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Veterinary Drug Residue in Food of Animal Origin in Switzerland: A Health Concern?

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Abstract: Many drugs are used in livestock farming, particularly antibiotics, with almost 80% of world antibiotic production used for animals. Therefore, veterinary drugs may be present as residues in food of animal origin even if their use is fully regulated to ensure the proper use of veterinary medicinal products, to protect consumers from undesirable residues, and to ensure the supply of quality, safe and effective veterinary medicinal products to safeguard animal health. Analytical techniques for residue analysis have improved significantly with routine use of liquid chromatography coupled with tandem or high-resolution mass spectrometry. This improvement enabled specific searches for several hundred target compounds within minutes in complex matrices such as milk, eggs, honey or meat and organs after slaughter. For many years, a national residue control programme in food has been effective. The rate of non-compliant samples remains low and shows that consumers do not have to worry too much about the presence of drug residues in food of animal origin produced in Switzerland. Despite the significant reduction in antibiotic consumption observed in recent years, the resistance rate and distribution of multidrug-resistant bacteria is steadily increasing, even in countries where drug consumption has decreased significantly. Reducing antimicrobial resistance will undoubtedly be one of the most difficult food safety challenges in the coming years.

Keywords: Antimicrobial resistance · Drug residue monitoring · Food safety · Meat product · National control plan · Switzerland

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1. Introduction

Human health is directly linked to the environment and in particular to the nature and quality of food. Pesticides or other chemicals used in agricultural or food production and veterinary drugs may be present as residues in food of animal origin coming from treated animals and therefore induce a potential public health problem.

After administration of a drug to an animal, the drug is most often metabolized to promote its elimination and, to a very large extent, its detoxification. In general, most of the parent products and their respective metabolites are excreted in the urine and faeces. However, such products can be found in food from animal origin such as milk, eggs, honey or meat as well as organs after slaughter. It should be noted, however, that the drug is administered to the animal at a therapeutic dose, and therefore, in principle, is slightly toxic, considering that it is the same active substances that is used in human medicine with a few exceptions.

As a result, it is certain that residues in foods of animal origin will be low in most cases, usually at the ppb level or sometimes hundreds of ppb but rarely above. Given that residues exist at very low concentrations, it is clear that their presence is not a cause of acute toxicity hazards. Despite this, consumers no longer accept the presence of chemical residues in their food, whether toxic or not.

However, there are still questions that arise in terms of public health in relation to the use of veterinary drugs in livestock. It has been shown that regular ingestion of small amounts of the same substance can lead to toxic symptoms over time (chronic exposure) and may promote the development of allergies.[1] A few classes of drugs cause allergic reactions more frequently, such as antibiotics (*e.g.* penicillins), which are precisely the drugs used extensively in animal production. Another emerging and very problematic issue for the medical community is the increase of antimicrobial resistance (AMR), which is the ability of microorganisms to resist antimicrobial treatments, especially antibiotics. The intensive use of antibiotics in veterinary medicine leads to an increase in multidrug-resistant pathogens.[2] The resistance rate and distribution of multiresistant bacteria, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) or extended-spectrum-beta-lactamase (ESBL) producing isolates,

has increased steadily in most European countries. According to the EU Health and Food Safety Commission: "AMR has a direct impact on human and animal health and carries a heavy economic burden due to higher costs of treatments and reduced productivity caused by sickness. AMR is responsible for an estimated 25,000 deaths per year in the EU. It is also estimated that AMR costs the EU 1.5 billion € per year in healthcare costs and productivity losses. Excessive and inappropriate use of antimicrobial medicines and poor infection control practices has transformed AMR into a serious threat to public health worldwide. If the trend of increasing AMR continues, we would revert to a world where simple infections are no longer treatable."[3] A comprehensive report on the status of AMR in Switzerland has been published[4] and a National Strategy on Antibiotic Resistance (StAR) is being developed.[5] The aim is to ensure that antibiotics remain effective in the long term and to curb the development of resistance.

The monitoring of drug residues in food of animal origin is therefore useful at several levels. This ensures (in particular) that prohibited substances, banned due to their toxicity, are not present or used and that authorised substances do not exceed maximum residue levels, at least with respect to withdrawal times between treatment and slaughter. In Switzerland, federal authorities in collaboration with cantonal enforcement authorities organised a National Control Program for detection of Contaminants in foodstuffs of animal origin (NCPC), covering the control of several thousand samples each year. Conducted annually, the NCPC provide a national overview of the presence of drug residues in food of animal origin produced in Switzerland (see Section 3.2).

1.1 *Regulatory Aspects*

Veterinary medicinal products are regulated in the same way as medicinal products for human use. This is described in the Therapeutic Products Act (TPA – RS 812.21) and the Ordinance on Medicines (OMed – RS 812.212.21). The latter defines the procedure for authorising medicinal products and their conditions of use, whether for human or veterinary medicine. There are currently about 1000 specialities authorised for veterinary use in livestock. The complete and updated list is available on the Swissmedic website.[6]

More specifically, for food and drug residues, there are two specific ordinances. The ordinance on veterinary medicinal products (OMedV - RS 812.212.27) has the following three main objectives: 1) Ensure the correct use of veterinary medicinal products, in particular the judicious and targeted prescribing, dispensing, and use of antibiotics; 2) protect consumers from unwanted residues of veterinary medicinal products in foodstuffs of animal origin; and 3) ensure the supply of quality, safe, and effective veterinary medicines in order to preserve animal health. The second one is the Ordinance on residues of pharmacologically active substances and feed additives in food of animal origin (OResDAlAn – RS 817.022.13). This ordinance sets the maximum limits for residues of pharmacologically active substances, which shall only be present in food of animal origin in quantities which are technically unavoidable and do not present a health hazard. It prohibits marketing of food containing residues that exceed the maximum limit which are banned or not authorised for veterinary use.

2. Veterinary Drugs Use

Worldwide, medicines are applied to numerous types of food-producing animals, such as cows, pigs, poultry, sheep, rabbits, horses, fish and even bees, which may sometimes require treatment even if, as in Switzerland, national regulations limit certain uses. Drugs for veterinary purposes are usually also used in human medicine.[7] There are a few exceptions, such as dihydrostreptomycin, which is a typical veterinary drug. The primary purpose of using a veterinary drug is to protect animal health. An animal should not suffer needlessly, and treatment should be brought to them if they contract diseases or infections. Unfortunately, intensive livestock farming too often involves treatment with medicines, which are sometimes preventively prescribed to prevent epidemics on farms. Fortunately, the use of medicines as growth promoters is now banned in all European countries, including Switzerland, even if this practice continues in some countries, particularly in the USA. There is very little data on the number of drugs used in animal husbandry, except antibiotics, for which much data is available due to mandatory monitoring and controls related to the AMR problem. It is estimated that nearly 80% of the world production of antibiotics is for veterinary use.[3]

According to regulation, all therapeutic treatment should be done under veterinary prescription. The choice and decision to dispense or use drugs is the responsibility of the attending veterinarian after the diagnosis has been made. The veterinarian must weigh the benefits and risks to the animal, humans, and the environment based on their knowledge and the regulations. While the rules are similar in all European countries, there is great disparity in the quantities used from one country to another (Fig. 1).[8] The amount of antibiotics expressed in mg of antibiotics used per PCU (Population Correction Unit; 1 PCU = 1 kg of biomass livestock) is adjusted in relation to the biomass of livestock population. It varies by a factor of more than 100 between Nordic countries, such as Norway (3 mg/PCU), and some southern countries such as Spain, Italy, or Cyprus (>300 mg/PCU). Differences between countries can be explained in part by differences in animal demographics, the climate, choice of antimicrobial agents, and veterinarian prescribing habits and prices. Switzerland is

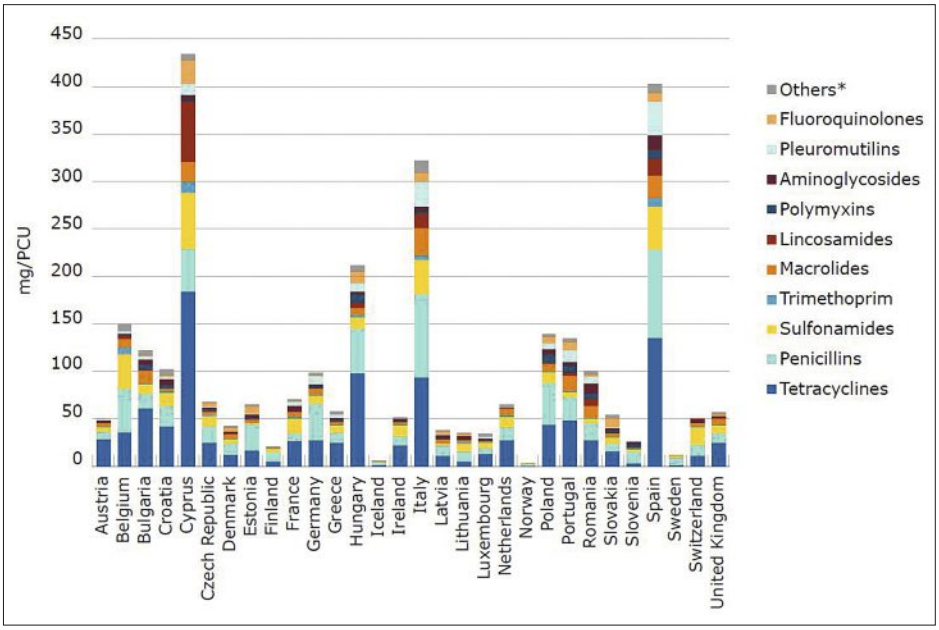

Fig. 1. Sales for food-producing species, in mg/PCU, for the various veterinary antimicrobial classes, for 30 European countries, in 2015. Figure reproduced with permission of Wiley.[7] *Amphenicols, cephalosporins, other quinolones, and other antibacterials (classified as such in the ATCvet system).

approximately in the middle of the ranking currently using ~40 mg/PCU and still has possibilities to improve the situation and reduce antibiotic use.

In Switzerland, sales of antibiotics for livestock use has fallen by more than half in ten years, from nearly 70,000 kg in 2008 to some 32,000 kg in 2017 (Table 1). The amount used has reduced from 86 to 41 mg/PCU. However, this reduction is not identical for all classes of substances. The top selling antibiotics includes sulfonamides, followed by penicillins and tetracyclines, which together account for more than 80% of all antibiotics used. Penicillin consumption has fallen by only 20% in ten years, while macrolide consumption has fallen by over 65% and Colistin consumption by nearly 80%. It is particularly encouraging to note that the sale of so-called critical antibiotics (macrolides and fluoroquinolones), those of first priority for human medicine, have decreased significantly. This decline may be explained by the reduction in sales of veterinary drugs administered with feed to treat whole herds of livestock. Macrolides represent the highest proportion in terms of quantity, as they are often administered as premixed medicated feed. A sharp decline was observed after the entry into force of the revised OMedV on April 1, 2016. Since that date, the release of critical antibiotics as stocks has been prohibited.

3. Control Plan and Monitoring of Drug Residue in Animal Products

3.1 *Analysis of Drug Residue*

Analyses of drug residues in food are very complex to carry out, because on the one hand there are a large number of drugs (and metabolites) to search for and, on the other hand, it is necessary to look for small amounts of substances in complex matrices such as meat, liver, eggs, milk and honey. Another difficulty is that chemists usually never know in advance whether the food sample comes from a treated animal or the nature of the drugs. Laboratories must therefore conduct a blind search, and analytical methods must be highly selective, sensitive and allow for targeting as many substances as possible. Since the 1970s, methods for detecting antibiotics residues were primarily inhibition tests by means of test cultures using various microorganisms, such as *Bacillus subtilis*, *Sarcina lutea*, *Streptococcus thermophilu*s and *Streptococcus lactis* (Fig. 2a). After incubation, the normal growth of the microorganism induces acid production, which turns the purple pH indicator to yellow. In the presence of inhibiting substances, the colour of the medium remains purple. While such rapid and cheap screening tests are still commonly used to detect the presence of antibiotics in milk, they are difficult to apply to more complex matrices such as meat, organs and honey, and they do not provide any indication of which inhibitory substance is present in the sample. Antibody-based screening tests (Fig. 2b) are more specific to a drug family. Such tests provide approximate information on the quantities and substances present or at least indicate which drug family is present. Antibody-based tests depend on each compound and lead to very different sensitivities for each compound. Therefore, interpretation of quantitative results is very hazardous because within the same family of drugs, detection limits, as well as maximum residue limits (MRLs), can be very different. In addition, complex matrices such as foodstuffs regularly lead to false positive results due to cross reactivity with the matrix. According to the European directive 657/2002/EC concerning the performance of analytical methods: "For the required specificity, a method shall be able to distinguish between the analyte and the other substances under the experimental conditions. Confirmatory methods for contaminants are therefore needed to provide information on the chemical structure of the analyte" and, consequently, results obtained by such techniques must be systematically confirmed by another analytical technique.

As regulations became more stringent with respect to MRLs, the need to develop analytical methods for quantitative and unambiguous identification becomes of greater interest. For many years, the use of complex sample preparation methods followed by liquid chromatography (HPLC) with UV or fluorimetric detection analyses, or, even GC-MS methods, after tedious derivatization steps, were the techniques of choice. The disadvantage was that due to the specificity of the sample preparation, specific methods had to be developed for each compound family. Many analytical methods were therefore needed for a single sample, resulting in a relatively low analytical throughput and higher cost of control laboratories.

Chromatography coupled to tandem mass spectrometry (MSMS) or high-resolution mass spectrometry (HRMS) has played an important role in residue analysis. The emergence of these techniques and their routine use in control laborato-

Table 1. Total sales in kg of authorized antibiotics for livestock by active ingredient class from 2008 to 2017.

	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Sulfonamides	29'088	27'231	25'672	23'118	21'556	18'942	17'009	14'959	13'130	10'181
Penicillins	10'827	10'226	10'793	11'023	10'582	10'437	9'893	9'573	9'249	8'644
Tetracyclines	16'704	15'546	14'746	13'731	12'038	11'626	10'398	8'679	8'172	6'851
Aminoglycosides	3'688	3'549	3'215	3'317	3'199	3'115	3'114	3'095	2'988	2'462
Macrolides	4'265	4'003	3'806	3'459	3'289	3'089	2'784	2'610	1'967	1'574
Trimethoprim	1'854	1'749	1'702	1'548	1'368	1'148	1'102	904	829	591
Colistin	1'577	1'543	1'489	1'454	1'057	854	773	502	372	327
Fluoroquinolones	408	403	388	371	335	384	379	384	282	207
Cephalosporins	169	203	237	249	237	228	241	234	190	163
Amphenicols						183	169	199	244	341
Other	263	271	303	616	449	310	241	197	152	181
Total	68'843	64'723	62'350	58'886	54'111	50'316	46'103	41'337	37'575	31'521

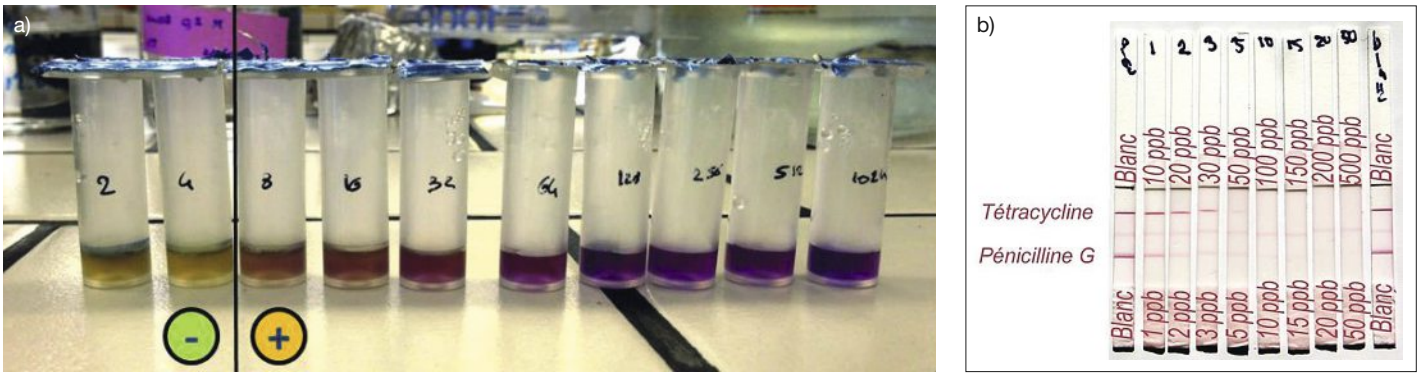

Fig. 2. Non-specific tests for antibiotics in milk. a) Example of an inhibitor test. b) Example of an antibody-based test for tetracyclins and penicillins. QC samples of milk spiked with 10 to 500 ppb of Tetracyclin and 1 to 50 ppb of Penicillin G.

ries since the early 2000s made it possible to develop methods for multitargeting residues with very high selectivity and precise quantification of trace compounds, even in complex food matrices. Nowadays, it has become the gold standard approach for multiresidue analysis, which is required to confirm the results of non-specific screening methods. Fig. 3 shows an example of a chromatogram obtained by UHPLC-HRMS on a real milk sample contaminated with two types of penicillins. Even at the ppb level, the signal to noise ratio is very good, illustrating the extreme sensitivity of these techniques. For specificity, no other peaks interfere and the example shown is quite representative of chromatograms that are usually obtained. Identification of the substances is confirmed by the match of the retention time, but mainly by the comparison of full scan high resolution mass spectra to the reference spectra in HRMS mode or with the fragmentation pattern and relative intensity of fragmentation ions in MSMS mode. Numerous publications[9–17] show that it is now possible to use single methods, allowing in a few minutes to search specifically for several hundred target compounds in complex matrices. The critical point of such methods still remains to find sample preparations that are generic enough to suit all compounds.

3.2 *National Control Plan for Contaminants in Food from Animal Origin*

Every year the Federal Office for Food Safety and Veterinary Affairs (FVO) organises a national control plan for the detection of contaminants in livestock and foodstuffs of animal origin (NCPC). The NCPC controls the use of veterinary drugs in live animals (blood, plasma, serum or urine samples) as well as the presence of residue in animal foodstuffs (meat, liver, kidney, milk, honey, egg). Under the bilateral agreement with the European Union, this allows Switzerland to export its animal products within the EU without constraints. The FVO uses annual slaughter statistics, herd size, and previous years' results to define the number and type of samples to be analysed. Each year, an average of 5000 samples, representative of all animal species, are analysed. Enforcement is the responsibility of the cantonal veterinary offices for sampling and the cantonal food control authorities for residue monitoring. The groups of substances to be analysed in accordance with Directive 96/23/EC are listed in Table 2 and divided into the following two groups: A) substances having anabolic effects and unauthorised substances and B) veterinary drugs and contaminants.

A full report on NCPC results is published annually on the OSAV website.[18] In 2016, 22 samples out of 5127 were found to be non-compliant. The NCPC 2016 non-compliance rate was 0.43% and well within European standards which is generally below 0.5%. Over the last ten years, approximately 100 cases of non-compliant samples have been detected. Half of the cases concerned exceedances of MRLs for antibiotic residues, mainly sulphonamides and tetracyclines, as might be expected according to their very important use. About 20% of the cases concerned the presence of excessive levels of heavy metals (Pb and Cd) and the remaining 30% were for problems related to the use of anthelmintics or thyreostatics.

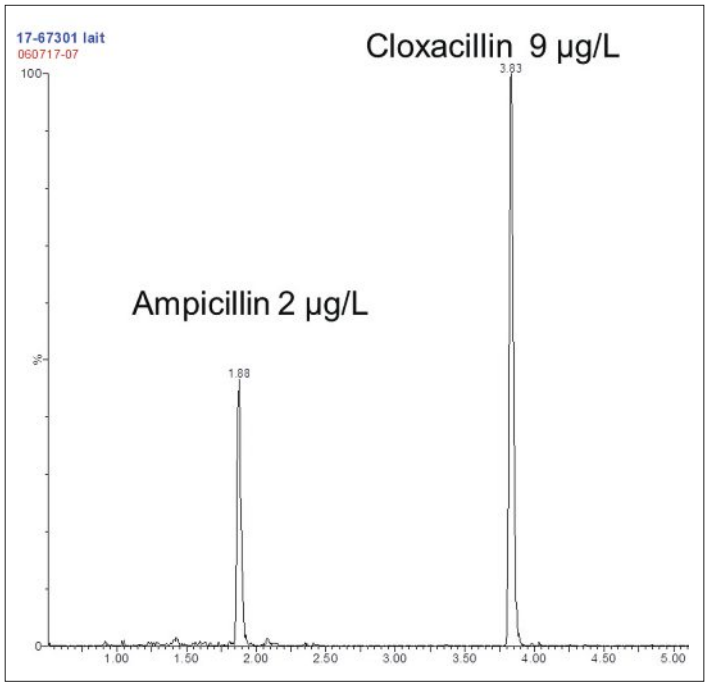

Fig. 3. Typical extracted ion chromatogram of UHPLC-HRMS screening for a milk sample contaminated with residues of two penicillins.

4. Conclusion

In recent years, the use of veterinary drugs in Switzerland has decreased significantly. Effective measures have been taken to reduce their use in animal husbandry as much as possible and a sharp decrease has been observed over the last ten years. Improved analytical techniques and monitoring programmes for drug residues in foodstuffs are effective. The rate of non-compliant samples remains low and shows that consumers do not have too many concerns about the presence of residues in foodstuffs of animal origin produced in Switzerland. Despite the signif-

Table 2. Groups of substances to be analysed in the NCPC according to Directive 96/23/EC

GROUP A — Substances having anabolic effects and unauthorised substances	**GROUP B — Veterinary drugs and contaminants**
A1 Stilbenes	B1 Substances with antibacterial effect
A2 Thyrostatic	B2a Anthelmintics
A3 Steroids	B2b Anticoccidials
A4 Resorcylic acid lactones	B2c Pesticides
A5 β-agonists	B2d Sedatives
A6c Chloramphenicol	B2e Non-steroidal anti-inflammatory drugs
A6n Nitrofurans	B2f Other pharmacologically active substances
A6ni Nitroimidazoles	B3a Organochlorine compounds (*e.g.* PCBs)
	B3b Organophosphorus compounds
	B3c Chemical elements (*e.g.* heavy metals)
	B3d Mycotoxins
	B3e Dyes

icant reduction in antibiotic consumption and the low presence of residues in foodstuffs, the problem of antibiotic resistance remains unresolved. The resistance rate and distribution of multiresistant bacteria has increased steadily, even in countries where there has been a sharp decrease in drug use. The reduction of the AMR will undoubtedly be one of the most difficult challenges to achieve in the food safety area in the years to come to preserve human health and maintain effective antibiotic use for the treatment of infections.

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