol (CHL)	> 16
Sulfonamides	Sulfamethoxazole (SSS)	> 256*
Diaminopyrimidines	Trimethoprim (TMP)	> 2
Tetracyclines	Tetracycline (TET)	> 8
Glycylcyclines	Tigecycline (TGC)	> 1
Polymyxins	Colistin (CST)	> 2

*: cut-off values not provided by EUCAST (http://www.eucast.org/mic_distributions_and_ecoffs/), values used on a proposal from the European Union Reference Laboratory (EURL)-Antimicrobial resistance (http://www.crl-ar.eu/)

- then the pieces of neck skin from three carcasses were pooled in order to form five units with the minimum weight of 25g required for the analysis.

The samples were sent to the analytical laboratories accredited for the detection and serotyping of *Salmonella*. The isolated *Salmonella* strains were then sent to ANSES in Maisons-Alfort for the analysis of their antimicrobial susceptibility.

Analytical methods

Salmonella detection and serotyping

Salmonella detection and serotyping in the isolated strains were undertaken according to reference method NF EN ISO 6579 "Microbiology of foods – Horizontal method for the detection of *Salmonella*". Equivalent alternative methods validated by AFNOR Certification were authorised if they had no restrictions for use.

Analysis of the antimicrobial susceptibility of the isolated strains

The antimicrobial susceptibility profile was determined by microdilution in a liquid medium according to the Sensititre® method. The Minimum Inhibitory Concentration (MIC) of fourteen antimicrobials, representing twelve antimicrobial classes, was measured. The interpretative thresholds used were those listed in Decision 2013/652/EU. These are the epidemiological cut-off values determined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). For non-determined (ND) values, the European Food Safety Authority (EFSA) provided temporary interpretative values (Table 1). These cut-offs may change as knowledge is improved and data are accumulated. A resistance phenotype is said to be "wild-type" when the bacterium shows no acquired resistance. Wild-type *Salmonella* are naturally susceptible to the 14 tested antimicrobials. "Multidrug resistance" is defined as the acquisition of resistance to at least three classes of antimicrobials (EFSA and ECDC, 2016).

Results

In total, 1183 samples from fattening turkeys and 1696 samples from broiler chickens were analysed, corresponding to sampling rates of 98.5% and 94% respectively for the plan. These samples were collected from a total of 131 slaughterhouses (20% of French slaughterhouses slaughtering poultry).

Contamination rates and verification of compliance with the safety criterion

Broiler chickens

Of the 1696 samples collected from broiler chickens in 122 slaughterhouses, *Salmonella* was detected in 210 samples from 26 slaughterhouses (21% of the included slaughterhouses), corresponding to an average carcass contamination rate of 12.4%.

Nineteen different serovars were identified; the most common were Derby (29%), Anatum (27%) and Indiana (20%). Serovar Typhimurium (including its monophasic variant 1,4,[5],12:i:-) was found in 10 samples and serovar Enteritidis was not isolated, corresponding to an estimated regulatory non-compliance rate of 0.6% for broiler chickens.

Fattening turkeys

Of the 1183 samples collected from fattening turkeys in 27 slaughterhouses, *Salmonella* was detected in 192 samples from 15 slaughterhouses (111 contaminated samples came from the same slaughterhouse), corresponding to an average carcass contamination rate of 16.2%. Sixteen different serovars were identified; the most common were Bredeney (41%), Anatum (14%) and Saintpaul (12%). Serovar Typhimurium (including its monophasic variant 1,4,[5],12:i:-) was found in 14 samples and serovar Enteritidis was found in one sample, corresponding to an estimated regulatory non-compliance rate of 1.3% for fattening turkeys.

Analysis of antimicrobial susceptibility

After the exclusion of isolates that were received in duplicate[2] or were contaminated[3], 169 *Salmonella* strains isolated from chicken carcasses and 173 *Salmonella* strains isolated from turkey carcasses were analysed to test their antimicrobial susceptibility.

Broiler chickens

In total, 154 wild-type strains (91.1%) were observed, 11 strains (6.5%) had a phenotype of resistance to one or two antimicrobial classes, and four strains (2.4%) were multi-drug resistant (Figure 2). Production of extended-spectrum beta-lactamases (ESBLs) was not observed. No resistance to third-generation cephalosporins (3GC) or carbapenems was detected. Resistance to ciprofloxacin accounted for 1.2% of strains. Resistance to colistin accounted for 2.4% (Table 2).

All the isolates of the main serovar, Derby, were wild-type. For Typhimurium, resistance was observed for ampicillin, sulfonamides and tetracycline, while the monophasic variants of Typhimurium were resistant to ampicillin and sulfonamides.

Fattening turkeys

In total, 54 wild-type isolates (31.2%) were observed, 79 strains (45.7%) had a phenotype of resistance to one or two antimicrobial classes, and 40 strains (23.1%) were multidrug resistant (Figure 2).

As in the chicken sector, no ESBL production and no resistance to 3GC or carbapenems were observed. Resistance to ciprofloxacin accounted for 6.9% of strains; as for colistin, 38.7% of strains had a MIC value just above the ECOFF.

Strains of the main serovar, Bredeney, displayed a variety of resistance profiles. It should be noted that a high percentage of strains were resistant to tetracycline (53/56) and/or had a MIC for colistin (37/56) slightly above the cut-off value.

For regulated serovars in the framework of the *Salmonella* controle and eradication l European programme in the poultry sector, the two *S.* Hadar strains were resistant to nalidixic acid and tetracycline. Strains of *S.* Typhimurium and its monophasic variant showed homogeneous resistance: they were all resistant to ampicillin, sulfonamides, tetracycline and gentamicin. However, one *S.* Typhimurium strain and one monophasic variant also had a MIC for colistin above the epidemiological cut-off value, classifying them as resistant to this antimicrobial.

The distribution of "resistance" to colistin was highly heterogeneous between serovars (majority of *S.* Bredeney and *S.* Brandenburg, some *S.* Anatum, *S.* Albany, *S.* Newport, *S.* Indiana, *S.* Montevideo, *S.* Eko). Most of the colistin-resistant strains had a MIC of 4 mg/L, i.e. the value just above the cut-off, which is not a result indicative of true resistance. Moreover, an antibiogram on agar medium did not provide confirmation of the colistin resistance of these *Salmonella*. However, a serovar Brandenburg strain had a MIC for colistin of 8 mg/L. For this strain, an antibiogram on agar medium showed an inhibition zone diameter of 9 mm around the 10 µg colistin disk. This is significantly narrower than what is typically obtained with *Salmonella* (approximately 15 mm) and suggests a colistin-resistance mechanism. Testing for the *mcr-1* gene, the sole mechanism of plasmid-mediated colistin resistance described before the summer of 2016 (Box), did not evidence this type of mechanism for this strain.

Discussion - conclusion

The average *Salmonella* contamination rate in poultry carcasses at the slaughterhouse was approximately 10% and appeared higher in the fattening turkey sector than in the broiler chicken sector. In this respect, the results obtained in 2014 were similar to the results of the 2010 surveillance programme obtained from fewer samples. Nonetheless, these results should be interpreted with caution, given the variability observed between slaughterhouses. Contamination rates in poultry carcasses

depended on several factors, such as slaughter volumes and rates, processes, farm contamination levels, etc. More in-depth studies into these risk factors would provide confirmation of these assumptions. Furthermore, several selection biases may have caused the results

2. All isolates from the same sample with the same serovar were considered as duplicates. In this case, only one copy was kept, which became a strain.
3. The stage of *Salmonella* detection in the sample must have been followed by a purification stage before sending to the NRL for Antimicrobial resistance for analysis of the resistance phenotype. Some cultures were found to be polymicrobial and could not be used.

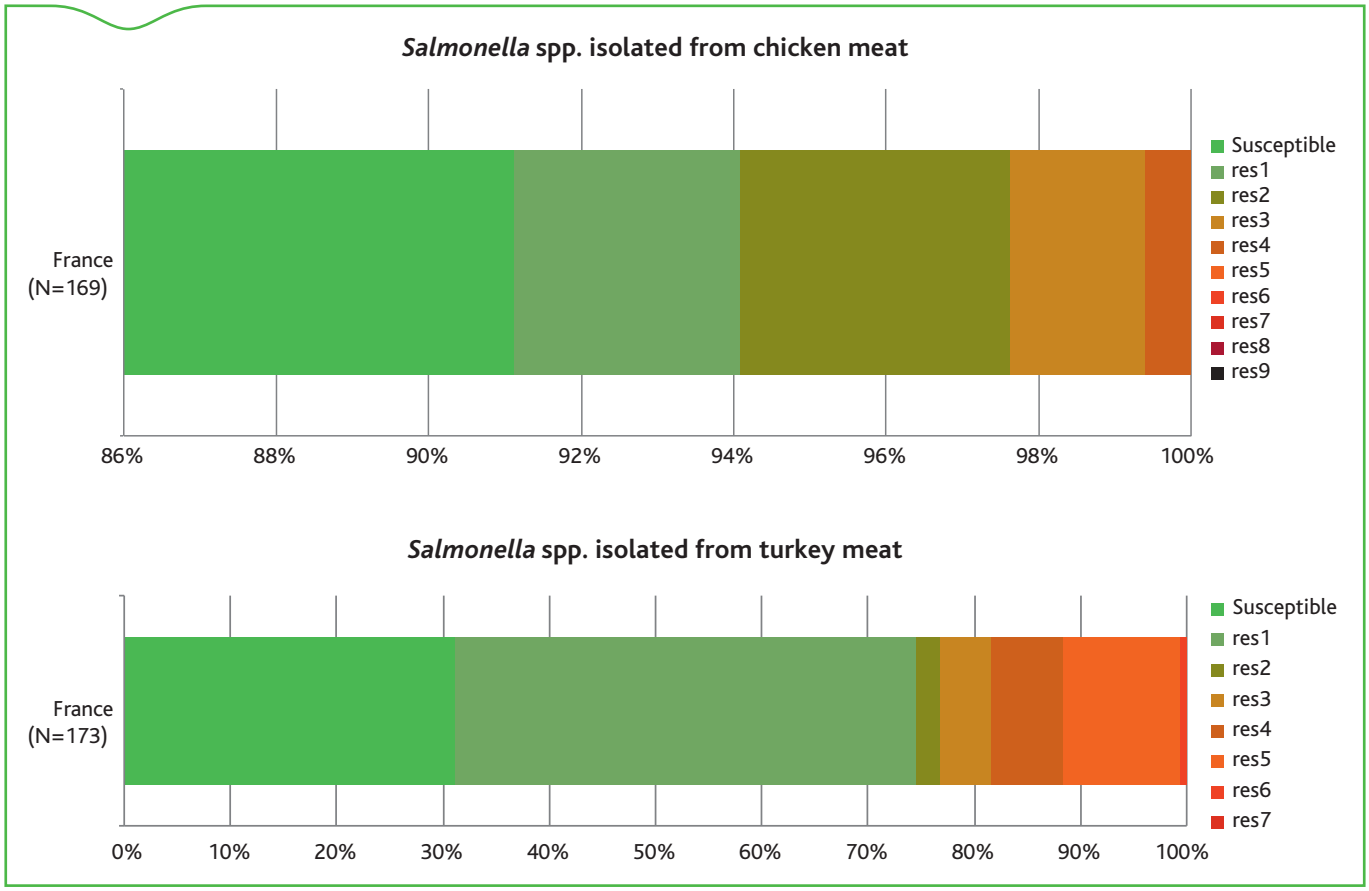

Figure 2. Distribution of resistance frequencies for *Salmonella* strains isolated from turkey and chicken carcasses in 2014 in France, expressed by antimicrobial class (according to EFSA and ECDC, 2016). Multi-drug resistance is defined as the acquisition of resistance to at least three classes of antimicrobials

to be overestimated, in particular possible non-compliance with the sampling strategy by some samplers preferring to sample batches from farms found positive for *Salmonella*.

It should be noted that control measures in the poultry sector seem to have limited the presence of the five serovars covered by eradication programmes (*S.* Typhimurium and its monophasic variant, *S.* Enteritidis, *S.* Hadar, *S.* Infantis, *S.* Virchow). These serovars were not those mainly found in poultry carcasses. This underlines the importance of taking all *Salmonella* serovars into account in the health control plans of operators downstream of slaughterhouses.

According to the results of the 2014 and 2010 surveillance programmes, the introduction of safety criterion 1.28 in Regulation (EC) No 2073/2005 in 2011 had no impact on *Salmonella* contamination rates in poultry carcasses. The rate of regulatory non-compliance for poultry carcasses (presence of serovar Typhimurium (including its monophasic variant 1,4,[5],12:i:-) or Enteritidis) was close to 1%. The management of non-compliant batches led to the withdrawal of their carcasses and cuts of meat, in accordance with the guide to the management of food alerts(4)[4].

As for the analysis of antimicrobial susceptibility, most of the *Salmonella* strains isolated in the chicken sector were wild-type. Rates of resistance and multi-drug resistance for

Salmonella strains were higher in the turkey sector. This finding appeared valid for all the Member States that reported data to EFSA for turkey and chicken meat (EFSA & ECDC, 2016).

It is reassuring to note that no ESBL phenotypes, 3GC resistance or carbapenem resistance were observed in *Salmonella* from the

4. http://agriculture.gouv.fr/sites/minagri/files/documents/pdf/_Guide_Gestion_Alerte_Revision_2_jlt_2009_COMPLETEE_VDef__cle09fc34.pdf.

Table 2. Rates of resistance for the isolated *Salmonella* strains by antimicrobial

Antimicrobial (Epidemiological cut-off value in mg/L)	Resistance rate (%, [95CI])	
	Broiler chickens N=169	Fattening turkeys N=173
Ampicillin (8) AMP	5.9 [3.2-10.5]	24.3 [18.5-31.2]
Cefotaxime (0.5) CTX	0.0 [0.0-2.2]	0.0 [0.0-2.2]
Ceftazidime (2) CAZ	0.0 [0.0-2.2]	0.0 [0.0-2.2]
Meropenem (0.125) MEM	0.0 [0.0-2.2]	0.0 [0.0-2.2]
Azithromycin (16) AZM	1.2 [0.3-4.2]	0.0 [0.0-2.2]
Nalidixic acid (16) NAL	0.0 [0.0-2.2]	6.4 [3.6-11.0]
Ciprofloxacin (0.06) CIP	1.2 [0.3-4.2]	6.9 [4.0-11.7]
Gentamicin (2) GEN	0.0 [0.0-2.2]	0.6 [0.1-3.2]
Chloramphenicol (16) CHL	0.6 [0.1-3.3]	10.4 [6.7-15.8]
Sulfamethoxazole (256) SSS	4.7 [2.4-9.1]	22.5 [17.0-29.3]
Trimethoprim (2) TMP	1.8 [0.6-5.1]	17.3 [12.4-23.7]
Tetracycline (8) TET	3.6 [1.6-7.5]	65.9 [58.6-72.5]
Tigecycline (1) TGC	0.0 [0.0-2.2]	1.7 [0.6-5.0]
Colistin (2) CST	2.4 [0.9-5.9]	38.7 [31.8-46.2]

chicken and turkey sectors at the slaughterhouse. There was also a low level of fluoroquinolone resistance; it was higher in the turkey sector (6.9%) but below the average rate for the other Member States (24.3%) (EFSA & ECDC, 2016). Comparison with EFSA's data is however limited to countries that reported results in these sectors and should also be considered in relation to the number of analysed strains. For example, out of 28 Member States, data from only nine Member States reporting a total of 726 analysed *Salmonella* strains were available for the entire turkey sector (farming environment and/or meat). For turkey meat specifically, only three countries (France, Germany and Hungary) reported data for 226 analysed strains. The observed colistin-resistance rate was apparently high, especially in the turkey sector. However, it should be analysed with caution due to the limitations of the method and the lack of perspective for these data. The MIC values measured by the micro-dilution method are accurate ata factor of 8, which means that 2 values measured can not be considered as different if they do not differ from a factor of 8 There were many measurements just above the cut-off value. Application of this factor of 8 did not show that these strains were definitively resistant to colistin. The collection of MIC data for colistin in future surveillance programmes, as well as further research into the topic, should shed light on these results and provide a clearer idea as to the risk of this resistance spreading. Lastly, the relevance of the cut-off value for colistin (> 2 mg/L) currently used for the "resistant" or "susceptible" interpretation should be reviewed as MIC data are accumulated.

This programme is designed to be reproduced every other year at European level. The experience acquired by the various EU Member States should facilitate the analysis and transmission of data in order to highlight any trends by country or even the circulation of antimicrobial-resistant strains.

Box. Colistin resistance

In November 2015, Liu *et al.* (2016) published the first mechanism of plasmid-mediated colistin resistance. Before that, colistin resistance had been considered as not horizontally transmissible between bacteria. The discovery of this *mcr-1* gene in China was quickly followed by descriptions of this gene all over the world, in various Enterobacteriaceae (*E. coli*, *Salmonella*, *Klebsiella pneumoniae*, etc.) and from various origins (humans, animals, food, etc.). Colistin resistance has been monitored in Europe only since the implementation of Decision 2013/652/EU on 1 January 2014. It should be noted that methods for the phenotypic characterisation of colistin resistance are still not very robust and are difficult to interpret.

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The *Salmonella* network: a surveillance scheme for *Salmonella* in the food chain: 2015 results

Vincent Leclerc (1), Frédérique Moury (1), Véronique Noel (1), Isabelle Berta-Vanrullen (2), Sabrina Cadel-Six (1), Renaud Lailler (1) (renaud.lailler@anses.fr)

(1) ANSES, Laboratory for Food Safety, Maisons-Alfort, France
(2) ANSES, Scientific Affairs Department for Laboratories, Maisons-Alfort, France

Abstract

For 20 years, the *Salmonella* network has been centralising serotyping results for *Salmonella* isolated on a voluntary basis in the food chain, in all industries and sectors. This outbreak surveillance supplements the official inspections undertaken every year. This massive volume of data collected by ANSES confirms the trends and emerging strains reported at European level. All origins combined, S. Typhimurium and its monophasic variants as well as S. Enteritidis are the main isolated strains. For many years, *Salmonella* has been a major microbiological contaminant responsible for foodborne epidemics in France and Europe. Optimising the assessment and management of the risk of salmonellosis in humans and animals requires the collection of high-quality data, over a suitable time period. In 2015, after a process was undertaken to evaluate its operations, this network launched a major campaign to modernise its analytical tools and tools for the management, interpretation, sharing and communication of information to better meet the needs expressed by the stakeholders and users of this surveillance system. In addition to being tested for their serovar, the *Salmonella* isolated through this network can be characterised for their potential epidemiological link. New typing methods based on genome sequencing offer highly promising prospects in this area.

Keywords

Salmonella, Surveillance, Zoonosis, Serovar, Emergence

Résumé

Le réseau* Salmonella*, un dispositif de surveillance des salmonelles sur la chaîne alimentaire : bilan 2015

Depuis 20 ans, le réseau Salmonella *centralise des résultat sde sérotypage de salmonelles isolées sur la chaîne alimentaire, de manière volontaire, dans toutes les filières et tous les secteurs d'activités. Cette surveillance événementielle complète les contrôles officiels réalisés chaque année. Ce volume massif de données collectées par l'Anses confirme les tendances et les émergences rapportées en niveau européen. Toutes origines confondues, S. Typhimurium et ses variants monophasiques ainsi que S. Enteritidis demeurent majoritairement isolées.* Salmonella *est depuis de nombreuses années un contaminant microbiologique majeur à l'origine d'épidémie d'origine alimentaire en France et en Europe. L'optimisation de l'évaluation et de la gestion du risque de salmonellose chez l'homme et l'animal implique la collecte de données de qualité, dans un pas de temps adapté. À la suite d'un processus d'évaluation de son fonctionnement, ce réseau a entamé en 2015 une action profonde de modernisation de ses outils analytiques mais également de pilotage, d'interprétation, de partage et de communication de l'information pour mieux répondre aux besoins exprimés par l'ensemble des acteurs et utilisateurs de cette surveillance. Au-delà du sérovar, les salmonelles isolées dans le cadre de ce réseau peuvent être caractérisées pour leur potentiel lien épidémiologique. Les nouvelles méthodes de typage basées sur le séquençage du génome offrentdes perspectives très prometteuses dans ce domaine.*

Mots-clés

Salmonella*, surveillance, zoonose, sérovar, émergence*

Salmonella are a microbiological hazard mainly transmitted to humans through food. This hazard has been known and monitored at the local, national and international levels for many years. In 2014, *Salmonella* was in second position, behind *Campylobacter*, in the ranking of bacterial agents isolated in humans in Europe. It is also the main microbiological contaminant causing food-borne outbreaks in which the responsible agent has been confirmed (EFSA-ECDC, 2015). In France, over the 2008-2013 period, the incidence of non-typhoid *Salmonella* was estimated at 307 cases per 100,000 inhabitants (90%CI: 173–611), resulting in 4305 hospitalisations per year on average (Van Cauteren *et al.*, 2015).

The foods most commonly contaminated by *Salmonella* are poultry meat, pork and beef. While table eggs (and egg products) are very seldom contaminated, they still represent the leading cause of *Salmonella* outbreaks in Europe due to their very wide consumption and the risk of consuming these foods raw or undercooked (EFSA-ECDC, 2015). The impact of *Salmonella* on human health and the economic consequences of management measures in the various animal production sectors underline the need to identify and characterise *Salmonella* throughout the food chain, in order to control this pathogen.

The scheme's objectives

The goal of the *Salmonella* network, created in 1997, was to provide scientific and technical support to partner laboratories in charge of detecting this pathogenic bacterium in animal and/or food matrices. The network now covers the entire country. Some partner laboratories were also located abroad. This support involved the phenotypic and even molecular characterisation of isolates with the aim of confirming the serovar and possibly distinguishing between the bacterial strains isolated. This activity generated the massive collection of descriptive data, associated with the sampling context. Given the network's stability, the relevance of monitoring isolation trends for the main serovars became increasingly obvious over time (Lailler *et al.*, 2012).

Today, this network's main objective is to detect the emergence of potentially problematic strains for public health and/or strains with a potential economic impact on animal production sectors. It aims to characterise contamination in animals, their environment, the ecosystem and foods in relation to the *Salmonella* hazard. Strains isolated by partner laboratories are submitted on a voluntary basis.

In this context, the data presented here are the results of serotyping by plate-agglutination tests only, obtained in 2015 by the ANSES

Box.

Objectives
Detection of the emergence of *Salmonella* serovars in a specific sector, monitoring of trends for every serovar isolated in the food chain, scientific and technical support for field laboratories for the characterisation of isolates.

Programming framework
The European regulations (Hygiene package) require *Salmonella* testing all throughout the food chain. Regulations (EC) No 178/2002 and No 2073/2005 (as amended) define the responsibilities of the various stakeholders in this chain and the microbiological safety and hygiene criteria that target, in particular, *Salmonella* in foods. In their most recent Opinions on *Salmonella*, EFSA (2010) and ANSES (2013) recommended the comprehensive serotyping of *Salmonella* isolated in the food chain to provide risk managers and assessors with accurate information.

In Europe, *Salmonella* and *Campylobacter* are considered to be the zoonotic agents responsible for most cases of zoonoses in humans *(Regulation (EC) No 2160/2003)*. To take into account the impact on animal health and health crises, which mobilise considerable financial and human resources, the competent authority defined *Salmonella* as a Category 1 health hazard for the *Gallus gallus* and *Meleagris gallopavo* animal species (Ministerial Order of 29 July 2013).

Protocol
- **Nature of the analysed contaminant:** *Salmonella* spp.
- **Affected products:** animal production and crops, production environments, animal feed, human food, ecosystem.
- **Stage of the food chain:** from farm to fork.
- **Definition of a "case':** isolation of *Salmonella* from a sample collected in the food chain.
- **Number of samples and sampling method:** 3465 *Salmonella* isolated as part of self-inspections, alerts, farm diagnoses and investigations (the total number of samples collected for self-inspections is unknown).
- **Sampling strategy:** random/targeted depending on the surveillance systems involved; data reporting on a voluntary basis.
- **Analytical method, nature of sampling:** potentially every matrix in the food chain. *Salmonella* testing using the methods validated by AFNOR Validation, reference method: NF EN ISO 6579-1 and NF EN ISO 6579/A1 (Annex D). *Salmonella* serotyping by agglutination: *FD CEN ISO/TR 6579-3*.

Laboratory for Food Safety. These results are associated with descriptive metadata regarding samples collected in the field. This report does not include serotyping results obtained by the network's partner laboratories (approximately two-thirds of the data centralised every year). The organisation and means implemented for this network do not currently ensure adequate responsiveness in reporting for the serotyping undertaken by partner laboratories or its integration into the network's database. In collaboration with all its partners, the network is undergoing major changes to improve this. The network is upgrading to allow responsiveness and to acquire more effective tools to meet its new surveillance objectives (see § Analysis of system's strengths and weaknesses).

Summary of operations

Voluntary partner laboratories

The *Salmonella* network is managed and coordinated by the ANSES Laboratory for Food Safety in Maisons-Alfort. The Laboratory for Food Safety is associated with the ANSES Ploufragan-Plouzané Laboratory, which acts as the *Salmonella* National Reference Laboratory (NRL), for the characterisation of *Salmonella*, all industries combined. In the framework of its reference mandate and according to Order 2015-1245 of 7 October 2015, the NRL is in charge of "providing the French State, accredited laboratories, and the platforms mentioned in

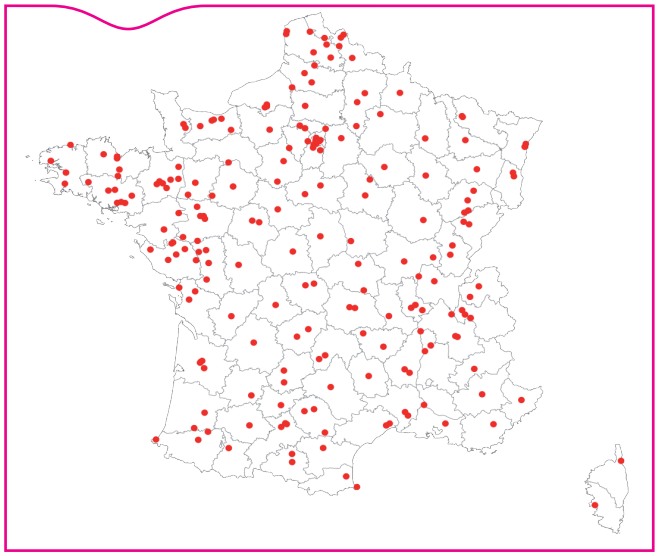

Figure 1. Geographic distribution of the *Salmonella* network's partner laboratories in 2015
Each red dot represents a partner laboratory. Laboratories located in French overseas territories and abroad are not shown on this map.

Table 1. Relative significance of the various sampling contexts associated with the strains received by the Laboratory for Food Safety as part of the *Salmonella* network

Sampling context	Number of strains	Proportion (%)
Product alert	34	1.0
Epidemic/alert	99	2.9
Farm diagnosis	288	8.3
Monitoring (own-checks)	3,039	87.7
Survey	5	0.1
General total	**3,465**	**100.0**

Sampling date between 01/01/2015 and 31/12/2015. Strains received by ANSES between 5/1/2015 and 6/6/2016

Section II of Article L. 201-14 with the scientific and technical support required for the collection, processing, accessibility, transmission and dissemination of epidemiological surveillance data. These laboratories can also provide support to other surveillance programme managers". The *Salmonella* network therefore works closely with the NRL to help it carry out this task and fulfil these requirements. In this context, the network offers a sample surveillance tool under the comprehensive management of ANSES.

The network's partners include both public and private laboratories; most of them are members of the Adliva, Aflabv and Aprolab associations. These three associations represent the following, respectively, in the *Salmonella* network:
- public departmental veterinary analysis laboratories,
- private veterinary biological analysis laboratories involved in primary production in particular,
- private environmental and food hygiene analysis laboratories.

In 2015, 131 partner laboratories sent strains and related data to the network (Figure 1). The number of strains submitted to ANSES by each partner ranged from one to 392. The sampling context associated with these strains mainly (88%) involved own-checks undertaken by professionals to monitor their activities, irrespective of the stage of the food chain (Table 1). Some strains were isolated in the framework of analyses undertaken for diagnostic purposes on farms. Less often, the *Salmonella* received were detected further to an alert, related to the contamination of a finished product, possibly during distribution, or following the occurrence of human cases of salmonellosis. Thus, the strains collected by the network were

isolated from a wide variety of matrices: from both sick animals and healthy carriers, in the farm environment, in slaughterhouses, in processing plants, and in human food and animal feed.

Description of the data collected

The health status of an animal or plant involved in the processing and production of human food must be monitored to prevent pathogens such as *Salmonella* from being transmitted to humans. Partner laboratories therefore test for *Salmonella* using samples collected in all stages of the food chain: from the importing of raw materials for animal feed to food intended for consumers in their homes or in restaurants. In this context, numerous analyses are requested every year involving samples taken on farms, at the slaughterhouse, or in other stages of the food chain as part of surveillance plans and official controls or own-checks by operators.

The serotyping results integrated in the database of the *Salmonella* network are obtained either by partner laboratories or by the ANSES Laboratory for Food Safety (the only data taken into account in this article, considering the main objective related to the detection of unusual signals and emerging contamination). These results are accompanied by epidemiological data that characterise the strain:

- the country, the *département,* and if possible the town where the sample was taken,
- the sampling "site" (holding, processing plant, slaughterhouse, etc.) and date,
- the "sector" (natural ecosystem, animal feed, animal health and production, human food) and any clinical signs observed in animals,
- the "context" (surveillance, diagnosis, epidemic, product alert, etc.),
- the "sampler" (self-inspection, official sampling, etc.),
- the "sample type" (animal feed, human food, environmental sample, animal sample, etc.),
- the nature of the analysed matrix,
- identification numbers for the investigation of situations when necessary (INUAV, DAP, EDE, EGET, Food-borne outbreak no., Guidance note no., etc.).

For every strain received, a form is completed by the laboratories and the collected metadata are entered in the network's ACTEOLab (Application for the centralisation and transfer of data dedicated to the operational epidemiological surveillance of laboratories) database. Serotyping background data provided by partners are systematically verified before being included in the database. These data can be discussed on the telephone with the laboratory shipping the strains to obtain additional information. When serotyping is performed by the Laboratory for Food Safety and the result has been validated by the technical team in charge of coordinating the network, an analysis report is sent to the requesting laboratory. For strains that do not agglutinate, which cannot be serotyped using conventional methods, an alternative method is implemented by the Laboratory for Food Safety, to be able to characterise these strains (Check & Trace *Salmonella* kit by Check-Points).

These data are useful:

- to partner laboratories, which can question the *Salmonella* network's team, for example to identify the main serovar found in a given matrix or environment, or determine trends for a serovar over the years,
- to risk managers, who have information about the presence of non-regulated serovars and the emergence of certain strains to be taken into account in the regulations where appropriate,
- to partners involved in the investigation of food-borne outbreaks or product alerts related to non-compliant products placed on the market. In this case, the network's contribution consists in the transmission of reports enabling (potentially) responsible serovars and/or foods to be targeted,
- for the detection of unusual events in the food chain, through the development of dedicated statistical tools (time-series analysis in particular).

Molecular typing methods (characterisation of Typhimurium variants by PCR, MLVA, PFGE, sequencing) can also be implemented by the laboratory. These methods are able to compare strains with one another and illustrate potential links between strains isolated from various types of samples. Indeed, the probability of two strains deriving from a recent common ancestor is even higher when these strains have similar or even non-distinguishable molecular profiles. In addition to sampling information (sampling context, date and site), these methods are of particular interest for monitoring strains on a holding/in a plant or for investigating food-borne outbreaks.

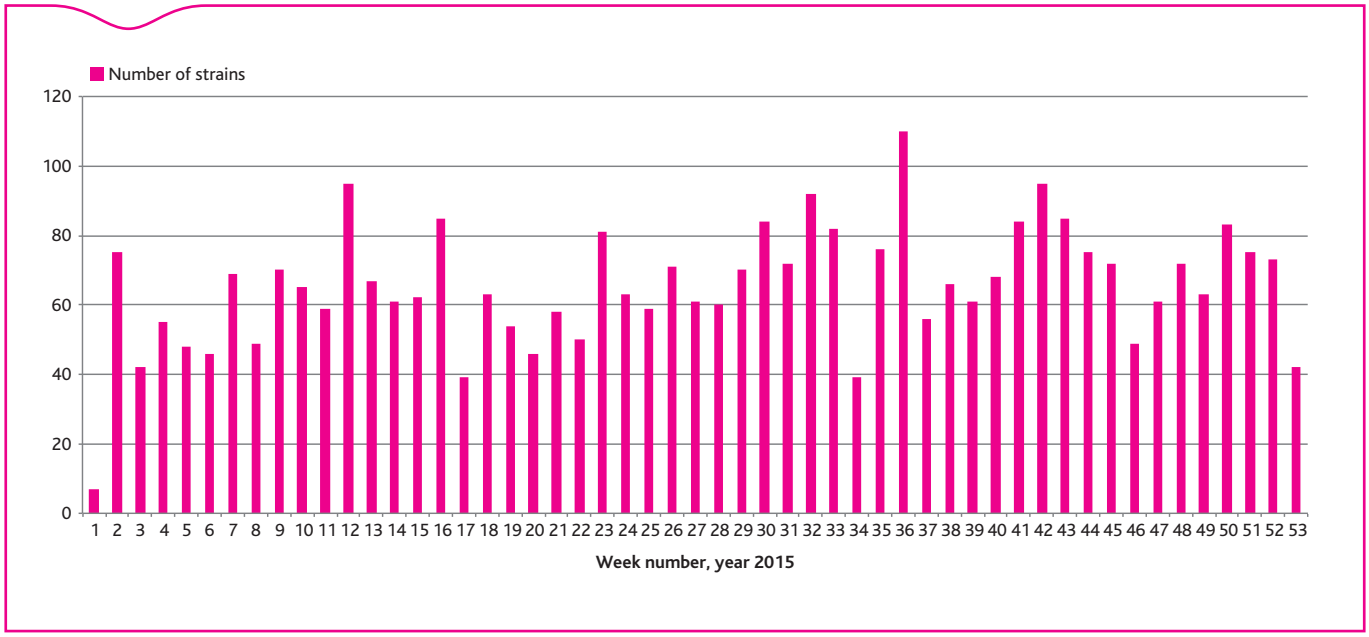

Figure 2. Breakdown of the number of strains submitted to the Laboratory for Food Safety in the framework of the *Salmonella* network, by sampling week (average number of strains isolated and submitted to the Laboratory for Food Safety = 68 strains/week)

Table 2. Main serovars of the strains received by the Laboratory for Food Safety by sector of activity, in the framework of the *Salmonella* network in 2015.

Human food (n=1503)	Animal feed (n=619)	Animal health (n=1236)	Ecosystem (n=107)
S. 1,4,[5],12:i:- (224)	*S.* Livingstone (162)	*S.* Enteritidis (154)	*S.* Veneziana (17)
S. Typhimurium (135)	*S.* Cerro (113)	*S.* Livingstone (71)	*S.* 4,5,12:i:- (10)
S. Enteritidis (131)	*S.* 1,3,19:z27:- (19)	*S.* 1,4,[5],12:i:- (64)	*S.* Enteritidis (9)
S. Derby (111)	*S.* Hadar (19)	*S.* Montevideo (56)	*S.* Typhimurium (7)
S. Bredeney (98)	*S.* Mbandaka (18)	*S.* IIIb 61:k:1,5,7 (55)	*S.* Albany (6)
S. IIIb 61:k:1,5,7 (93)	*S.* Anatum (16)	*S.* Mbandaka (45)	*S.* Newport (5)
S. Dublin (66)	*S.* Havana (13)	*S.* Kottbus (42)	*S.* Bovismorbificans (4)
S. Montevideo (49)	*S.* Tennessee (13)	*S.* IIIa 48:z4,z23:- (38)	*S.* Livingstone (4)
S. Mbandaka (41)	*S.* Agona (12)	*S.* Lille (35)	*S.* London (4)
S. Infantis (38)	*S.* Newport (12)	*S.* Typhimurium (35)	*S.* Napoli (4)
S. Kentucky (28)	*S.* Indiana (11)	*S.* Llandoff (33)	*S.* Weltevreden (3)
S. Livingstone (27)	*S.* Infantis (11)	*S.* Tennessee (28)	*S.* Agona (2)
S. Newport (27)	*S.* Llandoff (10)	*S.* Give (25)	*S.* Ajiobo (2)
S. Anatum (26)	*S.* Montevideo (10)	*S.* Newport (25)	*S.* Durban (2)
S. Rissen (25)	*S.* Typhimurium (10)	*S.* Veneziana (21)	*S.* Infantis (2)
S. Kedougou (25)	*S.* 1,4,[5],12:i:- (10)	*S.* Dublin (20)	*S.* IIIb 38:r:z (2)

Results obtained

In 2015, the Laboratory for Food Safety serotyped 3465 strains. On average, 68 strains were received per week by the Laboratory for Food Safety, for confirmation of the serovar (Figure 2).

Breakdown of the isolates received by the Laboratory for Food Safety by sector and matrix type

The inventoried strains were broken down as follows by original sector of activity: 1503 strains (43.4%) in human food, 1236 strains (35.7%) in animal health and production, 619 strains (17.8%) in animal feed, and 107 strains (3.1%) from the natural ecosystem (Table 2).

Human food

The strains collected in this sector primarily came from the "meat products" category (815 strains, i.e. 54.2%) and the "dairy products" category (545 strains, i.e. 36.3%). Other product categories (eggs and egg products, fruits and vegetables, seafood products) each accounted for less than 2% of isolates.

Pork (302 strains), chicken (166 strains) and turkey (96 strains) meat accounted for 69.3% of the meat products for which *Salmonella* was isolated by the Laboratory for Food Safety. Isolates from sheep, cattle and duck meat accounted for 8.2%, 8.0% and 2.3% respectively. Other meat (deer, horse, goat, wild boar, goose, game, rabbit, etc.) accounted for 11.6% of the isolates from meat products received by the Laboratory for Food Safety.

Milk and cheese from cattle (114 and 189 strains) and sheep (38 and 51 strains) were the two main sources of contamination for isolates from dairy products. They accounted for 71.9% of dairy products for which *Salmonella* was isolated by the Laboratory for Food Safety.

Animal health and production

The strains in this sector serotyped by the Laboratory for Food Safety were primarily isolated from the *Gallus gallus* species (546 strains, i.e. 44.2%), cattle (342 strains, i.e. 27.7%) and ducks (111 strains, i.e. 9.0%). Of the 546 strains isolated from *Gallus gallus*, 140 (25.6%) were isolated from laying hens and 385 (62.6%) from broiler chickens.

Animal feed

The strains in this sector serotyped by the Laboratory for Food Safety were primarily isolated from pet food (379 strains, i.e. 61.2%). For 84 of the 619 isolates processed by the Laboratory for Food Safety (13.6%), precise information was not available; they were noted as "all animal feed'. They were followed by compound feed for poultry (43 strains, i.e. 6.9%). The Laboratory for Food Safety also serotyped 43 strains (6.9%) from seed and fruit oils (soy, rapeseed, sunflower, etc.), 35 strains (5.6%) from raw materials of animal origin, and 13 strains (2.1%) from raw materials of cereal origin (barley, maize, wheat, etc.). The other strains were divided up between various other categories.

Ecosystem

The strains in this sector serotyped by the Laboratory for Food Safety were primarily isolated from water sources/catchments (54 strains, i.e. 50.5%) and water treatment plants (33 strains, i.e. 30.8%). Strains isolated from water distribution systems accounted for 4.7% (five strains), and eleven strains (10.3%) were identified as "other activities'.

Main serovars identified by the Laboratory for Food Safety

Of the strains received by the Laboratory for Food Safety in 2015, 42 were strains that do not agglutinate (rough serovar).

Human food

> "Meat" category

- Pork (n=302): the strains collected in this category belonged to 26 serovars. The three main serovars – the monophasic variants of Typhimurium (*S.* 1,4,[5],12:i:-) (43.7%), *S.* Typhimurium (17.9%) and *S.* Derby (17.9%) – accounted for 79.5% of the strains in this meat category.
- Chicken meat (n=166): *S.* Derby (14.5%), *S.* Infantis (13.7%) and *S.* Kentucky (13.3%) were the main isolated serovars out of 31 detected.
- Turkey meat (n=96): the three main serovars – *S.* Bredeney (31.3%), *S.* 1,4,[5],12:i:- (24.0%) and *S.* Brandenburg (14.6%) – accounted for 69.9% of the strains in this meat category. Fourteen serovars were found in total.
- Mutton (n=67): of the 11 serovars isolated in this meat category, the *S.* IIIb 61:k:1,5,7 serovar was the only major serovar found (64.2%).

> "Milk and dairy products" category

The main serovars isolated from cow's milk (n=114) were *S.* Montevideo (26.3%), *S.* Mbandaka (21.1%), *S.* Dublin (17.3%) and *S.* Enteritidis (14.0%). In total, 16 serovars were found. For cheese made from cow's milk (n=189), *S.* Enteritidis (31.2%), *S.* Dublin (21.7%), *S.* Typhimurium (14.8%) and the monophasic variants of Typhimurium (*S.* 1,4,[5],12:i:-) (9.5%) were the four main isolated serovars out of the 21 detected in this type of product.

Sheep's milk (n=38) was also occasionally a source of contamination. Over half of the isolated strains belonged to the *S.* IIIb 61:k:1,5,7 serovar (55.3%). Ten serovars were found in total. For cheese made from sheep's milk (n=51), the two main serovars found were *S.* IIIb 61:k:1,5,7 (27.4%) and *S.* IIIb 50:i:z (21.6%). For other dairy products, all types combined, *S.* Bredeney (40.5%) was the main isolated serovar of the 29 detected.

> "Eggs and egg products" category

The most commonly found serovar was *S.* Livingstone (35.7%) but very few of these matrices were processed by the Laboratory for Food Safety (n=28). In total, seven serovars were detected.

> "Seafood products" category

For seafood products (crustaceans and molluscs, n=11), nine different serovars were identified; there was therefore no major serovar.

> "Fruits and vegetables" category

The three most commonly isolated serovars were *S.* Typhimurium (21.4%), the monophasic variants of Typhimurium (*S.* 1,4,[5]:12:i:-) (14.3%) and *S.* Anatum (14.3%). In total, fourteen strains were isolated belonging to 10 different serovars.

Animal health and production

"Cattle" sector (n=342): the strains collected in the cattle sector were mainly isolated in samples from sick animals and their farm environment and belonged to 27 serovars, the main ones being *S.* Enteritidis (32.7%), *S.* Montevideo (13.5%) and *S.* Mbandaka (10.8%).

"Broiler chicken" sector (n=385): *S.* Livingstone (18.1%) and *S.* Lille (10.2%) were the two main isolated strains. It is interesting to note the wide variety of serovars (72 different serovars) detected representing these 385 strains serotyped by the Laboratory for Food Safety.

"Laying hen" sector (n=145): the most commonly isolated serovars were *S.* Enteritidis (11.4%), *S.* Havana (9.3%) and *S.* Banana (8.6%); 37 different serovars were found in total.

"Duck" sector (n=111): out of 30 identified serovars, the three main serovars isolated were *S.* Give (19.8%), *S.* Kentucky (8.1%) and *S.* 6,7:-:- (8.1%).

Animal feed

In this sector, pet food was the sampling category with the largest number of strains serotyped by the Laboratory for Food Safety (n=379). The *Salmonella* most frequently isolated in this sector belonged to the *S.* Livingstone (37.5%) and *S.* Cerro (28.0%) serovars, out of a total of 31 identified serovars.

Ecosystem

S. Veneziana (27.8%) was the main identified serovar out of the 25 detected for strains collected from water sources and catchments (n=54). In samples collected from water treatment plants (n=33), *S.* Albany was the most commonly isolated serovar (42.9%).

Analysis of the system's strengths and weaknesses

The systematic serotyping of isolated strains is recommended by EFSA (2010) to enhance surveillance in the various stages of the food chain or to refine the messages provided as part of the Rapid Alert System for Food and Feed concerning the *Salmonella* hazard. Agglutination serotyping is the official typing method for *Salmonella*.

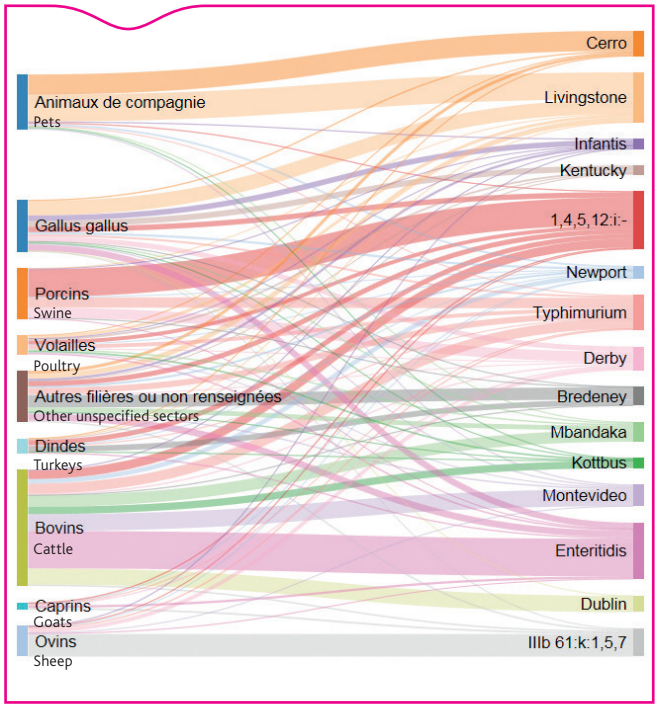

Figure 3. Distribution of the top 15 *Salmonella* serovars (n=2100, 61%) identified from the strains sent to the Laboratory for Food Safety in the framework of the *Salmonella* network in 2015, by sampling production sector. (Sankey diagrams illustrate the relative proportion of each isolated serovar in the various production sectors)

For uncommon or less common serovars, the isolation and identification of such strains provide valuable data for establishing a high likelihood of a relationship between strains. However, this traditional method is less relevant for the most abundant serovars found in separate sectors (monophasic variants of Typhimurium, Typhimurium, Enteritidis, Newport, Livingstone, Derby, etc.). It would be extremely beneficial to sequence the whole genome, for all or some of these serovars, to demonstrate the advantages of improving strain discrimination in preventive epidemiology. The *Salmonella* network is planning to undertake this study in 2017.

The characterisation of certain strains can be improved in response to an alert in a company or the investigation of a food-borne outbreak in order to assess the relationship between strains isolated from humans and those of non-human origin. This comparison of molecular profiles requires sound knowledge of the various strains circulating in the field that belong to the serovar in question. The network's extensive collection of strains provides access to a wide variety of isolation origins (in terms of location, time period, matrix and context) and can help confirm or refute assumptions involving epidemiological links between strains.

Data quality is ensured through the maintenance of expertise by the staff of the Laboratory for Food Safety and the network's member laboratories. Training activities on serotyping for the technicians in these laboratories are organised several times a year, but the audience remains limited (two or three people per session). In addition, every year, the Laboratory for Food Safety organises an Inter-Laboratory Proficiency Test (ILPT) in which over half of the network's partner laboratories participate and achieve satisfactory results. The aim is to assess their capacity to undertake, at the very least, the serotyping of regulated *Salmonella*. The Laboratory for Food Safety also participates in two ILPTs organised at the international level by the EU Reference Laboratory for *Salmonella* and by the World Health Organization.

As in previous years, the comparison of the annual reports prepared by the National Reference Centre (NRC) for *Salmonella* and the *Salmonella* network highlights similarities between the main

serovars isolated in the human food sector and those isolated from humans: the emergence of the monophasic variants of Typhimurium (*S*. 1,4,[5],12:i:-) since the early 2000s and the preponderance of *S*. Typhimurium and *S*. Enteritidis since the 1990s. These same serovars were the "top-3" *Salmonella* serovars identified in 2014 in Europe in these same sectors (EFSA–ECDC, 2015). More recently, based on the surveillance data collected by the *Salmonella* network and the NRC, the Kentucky serovar was provisionally included on the list of Category 1 health hazards, by Ministerial Order[1], to combat the establishment of multi-drug resistant strains during primary production in the regulated *Gallus gallus* and *Meleagris gallopavo* sectors.

The *Salmonella* network also collects data from the animal feed sector, primarily related to strains isolated from pet food, which is a potential source of human contamination by direct contact. The goal is therefore to reduce this route of animal contamination and monitor the carriage of *Salmonella* by pets, some of which are more exotic (reptiles, snakes, etc.) and are known to sometimes host several serovars without showing signs.

The scope of surveillance covered by this network is therefore very broad. However, it has some weaknesses, described below, which need to be corrected to improve its operations. It is reasonable to assume that first-line laboratories more easily determine *Salmonella* serovars they encounter on a regular basis or for which regulatory requirements are set and ILPTs are organised.

Moreover, the external assessment of the network, undertaken in 2015 using the "Oasis flash" method (Hendrikx *et al.,* 2011), underlined the lack of information regarding the representativeness of the data collected in relation to all of the *Salmonella* isolated throughout France. This surveillance system does not take into account the total number of analyses undertaken but considers only isolated strains voluntarily submitted by partners for serotyping. However, the prevalence of *Salmonella* in at-risk matrices could be estimated by enhancing the centralisation of analytical results, including negative results obtained in France.

Deadlines for the reporting of serotyping data by the network's partners to the system's central unit as well as deadlines for integration into the database must be compatible with the level of responsiveness expected by the network's users.

Furthermore, some serovars that are not commonly isolated, as well as others whose antigenic formula requires the use of uncommon sera, are probably overrepresented among the strains received by the Laboratory for Food Safety for serovar confirmation. More generally, even if laboratories are competent to perform this serotyping, a non-negligible proportion of strains isolated mainly on poultry farms (a regulated sector) are sent to the Laboratory for Food Safety, associated with the NRL, to confirm the result (for the purpose of an audit argument or to restore a client's confidence).

The network therefore needs to strengthen its actions for the development of tools facilitating the use of data and real-time communication between partners before it sets more binding reporting targets.

To be more effective, the wealth of data collected by the *Salmonella* network should be processed in near real-time in order to provide risk managers with information allowing them to anticipate the potential occurrence of human cases and plan official controls. This development is eagerly awaited, since the database is needed by the French Public Health Agency (SPF) to facilitate epidemiological investigations in the context of health alerts, which would ideally require recent analytical results, involving samples collected in a time window compatible with the timeline of cases. For this to happen, the characteristics of the suspected food matrix must also be considered: the product's shelf life, the complexity of the product's production and distribution process, etc.

1. Ministerial Order of 17 February 2015 amending Ministerial Order of 29 July 2013 on the definition of Category 1 and 2 health hazards for animal species.

The network is coordinated by a multidisciplinary team made up primarily of microbiologists and epidemiologists. Its coordinating team collaborates with the Agency's other entities to develop computing tools (database, algorithms, applications in the R-Shiny environment, etc.). Through its new tools, the network is diversifying its support for its partners and thus indirectly for professionals in the various sectors of the agri-food industry and risk assessors. Query tools are available for example to determine the nature of the most contaminated matrices for a given serovar. This information is extremely useful for guiding professionals in the management of a contamination situation. This feature, currently available only to the network's coordinating team, will soon be offered to the network's partners in return for their active participation in the health surveillance of *Salmonella* in the food chain.

This *Salmonella* surveillance system is thus undergoing major changes. Internal discussions are currently being held at ANSES on whether the means allocated to this system can meet the surveillance objectives currently set in France, in relation to the *Salmonella* hazard. The network's new operating procedures will be clarified by the end of 2016, after approval by the steering committee. The roles of all of the system's stakeholders will be specified. Through these efforts, the network is expected to strike a better balance between the acquired data (benchmarks, estimated representativeness of certain industries, etc.) and the expectations of end users (risk assessors and managers, agri-food professionals) of the information produced by the surveillance system.

Acknowledgements

We would like to thank all of the *Salmonella* network's partner laboratories for their voluntary participation in this surveillance system. The network's coordinating team relies on a group of scientists, technicians and administrative staff that goes far beyond the simple list of co-authors for this article. We are extremely grateful to these people for their valuable contribution.

Glossary

ADILVA: French Association of Directors and Executives of Public Veterinary Analysis Laboratories

AFLABV: French Association of Veterinary Biological Analysis Laboratories

Aprolab: Professional Association of French Analytical Laboratories

NRC: National Reference Centre

DAP: Support document for samples

EDE: Identification number for cattle farms

EGET: Identification number for fattening pig plants

ILPT: Inter-Laboratory Proficiency Test

NRL: National Reference Laboratory

EURL: European Union Reference Laboratory

MLVA: Multi-Locus VNTR Analysis

WHO: World Health Organization

PCR: Polymerase Chain Reaction

PFGE: Pulse Field Gel Electrophoresis

FCS: Food Chain Surveillance

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A shared molecular database for the surveillance of *Listeria monocytogenes* in the food chain in France

Benjamin Felix (1) (benjamin.felix@anses.fr), Damien Michelon (1), Bertrand Lombard (1), David Albert (1), Léna Barre (1), Carole Feurer (2), Sophie Roussel (1)

(1) University of Paris-Est, ANSES, Laboratory for Food Safety, *Salmonella-E. coli-Listeria* (SEL) Unit, Maisons-Alfort, France
(2) IFIP, French Pork and Pig Institute, Maisons-Alfort, France

Abstract

Listeria monocytogenes (*Lm*) is a ubiquitous bacterium responsible for a rare but serious infection: listeriosis. Transmitted through the consumption of contaminated food, listeriosis is fatal in 20% to 30% of cases. It mainly affects people with a weakened immune system. Therefore, the surveillance of strains isolated from the food chain and the environment is essential. An effective food chain surveillance system requires the centralisation of high-quality data and the production of useful and accessible information. ANSES, under its mandates as National Reference Laboratory (NRL) and European Union Reference Laboratory (EURL) for Lm, provides scientific and technical support prior to this data collection. In particular, it harmonises typing methods for strains isolated from the food chain, and organises training and inter-laboratory proficiency tests for laboratories in the French and European networks. In France, as part of the ARMADA Joint Technological Unit (UMT), ANSES and the French Pork and Pig Institute (IFIP) have been working for four years on the development of a national database for the centralisation and sharing of epidemiological and genetic data on the strains held by the two organisations. Over time, it will be shared with four other French technical institutes and the ANSES laboratories involved in Lm surveillance. This database is interconnected with the European database system developed by the EURL and the European Food Safety Authority, which makes it possible to report data collected nationwide at European level. The database of the ARMADA UMT currently contains 1,200 strains typed by PFGE, sharing 256 combined ApaI/AscI profiles. This tool is enhancing the surveillance of strains circulating in the various food sectors in France.

Keywords

Database, PFGE, *Listeria monocytogenes*, Molecular surveillance

Résumé

Une base de données moléculaires partagée pour améliorer la surveillance de* Listeria monocytogenes *dans la chaîne alimentaire en France

Listeria monocytogenes *(*Lm*) est une bactérie ubiquitaire responsable d'une infection rare mais grave: la listériose. Transmise par la consommation d'aliments contaminés, la listériose s'avère mortelle dans 20 à 30 % des cas. Elle touche principalement les personnes immunitairement affaiblies. De ce fait, la surveillance des souches isolées de la chaîne alimentaire et de l'environnement de production est essentielle. Un dispositif efficace de surveillance sanitaire de la chaîne alimentaire nécessite la centralisation de données de qualité et la production d'informations utiles et accessibles. L'Anses, au titre de ses mandats de Laboratoire de référence national (LNR) et de l'Union européenne (LRUE) pour Lm, fournit un appui scientifique et technique en amont de cette collecte de données. Elle assure notamment l'harmonisation des méthodes de typage des souches isolées de la chaîne alimentaire, l'organisation de formations et d'essai inter-laboratoires d'aptitude pour les laboratoires des réseaux français et européen. En France, dans le cadre de l'unité mixte technologique (UMT) Armada, l'Anses et l'Institut du Porc (Ifip) ont travaillé depuis quatre ans au développement d'une base de données nationale pour la centralisation et le partage des données épidémiologiques et génétiques des souches détenues par les deux organismes. A terme, elle sera partagée avec quatre autres instituts techniques français ainsi que les laboratoires de l'Anses impliqués dans la surveillance de Lm. Cette base de données est interconnectée avec le système de base de données européen mis en place par le LRUE et l'Autorité européenne de sécurité des aliments et permet la remontée au niveau européen des données collectées au niveau national. La base de l'UMT Armada contient actuellement 1200 souches typées par PFGE, partageant 256 profils combinés ApaI/AscI. Cet outil permet une surveillance plus fine des souches circulant en France dans les différentes filières alimentaires.*

Mots-clés

Base de données, PFGE,* Listeria monocytogenes*, surveillance moléculaire

This article describes the method of operation and features of a tool for collecting molecular typing data, used to:

- improve our knowledge of the structure of *Listeria monocytogenes* (*Lm*) populations circulating in France,
- monitor this pathogen nationwide.

Listeria and listeriosis: reminders

Lm is an environmental bacterium responsible for listeriosis. This infection is characterised by i) the severity of its symptoms, ii) the high disease mortality rate, ranging from 20% to 30% of cases, and iii) a preference for immune-deficient subjects, pregnant women, and their children (Tourdjman *et al.*, 2014). Listeriosis is contracted through the consumption of contaminated foods. Foods can be contaminated by a raw material of animal or plant origin, or by the food processing and distribution environment ("resident" bacteria). *Lm* can persist in

products all along the food chain, multiplying at refrigeration temperatures, resisting cleaning and disinfection procedures, and contaminating food processing plants. The principal food sectors are monitored, in particular the pork and pig sector, which has been affected by several health crises involving *Lm* (Giovannacci *et al.*, 1999; Hong *et al.*, 2007).

The French regulations require the withdrawal from the market of foods contaminated at concentrations above 100 CFU/g as well as foods contaminated at lower concentrations but enabling the growth of *Listeria* to values of above 100 CFU/g at the end of their shelf life. The high number of sporadic cases in France (Tourdjman *et al.*, 2014) provides grounds for improving knowledge of circulating strains and their reservoirs.

Pulsed field gel electrophoresis (PFGE) remains the reference method in France and internationally for surveillance of clinical and food-borne strains of *Lm* (Tourdjman *et al.,* 2014).

Methods based on whole genome sequencing (WGS) are expected to replace PFGE in the very near future. However, WGS typing is not yet routinely used by all of the laboratories involved in the surveillance of this bacterium.

The *Lm* species is divided into four phylogenetic lineages and 13 separate serotypes. Two of these lineages and four of these serotypes are primarily associated with human listeriosis: 4b, 1/2b (lineage I), 1/2a and 1/2c (lineage II). The genetic diversity of the species has been widely studied over the past few years, in particular by the research group of Institut Pasteur (NRC and WHO CC for *Listeria*) using the MLST (Multi-Locus Sequence Typing) technique. (Ragon *et al.*, 2008). This can characterise strains based on their "sequence type" (ST) obtained from the sequencing of seven housekeeping genes. STs can be grouped into clonal complexes (CCs). A CC is a group of STs with at least six alleles in common (Ragon *et al.,* 2008). These MLST data provided the foundation for a French and international reference nomenclature. They are now essential for analysing the structure of populations and for exchanging surveillance information. Certain clonal complexes (CC1, CC2, CC4 and CC6) are commonly found in cases of human listeriosis in France and around the world (Ragon *et al.*, 2008; Chenal-Francisque *et al.*, 2011). The strains in these clonal complexes were recently recognised as being hyper-virulent, with the particular capacity to attack the brain and foetus, whereas the strains of other CCs such as CC9 and CC121 have very little or no virulence (Maury *et al.*, 2016).

Under their mandates, the European Union Reference Laboratory (EURL) and the National Reference Laboratory (NRL)(1)1 for *Lm* have built an exceptional collection, by virtue of its size (more than 10,000 strains including 3000 that have been typed by PFGE) and the diversity of the field strains it contains (from a variety of food sectors, isolated for more than 20 years). These strains were received as part of self-inspections, French and European surveillance programmes (Roussel *et al.,* 2012; Roussel *et al.,* 2014), and research projects undertaken jointly with INRA, technical centres, and European NRLs. Part of the collection has been characterised genotypically, by serotyping and PFGE (Félix *et al.*, 2012a,b, 2013; Michelon *et al.,* 2014; Roussel *et al.,* 2014). Some strains have also been characterised phenotypically (antimicrobial resistance, ability of strains to survive in extreme conditions, ability to form biofilms, virulence).

Information from these collections has been structured in shared molecular databases, in collaboration with all the partners. In 2012, as part of the activities of the EURL for *Lm*, a database shared with the European NRLs, the "EURL for *Lm* DB', was created. The various stages of this tool's development, as well as its operation, are described in detail in two articles published in 2014 (Félix *et al.*, 2014 and 2015). The same approach was used to set up a French database during a five-year project undertaken in close collaboration with the French Pork and Pig Institute (IFIP), as part of the Armada Joint Technological Unit (UMT). This database is shared by the UMT's various stakeholders that will use the tool: French agro-industrial technical institutes (ITAIs) (IFIP, Aérial, Actalia La Roche-sur-Foron and Adria Développement) and three ANSES laboratories (Ploufragan-Plouzané Laboratory, the Boulogne-sur-Mer and Maisons-Alfort sites of the Laboratory for Food Safety). The objective of the tool is to centralise the main molecular profiles of *Lm* circulating in France in the various food sectors.

In this article, we describe the database of the Armada UMT; in particular, we explain how it works and the data it contains. Lastly, we illustrate potential uses of this database by providing a few examples.

1. Scientific and technical activities managed by the *Listeria* team of the SEL (*Salmonella-E. coli-Listeria*) Unit of the Laboratory for Food Safety, Maisons-Alfort site, ANSES.

Box.

Objectives
The database of the Armada UMT enables agro-industrial technical institutes (ITAIs) and agri-food professionals to pool, with ANSES, molecular profiles of *Listeria monocytogenes* (*Lm)* collected in France in order to track and detect sources of contamination in production chains. In this sense, the database will be used as a supporting tool for the definition of actions to prevent contamination. It will be easy to share the submitted profiles and validate their quality because of the validation criteria applied to the data submitted. This will ensure the immediate availability of typing data as needed.

Regulatory requirement
Molecular profiles of strains and related epidemiological information are submitted to the database of the Armada UMT on a voluntary basis. The NRL submits molecular profiles of strains resulting from the control and surveillance plans implemented by the monitoring authorities as described by Roussel *et al.* (2012). ITAIs submit profiles for strains in their collections, most of which have been isolated during specific studies.

Protocol
The Armada UMT's database centralises the PFGE molecular profiles of *Lm* strains and related epidemiological information. It provides users with access to data while ensuring the anonymity of the strains' origins and geographic regions of isolation as described in Felix *et al.* (2014; 2015; 2016). The data can be consulted, for example, to compare a molecular profile with those in the database. Submitted data are validated at the European level and made available at the national level *via* a cascading synchronisation system between the database of the Armada UMT and those of its users.

The Armada UMT's database currently contains the strain typing data from IFIP and ANSES and will be available to the ANSES Ploufragan-Plouzané Laboratory, the Boulogne-sur-Mer and Maisons-Alfort sites of the Laboratory for Food Safety, and four ITAIs (IFIP, Aérial, Actalia La Roche-sur-Foron and ADRIA Développement).

Definition of a "case'
The database contains molecular profiles and epidemiological information related to *Lm* strains isolated from samples collected from animals (asymptomatic carriage or sick animals), food or the food production environment.

Materials and methods

Harmonised typing methods between partners

The activities undertaken by the EURL over the past few years have made a major contribution to enhancing typing capacities in the NRL network, thanks to ongoing training, theory and practical courses, annual meetings, and Inter-Laboratory Proficiency Tests (ILPTs) (Félix *et al.,* 2012; Félix *et al.,* 2013). This experience has contributed to the nationwide organisation, by the French NRL, of training sessions on PFGE and PFGE profile interpretation, for the various partners of the Armada UMT. In addition, the two ILPTs organised by IFIP, in the framework of the UMT, provided validation of the typing capacities of the UMT's stakeholders and helped improve the quality of the profiles obtained following the corrective actions taken.

A technical platform organised and administered by ANSES

Data are exchanged between the various users *via* a web server (BN Server Web Edition, version 7, Applied Maths, Sint-Martens-Latem, Belgium). This enables several database networks to be managed simultaneously. It currently manages the EURL *Lm* DB and the Armada UMT's database.

ANSES is the administrator of these databases and is responsible for validating the PFGE profiles submitted to the

EURL *Lm* DB. ANSES is also in charge of the storage and continuity of the submitted data.

Who can submit profiles to the database?

Molecular profiles are submitted to the Armada UMT's database on a voluntary basis. Users must first have been i) trained in PFGE according to standardised protocols (Roussel *et al.,* 2014) and ii) assessed for the achievement of satisfactory results when participating in the ILPTs organised by IFIP every other year. In addition, IFIP and ANSES have established a charter of use for the database, whose users are the signatories. This charter defines the conditions under which users can populate the database and ANSES can make its data available. It also specifies data ownership and confidentiality.

Four ITAIs (IFIP, Aérial, Actalia La Roche-sur-Foron and Adria Développement) and two ANSES laboratories (Ploufragan-Plouzané Laboratory, the Boulogne-sur-Mer and Maisons-Alfort sites of the Laboratory for Food Safety) can currently use the Armada UMT's database.

The database is monitored by a steering committee made up of the founding members of the Armada UMT (ANSES, IFIP, Actalia La Roche-sur-Foron) and the database's administrators.

Two key points: management of sensitive data and nomenclature

BN Server randomly generates a registration number for each strain when data are submitted (a unique identification code containing 33 alphabetic characters); this number serves as an ID in the database. Strains are identified by two other fields: the first contains the identity of the user submitting the data, in the form of a numerical code, and the second is the strain number initially assigned by the user. To ensure the anonymity of the user providing the data, other users do not have access to the identifying numerical code or the initial strain number. Similarly, geographic data can be submitted but are not visible to other users. The pulsotype nomenclature has been established according to the PulseNet USA pulsotype format (Gerner-Smidt *et al.,* 2006) identified with the "EU" tag. For example, for a "GX6A16.0001.EU" *Asc*I profile, "GX6" means *Lm*, "A16" refers to the *Asc*I restriction enzyme, "0001" is the pulsotype number, and "EU" is the European tag. Each pulsotype is associated with information about its occurrence in the entire database (ratio of the number of strains belonging to the same *Asc*I + *Apa*I pulsotypes to the total database population).

An epidemiological classification in agreement with the European Food Safety Authority (EFSA)

Related epidemiological data are recorded according to a detailed classification (Figure 1) containing several consecutive fields associated with lists of choices predefined in the software. The structure of this epidemiological classification is based on all the data required by EFSA's epidemiological reporting system (EFSA, 2012). However, to simplify its use, the epidemiological data contained in the Armada UMT's database have been limited to the classification of foods generally used for the assessment of risks related to *Lm*.

A system for the automatic conversion of epidemiological data, based on the sample descriptors used at the European level by EFSA (FoodEx2) (EFSA, 2015), has been developed in close collaboration with EFSA. This system automatically generates a code and a standard description based on the descriptions in

the Armada UMT's database (e.g. A0EYM#F01.A057F Charcuterie meat products, SOURCE= Pig (live animals) corresponds to...). This system has been designed to evolve if new terms are added to the epidemiological classification of the Armada UMT's database. It anticipates the database's connection to the future database being set up by EFSA and ECDC, whose pilot was launched in 2016 (Figure 1) (EFSA, 2014).

Interconnection of database systems and validation of typing data

Molecular profiles of strains are submitted by the Armada UMT's members. The profiles are then sent to a European database (EURL *Lm* DB until 2017 and then the EFSA database (Box)). This system enables available molecular profiles to be grouped at the national level and then submitted at the European level (Figure 2). PFGE profiles are validated at the European level, together with the profiles submitted by other NRLs. A synchronisation system enables typing data to be returned after validation (modified molecular profiles, assessor comments and nomenclature). All of the changes made by the operator in charge of validating and integrating profiles in the database are tracked and can be downloaded by users for their own profiles. Thus, the Armada UMT's database regularly uploads data for validated profiles that have been submitted at the European level. Profiles appearing in the databases of national users can also be synchronised when they have been submitted to the Armada UMT's database (Figure 2).

What is a curator?

The operator responsible for validating each new molecular profile in a typing database is referred to as a "curator'. The curator can directly modify processing parameters for gel images and the marking of bands on profiles. Each profile is analysed and identified according to an innovative protocol for the interpretation of PFGE profiles developed by the *Lm* EURL (Felix *et al.*, 2012) and used and enhanced by EFSA (Roussel *et al.*, 2014). Curation occurs through an interpretation system divided into identification groups. The curator's technical competences, for the interpretation of PFGE gels, are regularly updated and verified as part of an internal evaluation process that determines suitability for the position of curator. After processing, the curator rates the profiles as follows: "confirmed" or "unsatisfactory'. In the current system, the interconnection of databases provides for centralised curation in the EURL *Lm* DB. This system will be transposed to the EFSA database in 2017.

Unlimited consultation of the database

All of the PFGE profiles available in the Armada UMT's database can be compared to the profiles appearing in user databases. For a given PFGE profile, users have access to the following information: 1) serotype, 2) food matrix, 3) sampling date, and 4) frequency at which the profile appears in the Armada UMT's database.

The database also contains a sub-set of 167 strains that have been typed by both PFGE and by Multi-Locus Sequence Typing (MLST). MLST data are available in two dedicated fields corresponding to the CC and ST.

In a study recently published by ANSES and DTU Food (Henri *et al.*, 2016), we achieved good congruence (ability of a method to predict the result of another method), for a panel of 396 strains, between the PFGE groups with 80% similarity (group established using the UPGMA dendogram construction method based on the average similarity of the *Asc*I and *Apa*I restriction profiles, Dice coefficient, tolerance and optimisation set at 1%) and the STs.

This sub-set may be used as a dictionary that gives to the users an indication of the CC and ST for some of their strains and enables them to link their PFGE results to MLST data.

Results

Key figures: current content of the Armada UMT's database

The Armada UMT's database contains the combined PFGE profiles of 1602 strains generated with the *Asc*I and *Apa*I restriction enzymes. Of these profiles, 1136 have been submitted at the European level, for validation of their quality and linking to a pulsotype number. The other profiles are undergoing validation. Of the profiles already validated, the strains have been divided up by food origin: meat products (524 strains including 241 isolated from pork products), dairy products (189 strains), fishery products (179 strains), composite

products (elaborated products combining at least two products of different food origins - 213 strains), plants (59 strains), and non-food animal and environmental samples (45 strains).

The validated profiles include 93 submitted by IFIP, 1030 submitted by ANSES, and three submitted by other users. The profiles of these strains have been subdivided into PFGE groups with 80% similarity. The 14 main PFGE groups account for 86% of the submitted profiles. They are all observed in the four main food origins. These groups have been associated with clonal complexes (Table 1).

Discussions

Example of a practical use of the Armada UMT's database

Understanding the diversity of strains in a given sector

The Armada UMT has strengthened relations between IFIP, the Laboratory for Food Safety (LSA), and the ANSES Ploufragan-Plouzané Laboratory. These three entities recently pooled their knowledge and know-how in order to better assess the diversity of *Lm*

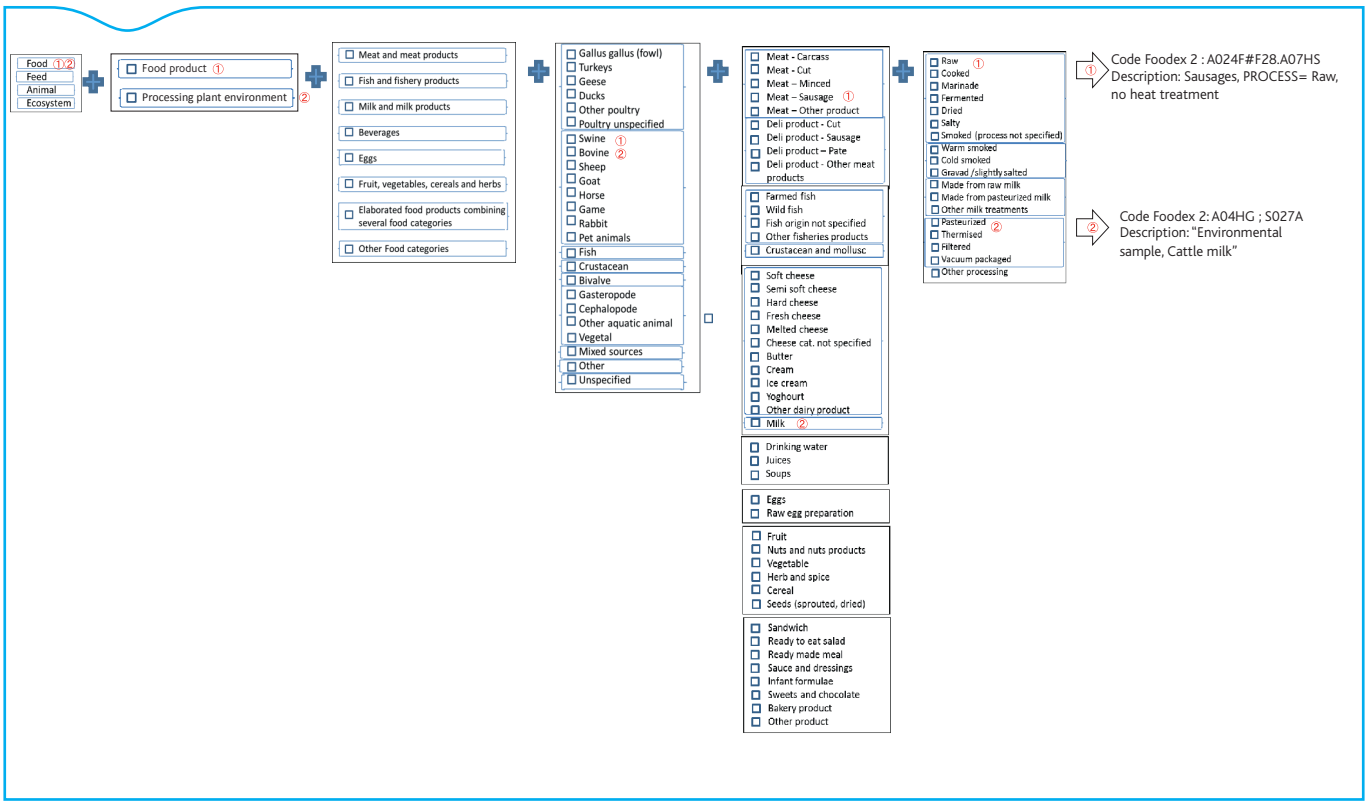

Figure 1. Standard food description used in the Armada UMT's database and automatic correspondence with the FoodEx2 epidemiological scheme (EFSA, 2015).

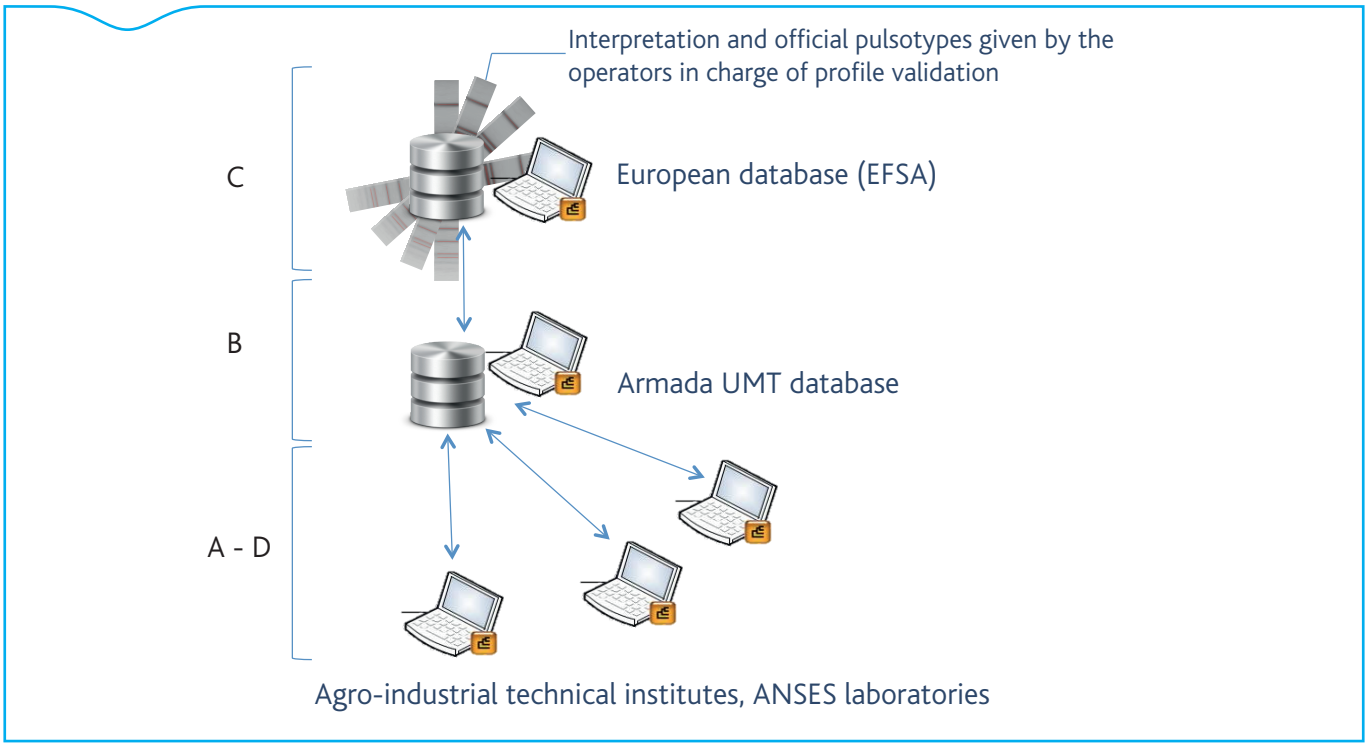

Figure 2. Information flows ensuring (A) the submission of molecular profiles, (B) the sharing of data between users of the Armada UMT's database, (C) data curation at the European level, and (D) the return of curation information to users

strain populations isolated from the pork and pig sector, from farm to finished product. The data provided by ANSES Ploufragan-Plouzané came from two field studies (Boscher *et al.*, 2008; Kerouanton *et al.*, 2011). The data provided by IFIP came from specific studies undertaken with professional partners. The data provided by the LSA in Maisons-Alfort came from DGCCRF surveillance and control plans for which strains have been typed since 2005 by the NRL, and from self-inspection strains collected between 2003 and 2016. Thanks to the Armada UMT's database, it was possible to compile, for the first time, the PFGE profiles of over 900 strains circulating in the pork and pig sector, from farm to distribution. The pooling of these strains collected in various isolation contexts helped broaden our knowledge of the genetic diversity of *Lm* over time and of the high number of colonisation sites for this bacterium in the pork and pig sector.

Example of use of the Armada UMT's database by ITAIs

For an ITAI, the Armada UMT's database can be used for the *Lm* surveillance undertaken by a corporate client of the institute. This surveillance can occur in its slaughter or cutting plants, or plants for the manufacture of processed products. This enables the company to characterise the diversity of strains circulating in its plant environments and link this diversity to that of strains isolated from raw materials or finished products, in order to better track sources of contamination for cuts of meat/finished products. Use of the multi-sector database allows the company to view the frequency at which the PFGE profiles of these strains are observed and compare this frequency to that of its own sector of activity as well as other food sectors. It can also enable it to assess the hazardous nature of the strains it isolates. Since the hyper-virulence of certain CCs has been described, it is possible to predict the CC based on the PFGE profile and obtain this information.

The Armada UMT's database can also be used for investigating contamination incidents on a production site. If the company monitors *Lm* in its plants every day, the detection of a contaminated product on a production line will be more quickly linked to a contamination source (equipment, raw material), by comparison with the typing data contained in the database. The company can then more quickly control the spread of this contamination and in some cases can report it to the supplier(s) of the contaminated raw materials.

This approach can also be used to assess the effectiveness of plant and equipment cleaning and disinfection procedures for circulating or persistent strains in a company.

Outlook

Several stakeholders with complementary roles are involved in the *Lm* surveillance undertaken by the public authorities in the food chain and the French population. The Armada UMT's database can be used by public stakeholders only as stipulated in Article R201-11 of the French Rural Code, i.e. when owners or analytical laboratories holding foodstuffs undergoing an epidemiological investigation further to a food-borne illness are required to submit samples or analytical results. It is therefore necessary to work with producers and their associated ITAIs to have access to the submitted data.

The Armada UMT's database has been designed to incorporate various types of data, especially those obtained using cutting-edge technologies such as whole genome sequencing (WGS). This new technology enables previously inaccessible genetic information to be used. It considerably increases the discriminating power of typing, and also eliminates genetic artefacts inherent in methods based on the analysis of molecular profiles such as PFGE. WGS thus promises to make the detection of outbreaks more relevant and advanced. Several European and international laboratories now use techniques based on WGS for the typing of clinical and food-borne strains. The methodologies used differ from one laboratory to the next (Moura *et al.*, 2016; Hyden *et al.*, 2016; Painset *et al.*, 2016; Gerner-Smidt *et al.*, 2016; Nielsen *et al.*, 2016). A current limitation of WGS is the bio-informatic processing of data. In the United States where WGS is becoming widespread, computer equipment with the computing power required to analyse WGS data is hosted by the laboratory responsible for surveillance (Jackson *et al.*, 2016; Allard *et al.*, 2016). This ensures harmonised data analysis for national laboratory users. In France, this solution could be proposed by ANSES for the development of the Armada UMT's database.

Box. The fate of the EURL *Lm* DB

The EURL *Lm* DB is an integral part of the *Listeria* surveillance system at the national and European levels. It is currently seen as a European surveillance tool for circulating clones. It can be accessed by European NRLs to direct their investigations to a food sector or product category. However, it is not meant to be used unless there is a suspicion of an international outbreak.

The development of this database has enabled the laboratory and ANSES to establish its expertise and position itself in relation to key stakeholders involved in *Lm* surveillance: i) in Europe (ECDC, EFSA, SSI), ii) in the United States (CDC), and iii) internationally (PulseNet International).

The database set up by EFSA and ECDC, currently in the pilot phase, should be operational in 2017 (EFSA, 2014). It will contain all the profiles of food, animal, and environmental strains as well as those of human strains of *Lm, Salmonella* and VTEC. It will thus replace the EURL *Lm* DB for the collection of typing data of non-human origin. Users of the EURL *Lm* DB will recover data related to their molecular profiles by synchronisation. They will then install the features of the EFSA-ECDC database and will submit the molecular profiles of their strains. The EFSA-ECDC database has been developed to maintain the same features as the EURL *Lm* DB, in particular by enabling profiles to be synchronised after curation. The EURL *Lm* DB curation team will be an integral part of the steering committee for the EFSA-ECDC database and will be in charge of curating data related to *Lm* of non-human origin in this new system.

In France, the competent authorities (DGAL, DGCCRF and DGS) are to appoint the laboratory responsible for submitting French molecular typing data to the EFSA-ECDC database. NRLs are the laboratories identified for this task. One possibility is to enable the grouping and submission of all of the national typing data of users of the Armada UMT's database.

Table 1. Breakdown of the main PFGE groups in relation to the main food origins of the strains submitted to the Armada UMT's database by ANSES and IFIP

PFGE group*	Fishery products	Dairy products	Comp-osite products	Meat products	Total	MLST clonal complexes demon-strated by PFGE
A	70	8	48	120	**246**	CC121
B	7	3	70	94	**174**	CC9
C	15	3	5	40	**63**	CC5
D	12	33	15	36	**96**	CC8
E	7	7	13	35	**62**	CC1
F	15	66	17	31	**129**	CC4
G	5	18	10	21	**54**	CC31
H	0	9	0	12	**21**	CC204
I	5	4	6	12	**27**	CC20
J	0	6	5	9	**20**	CC37
K	9	0	5	7	**21**	CC121
L	1	5	3	7	**16**	CC155
M	9	4	3	4	**20**	CC77 - CC54
N	11	2	8	4	**25**	CC14
Total	**166**	**168**	**208**	**432**	**974**	

* group established by UPGMA based on the average similarity between AscI and ApaI profiles above 80%, Dice coefficient, tolerance and optimisation set at 1%

Conclusion

The Armada UMT's database has encouraged the use of molecular typing for *Lm* in the French network. The development of this database was proposed as an innovative project created by ANSES in close collaboration with national stakeholders in the agri-food sector, in particular IFIP. This project helped establish the interconnection between the Armada UMT's database and the one currently being developed by EFSA and ECDC.

It was agreed with the database's users that profiles would be validated at the European level, in order to enable typing data to be reported to the European surveillance system. In France, the Armada UMT's database can be accessed by the authorities only when producers are officially required to report information as specified in Article R201-11 of the French Rural Code. Beyond the harmonisation of typing methods, the joint use of a database system is a way to rally *Lm* surveillance stakeholders at the national and European levels.

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Results of **histamine monitoring in refrigerated fish** with high histidine concentrations in France (2010-2012 and 2015)

Laurent Guillier (1) (laurent.guillier@anses.fr), Isabelle Berta-Vanrullen (2), Laurence Rudloff (3), Diane Cuzzucoli (4), Mathilde Saussac (5), Guillaume Duflos (1)

(1) ANSES, Laboratory for Food Safety, Department for Fishery Products and Department for Microbiological Contaminants, Boulogne-sur-Mer and Maisons-Alfort sites, France
(2) ANSES, Laboratory Affairs Department, Unit for the Coordination and Support of Surveillance, Maisons-Alfort, France
(3) Directorate General for Food, Office for Seafood and Freshwater Products, Paris, France
(4) ANSES, Risk Assessment Department, Maisons-Alfort, France
(5) ANSES, Laboratory Affairs Department, Unit for the Coordination and Support of Surveillance, Lyon, France

Abstract
Fresh fish with high concentrations of histidine are the main contributors to histamine risk. From 2010 to 2012, a monitoring plan for fresh fish with high concentrations of histidine was carried out. Sampling was established according to consumption data. It took into account both seasonal and regional distribution, in order to be representative of consumer exposure. Mean histamine concentrations showed little differences between sampled fresh fish. Probabilities of exceeding the regulatory limits or concentrations that have a known impact on consumer health appeared to be better indicators of food safety and quality. The species that most contributed to consumer exposure, with high concentrations of histamine, was chilled tuna. In addition, the 2015 results, obtained from a smaller sample, show there is greater uncertainty regarding the indicators, and possible changes in consumer exposure can thus no longer be estimated.

Keywords
Biogenic amines, Histamine, Surveillance, Fish

Résumé
Surveillance de l'histamine dans les poissons réfrigérés à forte teneur en histidine en France (2010 à 2012 et 2015)
Les poissons frais à forte concentration en histidine sont les plus forts contributeurs au risque histaminique. Une surveillance de l'histamine dans les produits de la mer est organisée chaque année depuis 2005 par la direction générale de l'Alimentation. De 2010 à 2012, l'échantillonnage pour les poissons frais à forte concentration en histidine, établi à partir des données de consommation (notamment de la répartition saisonnière et régionale des consommations), a permis d'obtenir des résultats représentatifs de l'exposition des consommateurs. Les contaminations moyennes en histamine présentent peu de différences entre les différents poissons frais suivis. Les probabilités de dépasser les seuils réglementaires ou les concentrations qui ont un impact connu sur la santé des consommateurs apparaissent comme un meilleur indicateur de la qualité sanitaire des aliments. L'espèce qui contribue le plus à l'exposition des consommateurs, avec des concentrations élevées en histamine, est le thon réfrigéré. En outre, les résultats de 2015 établis à partir d'un échantillonnage réduit par catégorie de poissons frais montrent que l'incertitude sur les indicateurs devient plus importante et ne permet plus d'estimer d'éventuelles évolutions de l'exposition des consommateurs.

Mots-clés
Amines biogènes, histamine, surveillance, poisson

Health context

Histamine

Histamine belongs to the class of biogenic amines, which are involved in metabolism in humans, animals and plants. With regard to food, these substances are non-volatile amines formed by the decarboxylation of amino acids by microbial and tissue enzymes. More than 200 bacterial species are capable of producing histidine decarboxylase, and can produce histamine depending on the environmental conditions.

Histamine is an essential physiological compound for humans. However, food can supply too much of it; it then disrupts the body and induces poisoning in the form of a "pseudo-allergic" reaction.

In France, there is no specific mandatory reporting for histamine-related poisoning, which is monitored through the reporting of food-borne outbreaks. The number of these outbreaks in which histamine's role was confirmed rose from nine in the early 2000s (Delmas *et al.*, 2005) to more than 27 in 2006 (InVS, 2007). Several unverified assumptions were put forward to explain this increase: changes in the affected products (fish species consumed, geographical fishing areas, etc.), changes in consumer practices, and improved operation of the reporting system (AFSSA, 2009).

The most recent data show a smaller number of food-borne outbreaks. In 2014 in France, histamine's involvement was confirmed or strongly suspected in respectively seven and 25 food-borne outbreaks, affecting 36 and 115 people (InVS, 2014). Histamine accounted for 3% of outbreaks whose agent was confirmed (InVS, 2014). In 2014, at the European level, 74 food-borne outbreaks involving histamine were reported (EFSA, ECDC, 2015).

Background of histamine surveillance in foods in France

Regulations

Histamine is regulated for fishery products only. The safety criteria are defined in Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. This regulation applies to the industry own control programmes undertaken by operators to verify the safety of the product batches they place on the market. In this context, they are required to collect nine samples per batch (n=9); the mean concentration for these nine samples must be less than or equal to 100 mg.kg-1 (m); no more than two samples (c=2) can have a concentration between 100 (m) and 200 mg/kg (M) but none can exceed 200 mg/kg (a factor of two is authorised for these values for products that have undergone enzyme maturation, such as anchovies). Regulation (EU) No 1019/2013 of 23 October 2013 provides some clarifications regarding the ability to consider only one sample (n=1) for the verification of foods at retail level; the concentration of histamine must not exceed 200 mg/kg. It adds a safety criterion for fish sauce produced by fermentation of fishery products (n=1; m=M=400 mg/kg) (EU, 2013).

Main foods containing histamine

Histamine can be found in fermented food products such as wine, beer, sauerkraut, cheese (Roquefort, Gruyère, Cheddar, Gouda, Edam, Emmental, Gorgonzola), delicatessen meat (salami, chorizo, dried sausage), chocolate, and hung game, as well as in non-fermented products such as spinach and especially certain fish (Suzzi and Gardini, 2003; Lavizzari *et al.*, 2007).

However, the large majority of food-borne outbreaks involving histamine (over 70%) are associated with fish and fishery products (FAO/WHO, 2013). Only some fish species can contain a large quantity of histamine, due to their high histidine levels. The fish most commonly involved in cases of poisoning belong to the Scombridae family. In fact, the term "Scombroid fish poisoning" is used to describe poisoning due to histamine in fishery products. Other classes of fish are recognised as presenting a risk (Table 1, Guillier *et al.*, 2011).

Surveillance plans

Given the increase in the number of food-borne outbreaks between 2000 and 2006, the Directorate General for Food (DGAL) submitted a request to AFSSA in 2008 to improve the surveillance plan organised every year since 2005. An Opinion was issued (AFSSA, 2009) and the DGAL surveillance plan for histamine was revised. The proposed plan (which was implemented for the 2010-2012 period) directly assessed consumer exposure to histamine. This plan relied on the risk levels of the various categories of seafood products associated with species with high histidine concentrations. The overall approach is described in detail in the AFSSA Opinion of 2009 and a scientific article (Guillier *et al.*, 2011). The plan focused on high-risk product categories (fresh fish). For each category, the sampling plan was then defined based on consumption data, in order to ensure spatial and seasonal representativeness. The samples were divided up proportionally for these two criteria between eight major regions (North, East, Paris region, West, Centre-West, South-West, Centre-East, South-East) and six periods of the year (January-February, March-April, etc.). Samples were taken in the distribution (supermarkets, fishmongers) and catering stages, respecting a distribution in proportion to the relative quantities of fish associated with places of consumption (at home and outside the home).

The surveillance plan undertaken in 2015 had the same objective, but did not adhere to the same constraints regarding the spatial and temporal distribution of the samples. The 212 samples of fresh fish were planned in the stage of direct delivery to consumers. The number of samples to be taken by region was established in proportion to the size of the human population. Samples were collected from various batches to ensure the representativeness of results. The variability of contamination levels could not be estimated for three categories (herrings, sardines and fresh salmon) in 2015 since the contamination levels were below the limit of quantification.

Data used and methods for characterising data on histamine contamination in products

Source of the data

Data on histamine contamination in fishery products provided by the DGAL were extracted from the Access database pooling public surveillance data developed during the prototyping of the proposed health section of the Food Observatory (OSSA).

These data come from the DGAL's surveillance plans covering 2010-2012 and 2015. In order to comply with the format and nomenclature requirements of the European database, the data have been recoded by ANSES according to "Standard Sample Description ver.2.0" (SSD2) and the FoodEx2 food description and classification system. Figure 1 shows the breakdown of the 1686 histamine concentration data between the four years (2010, 2011, 2012 and 2015) and the various categories of fresh fish. Only data related to tuna (yellowfin and

Table 1. All of the fish species potentially at risk for the histamine hazard (according to AFSSA (2009) and Guillier *et al.* (2011)). The fish categories analysed in the surveillance plan appear in dark red

Class	Species	English name
Arripidae	*Arripis trutta*	Australian salmon
Amodytidae	*Ammodytes tobianus*	Lesser Eel or Small Sandeel
Belonidae	*Belone belone*	Garfish
Carangidae	*Seriola dumerili (Risso)*	Greater amberjack
	Seriola lalandii	Yellowtail amberjack
	Caranx spp.	Jack or Blue Runner
	Trachurus spp.	Horse mackerel
Coryphaenidae	*Coryphaena hippirus*	Mahi-mahi
Clupeidae	*Sardinella sirm*	Sprat
	Amblygaster sirm	Spotted sardinella
	Sardinops sp.	Sardinella, Madeiran
	Sardina pilchardus	Sardine
	Clupea harengus	Herring
	Sprattus spp.	Sprat
	Harengula spp.	Herring, Pacific Thread
	Alosa pseudoharengus	Alewife or River Herring
	Spratelloides gracilis	Herring, Silver-stripe Round
Engraulidae	*Anchoa* spp.	Anchovy
	Anchoviella spp.	
	Engraulis spp.	
	Cetengraulis mysticetus	
	Stolephorus spp.	
Gempylidae	*Lepidocybium flavobrunneum*	Escolar
	Rivetus pretiosus	
Istiophoridae	*Makaira (Tetrapterus) audax (poey)*	Marlin
	Istiophorus spp.	Sailfish
Lutjanidae	*Aphareus* spp.	Snapper
	Aprium virescens	
	Pristipomoides spp.	
Pomatomidae	*Pomatomus saltatrix*	Bluefish
Sciaenidae	*Seriphus politus*	Queenfish
Scomberesocidae	*Cololabis saira*	Pacific saury
Scombridae	*Auxis thazard*	Bonito tuna
	Acanthocybium solandri	Wahoo
	Euthynnus alleratur	Little Tuna or Kawakawa
	Katsowonus pelamis	Skipjack tuna
	Sarda sarda	Atlantic bonito
	Scomber japonicus	Pacific mackerel
	Scomber scombrus	Atlantic mackerel
	Scomberomorus cavalla	King mackerel
	Scomberomorus maculatus	Atlantic Spanish mackerel
	Scomberomorus regalis	Cero
	Scomberomorus brasiliensis	Serra Spanish mackerel
	Thunnus alalunga	Albacore
	Thunnus albacares	Yellowfin tuna
	Thunnus obesus	Bigeye tuna
	Thunnus thynnus	Atlantic bluefin tuna
	Thunnus atlanticus	Blackfin tuna
Salmonidae	*Salmo salar, Oncorhyncus* sp.	Salmon
Serranidae	*Epinephelus* sp.	Grouper
Xiphiidae	*Xiphias gladius*	Swordfish

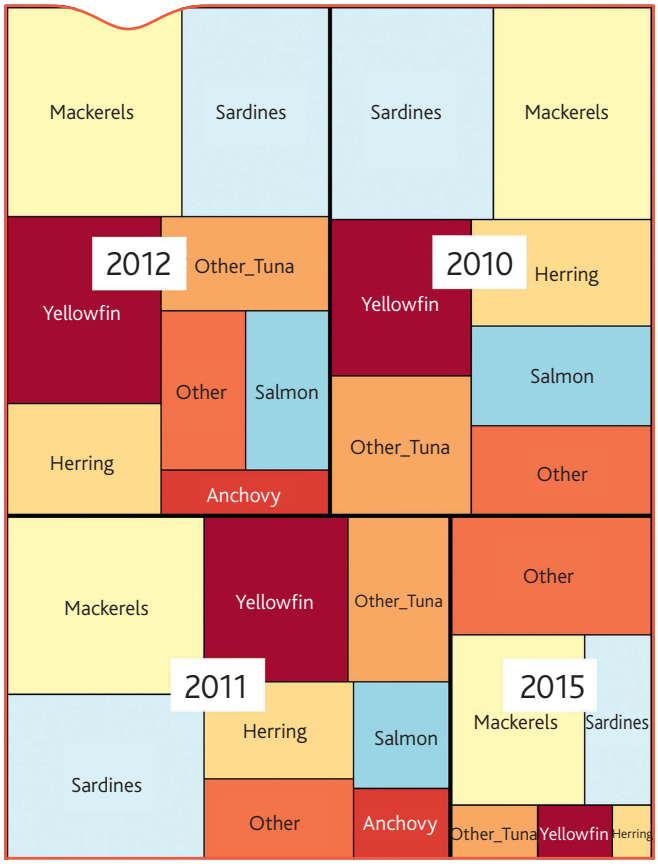

Figure 1. Hierarchical display of the breakdown of the 1686 data from the surveillance plan for refrigerated fresh fish in 2010, 2011, 2012 and 2015. The areas are proportional to the sampling distribution

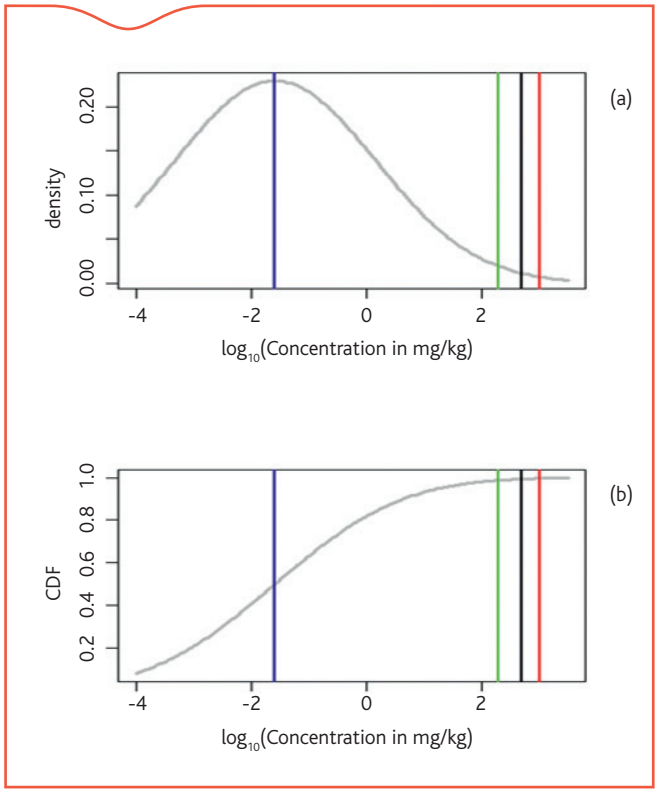

Figure 2. Illustration of a log-normal distribution used to characterise the histamine concentration data of the surveillance plan. (a) Density, (b) Cumulative distribution (CDF). Legend for the quantiles used to characterise the distribution: blue=median/mean, green=200 mg/kg, black=500 mg/kg, red=1000 mg/kg

other species of the *Thunnus* genus), mackerels, sardines, herrings and salmon are analysed here. The "Other" category contains data for various other fish species (e.g. horse mackerel, grouper, swordfish) for which the sample populations do not enable an analysis with sufficient statistical power. This option to monitor species other than those most commonly consumed had been proposed in the AFSSA Opinion of 2009, in order to provide the opportunity, as part of the surveillance plan, to study species and origins of seafood products subject to outbreak surveillance.

Statistical methods

Since most of the results of the histamine surveillance plans are below the limit of quantification, the use of descriptive statistics (mean, median, etc.) is of limited interest and would even lead to biases if data below this threshold were randomly set at the value of the limit of quantification (LOQ) for the method. In this context, data modelling is a genuine advantage to improve the overall description process for an empirical distribution. The methodology applied here for histamine is directly inspired by methodologies applied in microbiology (Busschaert *et al.*, 2010; Pouillot and Delignette-Muller, 2010).

The other methodological objective was to characterise uncertainty for the distributions used to improve knowledge of variability in product contamination. There are several available methods for assessing uncertainty, including bootstrapping (re-sampling technique) and the Bayesian approach (Commeau *et al.*, 2012). Bootstrapping has been used to characterise uncertainty for descriptive statistics based on distributions.

The statistical functions used to adjust the log-normal distribution for censored data and to characterise uncertainty for the quantiles of interest are those of the R package "fitdistrplus" (Delignette-Muller *et al.*, 2015). Figure 2 shows the values estimated from the surveillance data. Mean

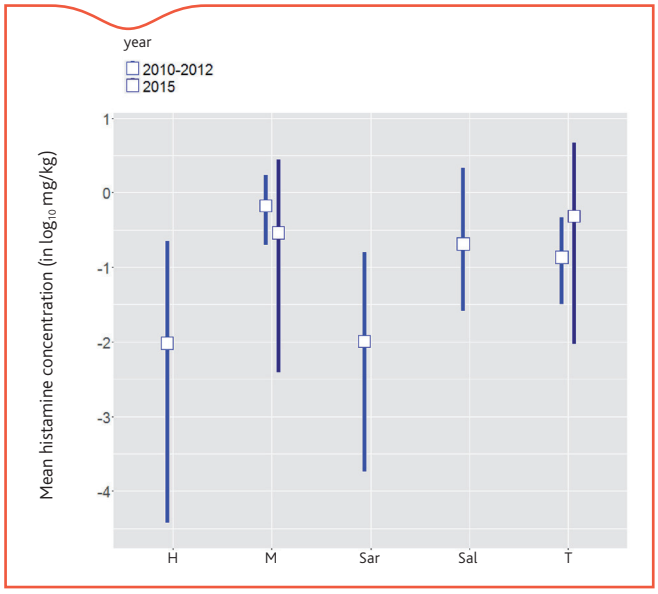

Figure 3. Mean concentrations in $\log_{10}$ (mg/kg) of histamine in the various seafood products monitored in the surveillance plans (H=Herrings, M=Mackerels, Sar=Sardines, Sal=Salmon, T=Tuna). The most probable values (dots), 95% credible intervals for the mean concentrations (error bars)

concentrations and probabilities of exceeding the respective thresholds of 200, 500 and 1000 mg/kg have been estimated.

Surveillance plan results and discussion

Since contamination levels did not differ significantly between the four years, they will be presented as a whole. Figure 3 gives mean contamination levels for the various categories of fresh fish. For

2010-2012, mean contamination levels were respectively 0.01, 0.76, 0.01, 0.18 and 0.15 mg/kg for herrings, mackerels, sardines, salmon and tuna. The uncertainty associated with these estimates confirms that the differences between fish in terms of mean contamination were low and generally insignificant (only the mean contamination for mackerels was significantly higher than that for sardines). There were fewer data for 2015, resulting in greater uncertainty regarding the results; this means that changes in contamination between 2012 and 2015 could not be estimated. Figure 4 shows the probability of reaching high levels (in relation to the regulatory threshold and those associated with a high probability of inducing poisoning) for each fresh fish. The data analysis shows that the probability of reaching high contamination levels is higher for tuna than for the other categories of fish with high histidine concentrations. As for the mean contamination levels, potential changes in probabilities of high contamination could not be estimated due to the small number of samples for 2015.

The contamination levels observed in fresh fish in the consumption stage in France were of the same order of magnitude as those provided in an international summary presented in a FAO/WHO report (2013). For example, in the Netherlands, the mean concentration of histamine in fresh tuna was 14 mg/kg in 2010 and the probability of exceeding 200 mg/kg was 2.9%. Other more recent publications report probabilities of exceeding the 200 mg/kg threshold of below 3.3% (Michalski, 2016; Petrovic *et al.*, 2016). However, the median contamination values and probabilities of exceeding the limit values are only representative of the foods analysed. As it is almost impossible from the survey reports to know whether sampling was representative of the country's consumption profile, the results cannot be compared among countries (FAO/WHO, 2013).

The decision to devote a certain number of samples to fresh salmon had been proposed in the AFSSA Opinion of 2010. There were still doubts regarding the potential involvement of this fish in cases of histamine poisoning (Emborg *et al.*, 2002). The analysis of the data from the 2010-2012 plans shows that histamine levels can be high in this type of fish. Surveillance plan data obtained for salmon confirm current knowledge on the possible contamination of this fish by histamine (Løvdal, 2015). The median contamination levels estimated for salmon under these surveillance plans are robust. The probability of exceeding higher concentration levels is much more uncertain. Unlike for other fish with high concentrations of histidine, it is not certain for salmon that microbial growth and/or the initial histidine concentration enable high contamination levels to be reached. In other words, the distribution used suggests high levels whereas actual histamine contamination might not exceed a certain level. The maximum concentrations observed for fish with high histidine levels exceed 2000 mg/kg. To our knowledge, this level of contamination has never been observed for salmon.

With a sampling plan that is not representative of consumption, the data must be adjusted to assess exposure. Statistical adjustment consists in taking the sampling plan's data into account to assign a particular "weight" to each sample based on its category. Weighting depends on the consumption of each fish; the weight is greater than 1 if its category is not sufficiently represented in relation to its share of consumption, and is less than 1 if it is overrepresented. However, it is difficult to adjust data if the plan includes several that are below the limit of quantification (Williams and Ebel, 2014). In this case, the data were not adjusted because for each product category, the samples they came from were directly representative of consumption data (Guillier *et al.*, 2011). Since a very high percentage of the analysed data are below the limit of quantification, it will be necessary to continue using sampling representative of exposure for future surveillance plans. The 2010-2012 plans used sampling representative of the seasonality and regional distribution of consumption (AFSSA, 2010). The data analysis shows that some factors have little influence on contamination levels. It therefore does not appear necessary to strictly index the distribution of samples to the seasonality of consumption or to all French regions.

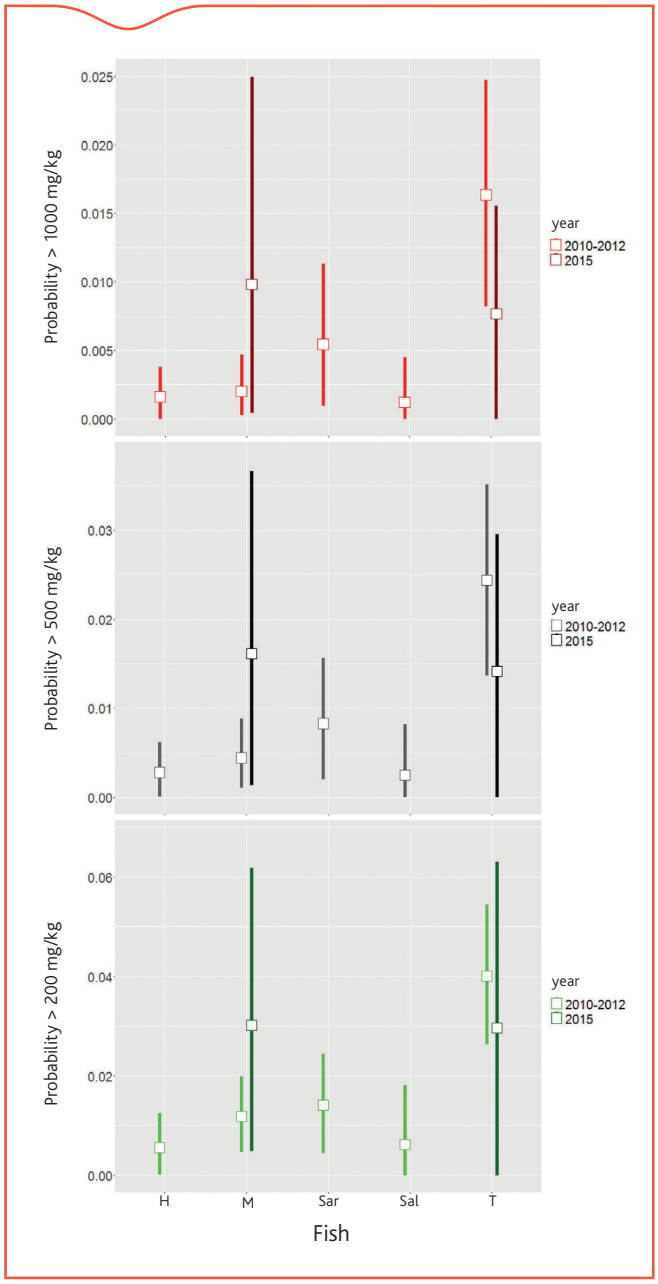

Figure 4. Probabilities of exceeding histamine concentrations in various seafood products of (a) 1000 mg/kg, (b) 500 mg/kg, and (c) 200 mg/kg (H=Herrings, M=Mackerels, Sar=Sardines, Sal=Salmon, T=Tuna). The most probable values (dots), 95% credible intervals for the mean concentrations (error bars)

The data analysis provides a classification of the fish that contribute most to histamine exposure. Tuna appears to be the most contributing species in terms of contamination levels. The assessment of histamine exposure undertaken through surveillance plans is paving the way for attributing cases of histamine poisoning in France to the various categories of fresh fish. Combining estimates of histamine exposure (concentration data from surveillance plans together with consumption data) with the dose-response relationship (used to calculate the probability of observing an effect in consumers based on the ingested hazard dose), including potential differences due to the specific susceptibility of sub-populations of consumers, would enable risk to be assessed as a relative or absolute number of human cases related to the various sources.

The FAO/WHO report (2013) raised the issue of the role of other biogenic amines (possible "potentiating" effect or not). Data need to be acquired to examine this issue. Thus, the accredited laboratories in the network of the National Reference Laboratory for histamine have been requested to submit not only histamine concentrations but

also concentrations of other biogenic amines (putrescine, cadaverine and tyramine). These data are essential to understand potential correlations between these amines and will make it possible to assess consumer exposure.

Conclusion

Future surveillance plans for histamine and biogenic amines will continue to monitor consumer exposure using the methodology proposed in the AFSSA Opinion of 2009. To monitor changes in this exposure, the sampling plan inspired by that used for 2010-2012 will be implemented, keeping the same fish categories. The results obtained in 2015 with a limited surveillance plan compared to 2012 indicate that it is preferable to keep only one category of fresh fish per year. This will provide sufficient statistical power to estimate changes in the exposure of French consumers to histamine in fresh fish.

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The role of **food alerts and food-borne outbreaks** in food chain surveillance

Anne Morand (1) (anne.morand@haute-corse.gouv.fr), Marie-Pierre Donguy (1), Nelly Fournet (2)
(1) Directorate General for Food, Mission for Health Alerts, Paris, France
(2) French Public Health Agency, Department for Infectious Diseases, Saint-Maurice, France

Abstract
This article briefly presents the two French systems for food alert management and food-borne outbreak surveillance as well as a specific annual report for both systems. The food alert management system and food-borne outbreak surveillance are considered complementary to optimise consumer safety.

Keywords
Food-borne outbreaks, Alert, Food

Résumé
Place des alertes alimentaires et des toxi-infections alimentaires collectives dans la surveillance de la chaîne alimentaire
Cet article présente succinctement les deux dispositifs nationaux de gestion des alertes alimentaires et de surveillance des toxi-infections alimentaires collectives (Tiac), ainsi qu'un bilan annuel spécifique de chaque dispositif. Les systèmes de gestion des alertes alimentaires et de surveillance des Tiac sont présentés comme complémentaires aux dispositifs de surveillance des aliments pour optimiser la sécurité des consommateurs.

Mots-clés
Toxi-infection alimentaire collective, alerte, aliment

The food alert management system and food-borne outbreak surveillance are two schemes set up place in France with a primarily operational objective of identifying poor practices and at-risk foods and products, in order to limit consumer exposure to a hazardous food and/or prevent new human cases.

Alert management

The harmonised management of alerts in France is the responsibility of the Mission for Health Emergencies (MUS) of the Directorate General for Food (DGAL), which receives alerts (from France and other countries) and ensures they are managed appropriately and proportionately nationwide.

A food alert ("product alert') is any information related to a food origin which, if not addressed, can lead to a situation jeopardising consumer safety. An "unsafe" foodstuff as defined in Article 14 of Regulation (EC) No 178/2002 can be detected by operators as part of their own-checks, by the authorities in France or other countries (information from the Rapid Alert System for Food and Feed, RASFF) as part of official controls, or by consumers themselves. When one of the parties (operators, professional organisations or the authorities) learns of an alert, it is required to inform the other parties.

The situation is first assessed by the operator that placed the product on the market as soon as they learn of the non-compliance. In accordance with Regulation (EC) No 178/2002, when the product is on the market, the professional must take actions aiming to protect consumers (product withdrawal or recall, see Box), inform the local competent authority, and ensure that normal production conditions are restored. After the information is received by decentralised services, the report is verified and the situation is assessed in terms of its hazardous nature to determine whether the report should be classified as a national or local alert and whether the management measures taken by the professional are appropriate and proportionate.

Box. Definitions

Withdrawal (Article 2(h) of Directive 2001/95/EU): "Any measure aimed at preventing the distribution, display and offer of a product dangerous to the consumer". Withdrawal operations are the responsibility of the professional holding these products, in all stages of the food chain.

Recall (Article 2(g) of Directive 2001/95/EU): "Any measure aimed at achieving the return of a dangerous product that has already been supplied or made available to consumers by the producer or distributor". A product recall, i.e. information for consumers, is determined according to the severity of the potential or confirmed risk to human health in order to prevent consumers from being exposed to the hazard, as quickly as possible, and to inform them of the risks related to consumption of the product in question.

Food-borne outbreak surveillance

Food-borne outbreaks are monitored at the national level by the French Public Health Agency, together with the Regional Health Agencies (ARSs) and in collaboration with the Departmental Directorates for Protection of the Population (DDecPPs), *via* a mandatory reporting system.

Physicians and managers of mass or social catering establishments are required to report a food-borne outbreak to the ARS and/or DDecPP. Reports can also be submitted by consumers or other people who have knowledge of an episode that could be a food-borne outbreak.

A food-borne outbreak occurs when there are at least two similar cases of generally gastro-intestinal symptoms that can be attributed to the same food origin.

Food-borne outbreaks are classified as follows:

- "confirmed': when a pathogen (bacterium, virus or parasite) is isolated in a sample of human origin (blood/stools), food leftovers, standard meals or the food's environment (e.g. fishing areas or surface samples),
- "suspected': when a pathogen has not been confirmed; it is then suspected using an algorithm for aetiological diagnosis taking into account the clinical signs, median incubation time and types of foods consumed,
- "of unknown aetiology': when a pathogen has not been confirmed or suspected.

When the ARSs and DDecPPs receive reports of food-borne outbreaks, investigations are undertaken to identify the responsible foods, the source of contamination, and any poor hygiene or food

preparation or storage practices where applicable. The ultimate objective is to take necessary measures (corrective measures, the closing of restaurants or zones, withdrawals, recalls) to prevent new food-borne outbreaks or new cases.

Report

"Product alert" report

This report is not an exhaustive inventory of all the non-compliances detected in France by operators or DDecPPs; it describes only those that have been reported at the central level, since they exclusively involve:

- products placed on the market,
- products distributed outside of their production *département* and/or recalled from consumers (regardless of the distribution scope).

In 2015, the MUS received 1082 food alerts: 952 of these originated in France (Figure 1) and 130 came from other countries. Of these 1082 alerts, the DGAL reported 117 *via* RASFF.

The main sources of alerts in France were: i) own-checks by French operators (retailers, producers), which accounted for over two-thirds of alerts, ii) official surveillance and control plans (SCPs), which accounted for 20% of alerts, and iii) consumer complaints, which were in third position, with almost 5% of product alerts (on the rise for the past few years).

In line with the regulatory targeting criteria (matrices and hazards explicitly covered by regulatory texts), the breakdown of alerts by product type places butcher's meat products at the top of the ranking, followed by fishery products and dairy products (Figure 2).

In addition, in line with the contaminants subject to regulatory criteria (in particular Regulation (EC) No 2073/2005), control pressure, and the assessment of the safety of contaminated products placed on the market, the five contaminants most commonly associated with product alerts were *Listeria monocytogenes* (32% of the dossiers processed by the MUS in 2015), followed by *Salmonella* (16%), heavy metals (9.1%, with over two-thirds detected as part of SCPs), pathogenic *Escherichia coli* and veterinary medicinal products (Figure 3). These dossiers led to 576 withdrawal operations and 272 recall measures in 2015.

Food-borne outbreak report

In the framework of the surveillance system for food-borne outbreaks, the identified hazards were mainly infectious agents and histamine. Other agents (toxins for example) were exceptional; they were generally monitored by a toxicovigilance programme.

An annual review of the food-borne outbreaks reported in France is available on the website of the French Public Health Agency: http://invs.santepubliquefrance.fr/Dossiers-thematiques/Maladies-infectieuses/Risques-infectieux-d-origine-alimentaire/Toxi-infections-alimentaires-collectives/Donnees-epidemiologiques.

In 2014, 1380 food-borne outbreaks were reported, affecting 12,109 people, including 649 (5%) who were hospitalised and two who died.

Food-borne outbreaks primarily occurred following meals in commercial or mass catering establishments (respectively 37% and 30% of the outbreaks reported). The proportion of food-borne outbreaks occurring further to family meals was 33% in 2014 (familial food-borne outbreaks increased by 22% compared to 2013 but were similar to the 2012 data).

The share of food-borne outbreaks where a pathogen was confirmed was relatively low (18%). For confirmed food-borne outbreaks, *Salmonella spp.* was the most commonly identified pathogen (43% of confirmed food-borne outbreaks). The other two most commonly confirmed/suspected pathogens associated with food-borne outbreaks were *Staphylococcus aureus* (30% of outbreaks) and

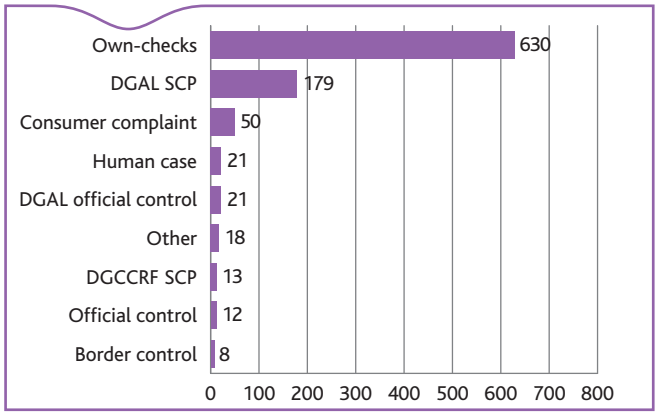

Figure 1. Breakdown of national alerts by detection source (2015)

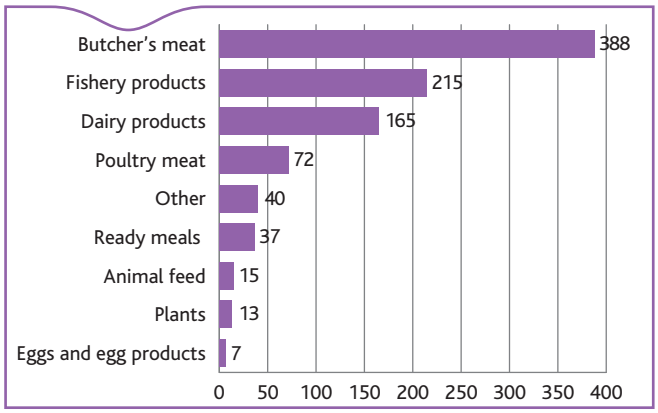

Figure 2. Breakdown of national alerts by product category (2015)

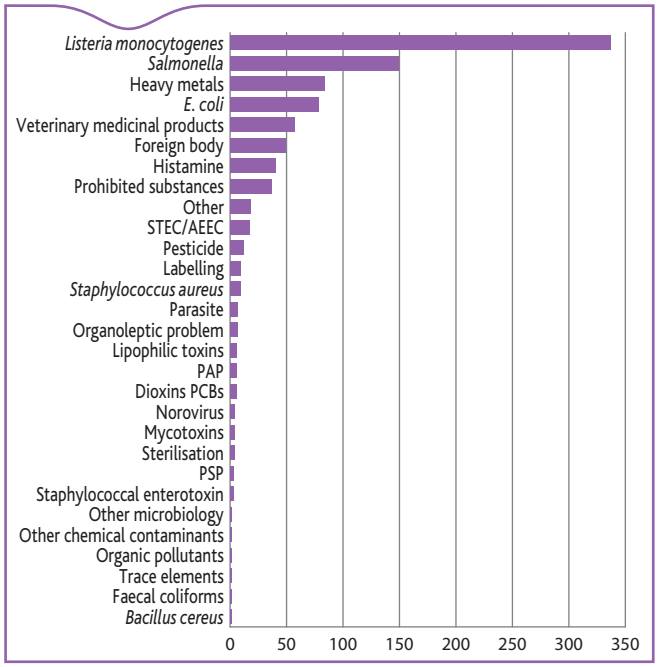

Figure 3. Breakdown of national alerts by responsible agent (2015)

Bacillus cereus (22%). In 13% of the reported outbreaks, no agents were detected or suspected.

In commercial/mass catering establishments, the most commonly encountered non-compliances were defective or unsuitable equipment, non-compliance with hygiene rules, poor handling by staff, and the contamination of materials (raw, intermediate or finished product).

Corrective measures were necessary for 490 (53%) food-borne outbreaks in commercial/mass catering establishments. The measures most frequently taken were employee information/training,

disinfection of the establishment, work in the establishment, and closing of the establishment. In 2014, 22 seizures and withdrawals/recalls were undertaken for foodstuffs.

Discussion - conclusion

The systems for food alert management and food-borne outbreak surveillance are capable of identifying situations involving a loss of sanitary control in food production and/or distribution processes, and rapidly responding. Over the long term, they also enhance knowledge regarding the origin and prevalence of contaminants in the food matrices most commonly associated with food-borne outbreaks.

Moreover, these systems help with the collection of information related to matrices and contaminants not taken into account in the planning of official controls (e.g. *Staphylococcus aureus* and its toxins, foreign bodies and labelling defects) and, when necessary, contribute to the detection of emerging contamination, in relation to the hazard analyses undertaken by operators as part of their own-checks.

However, these reports cannot be used to draw conclusions as to the safety of products placed on the market in France or to compare countries with one another, since they do not take into account the following in particular:

- differences between surveillance systems,
- production volumes and types,
- the number of samples to be analysed (own-checks or official controls),
- the definition, depending on the country, of a non-compliance giving rise to an alert. For example, there is a difference between Member States regarding the management of ready-to-eat products on the market that are contaminated by concentrations of *Listeria monocytogenes* below 100 CFU/g, leading to a high number of alerts in France,
- under-reporting for each system.

Solutions to optimise food chain control and surveillance can be considered by comparing various sources of information (alert and food-borne outbreak reports, results of other food chain surveillance systems). This analysis should highlight priorities for action in terms of sanitary control for the various stakeholders in the food chain, including consumers:

- relevance of the own-check plans of operators,
- planning priorities for official controls,
- optimisation of the reporting system for alerts and food-borne outbreaks,
- recommendations regarding compliance with the controlled temperature chain (hot or cold preparation),
- specific hygiene recommendations for consumers.

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Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

Regulation (EEC) No 315/93 laying down Community procedures for contaminants in food.

Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs.

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Report memo. Zoonoses, zoonotic agents and food-borne outbreaks in Europe in 2014

Note sur rapport. Zoonoses, agents zoonotiques et toxi-infections alimentaires collectives en Europe en 2014

Corinne Danan (corinne.danan@agriculture.gouv.fr) (1), Didier Calavas (2)

(1) Directorate General for Food, Sub-directorate for Food Safety, Support Office for Food Chain Surveillance, Paris, France
(2) ANSES, Lyon Laboratory, Epidemiology Unit, France

Keywords: Zoonoses, Europe, 2014/*Mots-clés : Zoonoses, Europe, 2014*

Every year for the past ten or so years, the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) have published a report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks. The report on the 2014 data is presented more concisely than the previous reports (EFSA & ECDC, 2015). Part of this report reviews the data collection background and is limited to a description of the most salient information and changes observed for certain zoonoses; the annexes contain hyperlinks providing access to data from various sectors (human, veterinary and food) used to write the annual reports. The data provided by each Member State (MS) (http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4329ax1.zip) as well as national reports (http://www.efsa.europa.eu/en/biological-hazards-data/reports) can be consulted on the EFSA website.

This report presents surveillance data from 32 countries (28 MSs and four non-EU countries). It provides a wealth of useful information regarding the epidemiological situation in Europe, at human and animal levels, and regarding the food chain. It describes over fifteen zoonotic agents and food-borne outbreaks, including eight regulated under Directive 2003/99/EC, in addition to rabies, toxoplasmosis, Q fever, infections related to the West Nile virus, yersiniosis, tularaemia, cysticercosis and sarcocystosis.

As in previous years, six food-borne zoonoses (campylobacteriosis, salmonellosis, yersiniosis, shigatoxin-producing *Escherichia coli* infections, listeriosis and echinococcosis) had the largest number of cases and the highest rate of incidence[1] for zoonotic infections in humans (Figure 1). Including trichinellosis and brucellosis, food-borne zoonoses accounted for 99.6% of the 343,256 human cases related to thirteen zoonoses reported in Europe.

*Campylobacter*iosis was the main cause of reported human cases; alone, it accounted for 69% of cases in 2014, with 236,851 confirmed cases and an incidence rate[(1)] of 71 per 100,000 inhabitants (Figure 1). This incidence has been on the rise since 2008, with a 9.4% higher incidence rate in 2014 than in 2013.

Salmonellosis was the second leading cause of reported human cases with 26% of cases, 88,715 confirmed cases, and an incidence rate of 23.4 cases per 100,000 inhabitants. For this zoonosis, the European surveillance data have shown a steady decrease in the number of human cases since 2008, which has been linked to the European *Salmonella* control policy in the poultry sector. However, the 2014 incidence rate was 15.3% higher than in 2013.

Of the significant trend analyses, there was also an increase in observed listeriosis cases from 2008 to 2014, but no connection was made to the level of food contamination. In 2014, 2,161 listeriosis cases were reported, with an incidence rate of 0.52 cases per 100,000 inhabitants. This incidence was 30% higher than in 2013.

These figures can be compared to the 0.4% of cases due to zoonoses that can be transmitted to humans through other routes (Q fever, West Nile virus, tularaemia, tuberculosis caused by *M. bovis*, rabies).

The mortality rate for the top twelve zoonoses (with the exception of tuberculosis caused by *M. bovis*), for confirmed cases, was 0.1% on average and generally below 1%, except for West Nile fever (3.4%), listeriosis (15.6%) and rabies (100%).

A total of 5,251 episodes of food-borne outbreaks, including those related to water, were reported in 2014. The causes, which were identified in almost two-thirds of cases, were mainly viruses, followed by *Salmonella*, bacterial toxins and *Campylobacter*. The foods most commonly associated with food-borne outbreaks were eggs and egg products, compound foods and seafood products (crustaceans, molluscs, shellfish and related products).

However, the limitations of this type of exercise should be considered. Furthermore, warning messages are reiterated all throughout the EFSA report, indicating that:

- the data come from surveillance systems of varying types and effectiveness between MSs,
- the sampling plans do not all rely on standardised sampling protocols, and the resulting data are not necessarily representative of national prevalence,
- not all MSs submit a comprehensive report to the European authorities.

Caution is thus required when interpreting the following:

- trends from one year to another, since procedures for reporting to the European authorities can vary and denominators are not adjusted for the age structure of populations, which also changes over time,
- relationships between cases of zoonoses in humans in a given country and the epidemiological situation of the corresponding zoonotic agent in the livestock of the same country, since it is impossible to distinguish between infections acquired in the country of origin and those acquired abroad or through the consumption of imported products,
- country's data in relation to the European data, since definitions are not always the same at national and European levels.

In any case, the information contained in this report is extremely useful for analysing and monitoring the epidemiological status of zoonoses and zoonotic agents in Europe. It is frequently referred to by the public authorities for defining or assessing the impact of management measures.

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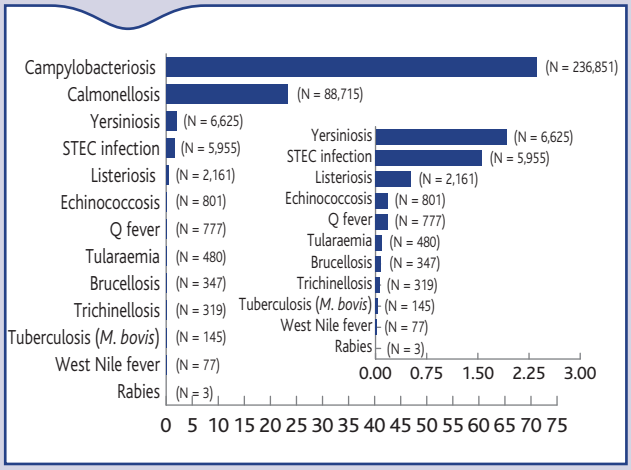

Figure 1. Number of human zoonosis cases, and incidence for 100 000 inhabitants, reported in Europe in 2017

1. It was assumed that these were incident cases and incidence (respectively "reported cases" and "notification rate" in the report).

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Anses - www.anses.fr
14 rue Pierre et Marie Curie
94701 Maisons-Alfort Cedex

Email: bulletin.epidemio@anses.fr
Design and production: Parimage
Photo credits: Anses, Parimage
Printing: Bialec
23 Allée des Grands Pâquis - 54180 Heillecourt
Circulation: 3,500
Legal deposit on publication/ISSN 1630-8018

Issue coordinated by: Corinne Danan (1), Julien Cauchard (2), Marion Bordier (3), Sabine Itié-Hafez (1), Isabelle Berta-Vanrullen (4), François Gauchard (5), Didier Calavas (2)

(1) Directorate General for Food, Support Office for Food Chain Surveillance, Paris, France
(2) ANSES, Lyon Laboratory, Epidemiology Unit, Lyon, France
(3) Directorate General for Food, Office for Management through quality and Control coordination, Paris, France
(4) ANSES, Laboratory Affairs Department, Unit for the Coordination and Support of Surveillance, Maisons-Alfort, France
(5) ANSES, Risk Assessment Department, Maisons-Alfort, France

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