

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

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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 que 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 anti-inflammatoires 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.

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