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affidia

THE JOURNAL OF FOOD DIAGNOSTICS

01 / 2020

/focus on

The threat of antimicrobial resistance

/case histories

Dioxins in food and feed

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Honey authenticity: scientific, normative, and analytical developments

SUPERBUGS ATTACK: antimicrobial use and antimicrobial resistance

VOL. 2 - NUMBER 1 - JANUARY/FEBRUARY/MARCH 2020
Affidia, The Journal of Food Diagnostics is published 4 times per year.

affidia
your food safety partner

Maurizio Paleologo

Founder and Chief Executive Officer of Affidia. He has nearly 30 years of experience in the field of food diagnostics. He was founder and director of Tecna, a company focused on food chemical contaminants detection.

"Modern factory farming methods could not be carried on without the use of antibiotics."
"The resistant bacteria can be passed direct from animals to man and secondly, they can be passed on in the food so produced."
These sentences are not from one of the authors of this issue of *Affidia*. I am quoting Ruth Harrison from 1964. Between 1960 and 1990, there was a sharp increase in global meat consumption. Western countries and Russia both exported the factory farming system, for which antibiotics were essential. Step by step, antimicrobial resistance (AMR) spread throughout different microorganisms, animal hosts, humans, and the environment. Residues from antimicrobial drugs, largely kept under control in western countries, became a serious problem, especially in imported seafood and honey from Low and Middle Income Countries (LMICs). Now, High Income Countries (HICs) blame LMICs for insufficient regulation of antibiotics. I do think, along with Claas Kirchhelle, that HICs "have a moral responsibility to contain the fallout of these systems in other parts of the world." Sustainable animal farming, molecular methods for tracing AMR, and new diagnostic technologies for the screening of residues are what the food chains need. I think this issue of *Affidia* provides a small contribution to help the community of food quality and safety managers understand the background of AMR risk. As usual, we have international insights about this topic in the US, Russia, and the EU.
In this issue, we start with honey. This gift of nature is for consumers one of the most pure and healthy foods and it has many useful properties. It is also an expensive product. Thus, due to the fact that it is a liquid, it was and is—like wine and olive oil—frequently adulterated. In recent years, antibiotic residues have been found in honey so honey is an important part of this discussion. The horse meat scandal taught us that when there is fraud and when processors or traders operate outside of ethical and regulatory boundaries, chemical contaminants are often a "side-dish" to the main crime.
For some years we have not had a dioxin scandal so I thought this would be a good moment to talk about this risk. I invited experts in this field to help remind us what happened in the past and why, unfortunately, it may happen again.
Last but not least, we have an interesting overview of food pathogens in Europe. This article helps us understand the recent cases of pathogens in the food chain and the growing number of food recalls. Official methods have been updated and whole genome sequencing (WGS) has moved from biotech research and life sciences to food control laboratories. This method gives us new tools to understand links between food born disease cases and we will soon begin using it much more frequently both to safeguard the food supply and to prevent food fraud, as well.
The next issue of *Affidia* will be monographic. We will explore the natural toxins that can contaminate food and feedstuffs, from plant alkaloids to aflatoxins. We are all aware of the risk of these contaminants and we will consider the hidden risks, whether or not regulations are appropriate, how food processors are coping with the issue, and what new analytical methods can offer us in order to monitor these risks in a cost-effective way.

Enjoy our work!

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OF FOOD DIAGNOSTICS

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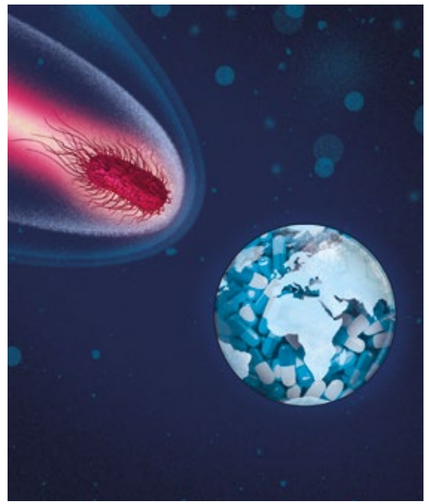
Superbugs attack: antimicrobial use and antimicrobial resistance



Editor-in-chief: Maurizio Paleologo

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Editorial staff: Maurizio Paleologo, Sara Moraca, Davide Zanelli, Laura Petrucco

Editorial contribution to this issue: Madhavi Annamanedi, Shamshul Ansari, Jovan Baryamujura, Peter A. Behnisch, François Bourdichon, Abraham Brouwer, Naomi Bull, Karen Constable, Lutz Elflein, Balázs Horváth, Mashkoor Mohsin, David Psomiadis, Maarten van Dongen, Vlad Vorotnikov, Gary M. Weber

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Illustrations: Paolo Pascutto

Editorial office: AREA Science Park, Padriciano 99, 34149 Trieste (Italy)
magazine@affidia.tech

Sales office: AREA Science Park, Padriciano 99, 34149 Trieste (Italy)
adv@affidia.tech

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The threat of antimicrobial resistance

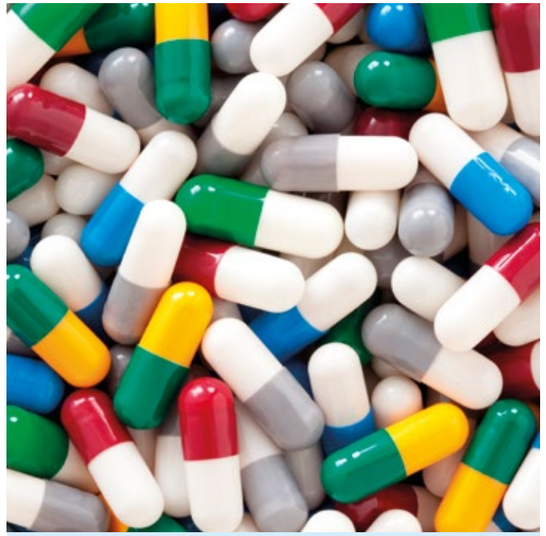
A lot of words, reports, and good will but where is the reduction in agricultural antibiotic use?

Maurizio Paleologo

Antimicrobial Resistance (AMR) is such a threat to human beings that it was described as a "ticking time bomb" (Walsh 2013) and a "global crisis that threatens a century of progress in health and achievement of the Sustainable Development Goals" (United Nations 2019). Every year, in the US, about 3 million people get an antibiotic resistant infectious disease and about 35.000 die (CDC 2019). In the EU, about 33.000 people die every year because of AMR (EU Commission 2020). Worldwide, the total number of deaths per year due to AMR is estimated to be 700.000 (O'Neill 2016) while the economic burden in the EU alone is about 1.5 billion euros/year (EU Commission 2020). The AMR trend is complex; between 2005 and 2015, the first signs that actions to counter AMR were having positive effects appeared in some High-Income Countries (HICs) . In Switzerland, the United Kingdom, Japan, Belgium, Germany, Iceland, and Canada, resistance across eight antibiotic-bacterium combinations fell by an average of 2.5 percentage points (OECD 2016). However, for other HICs during the same period, the index showed a significant increase. In Italy and the Slovak Republic there was an increase of 10.6% (OECD 2016). At the same time, AMR was growing quickly in Low- and Middle-Income Countries (LMICs). 40–60% of recorded bacterial infections in the Russian Federation, India, and Brazil are from antimicrobial-resistant pathogens, compared to an average of 17% in countries belonging to Organization for Economic Co-operation and Development (OECD 2016). If proper steps are not taken, the number of people who will die annually because of AMR by 2050 will reach 10 million (more than deaths due to cancer) and economic losses will reach one trillion USD annually by 2030 (World Bank 2017).

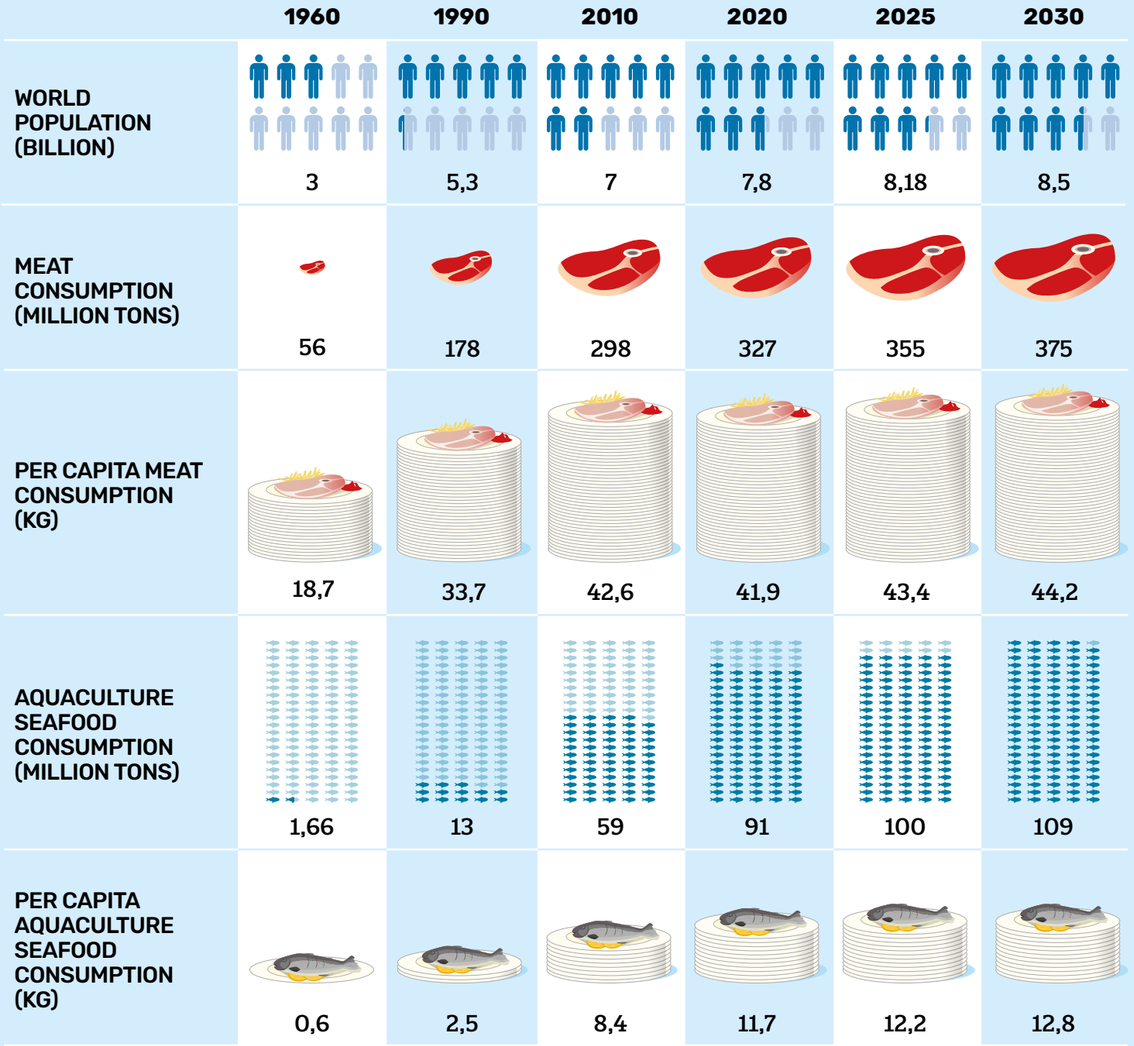
To what extent is AMR due to the use and misuse of antimicrobials in agriculture?

The growth of AMR is due both to the use of antibiotics in humans and on animal farms. While it is easy to understand the impact of therapeutic antibiotic treatment on the people, the pathways for passing antimicrobial-resistant pathogens from



ANTIMICROBIAL RESISTANCE

"Antimicrobial resistance is the ability of microorganisms, such as bacteria, to become increasingly resistant to an antimicrobial to which they were previously susceptible. AMR is a consequence of natural selection and genetic mutation. Such mutation is then passed on conferring resistance. This natural selection process is exacerbated by human factors such as inappropriate use of antimicrobials in human and veterinary medicine, poor hygiene conditions and practices in healthcare settings or in the food chain facilitating the transmission of resistant microorganisms. Over time, this makes antimicrobials less effective and ultimately useless." (EFSA 2019)



Sources: FAO, OECD

FOOD PRODUCTION FROM 1960 TO 2010

The negative consequences of the increase in intensive farmed livestock are:

- a strong contribution to water pollution, with consequent eutrophication of water sources, algal blooms in the sea, and cyanobacteria in fresh water;
- a significant contribution to greenhouse gas emissions (GHG) of at least 8% of the total (Poore and Nemecek 2018) to which must be added the direct and indirect impact due to "land use change";
- the race to productivity with the consequent use of growth promoters, including hormones and antibiotics;
- poor animal welfare that helps spread various infectious microorganisms, and therefore leads to frequent use of antibiotics for prevention;
- drug residues in meat, fish, milk, etc.
- the spread of antibiotics in the environment (see Moraca's article);
- the onset of antibiotic resistance in bacterial populations present on farms, in the environment, and, consequently, in foodstuffs, which has contributed to the overall AMR that endangers humans.

animals to humans are more complex. Resistant bacteria arise both directly in the treated animals and in the environment, especially in freshwater sources, polluted both by farm and human wastes. Antibiotic-resistant genes then move to humans in multiple ways: by direct contact between farmers and animals, through contaminated soil and water, and, even if to a lesser extent, through contaminated foodstuffs.

Data about the amount of Anti Microbial Resistant Bacteria (AMRB) in meat, milk, and aquaculture products are still not regularly produced even in some EU countries. Evidence exists that some food may be contaminated with these bacteria and/or antibiotic resistance genes (ARGs). See the box "AMR from Farm to Fork" for a schematic drawing of the consumer risk associated with AMR in foodstuffs (Jans et al. 2018; Perez-Rodriguez F et al. 2019; Mercanoglu T 2019; Ellis Iversen J et al. 2020).

The heavy toll of antibiotics on the environment is described more extensively by Sara Moraca further in this issue (*page 14*).

The way foodstuffs can contribute to cases of AMR in humans is described in the paper by Shamshul Ansari and the other authors of *Impact of antimicrobial use in animals on antimicrobial resistance in humans* (*page 20*).

Antibiotics in agriculture

The history of antibiotic use in agriculture is the history of factory farms. In the second half of the past century, the per capita consumption of meat grew substantially just as the human population was experiencing its own rapid growth (see infographic). The growth in meat consumption led to the growth in antibiotic use in animal husbandry. Since the 1960s, the concern over chemical residues and then, later, the concern over AMR gradually forced governments to put in place rules to reduce antibiotic use and misuse both for humans and animals. Most HICs are in the process of reducing the amount of antibiotics used by the farms but some countries are still using too many antibiotics and LMICs globally are not reducing the use of antimicrobials at all.

How much agricultural antimicrobial use is really occurring?

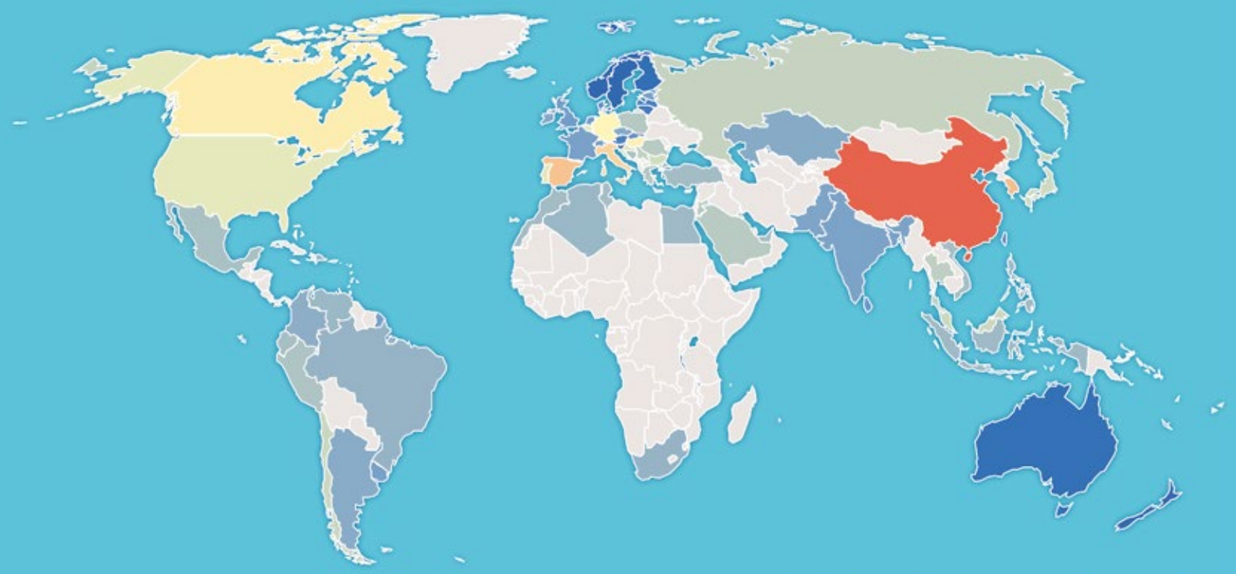
While some studies provide estimates, we don't know the real the amount of antimicrobials being used in agriculture. In many countries, this data is not collected. According to the FAO, just 42 countries collect agricultural antimicrobial use data (FAO 2020). Moreover, the presence in some countries of a black market for antimicrobial drugs makes this figure more difficult to calculate. However, the total amount is estimated at around 150.000 tons per year. A 2013 estimate put the amount used at 131.109 tons (Van Boeckel 2017) and there is general agreement that it was growing then and continuing to grow today, without even considering the black market. 150.000 tons is a lot, especially given the environmental impact of these chemicals, but the amount of farmed animals is also large and still growing. There are about 1.5 billion cows, almost a billion pigs, 1.2 billion sheep, and 23 billion chickens in the world today (FAO 2020).

Is using such an amount of antibiotics unavoidable when producing this much meat and seafood? The answer is no. This is evident when considering the huge differences in quantity used by different countries. Of course, since the number of animals in each country is different, the total amount of antibiotics used per country is not an accurate indicator. The best way to measure antibiotic use is to look at the ratio of the weight of drugs sold to the weight of the animals stocked in a country (mg/kg or mg/PCU, Population Correction Unit[1]). This ratio provides an impressive perspective. Inside Europe, even though there are common minimal requirements for animal welfare, there are large differences, from 3 mg/PCU in Norway to 273 mg/PCU in Italy. On average, in Europe, the value is about 100 mg/kg (ESVAC 2017). China, the main consumer of antibiotics in the world, also uses high amounts per animal at 318 mg/PCU in 2013 (Van Boeckel 2017). As reported in our interviews with representatives of some of the leading European food producers *(page 37)*, an important inter-professional UK association, RUMA, established targets in 2016, in mg/PCU, for the reduction of antibiotic usage in each animal species (RUMA 2017). For instance, in the pig sector, the plan is to reduce antibiotic use from 263 mg/PCU to 99 mg/PCU in five years; in poultry farming, the target for 2018 was 25 mg/PCU. Both sectors were able to reach their targets, as was the aquaculture sector (RUMA 2019). Total antibiotic consumption in Italy dropped from 421 mg/PCU to 273 mg/PCU in 7 years (2010-2017) and in Germany from 211 to 89 mg/PCU (ESVAC 2017). Given that this happened in just the last 10 years in Europe, it is clear that, even in intensive farming, it is possible to make substantial reductions in antimicrobial use.

According to the FAO, just 42 countries collect agricultural antimicrobial use data (FAO 2020).

What are antimicrobials used for?

Antimicrobials can be administered to animals to accomplish different goals *(Fig. 2)*. Apart from therapeutic reasons, the same drugs are used at lower dosages in order to prevent the spread of diseases that are already affecting part of the farm (metaphylaxis) or that could come from other farms (prophylaxis). In addition, there is a fourth reason that has nothing to do with the protection from infectious disease: the promotion of growth. At low dosages, a number of antibiotics and antiparasitic drugs increase animal yield. The use of Antibiotics as Growth Promotors (AGP), although at very low dosages, is a well-known



Reproduced with kind permission from the authors. The Center for Disease Dynamics, Economics & Policy. ResistanceMap: Animal use and resistance. 2020. Available from https://resistancemap.cddep.org/Animals.php. Date accessed: 12.03.2020. (Original consumption data from Van Boeckel et al 2015.)

Fig. 1: Antimicrobial consumption in Livestock, estimates for 2013.

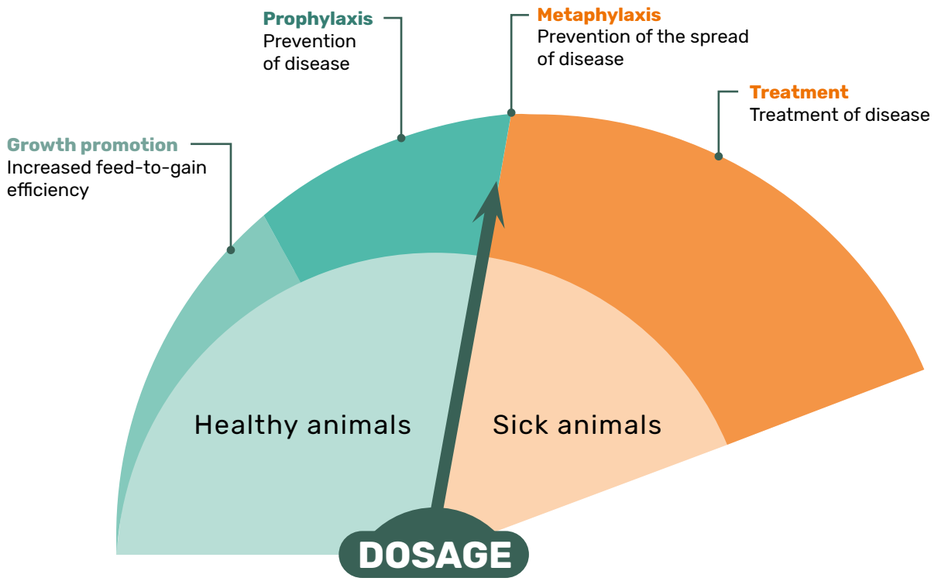


Fig. 2: Use of antimicrobial in livestock (redraw from O'Neil 2016).

source of AMR (You and Silbergeld 2014). The EU banned AGP in 2016 and the US has moved in this direction but AGP is still not illegal there (it was introduced as a so-called voluntary ban) but in the majority of countries this use of antibiotics is still allowed.

Which antimicrobials should be used?

Quantity is important, particularly when animal manure spread on fields contributes significantly to AMR pollution. However, which antibiotics are used is also an important consideration. Avoiding the agricultural use of drugs that are important for human therapy is a clear and urgent priority. The WHO has made this goal clear for years and, step by step, public health authorities, even in LMICs, are banning some antibiotics or asking veterinarians to prescribe them only in very special cases. The WHO introduced the concept of Critically Important Antimicrobials (CIA). Within the CIAs, some drugs have been classified as Highest Priority Clinically Important Antimicrobials (HPCIA) (WHO 2019). According to the WHO, the list is "to be used as a reference to help formulate and prioritize risk assessment and risk management strategies for containing antimicrobial resistance mainly due to non-human use." The HPCIA category includes quinolones, 3rd- and higher generation cephalosporins, macrolides and ketolides, glycopeptides, and polymyxins. In Europe, the EMA developed a categorization of all antimicrobials that can be used on animals to provide guidelines for veterinarians. In this table *(see fig. 4)* antimicrobials are divided into 4 classes according to their impact in terms of AMR:

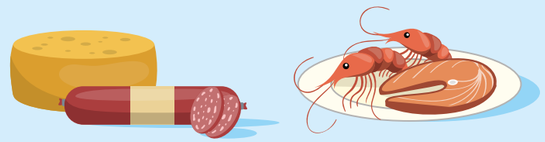
1) to be avoided
2) restricted
3) to be used with caution
4) to be used with prudence (EMA 2020).

1 - PCU is a standard unit of measure that takes into account the number of animals in a country and their average weight at the point they are most likely to be treated, providing an estimate of total kg of food-producing animals in a country.

AMR FROM FARM TO FORK

Several strains of Enterococchi, salmonella, campylobacter and other bacteria bearing multiple resistance to antimicrobials can contaminate foodstuffs. Assigned risk values are derived from evidence commonly cited in the literature.

High risk of AMR trasmission
We do not cook fermented, pre-boiled, and smoked foods.



Low risk of AMR trasmission
Vegetables are often eaten raw, but the occurrence of contamination is lower than in food from animals.

Low risk of AMR trasmission
Heating kills bacteria but DNA could move to our gut.

CATEGORISATION OF ANTIBIOTIC CLASSES FOR VETERINARY USE

(with examples of substances authorised for human or veterinary use in the EU)

A	**Amdinopenicillins** mecillinam pivmecillinam	**Carbapenems** meropenem doripenem	**Drugs used solely to treat tuberculosis or other mycobacterial diseases** isoniazid ethambutol pyrazinamide ethionamide	**Glycopeptides** vancomycin	AVOID
	Ketolides telithromycin	**Lipopeptides** daptomycin		**Glycylcyclines** tigecycline	
	Monobactams aztreonam	**Oxazolidinones** linezolid		**Phosphonic acid derivates** fosfomycin	
	Rifamycins (except rifaximin) rifampicin	**Riminofenazines** clofazimine	**Other cephalosporins and penems (ATC code J01DI), including combinations of 3rd-generation cephalosporins with beta lactamase inhibitors** ceftobiprole ceftaroline ceftolozane-tazobactam faropenem	**Pseudomonic acids** mupirocin	
	Carboxypenicillin and ureidopenicillin, including combinations with beta lactamase inhibitors piperacillin-tazobactam	**Sulfones** dapsone		**Substances newly authorised in human medicine following publication of the AMEG categorisation** to be determined	
		Streptogramins pristinamycin virginiamycin			
B	**Cephalosporins, 3rd- and 4th-generation, with the exception of combinations with beta-lactamase inhibitors** cefoperazone cefovecin cefquinome ceftiofur	**Polymyxins** colistin polymyxin B	**Quinolones: fluoroquinolones and other quinolones** cinoxacin danofloxacin difloxacin enrofloxacin flumequine ibafloxacin marbofloxacin norfloxacin orbifloxacin oxolinic acid pradofloxacin		RESTRICT
C	**Aminoglycosides (except spectinomycin)** amikacin apramycin dihydrostreptomycin framycetin gentamicin kanamycin neomycin paromomycin streptomycin tobramycin	**Aminopenicillins, in combination with beta lactamase inhibitors** amoxicillin + clavulanic acid ampicillin + sulbactam	**Amphenicols** chloramphenicol florfenicol thiamphenicol	**Macrolides** erythromycin gamithromycin oleandomycin spiramycin tildipirosin tilmicosin tulathromycin tylosin tylvalosin	CAUTION
		Cephalosporins, 1st- and 2nd-generation, and cephamycins cefacetrile cefadroxil cefalexin cefalonium cefalotin cefapirin cefazolin	**Lincosamides** clindamycin lincomycin pirlimycin		
			Pleuromutilins tiamulin valnemulin	**Rifamycins: rifaximin only** rifaximin	
D	**Aminopenicillins, without beta-lactamase inhibitors** amoxicillin ampicillin metampicillin	**Aminoglycosides: spectinomycin only** spectinomycin	**Sulfonamides, dihydrofolate reductase inhibitors and combinations** formosulfathiazole phthalylsulfathiazole sulfacetamide sulfachlorpyridazine sulfaclozine sulfadiazine sulfadimethoxine sulfadimidine sulfadoxine sulfafurazole sulfaguanidine sulfalene sulfamerazine sulfamethizole sulfamethoxazole sulfamethoxypyridazine sulfamonomethoxine sulfanilamide sulfapyridine sulfaquinoxaline sulfathiazole trimethoprim		PRUDENCE
	Tetracyclines chlortetracycline doxycycline oxytetracycline tetracycline	**Anti-staphylococcal penicillins (beta-lactamase-resistant penicillins)** cloxacillin dicloxacillin nafcillin oxacillin			
	Natural, narrow-spectrum penicillins (beta lactamase-sensitive penicillins) benzathine benzylpenicillin benzathine phenoxymethylpenicillin benzylpenicillin penethamate hydriodide pheneticillin phenoxymethylpenicillin procaine benzylpenicillin		**Cyclic polypeptides** bacitracin	**Nitroimidazoles** metronidazole	
			Steroid antibacterials fusidic acid	**Nitrofuran derivatives** furaltadone furazolidone	

< Fig 3: EMA classification of antimicrobial drugs (EMA 2020).

Still, while the US decided to ban the agricultural use of fluoroquinolones because they are useful in human medicine, Europe did not and even if the EMA classified them as "restricted", they are still used widely in the UK poultry sector. It is the same case with cephalosporins.

The trend

Recent studies find that the global use of antibiotics in animal husbandry will not decrease; rather, it is expected to grow, especially because of China's antibiotic practices (Van Boeckel 2017). According to the FAO, the amount of antimicrobials used in agriculture will double in the next 20 years (FAO 2019). In fact, the increase in antimicrobial consumption in Brazil, Russia, India, China, and South Africa was forecasted to be 99%, up to seven times the projected population growth in this group of countries (Van Boeckel 2015).

Considering this trend, actions are necessary in order to minimize the presence of antibiotic residues in foodstuffs and to prevent a situation where in 20 to 30 years AMR will cause more deaths than cancer.

China reacted quickly in the face of evidence of rapidly occurring antibiotic resistance to colistin (Liu YY et al. 2016), an HPCIA polymyxin antibiotic but much work has yet to be done in all LMICs in order to reduce the amount of antibiotic use just as much work remains everywhere to safeguard the effectiveness of HPCIAs for human use.

Ongoing actions

It is interesting to underscore that the concern over AMR and the calls for action are far from new. In the 1960s, more than half a century ago, particularly in the UK, scientists and public health authorities were well aware of the consequences of intensive animal farming and the misuse of antibiotics, especially after discovering "horizontal resistance proliferation". This is clearly documented in the Swann Report where it was recommended to limit veterinary prescriptions of antibiotics that were medically relevant for human health (Swann 1969).

However, only in the past 10 years have the WHO, the FAO, and the OIE begun taking the AMR problem seriously and begun issuing action plans and guidelines. A milestone is the Action Plan issued in 2015 by WHO, in which the "One Health" approach was launched (FAO 2015).

Still, at the national level, not much has been done in the agricultural sector. "While the majority of the top 10 chicken-, pork- and cattle-producing countries that responded to the survey (9 out of 10) have at minimum developed a national action plan..., the survey response shows that in almost all domains – surveillance, education, monitoring and regulating consumption and use – more activity can be seen in the human sector. There is an urgent need for resource prioritization and more action in the animal and food sectors.

According to the FAO, the amount of antimicrobials used in agriculture will double in the next 20 years.

Only 64 countries (41.6%) have limited the use of critically important antimicrobials (human and animal) for growth promotion in agriculture" (WHO, FAO, OIE 2018). When it comes to environmental contamination from antimicrobial use, only a few countries have regulations to limit this pollution. "This level of regulation is insufficient to protect the environment from the hazards of antimicrobial production" (ibid.). In many countries the quality, safety, and efficacy of veterinary medicines is not controlled and counterfeit medicines are even sold (ibid.). These conditions contribute to the increasing use of antibiotics in agriculture globally, as seen in *Figure 4* (Van Boeckel 2017).

Conclusion: what really matters

Although slowly, governments are now issuing regulations that should reduce AMR. However, if consumers are not properly informed, they will not care whether regulations are strict enough or how they are implemented. A clear example comes from Russia, where rules about the sales of drugs are largely ignored *(see the article by Vorotnikov in this issue, page 31)*. For this reason, the media and consumer associations should play a key role in informing consumers. As Dr. Alborali points out in this issue *(see interview on page 36)*, training and education, particularly at the

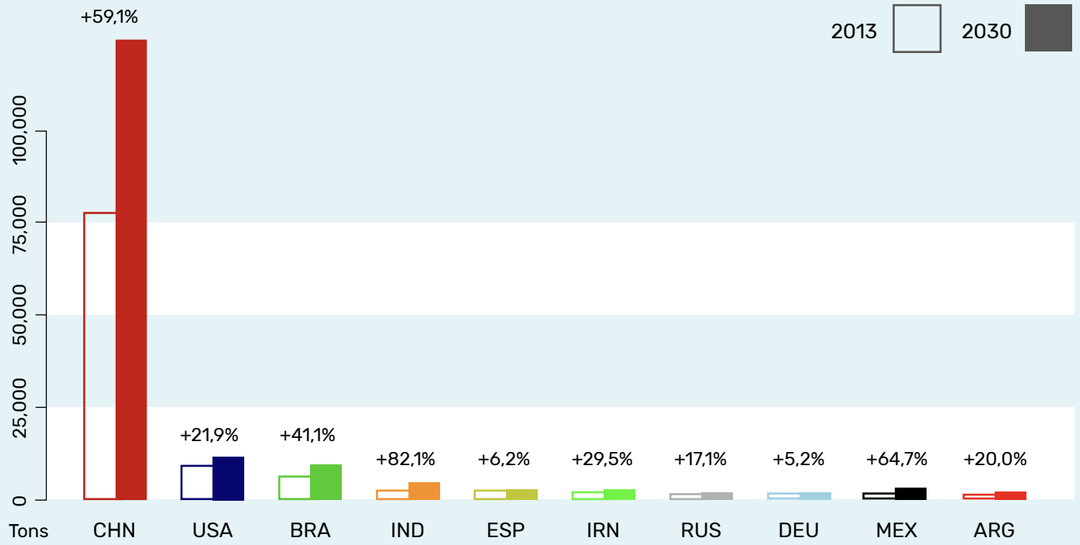


Fig. 4: Antimicrobial consumption in largest consumers in 2013 and 2030 (Van Boeckel 2017).

farm level (often it is just technicians who are making treatment decisions), are the keys for ending AMR. We do believe that more research and more education of operators will be more effective than user fees on drugs or other actions recently suggested in the literature (Van Boeckel 2017). At the same time it would be important to control more AMRB and AMRG on foodsuffs, particularly on food imported from countries where regulations are still not in place or not implemented, as well to use Whole Genome Sequecing (WGS) to map and traceback the spreads of Multi Drug Resistant (MDR) pathogens, as suggested by other recent studies (Baker et al. 2017).

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The environmental impact of antimicrobial use and abuse

The presence of antibiotics in fresh water: the global antimicrobial resistance threat, inadequate legislation, and technical gaps in water treatment systems.

Sara Moraca

Sara Moraca is a PhD Scholar at University of Bologna and a scientific journalist. She collaborates as expert of science communication with newspapers, magazines, universities and institutions.

The worldwide use and abuse of antimicrobials in the past 50 years has had a heavy impact on the planet's ecosystems. Antimicrobial-resistant genes, already present in bacteria, are now more widely present and threaten humans by contaminating foodstuffs and sources of drinking water. The One Health approach is the only plan that will lead to a safer environment.

In a recent literature survey of 236 published reports from 41 countries, water contamination by pharmaceuticals including antibiotics was extensive due to widespread consumption and subsequent disposal to rivers. Of the 61 substances considered, 25 were antibiotics; ciprofloxacin had the highest concentration among antibiotics at 6.5 mg/l (Goel 2015).

According to the literature, most antibiotics flow into fresh water via effluents from three sources: wastewater treatment plants (WWTP), chemical manufacturing plants, and animal husbandry and aquaculture.

Global usage of antibiotics for human therapy grew by around 65% between 2000 and 2015. Defined Daily Doses (DDD) of antibiotics have increased from 21.1 billion to 34.8 billion for humans (Klein 2018). Additionally, more than half of global antibiotic production is for farm animals (WHO 2015).

Despite the wide-scale adoption of antibiotic use in food animals, official data about the quantity and patterns of worldwide use are not available, just as there are no estimates in the literature regarding the discharge of antibiotics into the environment from these activities. However, according to a well-documented estimate reporting data back to 2013, total annual worldwide consumption of antibiotics by animals is around 130.000 tons (van Boeckel 2017). In the United States, according to data gathered about 10 years ago by the pharmaceutical industry-sponsored Animal Health Institute, about 8.000 tons of antimicrobials were used for animals, of which 1.400 were for non-therapeutic use (Mellon et al. 2001). In the last 30 years, use of penicillin-type drugs in farm animals has increased by 600% and use of tetracyclines by 1500% (Mohanta et al. 2012).

Many studies have also shown the increasing use of antibiotics in aquaculture but to date there has been no harmonization between the various existing surveillance systems and

Despite the wide-scale adoption of antibiotic use in food animals, official data about the quantity and patterns of worldwide use are not available.

a clear communication limit between institutions remains (Manage 2018).

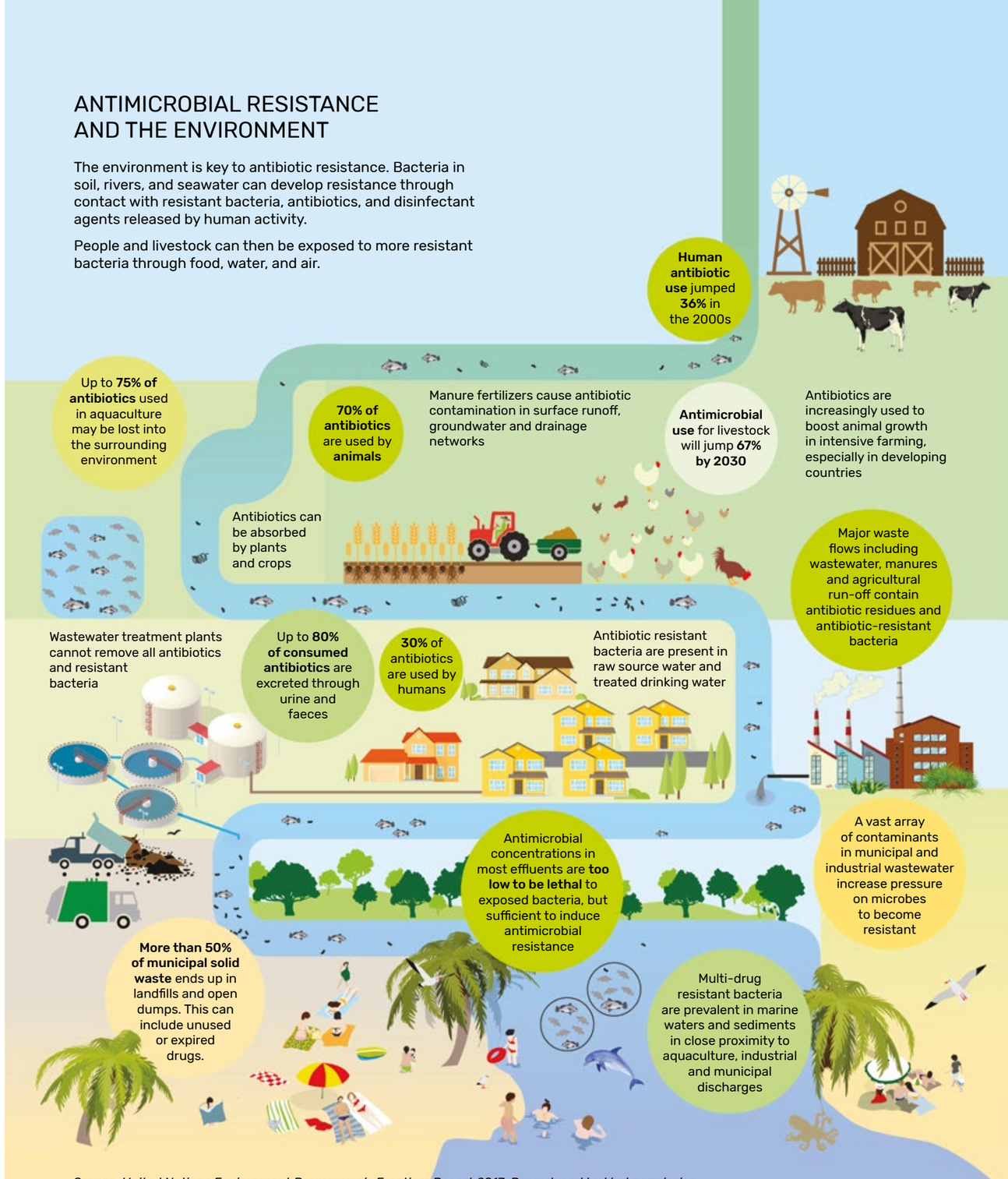
As for WWTPs, some studies provide evidence that between 50 and 80% of the antibiotics taken by people end up in the sewage system because they are expelled from the body through catabolic processes (Kummerer and Henninger 2003, Kummerer et al. 2009). These rates vary according to the type of antibiotic: ciprofloxacin ranges from 50% to 80% and tetracycline ranges from 80% to 90% while lower excretion rates are observed for antibiotics like erythromycin (5 to 10%), sulfamethoxazole (15 to 30%), and clarithromycin (25%). Even if purification systems use abiotic or biotic degradation systems, antibiotic molecules are often stable enough to bypass the degradation systems and be released into the environment. Although the environmental concentration is much lower than those that are pharmacologically active in the human body, it is clear that the presence of antibiotics in fresh water can have an impact on human health and ecosystems.

Research indicates that antibiotics residing in sediments can

seriously alter the microbial and microalgae flora of the ecosystem. Particular antibiotic cocktails could pose elevated ecological risks for aquatic ecosystems (Pleither et al. 2013).
A study published last May, coordinated by the University of York, provided evidence that streams worldwide are highly contaminated with antibiotics and in some cases the concentration is 300 times higher than what the AMR Industry Alliance, an organization that brings together pharmaceutical companies in more than 20 countries, considers safe (SETAC 2019). The AMR Industry Alliance, in fact, has developed Antibiotic Discharge Targets, a unified approach for establishing discharge targets in antibiotic manufacturing. These targets are referred to as Predicted No-Effect Concentrations (PNECs) for use in environmental risk assessments of antibiotics. The thresholds are just a few µg/l for most of the antibiotics except for Sulfadiazine, which is set at 720 µg/l. The situation is worst in Asia and Africa where the infrastructure for waste and waste water treatment is lacking or not working. The problem, however, is global and poses a threat to

It is clear that the presence of antibiotics in fresh water can have an impact on human health and ecosystems.

humanity's future. The presence of such concentrations can, in fact, cause some bacteria to develop a resistance to life-saving medicines and make them ineffective. If no immediate action is taken, more than ten million people could die annually from antibiotic-resistant bacteria by 2050 (United Nations 2016).
An analysis of 15 Chinese recharge sites for analysis of the presence of the 20 most commonly used antibiotics shows that sulfamethoxazole, erythromycin, and ofloxacin had the top three hazard quotient values (Ma et al. 2016). Scientists measured the presence of antibiotics in 711 rivers in 72 countries including the Tiber, the Thames, the Danube, the Tigris, and the Mekong, the most important watercourse in Indochina (SETAC 2019). Many of these had never been monitored before. Teams collected samples, froze them, and sent them to York for analysis. The results showed that 65% of the rivers were contaminated and in 111 cases the concentration was higher than what is considered safe. The worst situations were found in Ghana, Pakistan, Nigeria, Kenya, and Bangladesh. In the latter case, researchers found that the levels of metronidazole, an antibiotic used in aquaculture systems, was 300 times higher than normal.
In Europe it is a little better. Researchers found quantities considered dangerous in only 8% of cases. In the Danube, for example, researchers found that levels of antibiotics, including clarithromycin, were enough for bacteria to develop resistance to certain medicines normally used to treat respiratory tract infections, such as bronchitis and pneumonia.
The researchers found that the most contaminated rivers are those found in conflict zones, alongside waste or sewage landfills, and in countries where waste management and wastewater treatment infrastructure often don't work. In Kenya, for example, where sewage and waste - often untreated - are thrown directly into the sea, antibiotic levels were 100 times higher than those considered safe. John Wilkinson, a researcher in the Department of Environment and Geography at the University of York and co-author of the study, says that it is impossible to solve the problem without investing in infrastructure for the treatment of waste and waste water, cleaning already-contaminated sites and adopting more strict regulations.
Another recent literature review on the topic (Danner 2019) shows antibiotic concentrations of up to 15 µg/l in freshwater in the Americas with lower concentrations reported in European countries (over 10 µg/l) and higher concentrations in African and Asian-Pacific countries (50 µg/l and over 450 µg/l, respectively) .
A study published in *The Journal of Microbiological Methods* and led by the University of Central London found that bacteria in central London's freshwater sources contain high levels of Antibiotic Resistant Genes (ARG) with the River Thames containing the highest amount of bacteria resistant to common antibiotics such as penicillin, erythromycin and tetracycline (Xu et al. 2019). The presence of antibiotics in these rivers and ponds provides a chance for microbes to develop resistant genes, multiply quicker, and share their resistance with other microbes. According to the AMR Industry Alliance 2020 progress report: "*The spread of antimicrobial resistance (AMR) is undermining the efficacy of these medicines and procedures and presents a growing threat to global public health and human development. Antimicrobial resistance is a natural process. It occurs when a micro-organism harmful to humans (e.g. bacteria, viruses, fungi, and parasites) evolves to prevent an antimicrobial (e.g. an antibiotic, antifungal, antiviral, or antiparasitic) from working against it. However, this natural process has been accelerated by the inappropriate use of antimicrobials, in both humans and animals.*"
This emphasizes the need for more research on how water is treated, especially concerning methods of removing antibiotics. According to the literature, there are few technologies suitable for removing antibiotics from the environment today. There is also no legislation establishing that it is necessary to remove antibiotics or microbes with Antibiotic Resistant Genes (ARGs) from water sources. As a result, antibiotics and microbes with antibiotic-resistant genes could be present in drinking water, although testing is required to establish this. Antibiotic-Resistant Bacteria (ARB) and ARGs pose a public health concern when they transfer Antibiotic Resistance (AR) to human pathogens. Very few regulations exist that specifically limit the discharges of active pharmaceutical ingredients. This is true not only for countries that have a high rate of emissions and pollution such



Source: United Nations Environment Programme's Frontiers Report 2017. Reproduced by kind permission.

Antibiotics and microbes with antibiotic-resistant genes could be present in drinking water, although testing is required to establish this.

as India and China, but also in Europe and the United States. There are also no reports of major industrial discharges of pharmaceutical products from these regions.

To calculate the risk to human health, precise estimates of exposure are necessary, but there are still no unequivocal data on the correlation between the presence of ARB and ARGs in source water or drinking water treatment plants and the risk of human exposure to pathogenic ARB. Antibiotics can also find their way into the environment from pharmaceutical production plants. A study conducted at a pharmaceutical plant in Lahore (Pakistan) made it possible to establish that antibiotic contamination in the discharge water in that area was 1000 times higher than elsewhere (Hussain 2016). An important report published by the campaigning organization Changing Markets in 2016 has revealed for the first time the presence of drug-resistant bacteria at pharmaceutical manufacturing sites in India. The team, under the supervision of Dr. Mark Holmes from the University of Cambridge, sampled 34 sites close to Hyderabad, New Delhi, and Chennai: 16 of these sites were found to be harboring bacteria resistant to antibiotics (Changing Markets 2016). The effects of antibiotics are extremely context-dependent, with implications for the microbial food web, larger organisms, and ecosystem health. To date, very little is known about the redistribution of antibiotics in the trophic network because laboratory experiments cannot fully capture what occurs in a marine or river ecosystem where predators are also present and where multiple classes of antibiotics are detected simultaneously. A mix of substances, even if found only in low concentrations, can be lethal to organisms where the presence of just a single antibiotic may not be. The antibiotic resistance of the microbial network should be assessed over time and should include consideration of water temperature and any anthropogenic stressors. In this context, therefore, there is a need for research that is able to delineate the effects of antibiotic cocktails, a need to carry out context analysis on the trophic chain for the reactions that would take place in the real world and not in the laboratory, as well as a need to push for a new regulatory framework that lays the foundation for a momentum of innovation for more complex detection and removal systems than the few existing today.

This is not enough: with an estimated global mortality of around 700.000 deaths per year (O'Neill 2016), antimicrobial resistance is becoming a global threat, which national and international health organizations are preparing to face. To highlight this emergency, a list of antibiotic-resistant "priority pathogens" has been published by WHO, it contains 12 families of bacteria that represent the greatest danger for human health (WHO 2017).

It is important to remember that some of the genes that made it possible to develop antibiotic resistance have been present in nature since before antibiotics made their appearance. Some scholars emphasize that this may have resulted from human exposure to antibiotic substances - such as tetracycline - through nutrition or ancient natural remedies. From this emerges the need to identify pathogens with greater precision and limit the use of antimicrobials that could favor the acceleration of new resistance mechanisms. Bacteria have four billion years of evolution behind them and some researchers suggest it is necessary to study this complexity in order to respond appropriately (Aminov 2010).

One strategic approach, which is increasingly necessary, is "One Health". The goal of One Health is to obtain positive human and animal health outcomes while also taking into account the surrounding environment. It is a holistic approach and it is urgent that it be implemented at every level of the supply chain, especially considering that six out of ten human infections are of animal origin (WHO 2008).

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/focus on

Impact of antimicrobial use in animals on antimicrobial resistance in humans

Antimicrobial resistance is selected for in human and veterinary medicine alike, and resistance may be transferred from animals to humans and vice versa.

Shamshul Ansari[1], Naomi Bull[2], Madhavi Annamanedi[3], Jovan Baryamujura[4], Mashkoor Mohsin[5], Maarten van Dongen[6]

1 Department of Microbiology, Chitwan Medical College, Chitwan, Nepal;
2 Department of Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom;
3 National Institute of Animal Biotechnology (NIAB), Hyderabad, India;
4 Infectious Diseases Institute-IDI Knowledge Centre, Makerere University, Kampala, Uganda;
5 Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan;
6 AMR Insights, Utrecht, The Netherlands.
The authors are all members of the global network of AMR Insights Ambassadors.
For more information about this network: www.amr-insights.eu/about-us/ambassadors

Introduction

Antimicrobial (antibiotic, antifungal, antiviral, and antiparasitic) resistance (AMR) is defined as the development in microorganisms such as bacteria, fungi, viruses, and some parasites of traits that prevent antimicrobials from working against these microorganisms. Antimicrobial resistance occurs naturally. However, (mis)use and overuse of antimicrobials in humans as well as in animals has been shown to accelerate the selection and emergence of resistant microorganisms. Antimicrobial resistance may be selected for in human and veterinary medicine alike, and resistance may also be transferred from animals to humans and vice versa. AMR is currently considered one of the biggest threats to global health and food security.

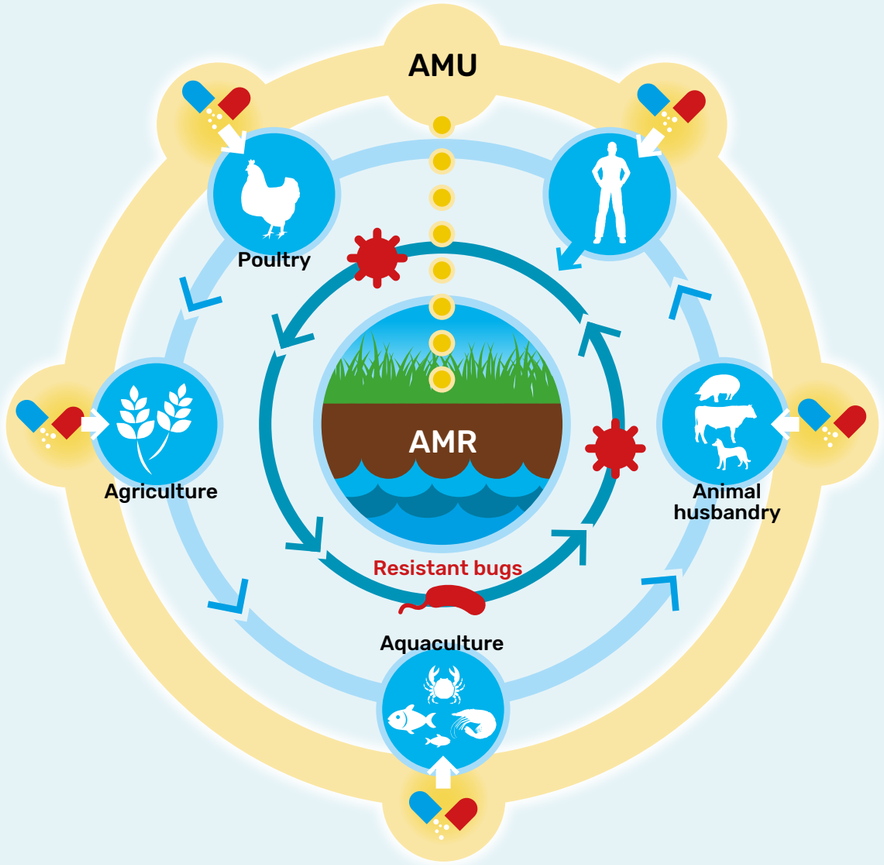
Use of antimicrobials in animals

The vast majority of antimicrobials administered to animals are used in the livestock sector where they are given for three major reasons: treatment of infection, prevention of infection (prophylaxis), and for growth promotion to increase weight gain in animals reared for food. In some parts of the world, use in the livestock sector has risen in the last century due to increasing intensification of livestock production. This is primarily due to a historical trend toward highly intensive animal production systems using more antimicrobials rather than less intensive systems. However, in many parts of the world the use of antimicrobials in animals has substantially decreased in recent times. In Europe, for example, the use of therapeutic and prophylactic antimicrobials in animals fell by more than 32% between 2011 and 2017 (European Medicines Agency 2019). This has been possible due to the implementation of higher biosecurity measures alongside improved husbandry and management practices, which together have led to a reduction in antimicrobial use in many countries (Laanen et al. 2013; Postma et al. 2016).
The use of antimicrobials for prophylaxis and growth promotion in livestock is a controversial practice as it involves administration of antimicrobials to healthy animals. Since any administration of antimicrobials increases the chance of resistant microbes developing, it is vital that their use is prioritised to those situations where they are most needed. In Europe, the use of antimicrobials for growth promotion was banned in 2006 and a large number of Asian countries have also already banned, or are in the process of banning, the use of antimicrobial growth promoters.
Antimicrobials are also used in companion animals; however, overall quantities administered to pets are significantly lower than those used in the livestock sector. Generally, antimicrobial use for pets mirrors use in humans, with administration most common for treatment of infections as well as pre- and post-surgery. Strategies similar to those proposed for reduction of antimicrobial use in humans should also be effective in this sector, with higher rates of vaccination, better rapid diagnostics, and improved awareness for veterinarians and pet owners all predicted to help reduce unnecessary use.

It has long been established that farmers and farm workers have higher levels of AMR than people who do not live in the proximity of livestock.

Transmission of AMR from animals to humans

Antimicrobial resistance arises as a result of natural selection. Bacteria and other microorganisms begin counteracting the



TRANSMISSION OF ANTIMICROBIAL RESISTANCE BY SPREADING RESISTANT MICROORGANISMS

effects of antimicrobials and develop resistance under selective pressure: among the population of microorganisms, some strains acquire resistance genes via mutations in the genetic material. These mutations are further carried or transferred to other bacteria through horizontal gene transfer and also passed on to the offspring by vertical transfer. The transfer of resistance between animals and humans has been studied extensively.

There are actually three different transmission routes, by which an exchange of resistance may come about:

_ 1. Direct Transmission

Resistant traits (bacteria or genes) may be transferred from animals to humans and vice versa through direct contact. This comes as no surprise, since every contact between living beings results in an exchange of bacteria and other microorganisms. Whether milking cows or handling pigs, whenever a human touches an animal or has close contact with animals, bacteria and microbial resistance genes alike will be exchanged. It has long been established that farmers and farm workers have higher levels of AMR than people who do not live in the proximity of livestock. Likewise, hospitals can act as a hotspot for AMR, exposing both humans and animals that live nearby. Companion animals should not be overlooked in the whole debate concerning transmission of resistance either. Direct contact between pet owners and their pets is very natural, but it also provides an excellent opportunity for transmitting resistance. It therefore comes as no surprise that an increasing amount of scientific literature describes the resistance transmission from companion animals to humans and vice versa. For methicillin resistant *Staphylococcus aureus* (MRSA), this direct contact between animals and humans may be the major route of transmission.

Vegetables are not exactly safe either; bacteria harbouring resistance genes have been found on and in vegetables.

_ 2. Transmission via food

AMR can also make its way to humans through the consumption of food that contains resistant microorganisms or genes. The most obvious route of foodborne transmission seems to be the consumption of meat, milk, or eggs. Yet, if these animal products are for instance cooked or pasteurised, and if hygienic measures are well respected in the kitchen, there will be little or no transfer of (resistant) microorganisms. The consumption of raw animal products, however, involves a higher risk of transfer. Vegetables are not exactly safe either; bacteria harbouring resistance genes have been found on and in vegetables. This may be caused, for instance, by using manure as a fertiliser or by irrigating with contaminated water. Eating raw vegetables is thus not totally risk-free. The food-borne route is perhaps the most important for enteric bacterial pathogens, such as *Salmonella enterica, Campylobacter jejuni,* and *Yersinia enterocolitica.*

_ 3. Transmission via the environment

A final route of transmission is through the environment. Bacteria living in the soil, for instance, may become antibiotic resistant through the transfer of resistance genes from human or animal bacteria or through residues of antibiotics (e.g. in manure) that end up on the land. Whenever contact with a contaminated environment occurs, resistance may be transferred.

AMR in humans due to animal/veterinary use of antimicrobials

There is evidence of adverse human health consequences due to resistant microorganisms resulting from usage of antimicrobials in animals. Three important bacterial genera - Enterococcus, Escherichia, and Campylobacter - and, to a lesser extent, Salmonella and Clostridium, are normal gut flora of food animals though they can also be serious human pathogens. The majority of studies have investigated the transmission of antibiotic-resistant bacteria from animals to farm workers, frequently before and after the introduction of antibiotics at their workplace. Direct spread of resistant bacteria from animals to people was first reported by Levy et al. who found strains of tetracycline-resistant *E. coli* in the gut flora of chicken caretakers similar to the strains found in the chickens receiving tetracycline-laced feed (Levy et al. 2019). Advances in genetic methods of analysis offer stronger evidence for the animal origin of bacteria that inhabit or infect humans. The rise of antibiotic-resistant bacteria in farm animals and consumer products like meat and fish has been documented. One study found ciprofloxacin-resistant *Campylobacter spp.* present in 10% to 14% of consumer chicken products tested (Gupta et al. 2019). There was also a correlation found between contamination of retail chicken with ceftiofur-resistant bacteria *Salmonella enterica* and incidence of human infections related to this type of isolate across Canada (Dutil et al. 2010). In three countries (the United States, Spain, and the Netherlands) a close temporal relationship has been recognized between the introduction of fluoroquinolone (sarafloxacin and enrofloxacin) therapy in poultry and the development of fluoroquinolone-resistant Campylobacter in human infections (Smith et al. 1999). Molecular and epidemiological tracking support the hypothesis that the resistance genes present in Salmonella outbreaks in humans and animals in Europe and the United States likely originated in aquaculture farms in East Asia (Cabello 2006).

Impact of AMR in humans due to animal use of antimicrobials

Development of AMR in pathogenic microorganisms is a major public health problem that demands the most urgent attention in global health security. According to the WHO, diseases caused by foodborne pathogens are becoming more difficult or even impossible to treat because of their increasing resistance to antibiotics (WHO 2018). The food animal pathogens which commonly cause livestock-associated infections of the gastrointestinal tract as well as other parts of the body, such as *Staphylococcus aureus (S. aureus), Campylobacter spp., Salmonella spp. and Escherichia coli,* can cause more serious diseases if the strain is multi-drug-resistant. In the United States alone, there are an estimated 1.5 million cases of infection with *Campylobacter spp.* and 1.35 million cases of infection with *Salmonella spp.* each year, costing USD 270 million and USD 400 mil-

According to the WHO, diseases caused by foodborne pathogens are becoming more difficult or even impossible to treat because of their increasing resistance to antibiotics.

lion respectively in direct medical costs. The European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority (EFSA) have suggested that Campylobacteriosis and Salmonellosis continuously contribute resistance to common antimicrobial drugs. In addition, 28.3% of human *Salmonella spp.,* particularly *Salmonella Typhimurium*, were multi-drug resistant (resistance to three or more antimicrobials) (EFSA and ECDC 2017). People infected with drug resistant pathogens untreatable by first-line antibiotics need more expensive drugs and longer duration of treatment in hospitals, which increases the financial burden to their families and society as a whole.

Countermeasures to control animal use of antimicrobials

To reduce antimicrobial use (AMU) globally, a collaborative approach across all the One Health sectors (human health, animal health, and the environment) is required. At a national level, data on sales of veterinary antimicrobials can be useful for guiding policy decisions aimed at reducing overall consumption or, more specifically, sales of particular antimicrobial classes such as the critically important antimicrobials (CIAs) for human medicine. Several international and national guidelines have been proposed for prudent use of antimicrobials in veterinary medicine. The World Organization for Animal Health (OIE) provides guidelines for the responsible use of antimicrobial agents in veterinary medicine with the aim of reducing overall AMR burden. In its guidelines, OIE also defines the respective responsibilities of the competent authority and stakeholders (OIE 2019).

In the United States, the Center for Veterinary Medicine (CVM) of the Food and Drug Administration (FDA) approves and monitors all drugs intended for veterinary use, in both pets and food-producing animals. New drugs purposed for use in animals are authorized under three marketing statuses: veterinary feed directive (VFD), veterinary prescription (Rx), and over-the-counter (OTC). VFD and Rx drugs are restricted to use only under a veterinarian's prescription. The FDA considers that "improved feed efficiency" or "increased weight gain" are not applicable conditions for use of any medically important antimicrobial in animals; however, they can be used for treatment, control, or prevention (FDA 2013). CVM's guidance document (GFI#152) classifies all medically important antimicrobials as critically important or highly important as per their relevance to human medicine (FDA 2003). FDA's VFD rule ensures a veterinarian's recommendation in order to use medically important antimicrobials in feed or water (FDA 2015).

At the national level, European Union (EU) countries have implemented the European Commission "Guidelines for the prudent use of antimicrobials in veterinary medicine" (2015/C 299/04) as part of their National Action Plans. The EU has banned the use of antimicrobials as growth promoters as of the European Parliament's regulation (EC) No 1831/2003 (European Parliament 2003). Stricter rules are being devised which will require EU trading partners to abstain from utilizing antibiotics as growth promoters in order to continue trading with the EU (EU Parliament 2018). Furthermore, according to new rules, metaphylactic (group treatment of animals when one is found to be infected) antimicrobial use will not be banned but permitted only if no alternative option is available (EU Parliament 2018a). EU guidelines on judicious use of antimicrobials also put emphases on treatment of individual animals after appropriate diagnosis and on raising farming standards to control infection in the first instance. Antimicrobials listed as critically important by the WHO, which are not authorized to be used in food animals, can only be used off-label (EU Commission 2015).

South Africa has drafted technical guidelines for the prudent use of antimicrobials in veterinary medicine that emphasize that antimicrobials should be used under the control of a veterinarian, who should encourage judicious use (Schellack et al. 2017). Currently, there is no legislation limiting the use of antimicrobials as growth promoters in South Africa. The South African *Antimicrobial Resistance National Strategy Framework* suggests the use of narrow-spectrum antimicrobials that are not used in human medicine to be used as growth promoters. The strategy framework also recommends devising legislature and policy reforms to limit the use of critical important antimicrobials as growth promoters by 2020 (DAFF 2018). In Kenya, the Ministry of Agriculture, Livestock, Fisheries and Irrigation devised *Guidelines for the Prudent use of Antimicrobials in Animals* in 2018 based on 2015 EU guidelines emphasizing individual animal treatment, improving farming practices, and veterinarian oversight (MALF Kennya 2018).

In India, antimicrobials are extensively used for prophylaxis, treatment, and growth promotion. Although the Department of Animal Husbandry, Dairying and Fisheries (DAHDF) requested States to advise veterinarians on careful use of antibiotics and ban the mixing of antibiotics in feed, there is no uniform policy or guidelines for judicious use of antimicrobials (Moa 2014). Similarly, in Pakistan, antimicrobials are broadly used for prophylaxis, treatment, and growth promotion in veterinary practice without the constraint of any governing law (CDDEP 2018).

In low- and middle-income countries, Thailand is leading in terms of observing strict regulations limiting antimicrobial use in animals, including implementing a ban on the use of antimicrobials as growth promoters in 2015. Likewise, Indonesia and Vietnam also banned the use of antimicrobials as growth promoters early in 2018 but they have comparatively fewer restrictions on antimicrobial use in animals and antimicrobials are frequently used for both prophylactic and treatment purposes in these countries (Coyne et al. 2019).

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/country focus

Use of antibiotics and AMR: U.S. perspective

Antibiotic use and antimicrobial resistance; personal experience, policies and regulatory response.

Gary M. Weber

Gary Weber's, PhD, career spans three decades. Not only does he have experience working on a family dairy and swine farm, he also has served in the following capacities: Michigan State University Adjunct Assistant Professor of Animal Science/Area Livestock Agent; National Program Leader for Animal Science, U.S. Department of Agriculture in Washington, DC; Executive Director of Scientific and Regulatory Affairs for the cattle industry in Washington, DC; President of Bioniche Food Safety U.S., and Prevention Manager for the Food and Drug Administration's Coordinated Outbreak Response and Evaluation Network. He now serves as a food safety consultant and the Senior Director of Food Safety and Contamination Prevention for WorldAware (Annapolis, Maryland), a global integrated risk management firm. The opinions shared here are his own.

Background

Antimicrobial compounds are a natural phenomenon in any microbiome. As an example, in the human microbiome it is estimated as many as 10^{11} organisms per milliliter are found in the large intestine. A healthy human microbiome, immune system and stomach acidity are the first lines of defense against any pathogenic organisms on food or in water.

In nature, antibiotic-producing gene clusters are common. It is estimated screening 10,000 common soil actinomycetes would result in identifying 2,500 that produce various antibiotics (Clardy et al. 2009). It is understandable why many bacteria naturally have and express antibiotic resistant genes. Using an antibiotic doesn't create antibiotic-resistant genes in and of itself. Bacteria naturally evolved to have genes that allow them to survive natural antibiotic warfare in response to competition from other bacteria and fungi.

The key to fighting antibiotic resistance is to use these tools carefully, including not overusing any one antibiotic. Any level of antibiotic use in humans or animals will select for microorganisms that are already resistant. Microorganisms can transfer resistance genes to other organisms through various mechanisms, such as plasmids.

Antibiotic use in livestock becomes an issue

Antibiotic use in animal agriculture has been of interest to me for over 40 years. My interests increased as I administered antibiotics on a family dairy and swine operation.

When I completed my PhD at Michigan State University, I accepted a position as an Area Livestock Agent for the Michigan State University Cooperative Extension Service. In this role I received training to help swine producers deal with "sick building syndrome." We knew high death losses were an issue on some farms. Despite great veterinary care and a lot of antibiotics, nothing was slowing the suffering and death loss on these farms.

The root cause was poor building design. Improper airflow was causing pigs to become sick and die. The solution was science-based air flow systems. This opened my eyes to the motives behind using too many antibiotics. In some cases, antibiotics were being used as a virtually worthless crutch.

Bacteria naturally evolved to have genes that allow them to survive natural antibiotic warfare in response to competition from other bacteria and fungi.

In 1987, I accepted an appointment as National Program Leader for Animal Science with the United States Department of Agriculture (USDA) Cooperative Extension Service in Washington, DC. In this role, I helped the USDA Food Safety and Inspection Service (FSIS) deal with a spike of violative sulfamethazine residues in market hogs. As I investigated the root cause, I realized that while raising pigs myself I was not aware sulfamethazine was to be withdrawn 15 days before harvest. I also learned that if pigs defecated outside, sulfamethazine residues in soils associated with areas that held water pigs could drink were sometimes high enough to result in violative tissue residues. These experiences reminded me of the importance of proper antibiotic use education.

This awareness motivated me to serve as the USDA representative to the Food Animal Residue Avoidance Data Bank (FARAD) (CDC NARMS 2020). To this day, FARAD continues to provide free information to veterinarians and livestock professionals to ensure antibiotics are used judiciously.

Animal rights and antibiotics

In the 1990, animal rights groups began raising concerns about the threat of antimicrobial resistance, and these discussions continue today (Eckholm 2010). Calls for action were joined by several consumer groups. Their concerns regarding antibiotic resistance were appropriate. The groups requested the U.S. Food and Drug Administration Center for Veterinary Medicine (CVM) to require animal health companies to publish total antibiotic sale volumes. The groups viewed all antibiotics as a threat.

As a scientist, I knew the amount of antibiotics used was not an appropriate metric alone for determining the selection pressure risk favoring antimicrobial-resistant enteric pathogens. To be effective, one should monitor actual antibiotic resistance to medically important antibiotics in enteric foodborne pathogens.

In addition, veterinarians and scientists knew that antibiotics like ionophores posed no threat to public health through selection pressure in favor of antibiotic-resistant enteric foodborne pathogens. The amount of these antibiotic used would be in the total use statistics. These ionophores improve animal efficiency and significantly reduce the emission of methane during rumen fermentation.

To be effective, one should monitor actual antibiotic resistance to medically important antibiotics in enteric food borne pathogens.

We know antibiotic resistance is a problem and it is the responsibility of regulatory agencies, veterinarians, scientists, and those who care for farm and companion animals to prevent the development of resistance that could threaten public health. Antimicrobial resistance is not only a threat to public health but also to animal health and well-being.

The agreed-upon approach was to develop the National Antibiotic Resistance Monitoring System, NARMS (CDC NARMS 2020). NARMS is an interagency partnership of the U.S. Centers for Disease Control and Prevention (CDC), CVM, and FSIS. Human surveillance for antibiotic-resistant enteric pathogens began in fourteen sites in 1996 and became nationwide in 2003. NARMS now supports a searchable database of human enteric pathogens and the level of antimicrobial resistance to medically important antibiotics (FDA 2012). For instance, resistance to nalidixic acid in *Salmonella Heidelberg* has been monitored since 1996. As of 2020, this pathogen shows virtually zero resistance. Today NARMS data guides decision-making by veterinarians, livestock producers, animal health companies, CVM, FSIS, and CDC. No one wins if antibiotic resistance puts animal health and well-being, and especially public health, at risk.

FDA guidance for industry: the judicious use of medically important antimicrobial drugs in food-producing animals

In 2012, CVM finalized a document titled *The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals* (Judicious Use Guidance, GFI #209)[6]. The final guidance discusses the FDA's concerns regarding the development of antimicrobial resistance in human and animal bacterial pathogens when medically important antimicrobial drugs are used in food-producing animals in an injudicious manner.

The Center for Infectious Disease Policy and Research (CIDRAP) at the University of Minnesota, summarized the results of a CVM report regarding antibiotic use in livestock (FDA 2013). The report showed domestic sales and distribution of medically important antibiotics for use in livestock decreased by 33% from 2016 through 2017, and 43% since 2015. Since 2009, the first year the FDA started collecting and reporting the data, sales have declined by 28%.

The 2017 summary report is the first issued since the CVM's rules on the use of medically important antibiotics in food-animal production were fully implemented. Under Guidance for Industry (GFI) #213 (FDA 2018) which went into effect Jan 1, 2017, antibiotics that are important for human medicine can no longer be used for growth promotion or feed efficiency in cows, pigs, chickens, turkeys, and other food animals.

As a result of CVM rulemaking it is estimated 95% of the medically important antibiotics used in animal water and feed for therapeutic purposes now require veterinary oversight. They can no longer be purchased over the counter.

Antibiotics that are important for human medicine can no longer be used for growth promotion or feed efficiency in cows, pigs, chickens, turkeys, and other food animals.

Food and Drug Administration Center for Veterinary Medicine Antibiotic Stewardship: the CVM published their Antibiotic Stewardship in Veterinary Medicine Goals in 2018 for fiscal years 2019-2023. The following is a summary of their approach (OIE 2015).

Pre-approval Review and Effectiveness: the drug sponsor must demonstrate that the drug works when administered to animals according to the label.

Target Animal Safety/User Safety: assessment of both the safety of the drug for the animals being treated as well as for the person administering the drug.

Environmental Safety: under the National Environmental Policy Act of 1969 evaluation of animal drugs on the environment.

Human Food Safety: for drugs intended for use in food-producing animals, the safety of potential drug residues in the food (meat, milk, eggs, and honey) derived from treated animals must be evaluated. This includes evaluating the impact of antimicrobial drug residues on the intestinal microflora of humans.

Antimicrobial Resistance Risk Assessment: antimicrobial drugs intended for use in food-producing animals are subject to a qualitative risk assessment to evaluate the potential for an antimicrobial drug to impact antimicrobial resistance of enteric pathogens in humans.

Chemistry, Manufacturing, and Controls: evaluation of methods used in facilities and controls used for manufacturing, processing, and packaging the drug are adequate to preserve identity, strength, quality, and purity.

Label Review: ensure accuracy and prevent misleading claims. Labels must inform consumers of appropriate use of the product, including safety considerations, storage, and handling.

Post-approval Surveillance and Monitoring, Adverse Event Reporting: review of adverse event reports submitted by the drug sponsor, veterinarians, and the public to determine if any post-approval actions (e.g. updates of product labeling) are warranted.

Drug Labeling, Promotion, and Advertising: evaluation and update of animal drug product labeling, including antimicrobial drug labeling to ensure continued safe and effective use. Evaluation of promotional and advertising materials used by drug sponsors to ensure it is truthful and consistent with approved product labeling.

Antimicrobial Sales and Distribution Data: review of annual reports submitted by drug sponsors detailing the amount of each antimicrobial drug product sold or distributed for use in food-producing animals.

Antimicrobial Resistance Monitoring: monitoring of resistance trends in enteric pathogens through use of NARMS (CDC NARMS 2020).

The World Organization for Animal Health

The World Organization for Animal Health (OIE) approach to antibiotic stewardship is in harmony with the CVM approach. The OIE International Committee unanimously adopted a List of Antimicrobial Agents of Veterinary Importance at its 75th General Session in May 2007 (Resolution No. XXVIII) (FSIS 2019, 2020).

Regulation of Antibiotic Residues in Foods

In the U.S. both the CVM and FSIS monitor foods for antibiotic residues. If a violative residue is found, the FDA aggressively investigates the situation using a risk-based prioritization approach (CDC 2015; FDA 2020). There are notable instances where violators were required by the courts to take specific actions to prevent future occurrences.

Antibiotic Use in Human Medicine

We know from a One Health perspective antibiotic-resistant pathogens can be passed from animals to animals, animals to people, people to animals and people to people. We would be negligent if we ignore the use of antibiotics in human medicine as it too plays a role in the health of both people and animals.
The CDC reported in 2014 that over 266 million courses of antibiotics were dispensed to outpatients in U.S. community pharmacies. This equates to more than five prescriptions written each year for every 6 people in the United States (CDC 2014; Chen et al. 2016). This is equivalent to 835 antibiotic prescriptions per 1000 persons.

Antibiotic-resistant bacteria in humans is a global health threat

In January of 2017, the CDC reported the death of a women caused by *Klebsiella pneumoniae*. In this case, it was a carbapenem-resistant *enterobacteriaceae* (CRF) Friedrich 2019). The carbapenem class of antibiotics are viewed as the last line of defense when other antibiotics fail. They are not approved for use in livestock anywhere in the world.
In this illness case, it is believed the patient became infected in India after a bone injury. The specific bacterial pathogen, *Klebsiella pneumoniae*, was reported to be resistant to the 14 drug options the hospital had on hand. While we cannot interpret this as resistance to all possible antibiotics, it is nonetheless a significant observation and warning.

Summary

Antibiotic-resistant pathogens in farm, companion animals and people, are a global health threat. Fortunately, efforts have been underway for decades to manage and reduce the risk posed by antibiotic use in animals. There are concerns regarding the number of prescriptions provided to people in the United States. Hospital acquired antibiotic resistant pathogens remains a significant global concern[16]. Awareness and understanding of the risk and mitigation strategies are critical to protect the health and well-being of people and animals.

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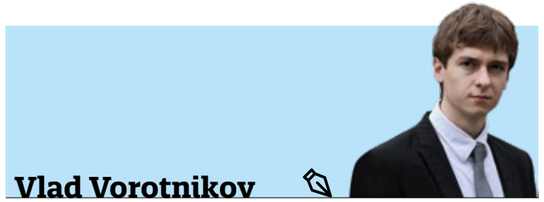

Vlad Vorotnikov

Vlad Vorotnikov is a Moscow-based multimedia freelance journalist who has been writing about the veterinary system in Russia & the CIS region since 2008. Occasionally, he reports about the health care service industry.He holds a master's degree in journalism from the Voronezh State University.

/country focus

Anti-antibiotics campaign is needed in Russia

Russian authorities claim they want to decrease antibiotic use both in veterinary and healthcare systems but any changes have been delayed either because of the cost to the federal treasury, potentially amounting to billions of rubles, or because local businesses do not welcome these changes.

Anti-antibiotics campaign is needed in Russia

On 3 December 2019, a draft bill titled *On Biological Safety* was submitted to the State Duma, the lower chamber of the Russian Parliament. That regulation called for enhanced government control of antibiotic consumption in the country in order to combat antibiotic resistance, according to Leonid Ogul, chairman of the State Duma's healthcare committee.
"Among other things, the draft bill brings together all of the measures that call for decreasing antibiotic resistance. It prohibits pharmacies from selling [some] antibiotics without prescriptions from physicians and prohibits physicians from administering antibiotics without a confirmed diagnosis," Ogul said. "As of today, over-the-counter sales of most antibiotics are already prohibited in Russia," Ogul continued, adding that he hoped that with the adoption of the new law, all pharmacies would begin complying with the requirement.
Yet, despite that, analysts don't believe the authorities are eager to enforce measures aimed at dealing with antibiotic resistance and superbugs. The law, if adopted, would simply bring togeth-

Almost all analysts agree that antibiotic consumption in Russia is a serious problem. The existing regulations are weak and they are regularly violated both by pharmacies and physicians.

er all of the rules contained in a dozen other regulations but there would be no new rules. More importantly, nothing new would be done to improve enforcement of these rules. Today, almost all antibiotics can be easily purchased over-the-counter in Russia.

"Despite the legal prohibition against selling antibiotics over-the-counter [in Russia], the fact is that everybody can buy them anywhere and whenever needed," said Vitaly Zverev, director of the Moscow-based Mechnikov's Research Institute of Vaccines and Serums. "I've checked this myself. I bought them in pharmacies without a prescription. And if a patient takes antibiotics without physician supervision, what happens then? He takes the drug for a day or two and stops at the first signs of improvement. The disease returns and so he starts over again. This is where the resistance comes from."

An opinion poll conducted by the Russian consumer rights and human well-being watchdog Rospotrebnadzor showed that most Russian citizens purchase antibiotics frequently, using them without hesitation. 44% of respondents said that they were buying antibiotics without visiting physicians, 26% said that they would terminate therapy at the first signs of improvements, and 67% expressed confidence that a common cold could be treated with antibiotics. Those findings were in line with the results of earlier research showing that Russians used to treat everything from diarrhea and rash to different types of viruses with antibiotics.

Almost all analysts agree that antibiotic consumption in Russia is a serious problem. The existing regulations are weak and they are regularly violated both by pharmacies and physicians.

"Antibiotic resistance comes from unsupervised short-term therapy. Despite the prohibition against selling antibiotics over-the-counter, they can still be bought in pharmacies. People self-administer antibiotics when they have a health issue and quite often they do it incorrectly," said Vladimir Nikiforov, PhD, Head of the Department of Infectious Diseases at the Moscow-based Pirogov's National Research Medical University.

Improvements are too expensive

Various environmental-protection organizations have repeatedly asked the government to completely prohibit pharmacies from selling antibiotics over-the-counter and to ensure compliance with this requirement. The truth is that this measure would cause a collapse of the entire Russian healthcare system.

"We predict that by requiring prescriptions for most drugs on the market amidst both a shortage of physicians and long waiting lists in the primary care units, patients' lives would be way more complicated," said Edward Gavrilov, chairman of a Russian patients' rights advocacy group.

"In my opinion, the introduction of strict prescription controls over antibacterial drugs on the market would be absolutely counterproductive. The number of primary care physicians simply would not be able withstand the increased burden of patients who are currently treating themselves or being treated by pharmacy employees," added Nikolay Bespalov, development director of the Moscow-based think tank RNS Pharma.

The Russian government is set to invest Rub800 billion (12.8 billion USD[1]) into the primary care segment of the national healthcare system in the next three years, but analysts don't believe that figure would be enough.

"The government would need to double the figure of planned investments in order to hire enough physicians to administer antibiotics to all patients who need them. The truth is that this is not realistic," said a source in the Russian healthcare system who wished to not be named.

The Russian Healthcare Ministry requires that primary care waiting times not exceed 24 hours. However, Russian patients have repeatedly complained that it sometimes takes 2 to 3 days to get an appointment with a physician. This is believed to be one of the reasons why the authorities have not banned over-the-counter antibiotic sales. In cases of acute bacterial infection, long waiting times can lead to serious health issues.

Sales remain high

The overall supply of systemic antibacterial drugs on the Russian market amounted to Rub52.3 billion (831 million USD[1]) in retail sales, including VAT, in 2017 and Rub54.2 billion (861 million USD[1]) in 2018. In the first 11 months of 2019, sales reached Rub53.4 billion (848 million USD[1]), up 8% compared to the same period the previous year, RNS Pharma estimated. Those figures included both domestic production and import.

In physical terms, supply reached 396.3 million packs in 2017, 330.6 million packs in 2018 and 337.5 million packs in the first 11 months of 2019 – up 13% from 2018 on a year-to-year comparison.

"It is important to understand that a pack is a measure that is not constant. In certain time periods, due to regulatory or other reasons, average pack size can vary. Thus, in 2017, supply amounted to 2.3 billion doses of antibiotics while in 2018 this figure reached 2.4 billion doses," said Bespalov.

The average Russian citizen spends around Rub250 ($4) per year on antibacterial drugs. This figure remains stable with a slight tendency to grow, Bespalov said.

"The share of antibacterial drugs of Russian origin on the domestic market was 45% in monetary terms and 58% in physical terms," Bespalov estimated.

"In 2018, Russia produced 386 tonnes of antibiotics for human consumption, down 17% compared to the previous year," said Yulia Kaulkina, Head of the Alto Consulting Group Analytical Department, an independent analytical company focused on high-quality market research and business plan development for industries and regions, both in Russia and in other countries. During the first 11 months of 2019, production reached 497 tonnes, Kaulkina added.

Despite some year-to-year volatility, "over the past three years, the production of antibiotics for human consumption increased by 12%. We forecast that between 2020 and 2023 the production would grow with a CAGR of 11%," Kaulkina said.

Russian analysts estimate the country imported around 1,800 tonnes of antibiotics for human consumption in the first 11 month of 2019.

"Russia has been increasing antibiotic imports by 8% per year on average over the past three years. The share of imports during that time shrank from 82% to 77%," Kaulkina said.

Similar picture in the veterinary system

When it comes to antibiotic use in the livestock industry, there is a lot to be desired. The veterinary system in the country is built in such way that farmers are free to use antibiotics on their livestock as they see fit. The only requirement is that the animals have no antibiotics present at the time of slaughter. It is widely believed that, despite the problems in the national healthcare system, it is veterinary antibiotics that contribute most to the proliferation of superbugs.

"We encounter most antibiotics through food, because they are widely used in industrial livestock and poultry operations," said Anatoly Martynov, chairman of the Russian union of physicians.

Over the past decade, Russian government officials and lawmakers have been declaring their intention to change that situation, but no improvements have been made so far.

In a statement posted in the *Russian Gazette*, the official publication of the Russian government, Sergey Dankvert, director of

1 - Exchange rate on February 6th, 2020.

the Russian veterinary watchdog Rosselhoznadzor explained that the meat producers using antibiotics were able to raise larger animals on their farms and were thus able to achieve higher profits.

"Antibiotics are being sold without any limitations, with every veterinary official, farmer, or feed producer able to purchase them," Dankvert said, adding that Russian business is against any changes in this field. "We are experiencing a strong resistance from those who don't wish to be subjected to any serious control over antibiotic use in the livestock and poultry industry," Dankvert added.

Rosselhoznadzor has submitted a set of amendments to the federal law titled 'On veterinary medicine' proposing to prohibit the preventive use of antibiotics, Dankvert disclosed, not saying when the new amendments might be adopted. There were several attempts to prohibit preventive use of antibiotics in Russia over the past years, but all of them eventually failed.

However, there are some positive changes in this area. For instance, some companies in Russia have recently begun manufacturing food products carrying an "antibiotic-free label" indicating that no antibiotics were used in animal feeding. In most cases, changes like this are due to growing consumer awareness about antibiotic resistance both inside and outside Russia. Most analysts believe that this trend will gain momentum in the coming years. 84% of Russian citizens are aware of antibiotic resistance and the threat it presents, Rospotrebnadzor said. The problem is that the products carrying the 'antibiotic-free label' might not satisfy market demands because this products may be 20% to 60% more expensive compared to the same goods without the label so it might only be in demand by a small proportion of Russian citizens.

There were several attempts to prohibit preventive use of antibiotics in Russia over the past years, but all of them eventually failed.

In 2018, Russia manufactured 341.8 tonnes of veterinary antibiotics, down 13.6% compared to the previous year, Kaulkina said. However, Russian companies produced 35.2 tonnes of in-feed antibiotics, 40.2% more than during 2017, she added.

Russia is importing around 3,000 tonnes of veterinary antibiotics, including 700 tonnes of in-feed antibiotics per year. Imported in-feed antibiotics accounted for 95% of in-feed antibiotic sales on the Russian market, research conducted by the Russian consulting agency Abercade found.

"When it comes to antibiotic use, the leaders of the domestic meat industry turn out to be rather responsible. The companies exporting their products to Europe, Asia, and the Middle East often have to prove that they are complying not only with the Russian, but also with the Western, veterinary standards. At the same time, there is a huge proportion of companies who continue using antibiotics and do so in really big quantities, but they will never admit that to the public," said a source in the Russian veterinary system who wished to remain unnamed.

Expanding export programmes in the Russian meat industry could become one of the factors prompting companies to move away from excessive antibiotic use on the farm, the source added.

"For example, we are trying to get a green light to export our products to China. This country is checking production for 67 types of different antibiotics. In our country there is no single laboratory that is able to check for the presence of all of them. One laboratory is able to test 5 antibiotics, another can test 20, and a third can test 15. In order to certify that our products are free from all antibiotics we have to transport our products all over the country," Artur Holdoenko, director of a St. Petersburg-based poultry farm said in a statement posted in the Russian government publication.

It is crucial for Russian poultry farmers to guarantee they are not using antibiotics in production process when they are exporting their products to the Middle East and Europe, Holdoenko said. Now there is a new state-of-the-art laboratory under construction in St. Petersburg that will become the first facility of its kind to check livestock products for traces of any veterinary antibiotics.

The truth is that nobody knows how many companies use antibiotics and in what quantities they are using them. Over the past few years, some companies claiming they were not using any antibiotics have been caught purchasing them.

The use by some large Russian manufacturers of the label 'grown without antibiotics and growth promoters' label is simply a marketing trick aimed to confuse customers since they are purchasing large quantities of antibiotics through competitive bidding procedures, Lybov Savkina, commercial director of the Russian think tank Feedlot said.

In 2015, Russian scientists designed an online map of antibiotic resistance, a tool to put together all of the research data collected from hospitals and primary care units. So far, the data is rather segmented due to a lack of studies of this kind in Russia. While this data reports on antibiotic resistance in the healthcare system, there are so far almost no studies related to superbug occurrence in the wild and among farmed animals.

"In Russia, the problem of antibiotic resistance is worse than in Europe. Their uncontrolled use in veterinary medicine, allowing antibiotics to make their way into food products, is one of the reasons for that," said Valery Danilenko, director of the biotechnological department of the Russia's Vavilov Institute of General Genetics.

/country focus

How European public health authorities and food businesses are working to reduce AMR by reducing antibiotic consumption

Insight to the point of view of animal health authorities, food producers and retailers.

The point of view of animal health authorities in one EU country: education and training are very important, aside the regulations, in order to reduce antibiotic use

In the EU, total antibiotic use on average has decreased significantly over the past 5 years. Non-therapeutic use of antibiotics has been forbidden since 2006. Residues are almost always below the regulatory limits but are often still present in minute quantities. The risk that we are exposed to AR genes through food or water is under investigation. Education and training are the only way to make the fight against AMR more successful.

G. Loris Alborali

Director of the Brescia Diagnostic Section of the Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna (Italy)

The consumption of antibiotics in livestock has decreased in recent years in Italy and in several other countries, sometimes drastically. How was this possible? What were all those tons of extra drugs used for before? Are there consumption data by type of animal available? Has a portion of the treatments and, therefore, of consumption become "submerged"?

Until a few years ago, antimicrobials were used both for the treatment of pathologies and for the prophylaxis of the most common diseases in breeding and often represented a way to circumvent necessary structural and manage improvements. The strategy to reduce the consumption of antimicrobials began at different times in various EU countries. Denmark was the first country to start a national plan, followed by other countries such as Holland. In Italy, this strategy was started after 2010, particularly in the poultry sector, but today it is also felt in other supply chains. However, the reduction in consumption is real because the veterinary surgeon and the breeder have understood that when priority is given to biosafety and welfare, not only are animals healthy and producing better but costs are also reduced. I believe that justifying the improvement in antibiotic practices with an increase in black market sales is very reductive and diminishes the real change that our animal husbandry is making.

Is it really possible to limit the use of drugs to therapeutic purposes only? If, however, a preventive use is legal (that is, group antibiotic administration without illness in the group because of fear that a pathology will arise), what distinguishes this use from auxinic use? The type of antibiotic or the dosage?

First of all, we must reiterate that the use of antibiotics as growth promoters in our country has been prohibited since 2006 and that the checks carried out every year under the National Residual Plan say that the positivity to antimicrobials is very low. Limiting the use of drugs to therapeutic purposes today is possible and numerous farms are working in this direction, obtaining very good results. It is necessary to start setting the selective dry treatment in dairy cows and to reduce the mass treatments of pigs in favor of treating individual animals.

There is no certain answer to the negative effects caused by residues of antibiotics below MRLs.

Antibiotic residues in meat and milk are almost always under the Maximum Limits permitted by law (MRL). However, some of these MRLs were set in the 1990s in a somewhat hasty manner. Is there a risk that "sub-MRL" concentrations could be harmful, if only because they alter the balance of the microbiota? Given the very low concentrations, does it make sense to fear that this exposure may even contribute to the onset of AMR forms?

The problem of MRLs is now a hotly debated issue and there is no certain answer to the negative effects caused by residues of antibiotics below MRLs. In order to counter AMR, however, it is important that we continue to study, deal with, and resolve situations in which we become aware of the presence of antimicrobial concentrations even if they are below the MRLs.

Are tests performed to check whether imported food products (e.g. shrimp from aquaculture or chicken meat) carry drug resistance genes?

In recent years, the study of AMR-carrying genes in the microorganisms found in humans, animals, food of animal origin, and the environment has significantly increased. In particular, these studies have focused on the genes that convey antimicrobial resistance considered critical to humans. Examples are the genes that carry resistance to beta-lactams (ESBL) and colistin (MCR).

"Antibiotic-free" brands of meat are spreading. Is it true that in the specifications of these productions, however, coccidiostats are allowed (for example, in chickens), which would also have a certain amount of antimicrobial activity and which could, in turn, promote the onset of AMR?

The world of "antibiotic-free" products is complex and today has a very commercial connotation. I think that the advent of electronic invoicing and the monitoring of consumption in individual farms through ClassyFarm[1] constitute a great step forward to make this world more transparent.

What is the difference between meat without antibiotics and organic meat? Given that all meat should be without antibiotics, wouldn't it be better to aim for less intensive farms?

It is meat produced with different specifications, the first of which provides that the meat is produced without the use of antibiotics while the second has separate requirements for the organic certification. Organic production requirements cannot be applied on a large scale and they do not allow for production levels of meat in line with market demands. Today, animals can be intensively bred while respecting high standards of welfare and antibiotic drug use.

In some countries (France, for example), veterinarians can sell antibiotics to farmers while in Italy they prescribe them but do not sell them. Is there a risk that the veterinarian may be incentivized to keep consumption high, which is notoriously the case in human medicine, even if the drug itself is sold by the pharmacy?

The problem that occurs in France is real and makes the path of reducing antibiotic consumption more difficult. In Italy, the strategy set by the Ministry of Health with the National Plan for Combatting Antibiotic Resistance has meant that the veterinary physician today is increasingly personally committed to reducing the use of the drug. The fact that now the consumption of antibiotics on all farms is known makes the veterinarian even more involved in this strategy.

What more could be done, in your opinion, to reduce the risk of AMR causing an increasing number of deaths in Europe and worldwide?

The aspects that should be improved today concern above all training and communication. In particular, training should be directed to farmers, owners, and veterinary doctors and should include all aspects and systems that can be put in place today to reduce and target the use of antibiotics. The fact remains, however, that there is much to do to communicate with consumers who are often confused by commercial messages and who know little about the real requirements for products of animal origin.

1 - ClassyFarm is a database where information about biosafety, animal welfare, animal nutrition, drug use, health state of animals, production data, and any wound detected at the slaughter house are recorded. Data from farms are anonymous. The farm vet can choose whether to provide the data or not; however, the system will classify every farm by means of the data available. ClassyFarm scores allow comparisons of each farm's risk level with respect to animal health.

Point of view: European food producers and retailers

Last October, the European Medicines Agency (EMA) published the ninth annual report on European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). The EMA Report analyzes the data sent to ESVAC, in 2017, from 31 European countries, for all food-producing animal species, including horses. The EMA, for the first time, emphasized the results of a constant commitment to prudent use. The consumption of polymyxins, one of the critical antibiotic classes, decreased sharply, 3rd and 4th generation cephalosporins also have decreased. Between 2011 and 2017, sales of veterinary antimicrobials in Europe decreased by 32%.

"This data shows that national campaigns promoting prudent use of antibiotics in animals to combat antimicrobial resistance are having a positive effect. The differences that emerge between the 31 countries are largely due also to the differences that occur in the onset of bacterial diseases or are related to the composition of the animal population and the different production systems," stated a representative from UNA Italia, a trade association that protects and promotes the Italian agri-food chains of meat and eggs. Reducing consumption in Europe was made possible thanks to an increased awareness within the livestock sector of the issue of antibiotic resistance, a multifactorial and environmental problem that requires commitment from all parties involved. Helen Sisson, technical director at Two Sisters Food Group, a leading UK food company, says, "Adopting national targets for each farm animal species sets a bar for reductions and encourages all farmers to benchmark their antibiotic usage, which can be a powerful tool in facilitating change."
In the UK, the emerging topic of antimicrobial resistance has led to the formation of Responsible Use of Medicines in Agriculture (RUMA) (1997) and the Food Industry Initiative on Antimicrobials (FIIA) (2017). Such organizations bring together retailers, manufacturers, processors, and food service companies to promote and support responsible antimicrobial use and action on

antimicrobial resistance. By 2017, RUMA established targets for the reduction of antibiotic usage for each sector, for all farmed animals. Data about antimicrobial usage per animal group, as well as data about the use of Highest Priority Critically Important Antimicrobials (HP-CIA) have been regularly collected for several years for pigs, poultry, salmon, and trout.

In the UK, during the last week of February 2020, an industry standard for measuring and monitoring antibiotic use on beef farms based on research from the University of Bristol and developed by the Cattle Health and Welfare Group's (CHAWG) Antimicrobial Usage (AMU) subgroup was adopted following extensive industry consultation.

In Italy, official drug consumption data will also soon be available for each farmed species. Beginning last year, the electronic veterinary recipe began and all the data flow in real time to a centralized database managed by the Ministry of Health. However, in general, an analysis of the literature shows that data don't exist on a European scale for each individual species.

The European Union banned the use of antibiotics, which were administered in low doses in feed, as growth promoters, in 2006 but outside of Europe this practice is still widely allowed. This has been shown to contribute significantly to the development of resistant bacterial strains. These bacteria can be present in food of animal origin and their antibiotic resistance properties can be transferred to pathogenic microorganisms that infect humans.

Generally, a reduction in the use of antibiotics is underway in the EU and US countries but in the rest of the world this trend is not yet consolidated. How then should importers behave in this case?

Helen Sisson states that importers demand assurances that imported food production complies with UK standards. Chiara Faenza, Sustainability and Values Innovation Manager for Coop Italia, said that they make a careful risk evaluation before planning laboratory controls. Moreover, there are other issues that have to be taken into account, according to Ursula Lavery of Moy Park, one of Europe's leading poultry producers. "One of the critical issues with comparing global use is around understanding the measurement and metrics that are used. In particular we need to look at overall use of HP-CIA to ensure we are on a level playing field in terms of the metrics."

For a few years, some meat products (e.g. ham) have been placed on the market with labels like "antibiotic-free" or "reared without antibiotics." Does it mean consumers can avoid contact with antibiotic-resistant bacteria? Consumer association surveys suggest that the answer is no. (Altroconsumo 2019). "The detection of antibiotic-resistant bacterial genes can occur in all types of farms and their products. In fact, bacteria with antibiotic resistance genes are ubiquitous and, pathogenic or not, they may have come into direct contact with the antibiotic and have developed antibiotic resistance or have never encountered antibiotics and received this characteristic from other bacteria because transmission of resistance from one bacterium to another is possible. Therefore, it is possible to find these genes both in animals and in products from antibiotic-free farms where antibiotics have not been used but, obviously, the risk is considerably lower. Of course, the contamination of ham can have occurred along the entire supply chain, both in breeding and in the transformation or distribution phase," says Faenza.

There are other factors that should also be taken into account when the issue of antibiotic resistance is treated, says Lavery. "From a scientific point of view, we are also aware that resistance can occur due to other stress factors even when there is no evidence of any antibiotic usage. AMR occurs normally in nature and goes hand in hand with bacterial evolution. There will always be animals that get sick and if they are clinically confirmed as sick, the responsible thing to do from a husbandry point of view is to treat them. The key is treating them responsibly. Responsible use of antimicrobials and a One Health approach is our best chance to reduce AMR risks."

Sisson added another point related to the One Health approach and animal welfare, "There is a concern that withholding antibiotics throughout an animal's lifetime may present welfare issues and suffering; this would not be acceptable." Furthermore, official analyses of standard supply chains show that 99% of the meat, milk, and cheese tested is free of antimicrobial residues above the legal limits due to withdrawal times, thus representing a decreased threat to human health.

"The fight against antibiotic resistance depends on the conscious use of the drug in the different breeding phases and it is there that we must intervene. Respect for biosecurity rules, prevention protocols, hygiene and health standards, and the application of good breeding practices favors the significant reduction of the use of antibiotics even in conventional breeding and consequently counteracts the onset of antibiotic resistance," explains Faenza. Accordingly, the European Medicines Agency has provided analysis of the possible impact on public and animal health of the use of certain antibiotics (such as colistin or 3rd and 4th generation cephalosporins).

"Following the decisions made by the EMA, there have been precise indications in this regard by the Italian Ministry of Health to limit their use as far as possible in order to minimize the potential risks associated with more widespread use. In the poultry sector, these indications have been meticulously accepted and, on a voluntary basis, thanks to the plan for the rational use of the drug in our supply chains, there has been no use of 3rd and 4th generation cephalosporins since 2009 or of colistin in chicken since 2017," a representative from UNA Italia explained.

Consumers have been sensitive to the problem of antibiotics on the plate, especially in meat, for many years. So are antibiotic residues still an issue for food safety?

All European National Residue Plans Reports show extremely low percentages of non-compliant samples (EFSA 2019). "A risk-based sampling programme is in place to collect meat samples from processing facilities and test these for antibiotic residues and other substances that may be of food safety concern," says Lavery, commenting about Moy Park's experience with the issue.

Much attention in this situation must be paid to the type of information that is given to the consumer about "antibiotic free" products, as Sisson explained. "We would caution that labelling products in this way has the potential to mislead the consumer by implying that the meat or milk not marketed as such contains antibiotics, which is not the case, as there are strict rules governing the administration of antibiotics to farm animals in the UK."

According to a representative of UNA Italia, the situation is not so different in Italy. "The wording *bred without the use of antibiotics* has nothing to do with the presence of residues in the meat product, as even in the case of drug administration, the withdrawal time before slaughter is always respected. Therefore, *breeding without antibiotics* is only additional and voluntary information that has nothing to do with the quality and food safety of meat, which is guaranteed for all products on the market." Breeding without the use of antibiotics is possible but it is a result, rather than an objective. "In any case, it is necessary to invest in animal health and welfare and this is the main tool for giving guarantees to consumers. Antibiotics are a cost, which can be avoided when animals do not need to be treated, as well as investments in biosecurity to reduce the use of the drug. The primary objective is animal health; when an animal gets sick, it is treated, regardless of the method by which it is raised. In these cases, the farm is downgraded starting from the database where the farms and production cycles are recorded and it will not then be possible to market the meat with the wording *bred without the use of antibiotics*."

Many challenges still remain, such as the need to be aligned across the industry in terms of both the measurement and collation of data. "This is something we are looking at within the FIIA; what data do we measure and how?" says Lavery. "How do we collate and anonymise the data so it can be used by farmers to measure, benchmark, and see how they compare with their peer group? If we have a means of comparing the various different livestock sectors, we then also need to examine how we can support those farms which, for whatever reason, have needed to use higher levels of antibiotics and provide support, training, and education to help them reduce their usage. This is where government can step in and possibly use its veterinary expertise to guide farmers."

THE INTERVIEWEES

G. Loris Alborali is Director of the Brescia Diagnostic Section of the Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna (IZSLER). IZSLER is the biggest Italian laboratory in the network that controls animal health and the safety of food of animal origin. Alborali was President of the Italian Society of Pig Breeding Pathology (SIPAS) and Graduated from the European College of Porcine Health and Management (ECPHM). He works in the field of animal health and public health and participates in national and European research projects.

Coop (Coop Italia) is a system of Italian consumers' cooperatives which operates the largest supermarket chain in Italy. With respect to the issue of antibiotic resistance, Coop's risk analysis strategy takes into consideration the countries of origin of the various goods it sells. All fresh meat from both land and aquatic animals under the Coop brand comes from Italian or European farms. Coop Italia has completely eliminated the use of antibiotics in its chickens and laying hens. In its adult pig and cattle farms, the company stopped using antibiotics 4 months ago. No Coop farmed fish undergo antibiotic treatments in the last 6 months of life. Coop is in the course of extending this initiative to other products.

Moy Park is one of the UK's top 15 food companies, Northern Ireland's largest private sector business, and one of Europe's leading poultry producers. As a business, Moy Park is committed to responsible welfare for poultry. Many of their farms have not needed to use antibiotics and their policy remains to ensure appropriate use of antibiotics where required. This policy includes no prophylactic use; i.e. only sick birds are treated and only after those birds have been diagnosed by a vet and medicines have been prescribed by a vet.

2 Sisters Food Group is a privately owned food manufacturing company based in Birmingham, England. In December 2013, the company was ranked as Britain's 4th Most Admired Company (food producers sector) in the Management Today Most Admired Company list, voted for by its industry peers. The company is in full support of and are adopting the policy and guiding principles of the FIIA. They are also pushing for a common strategy and approach in the important area of AMR.

Unaitalia is a trade association that protects and promotes the Italian meat and egg agri-food chains. It represents over 90% of the entire Italian poultry sector as well as a large part of the pig sector. With respect to AMR, the association clarifies that consumer safety is a priority for companies that have a duty and interest in providing healthy, safe, and quality products.

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/focus on

Pathogen outbreaks in Europe: growing concern or more efficient source-tracking?

The European food production industry has been shaken over the past two years by multiple reports of foodborne outbreaks. Food safety authorities also report a trend in the growing number of outbreak-related recalls. Is something going wrong?

François Bourdichon

François earned a Master's degree in microbiology from the Pasteur Institut (Paris) and a PhD from Università Cattolica del Sacro Cuore (Milan). After 15 years working formajor dairy (Nestlé, Danone, Savencia) and confectionary (Barry Callebaut) food business companies, he is the principal consultant for Food Safety, Microbiology and Hygiene.

Introduction

The summer of 2019 saw numerous foodborne outbreaks linked to *Listeria* and *Salmonella* in Europe, implicating different sectors of the food industry. The *Listeria* outbreak in Spain was the largest ever recorded in that country (though still outweighed by the 2017 South African episode). An episode of *Salmonella* contamination of infant milk formula (Spain, rice-based) was linked to the same strain (*Salmonella* Poona) found in a 2010 outbreak, although the contaminant itself could not be isolated either from the incriminated food product or from the processing environment.

The technology and the tools to track foodborne outbreaks has improved drastically in the past twenty years. But is this enough? In 1986, while dealing with the contamination of *Salmonella* spp. in Powdered Infant Formula (PIF), Habraken and his team (Habraken and Mossel 1986) published a reference paper that is still the basis of the statistical approach for microbial sampling in PIF. But one of his statements should be highlighted today: "The lack of reliability of the mere examination of finished products when evaluating the microbiological wholesomeness of food products has been known to microbiologists for a long time." The *Salmonella* Poona outbreak is one of the latest and best examples to substantiate this statement.

Regulation (EC) No 2073/2005 Microbiological criteria for foodstuffs

Food business operators too often rely on the criteria set forth in article 4 of Regulation (EC) No 852/2004 to ensure product safety. While it is true that those criteria are necessary to validate and monitor the implementation of Good Handling Practices (GHP) and the Hazard Analysis Critical Control Point (HACCP), 15 years ago, in 2005, the regulation already suggested that investigations should focus on the processing environment (Regulation (EC) 2073/2005, Whereas #22): "Sampling of the production and processing environment can be a useful tool to identify and prevent the presence of pathogenic micro-organisms in foodstuffs".

Since the first version of Commission Regulation (EC) No 2073/2005 and its numerous updates, the Codex Alimentarius has also updated its principles and guidelines for the establishment and application of microbiological criteria related to foods (Codex Alimentarius 2013). When focusing on the detailed components of the microbiological criteria, analytical methods and their performance parameters are often forgotten. However, the importance of using appropriate reference methods should not be underestimated when applying statistical approaches to determining microbial limits.

The International Standard Organisation, Technical Committee 34 – Food, Sub Committee 9 – Microbiology (ISO/TC34/SC9)[1]'s "Horizontal methods in the field of microbiological analysis of the food chain from primary production stage to food and animal feed products, including the environment of food production and handling" is one of the most influential reference bodies for defining appropriate methods. Recently, using the ISO Standard 17468[2], the ISO/TC34/SC9 updated 15 reference methods for pathogenic and hygienic microorganisms of interest for the food industry. All those reference-validated methods have been updated accordingly in the Regulation (EC) No 2073/2005, with implementation in the food analytical laboratories from about 2018 onward. Could this be the reason for the apparent growth in the number of recalls and reported outbreaks? It might presumably be one of the factors. This point, largely debated, was considered during the *Salmonella* Agona outbreak in France late 2017 when the service analytical laboratory claimed that the strain could not be isolated with the previous version of ISO 6579 for Detection of *Salmonella* spp. Because ISO/TC34/SC9 stated that the modifications from ISO6579 to ISO6579-1 were minor, this point remains highly controversial today.

What is certain, nevertheless, is the increase in testing by the food industry in Europe and globally as well in recent years,

1 - https://www.iso.org/committee/47920.html
2 - ISO 17468:2016 Microbiology of the food chain - Technical requirements and guidance on establishment or revision of a standardized reference method

With the recent outbreak episodes in Europe, no food business operator can ignore the importance of having a holistic approach in the processing environment.

following "unexpected" outbreaks caused by various food products. This increase in testing is followed by an increase in recalls and reported contamination as sampling efficiency improves. France has even reinforced its legislation further in the new EGALIM Law, article 50, where food business operators are expected to declare any positive pathogen testing results if the investigation shows a potential risk for the consumer population.

Beyond Regulation (EC) No 2073/2005 The General Food Law 178/2002

In March 2019, the Danish Veterinary and Food Administration (DVFA) established that an Estonian salmon fish producer was responsible for a lethal *Listeria monocytogenes*-related outbreak that had been ongoing since 2016 in Scandinavian countries. The producer claimed to have a record of compliant finished-product testing according to the European Regulation on microbiological criteria. However, there was no evidence that records had been kept when testing the processing environment or that corrective or preventive action had been taken following positive surface swab results (according to ISO 18593, modified in ….2018!). Since this first outbreak, investigations by the Estonian food safety authority has confirmed the presence of *Listeria monocytogenes* in other salmon facilities in the country.

In Europe, the general food laws put the responsibility of ensuring food product safety on the food business operator. It has been known for at least 40 years, and maybe longer, that finished-product testing is not sufficient for effectively monitoring the implementation of GHP and HACCP. For the past 15 years in Europe, laws have required investigating processing environment and searching for pathogen harbourage sites or growth niches. With the recent outbreak episodes in Europe, no food business operator can ignore the importance of having a holistic approach in the processing environment and must investigate to find the pathogen of interest (*Salmonella* spp., *Listeria monocytogenes*, *Cronobacter* spp.) depending both on the microbial ecology of the product and on the microbial ecology of the processing environment.

Focusing microbial monitoring plans only on finished products is neither in line with current regulation nor scientifically relevant. The recent episodes are a tough reminder to the food industry that safe food production is an endlessly evolving challenge.

Whole Genome Sequencing (WGS) The game changer for microbial source tracking

If neither the new methods nor the emergence of new food safety concerns can explain the high number of recalls and record number of outbreaks, what else could have changed in the landscape to explain, at least in part, the situation?

For many years, the supervision and tracking of microbial contamination has been done through serological testing, which has definitively improved food safety, but this alone has not been sufficient to identify much beyond low-prevalence food contamination and has not been able to identify related sporadic cases of infection. The *Listeria* salmon-related outbreak in Scandinavian countries is probably the best example to highlight what has changed most drastically: microbial source tracking. While in the mid-2000s, sequencing entire microorganisms became feasible, though technologically highly sophisticated, beginning in 2012, the discovery of new typing techniques through next generation sequencing (NGS) approaches has changed the game. WGS techniques have proven incredibly more efficient (timing, reproducibility, information sharing) than the previous standard, Pulsed Field Gel Electrophoresis (PFGE). Using a library of clinical isolates, and possibly food isolates as well in the near future (as is already the case in the United States), European Food Safety Agencies now have the capacity to track low-prevalence contamination for years following incidents, a method currently unavailable to food business operators that rely on finished-product testing.

"Classical" outbreaks, when a clear cause of deviation can be investigated at a specific food business operator (which should have detected and alerted the authorities), are reported more efficiently and quickly as epidemiological investigation is initiated sooner and is more focused. Low-level contaminations, which until recently remained untracked, can also be investigated to their sources.

In the investigation of the *Salmonella* Agona infant formula outbreak in early 2018, the Pasteur institute retroactively linked 25 cases of "sporadic" contaminations between the outbreaks of 2005 (141 cases) and 2017 (38 cases) (Santé Publique France 2018). The contamination went unnoticed despite a great deal of finished-product testing but as the first investigation team stated in 2007, "Routine microbiologic controls are insufficient to detect a low-grade contamination" (Brouard et al. 2007).

The push for new standards of food safety management certification

The first Food Safety Management System (FSMS) certification standards were initiated in the mid-1990s following a wave of foodborne incidents in Europe (the most well-known one being the mad cow disease episode). Since then, aligned in their approach under the umbrella of the Global Food Safety Initiative (GFSI), these standards have contributed greatly to improving food safety within the entire food production industry.

In the latest version, these standards focus on two new domains where it is believed major improvement will be made in ensuring safe food production:

- **Food Safety Culture:** The attitudes, values and/or beliefs which are prevalent at the site, relating to the importance of product safety and the confidence in the product safety systems, processes and procedures used by the site (BRCGS, Global standard food safety issue 8).
- **Processing Environment Monitoring:** The design of the environmental monitoring programme shall be based on risk, and at a minimum include sampling protocol, identification of sample locations, frequency of tests, target organism(s) (e.g. pathogens, spoilage organisms and/or indicator organisms), test methods, recording and evaluation of results (BRCGS, Global standard food safety issue 8).

This new certification standard is completely aligned with the expectations of the constantly evolving EC Regulations. The ability to collect scientific evidence of pathogens of concern has been highly improved with new technology for investigation and improvement of microbiological reference methods. This requires a change in the design and implementation of FSMS within the food process to implement a "food safety by design" approach, from hygienic engineering to validated control measures. Relying only on supervision by regulatory bodies is insufficient and the education of the food sector will ensure that producers have the ability to produce safe food.

Conclusion

While the investigation system has gained a lot of efficiency in epidemiology for the source-tracking of food borne diseases, food business operators, on the other hand, should reflect on the limitation of finished-product testing to ensure the entire food system is in compliance (whether it is microbiology, chemistry, physical bodies, or allergens).

New requirements for certification focus on two major points of improvement: food safety culture (food safety happens when no one is watching) and processing environment monitoring (search for pathogen harbourage sites or growth niches before contamination enters the food process).

Food safety authorities and reference laboratories are making the best use of the newly available techniques (validated reference methods, microbial source tracking, WGS). Most industrial actors in the food chain still need to implement these new tools to ensure safe food production and fulfil the Regulation (EC) No 178/2002 requirement.

Disclaimer

This article represents the opinions of the author and not those of the organizations or bodies he represents.

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/case histories

Dioxins in food and feed

A never-ending story and lessons learned.

Peter A. Behnisch and **Abraham Brouwer**

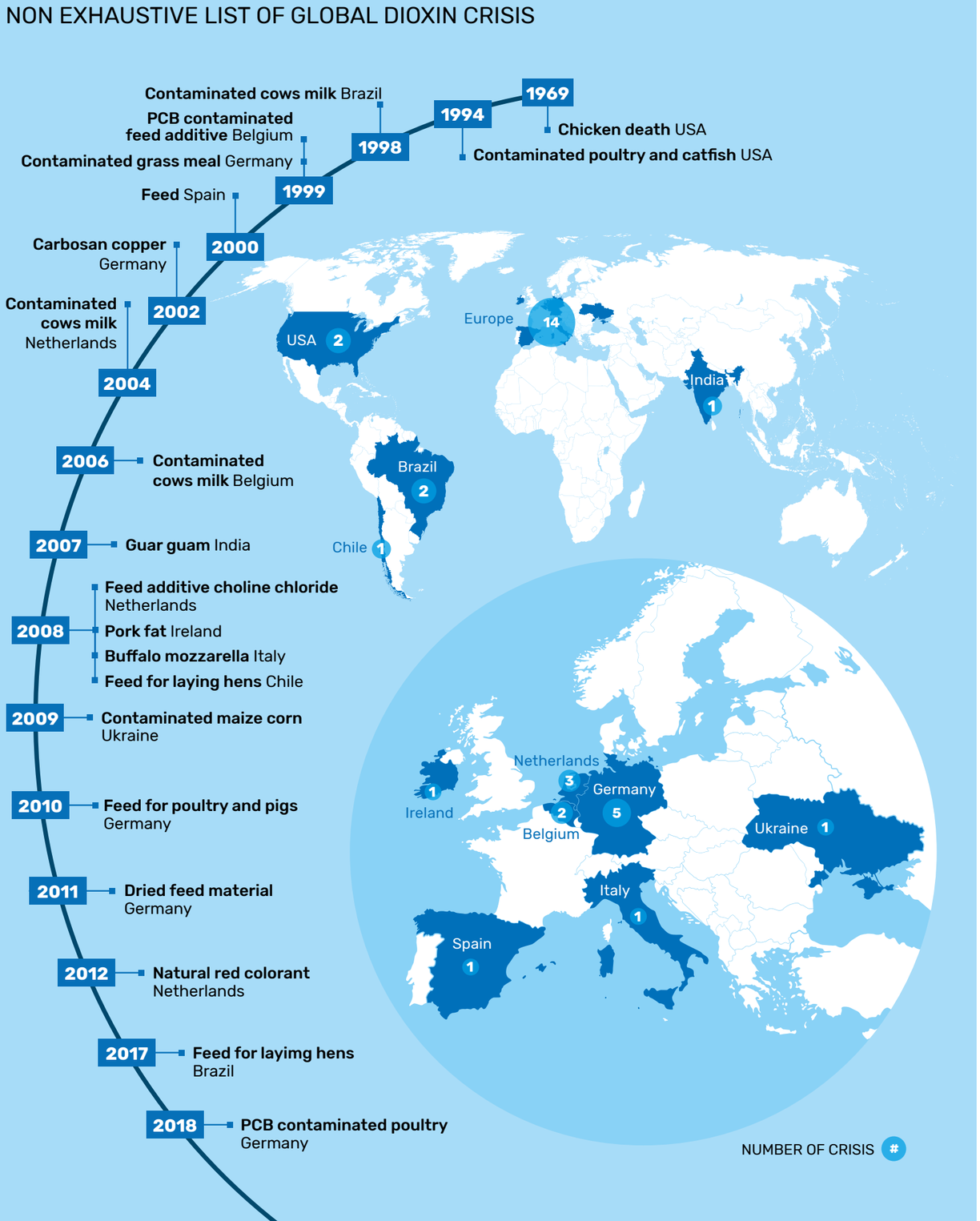
Peter A. Behnisch, PhD, *has been the Director of BioDetection Systems BV in Amsterdam (The Netherlands) since 2006. He has been involved in testing dioxins/PCBs since his. PhD at Eberhard-Karls University of Tübingen, Germany (1993). He has additional professional working experience in dioxin testing in England (Lancaster University, 1997), Japan (Kaneka, 1998-2002), and Germany (SGS and Eurofins,2002-2006).*

Abraham Brouwer *is a distinguished professor of environmental toxicology and ecogenomics at Vrije Universiteit Amsterdam and has 30 years of professional experience as a licensed toxicologist. He is the founder and CEO of BioDetection Systems and for the last 25 years he has initiated and promoted the development and widespread application of effect-based bioanalytical methods for safety assessments of food, feed, environmental matrices, chemicals, food contact materials, and natural compounds.*

At the beginning of the first dioxin crises at the end of the last century, we did not anticipate that they would continue to pop up as a problem in the world for so long and that so many unexpected dioxin sources existed in the feed and food sector (*see the figure on page 46*). Over time, we have learned many lessons about how dioxins can enter the feed/food chain through many different industrial processing steps occurring in many countries. There have been many multi-national dioxin crises with global implications (Behnisch 2005, 2011; Codex 2017, 2018; EFSA 2012; Fink-Gremmels 2012; Motarjemi 2013; Petrlik 2018, 2019; Weber 2018; Malisch 2017; McEvoy 2016; Vugt-Lussenburg 2013). During the first few dioxin crises, the individual countries where they occurred had not yet established national legal dioxin limits. After the EU guidelines appeared in 2001, regular monitoring programs (by national or industrial associations) using screening and confirmatory analysis were instituted, resulting in the detection of many new and unexpected dioxin/PCBs incidents that led to international feed/food scandals. Other non-EU countries either set up strict limits (e.g. USA, see FDA 2019) or used EU standards as an import benchmark (e.g. Russia, China). From these first experiences, different formation and distribution patterns of dioxins and dioxin-like compounds (e.g. PCBs, PCNs, PBDD/Fs, see Behnisch 2001) in the environment and food chain were identified. At the beginning of nearly every dioxin/PCB crisis, the source of contamination was unknown and the original source was detected only by the intensive detective efforts of food dioxin experts without the benefit of cooperation with environmental experts in most cases. The surprising and unforeseen impact of many kinds of dioxin contamination sources (e.g. PCP-treated wood as a fuel source for feed drying, PCB oils mixed with plant oils, several minerals from mining, all kinds of thermal processes with dioxin precursors, waste burning, or pesticides; see Behnisch 2005, Codex 2017 and 2018, Malisch 2018, Weber 2018), which also play a role in feed/food recycling, has been to cause further dioxin scandals affecting the global feed and food chain, making it very difficult to predict and protect populations from future dioxin scandals.

In all these incidents, the demand for dioxin analyses temporarily increased dramatically, often beyond the capacities of local or national laboratories. Only a few accredited international laboratories have the capacity to process more than 300 samples per week. Compared to time- and cost-intensive confirmatory chemical analyses (by HRGC/HRMS or GC/MS/MS), screening tests such as CALUX reporter gene assays require only minimal effort and cost to accelerate and extend laboratory capacity (Behnisch 2005, 2010, 2011, 2018), rendering these screening tests advantageous both for bigger feed/food exporting countries as well as for countries equipped with less analytical capacity (see India, UNIDO 2011; Codex 2018). Fortunately, in most of the dioxin crises described here, only a small percentage of samples tested positive, making screening tests ideal in these situations. Despite many dioxin crises in the feed/food sector, there has been no significant increase in environmental testing to avoid further dioxin crises. Industrial processes in the metal industry, PVC production, mining, waste incineration, and the handling of chlorinated aromatic compounds (e.g. PCB oils) often lead to environmental contamination (see Behnisch 1997, 2005;

Fortunately, in most of the dioxin crises described here, only a small percentage of samples tested positive, making screening tests ideal in these situations.



Petrlik 2019; Codex 2018) but these processes are usually only sanctioned in the few cases where it is possible to manage the whole cascade of events in dioxin crises. Therefore, we expect that dioxin crises will be a never-ending story.

Although very large volumes of feed/food are traded on the global market, only a small percentage is tested for dioxins. In order to better manage food/feed contamination issues and reduce the number of dioxin crises, it is paramount to develop easier and faster screening methods that are mandatory on a global scale (rather than country by country). This requires the urgent acceptance of innovative modern food analysis approaches by an international association/federation (e.g. EC/644/2017 and EC/771/2017) or inclusion in the Codex Alimentarius FAO/WHO reports (e.g. Codex 2018) which are accepted around the globe (e.g. USA, Asia, Africa, South-America). It is also necessary that such screening tests reach international acceptance for other relevant matrices (e.g. soil, sediment, sludge, ashes, mother milk and blood).

All crisis situations demonstrate the need for analysing a large number of samples within a short timeframe early on to prevent the occurrence of more widespread contaminations. Here we will present the key role of combinatorial detection efforts using biological effect-based screening and confirmatory chemical analytical investigations. There are now several cost-efficient biological and chemical screening methods available and accepted by EU guidelines in addition to the chemical confirmatory methods (EU/644/2017 and EU/771/2017, also mentioned in Codex 2018). These lower-cost methods make it easier for governments, companies, and citizens to ensure the safety of their food through more frequent monitoring. The cost of routine food analyses is generally small in comparison to the economic costs of dioxin incidents. Through the industry implementation of several quality systems in a few countries (e.g. QS Qualität und Sicherheit GmbH, Verein für kontrollierte alternative Tierhaltungsformen e.V. KAT and GMP+ in central Europe), repeated sampling and analysis throughout the year is now mandatory and, as a result, the number of dioxin crises has declined in these countries. By labeling feed/food products with quality symbols, citizens can be assured that at least once a year some testing is done, which can lead to more trust in, and a higher value for, these compliancy-testing products. Higher annual testing frequency is urgently needed in more countries because if "you don't search, you won't find" these highly toxic ultra-trace pollutants. In addition to large scale incidents, there are also general problems in environments currently contaminated or contaminated in the past that have led to locally contaminated free-range eggs (e.g. Petrlik 2019) as well as to PCBs in eels in polluted rivers (e.g. Behnisch 1997), in meat and milk from older animals (e.g. mother cows), and in many wild apex predator species (e.g. Behnisch 1997, 2018; Schwarz 2016).

Overview of dioxin crises - continued environmental monitoring is required

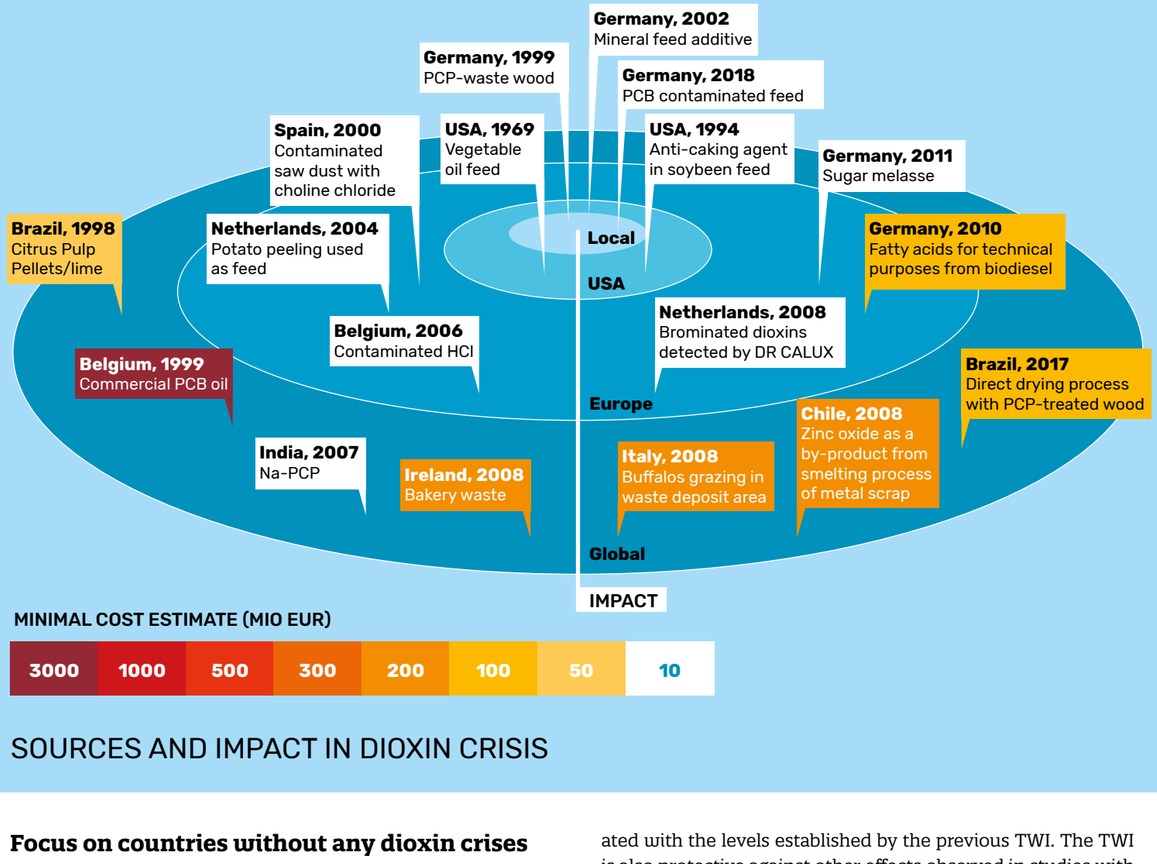
Recently, the Codex committee on contaminants in food released an overview of current PCB and dioxin sources as well as a recommendation on how to handle them (Codex 2018). They described the release of PCBs from leakages, accidental spills, and illegal disposal and through emissions via air from thermal processes. The emission of PCBs from paints and/or sealants into the environment (e.g. during demolition and reconstruction of older buildings) appears to be of some importance as a source. Dioxins are formed as unwanted by-products from several human activities, including certain industrial processes (e.g. production of chemicals, metallurgical industry) and combustion processes (e.g. waste incineration, for further information see the UNEP dioxin toolkit). Accidents at chemical factories have been shown to result in high emissions and contamination of local areas. Other dioxin sources include domestic furnaces, agricultural burning of harvest residues, and backyard burning of household waste. When released into the air, dioxins can be deposited locally on plants and on soil, consequently contaminating both food and feed. Sources of dioxins in soil include deposition from atmospheric dioxins, application of contaminated sewage sludge or waste incineration residues to farm land (e.g. Petrlik 2017), flooding of pastures with contaminated sludge, and prior use of contaminated pesticides (e.g. 2.4.5-trichlorophenoxy acetic acid) and fertilizers (e.g. certain composts). Other sources of dioxins in soil may be of natural origin (e.g. ball clay).

Global red alert - cows and chickens grazing on waste dump sites and soils contaminated through former pesticide usage

Recently, in several countries (Ghana, Nigeria, China, and Indonesia), poorly controlled waste dump sites and badly managed incinerators (e.g. burning plastic waste or e-waste, inadequate management of ash residue) have led to high levels of all kinds of dioxin-like compounds (chlorinated and brominated dioxins, PCNs, Petrlik 2018, 2019). Also, several cases have been reported of food contaminated by animals living in areas where war crimes have been committed (such as Agent Orange, see e.g. Weber 2018). Also, fire areas need more serious monitoring efforts from local authorities (as done, for example, in the case of the "Land of Fire" in several regions in Italy, see Behnisch 2018; see also Costner 2005).

Perspectives for laboratories: a few dioxin congeners or a total dioxins approach - what is safer?

From the beginning, EU regulations have focused on a very limited number of dioxin-like compounds (17 PCDD/Fs and 12 dioxin-like PCBs) while other dioxin-like compounds with similar toxicity have only been irregularly monitored (polybrominated dioxins/PBBs, mono-tri-chlorinated dioxins, PCNs, N-aromatic dioxins). Therefore, many industrial processes emitting other dioxin-like compounds (e.g. PCNs, brominated dioxins) still require monitoring to be designated dioxin-free, again with additional costs, while total dioxin effect methods, like the cell-based reporter gene bioassays always cover both regulated and non-regulated dioxin-like compounds!



SOURCES AND IMPACT IN DIOXIN CRISIS

Focus on countries without any dioxin crises

Globally, most countries (Asia, Africa, East-Europe) never took dioxin contamination in feed/food seriously at a national level and, as a result, environmental and food-related dioxin crises occur daily without public notice (e.g. see many examples from IPEN, such as the China POPs report or recent Ghana e-waste recycling, Petrlik 2018, 2019).

New tolerable weekly intake for dioxins and dioxin-like PCBs seven times lower: call for more testing

Recently, EFSA has confirmed the conclusions of previous assessments that dietary exposure to dioxins and dioxin-like PCBs is a health concern (EFSA 2018). Data from European countries indicate exposure in excess of EFSA's new tolerable intake level across all age groups. The EFSA Panel has set a new tolerable weekly intake [TWI] for dioxins and dioxin-like PCBs in food of 2 picograms/per kilogram of body weight. The new TWI is seven times lower than the previous EU tolerable intake set by the European Commission's former Scientific Committee on Food in 2001. The main reasons for reducing the TWI were the availability of new epidemiological and experimental animal data on the toxicity of these substances and more refined modelling techniques for predicting levels in the human body over time. This new TWI is protective against effects on semen quality, where adverse health effects had been associated with the levels established by the previous TWI. The TWI is also protective against other effects observed in studies with human subjects: lower sex ratio of sons to daughters, higher levels of thyroid-stimulating hormone in newborns, and developmental enamel defects on teeth. The main contributors to average dietary exposure for most age groups in European countries are fish (in particular, fatty fish), cheese, and livestock meat. Average and high exposures were, respectively, up to five and 15 times the new TWI in adolescents, adults, and the elderly. Toddlers and other children up to 10 years of age exceeded the new TWI by similar ratios.

Dioxin crisis situations and lessons learned

Certainly, one of the most important methods for preventing dioxin crises was to establish international alert systems such as the EU Rapid Alert System for Food and Feed (RASSF), which has generated a steady number of dioxin alerts in the last few years (e.g. RASFF notifications in 2012: 24, in 2013: 16, in 2018: 15 and in 2019: 11). However, new dioxin sources remain a never-ending story, including both the most recently-reported sources (e.g. 2018: calcium chloride with fat coating impacting Benelux countries; 2019: herbs from Germany; 2019: silkworms from China; 2019: ginkgo biloba leaves from China) along with the previously known sources that remain a concern (2019: canned cod liver, horse meat, tuna slices, lamb, copper sulphate). It is promising to see a low number of positive samples in non-EU countries (such as Brazil and Chile) as verified through intensive continuous dioxin/PCB monitoring of many kinds of feed/food with reporter gene CALUX screening (see e.g. Behnisch 2010; PNCRC 2016).

Global perspectives

Only a few countries outside of the EU have established dioxin guidelines. A few of those countries also include dioxin-like PCBs while only a very few countries also demand monitoring of non-dioxin-like PCBs. Globally, only a few industrial quality systems (such as QS, KAT, GMP+ in the EU) have required a specific frequency of dioxin testing. In most cases outside of the EU, dioxin testing is only done for products that are produced for export to the EU and, therefore, EU import regulations must be fulfilled. Consequently, many of the industrial practices described in this article still pollute feed/food in these countries with dioxins on a daily basis. This is mostly due to the high investment costs necessary for analytical chemical instruments as well as the associated maintenance costs even though cheaper screening tests have been thoroughly evaluated and are well-suited for this purpose (reporter gene assays, for example, see EC/644/2017, see also Codex 2018). Most countries only invested in limited infrastructure with just one or two national laboratories and a few experts and limited expertise rather than building a network around easier, lower-cost screening labs with local experts and expertise to manage both the constant, locally-occurring feed/food pollution by various dioxin-like compounds as well as the continuously-repeating, globally-occurring dioxin/PCB crises. Exemplary intensive monitoring programs outside the EU to monitor and protect feed and food from dioxins by using CALUX bioassays exist in Brazil (PNCRC 2016), Chile (Behnisch 2010), Kuwait (Husain 2014), Russia, Taiwan (Lin 2014), Turkey, and Vietnam (Hoang 2014).

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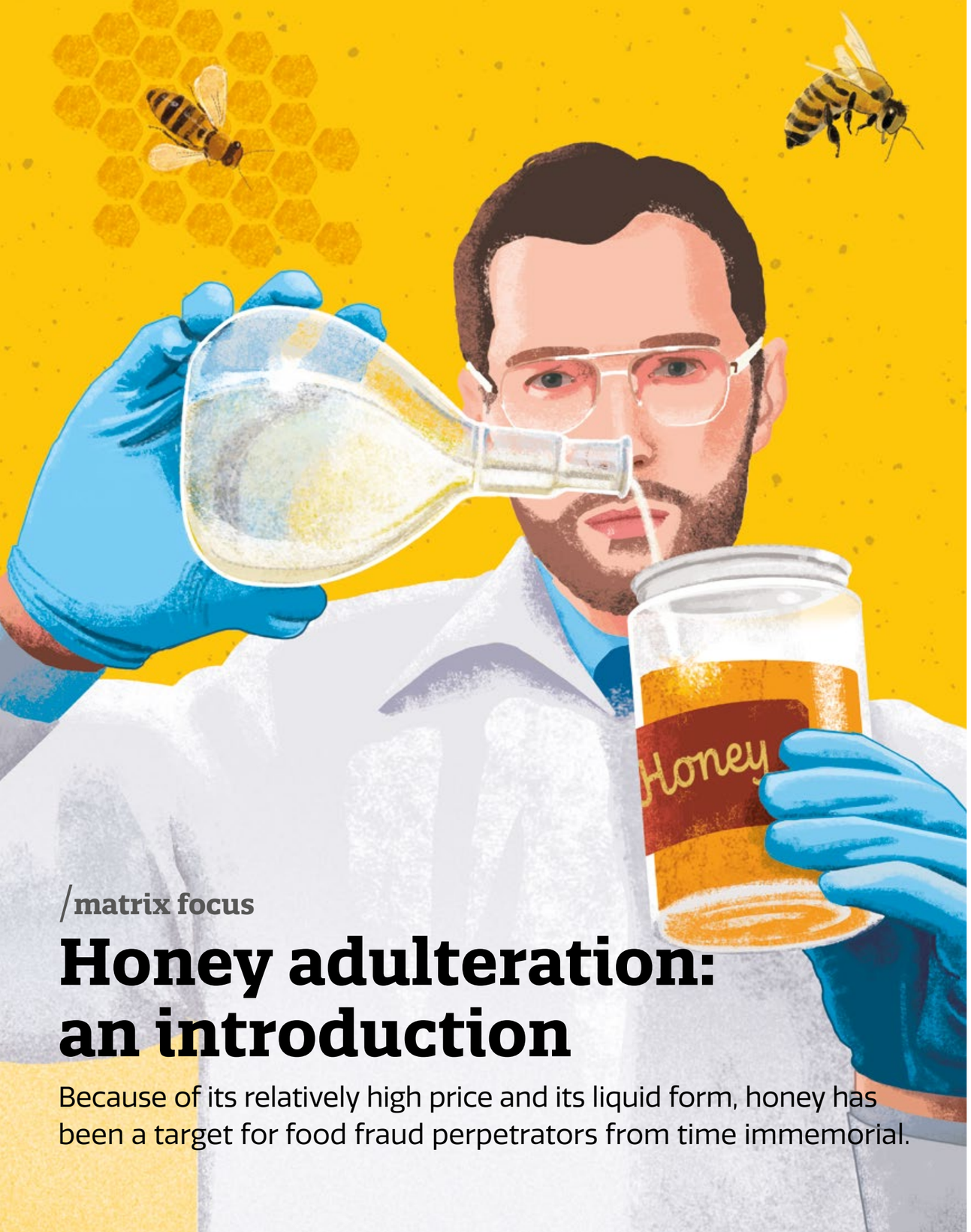

/matrix focus

Honey adulteration: an introduction

Because of its relatively high price and its liquid form, honey has been a target for food fraud perpetrators from time immemorial.

Karen Constable

Karen Constable is the principal consultant for Authentic Food and Food Fraud Advisors. She has more than 20 years' experience in food manufacturing, quality assurance, food product development, and food safety. She is an accredited adult trainer, a professional member of AIFST (Australian Institute of Food Science and Technology), and a member of the IAFP (The International Association of Food Protection) Food Fraud Professional Development Group.

Honey. Naturally delicious, syrupy and sweet, with a delicate, unique flavour, honey has been prized as a special food since pre-history. Cave paintings in Europe that date back more than 8000 years depict humans foraging for honey.

Honey is a sweet, viscous liquid produced by bees, using nectar from plants and their own enzymic secretions. Bees make and store honey in honeycombs within beehives. Human beekeepers collect the honey from semi-domesticated bee colonies that live in man-made hives fitted with removable frames upon which the honeycomb is formed. Each beehive produces between 30 and 40 kg of honey per year. After honeycombs have been collected from the hive, raw honey is removed from the honeycomb, filtered, and sometimes pasteurised.

Because honey is a natural food, it can vary significantly between different locations and at different times of the year. Major honey producers carefully select and blend honey from different sources so that they can sell a final product with colour and flavour that is relatively consistent from batch to batch. Honey is used as a food and food ingredient, as well as an ingredient in cosmetic products such as face creams, lotions, and lip balms. Honey has antimicrobial properties imparted by enzymes which have been added to the honey during its production in the beehive. Its antimicrobial and humectant properties make it useful in topical medications and wound treatments. Manuka honey, made by bees that have fed mostly on the flowers of the Manuka plant, exhibits especially strong antimicrobial properties and this makes Manuka honey highly prized by consumers. Manuka bushes grow only in Australia and New Zealand. Because of its relatively high price and its liquid form, honey has been a target for food fraud perpetrators from time immemorial. In recent times, bee mortality from a phenomenon known as "colony collapse disorder" has reduced honey production in many parts of the world (Oldroyd 2007; EPA 2018), driving up prices and making honey fraud even more attractive for criminals. Organic honey supplies have been particularly affected as beekeepers are forced to treat hive diseases with pesticides to keep their bees alive. Pesticide-treated hives cannot be used to produce organic honey.

Food fraud includes adulteration, in which something is added to a food; misrepresentation, in which a food is claimed to be something that it is not; simulation; and counterfeiting. All these fraud types affect honey. Extending honey by adding water is the simplest way for a dishonest honey seller to increase profits and this type of honey fraud is thought to have been common from the earliest days of trade. Once diluted, the honey may have its sweetness and viscosity increased by the addition of sugars. A 2018 round table discussion by honey stakeholders in Europe described the most common fraud as being adulteration with sugar syrup (Whitworth 2018).

Honey's liquid form makes it easy to extend with water and adulterate with sugar. But it is also relatively easy to make 'fake' honey that contains no bee products at all. Fake honeys can be made from sugar syrups, colours, and flavours. It is also possible to make honey by harvesting "unripe" honey from beehives (Tamma 2020), while it

In recent years there have been a number of media reports alleging fraudulent adulteration in well-known honey brands.

still has a high water content, then artificially drying it to a lower water content that mimics authentic, "rip" honey. An indirect type of honey adulteration is achieved by feeding the bees on sugar water, rather than letting them forage on flowers.
The most expensive honeys are those from singular botanical sources or special geographical sources. It can be difficult or impossible for purchasers to verify claims made by the supplier about such sources, making misrepresentation attractive to fraudsters.
As an example of geographical mis-representation, it has been claimed that some European countries have increased their honey exports at about the same rate as they have increased their imports from China, while there have been no increases in local production. It is alleged that the honey is being fraudulently re-labelled as European-grown honey when it originated in China (Tamma 2020). Along with geographical misrepresentation, fraudulent claims about the botanical sources of honey are another very common type of food fraud. Manuka honey is a single-botanical source honey that is often affected by such fraudulent claims. In fact, a New Zealand honey grower association reported in 2016 that only around 1,700 tonnes of genuine manuka honey was produced each year, while 10,000 tonnes of "manuka" honey was sold worldwide (NZHerald 2016).
Many countries have standards for honey which stipulate that it must be pure, natural, and free from added water or sugars. Sellers of honey are also required to be accurate in any claims they make about the geographical or botanical sources of honey. In recent years there have been a number of media reports alleging fraudulent adulteration in well-known honey brands. Research studies and surveys of honey consistently find high levels of adulterated or "suspect" samples. In one study of 95 honey samples from 19 countries, 27 percent of them were found to be adulterated according to the official AOAC International C4 test (Zhou et al. 2018).
The C4 test measures the ratio of carbon-12 and carbon-13 isotopes in a honey sample to identify the addition of C4 carbohydrates from cane sugar or corn syrups. The plants from which honey is derived are usually C3 plants.
Although it is acknowledged as being very widespread, honey fraud is rarely the target of food standards enforcement activities. Adulterated honey has resulted in few direct health issues for consumers. For this reason, food safety and regulatory authorities may be inclined to overlook honey fraud for other, more dangerous food safety and quality problems. Because testing occurs infrequently in the marketplace, and only sporadically, there is little risk of a perpetrator being caught. In addition, the penalties for food fraud are often not severe.
However, while it usually does not pose a direct health risk, fraudulent honey can be lacking in nutrients and other components of honey that can impart health benefits, so consumers will not receive the expected benefits of honey if it has been affected by food fraud.
It is not only consumers that are hurt by honey fraud: food businesses risk damage to their brands if the honey they purchase is inauthentic. It has been alleged that food fraud perpetrators have learnt how to 'trick' the commonly used C4 test for honey authenticity (Scimex 2018). This means that purchasers of bulk honey who use the C4 test to check for authenticity are at risk of buying honey that has been adulterated. If those purchasers use the honey as ingredients in food products, or pack and resell the honey, their brands can suffer damage and loss of consumer trust. Market withdrawals of affected product can cost hundreds of thousands of dollars.
Purchasers of honey should beware of the following fraud types:

- direct adulteration by the addition of sweeteners such as sugar syrup, corn syrup, invert syrup, fructose;
- direct adulteration by the addition of colourants;
- indirect adulteration - this occurs when bees are fed on sugar water rather than obtaining their food from flower;
- "unripe" honey production methods; the honey is harvested; from the hive while it still has a very high water content and is then artificially dried;
- dilution with water;

Food fraud perpetrators have learnt how to 'trick' the commonly used C4 test for honey authenticity.

- false claims about botanical source (note, some problems with botanical source claims are due to genuine errors on the part of the beekeeper rather than deliberate frauds);
- false claims about geographical origins;
- misrepresentation of organic status;
- addition of honey fragrance, pieces of beeswax and bee bodies to make fraudulent honey appear authentic (more likely for artisan and "farmhouse" honey);
- honey may also be affected by undeclared or illegal levels of antibiotic and pesticide residues.

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Multiplex binding assays for the detection of antibiotic residues in food

Although multiresidue detection seems like the ideal screening test for antibiotic residues, just a few platforms that accomplish this are actually available. Advantages and limitations of the commercially available systems are hereby presented.

Maurizio Paleologo

Although multiresidue detection in food and feed seems like the ideal screening test, and although a hundred million euros have been spent in the past 30 years to develop a high-sensitivity biosensor capable of detecting all chemical contaminants in a single test, just a few platforms that accomplish this are actually available. Advantages and disadvantages of the commercially available system are hereby presented.

Why is multi-target screening for antimicrobials needed?

As described in the first paper of this issue, antimicrobials have been and continue to be widely used—and sometimes abused—both in livestock and seafood farming. Residue concerns arose in the 1970s in some European countries but it wasn't until the end of the 1980s that the US and the EU established maximum residue levels (MRLs). Food Business Operators (FBO) in every food chain have the responsibility to guarantee that their products are compliant with these limits (EU Commission 1990). During the "tech era" (1970-1990), control of antibiotic residues was generally driven by technological issues (e.g. residues in milk hinder the fermentation in the cheese production process) but then, during the "regulatory era" (1990-2020), a need to cope with legal limits arose and the list of target molecules became much longer. With the exception of a few cases, during the "tech era", Microbial Inhibition Assays (MIAs), like the well known *Delvotest*, were fit for the purpose. After 1990, residue control became necessary even when milk was sold as such, not just for cheese production. A huge market for rapid diagnostic tests was created quickly. *Lac-tek*, a simplified ELISA seven-minute assay in a test tube and then *SNAP*, both by IDEXX, made milk controls fast enough to be used in milk production facilities. Of course, the end-users were looking not only for speed, but also for simplicity. Moving the Lateral Flow ImmunoAssay (LFIA) from clinical applications to the food sector, a Belgium company, UCB Bioproducts, launched the first Lateral Flow Device (LFD) for milk testing in 1996. In the subsequent 20 years, many other manufacturers developed similar products (*ROSA* by Charm, *Tetrasensor* by Unisensor, many Chinese brands). Because this occurred during the "regulatory era", many of these products incorporated 2 or more binding reagents in order to enlarge the number of classes of residues detected. However, with the established LFD technology, it was difficult to accommodate more than three or four groups of molecules on a single test.

With the established LFD technology, it was difficult to accommodate more than three or four groups of molecules on a single test.

In the meat, seafood, and honey sectors, the interest in residue screening only arose during the "regulatory era". In this case, however, particularly for seafood and honey, the main target was not represented by beta-lactams and turnaround time (TAT) was less important so ELISA kits have generally been used for screening (the only exception was *Charm II*, a battery of radio-immunoassays and radio-receptor assays). Manufacturers offered an increasing number of "broad-range" test kits, but often such assays suffered from an increase in matrix effects. Moreover, it is difficult for food processors to use five to ten different screening assays, each with different sample preparation procedures.

What changed then? For about 15 years an increasing number

of official control labs have been able to screen food of animal origin (FOAO) with fast multiresidue LC-MSMS methods. The pressure to be able to improve the controls became quite serious. At the same time, because of the AMR issue, food retailers started to ask for stricter monitoring, even at concentrations lower than the MRLs. Test kit manufacturers understood that the industry was entering the "residue-free era". The demand for multiresidue screening methods was clearly growing.

The way to a multiresidue screening system

Since the 1990s, several research groups have developed multiplex binding assays for the detection of food contaminants. Some were based on end-point binding assays and some employed a biosensor. However, only a few commercial platforms made it to market. The first to detect antibiotic residues was *Parallux*, an immunoassay-based instrument developed in the nineties by IDEXX in the US, patented in 1996 (WO 1998, Hut et al. 2002). The detection was based on an end-point competitive immunoassay with fluorescent labels. This system was not successful and disappeared after a few years. *Biacore*, an automatic immune biosensor platform was on the market for a few years, too, until GE decided to withdraw it. More recently, the Technical University, together with the Ludwig-Maximilians University of Munich (Germany) developed a biosensor for the detection of veterinary drugs in milk (Kloth et al. 2009). R-Biopharm AG was considering the possibility of commercializing it but this multiplex test never made it to market. It became clear that if a multiplex system required expensive instruments to perform the tests, very few industries or laboratories would choose it. Using external labs equipped with LC-MS/MS was still the more attractive option.

While many research groups in the academy developed and are continuing to develop platforms with many different technologies, often using complicated and expensive biosensors, the food diagnostic companies soon moved to the competitive immuno-array assays as the method of choice, primarily in the end-point format.

The food diagnostic companies soon moved to the competitive immuno-array assays as the method of choice, primarily in the end-point format.

The solutions on the market

Two companies are already selling validated platforms for the screening of antibiotics in FOAO: Unisensor (Belgium) and Randox (UK). Unisensor is manufacturing two different products, one dedicated to the milk industry, using an immuno-array version of the LF immunoassay (*Extenso*) while the other, dedicated to other food chains, is based on the bead-array immunoassay (*Beadyplex*). Randox is manufacturing just one product line and its principle method is a planar immuno-array (*Evidence Biochip Array*).

Extenso, the "broad range" LFDs for milk

Due to its extensive experience and well-known reputation for LFDs for milk testing, Unisensor was able to scale up this technology. Thanks to many spots for different specific binding reagents on the same strip, the *Extenso* system can detect more than 100 antibiotics with an assay time short enough for the milk industry (13 minutes). Running an assay is as easy as running a common LFD, so there is no need for a skilled lab technician as there is for the other multiplex platforms. With this kit, the end-user knows immediately to which group detected drugs belong. The color spots are very small so the results cannot be read by eye, as with a classical LFD for milk testing. *Extenso* requires a dedicated reader. However, the *Extenso* reader also makes it possible to customize the assay method based on end-user analytical needs. End-users choose the analytes needed and pay only for those targets. Another interesting characteristic is that with *Extenso* the milk factory can also screen for aflatoxin M1 and melamine. Sensitivities, according to the manufacturer claims, are all sufficient for controlling milk at the FDA and EU MRLs (apart from aflatoxin M1, whose LOD is not compliant with the EU limit). AFSCAS, the Belgium Food Safety Agency, added *Extenso* to the list of approved method for the control of antibiotics in milk (AFSCAS 2018). ILVO (an official Belgian laboratory recognized by AFNOR in 2017 as an expert in test kit validations) conducted a verification study that confirmed Unisensor claims. Thanks to this study, AFNOR granted to Unisensor an AF validation certification (AFNOR 2018).

Fig. 1: Extenso reader.

Beadyplex, the multiplex for lab testing of meat

To cope with the need to control antibiotic residue in other food chains, where rapid results are unnecessary, Unisensor produces a three-dimensional immuno-array. The binding partners in this case are both dispersed in liquid. The solids phase is made up of nano particles, whose fluorescence signal is different for each analyte. This kind of multiplex is a typical laboratory assay, requiring an experienced skilled technician with a complex instrument to read the results. The assay time is about one hour, the sample preparation is based on a water based extraction, in case of solid samples. According to Unisensor, with the *Beadyplex* kit it is possible to determine up to 80 veterinary drug residues qualitatively. Since 10 assays are performed in the same well with different coded beads, any positive result immediately shows to which antibiotic group the contaminant belongs.

The results of the assay can only be read by the Unisensor Flow Cytometer. There are protocols available for meat (porcine, bovine, poultry), seafood, eggs, and milk. At the moment there is no third-party validation available.

Fig. 2: BeadyPlex assay kit.

Fig. 3: Randox "Bio-chip".

Randox *Evidence*

The third platform commercially available for the screening of antimicrobials in FOAO is the Randox *Evidence*. Randox call this system "Biochip array". This may sound like a biosensor but that is not the case. The solid phase where the binding assay is performed has no direct link to any electronic interphase as, for instance, in the GE *Biacore*. The assay is an end-point immunoassay, multiplexed by positioning several different reagents on the bottom of a dedicated well, with sophisticated binding chemistry. The labeling technology is chemiluminescence. After a sequence of pipetting and washing, as in the single-plex ELISA, the test device must be positioned in a dedicated reader where the luminescence reaction is recorded by a high-sensitivity camera. The assay time is about 2 hours.

The assay device is an original-design, square, nine-well patented cartridge rather than a strip-shaped design as found in the well-established ELISA modules *(Fig. 3)*.

Instead of purchasing 12 strips of 8 wells (up to 96 tests), as in an ELISA kit, with an *Evidence* kit 6 devices with 9 wells are purchased. Thus, each kit allows for analysis of up to 54 samples/calibrators/controls. As with the *Beadyplex*, the competing meat-testing system, the test kits based on the Randox array require a relevant instrumental investment and a skilled technician. Randox claims results are quantitative, but, as it is common also in our experience, end-users often prefer to save chips for samples and make just a qualitative determination (see Gaudin et al. 2016).

Randox offers several different kits. There are five multiplex antimicrobial array kits (Array *I Ultra, II Plus*, *II, IV and V*) and one single-plex kit (*Array III CAP*).

With these four multiplex array kits, the end user, according to the manufacturer's claim, can check the compliancy of the test material for the majority of veterinary drugs.

Randox has invested quite a lot in this area and their antimicrobial array chips have been validated by ANSES.

The Evidence *Biochip* kits for antibiotics can be used to control meat, seafood, milk and honey samples. The sample preparation, like for BeadyPlex, is a simple water-based extraction.
Another *Biochip*, called the *InfiniPlex*, was recently developed by Randox and is especially dedicated to milk testing. With this multiplex assay it is possible to detect up to 130 veterinary drugs. As with the *Extenso*, any positive result is immediately classified as belonging to one of the antibiotic groups (with the *Evidence* kit, there are up to 44 different parallel assays).
The Array *I AMA Ultra* (the test for sulphonamide residues) is validated by AOAC RI (AOAC 2020). The other *Biochip* array kits are not certified but several scientific publications report satisfactory results using the Randox multiplex system for antibiotics (Gaudin et al. 2014, 2016).

To what extent do the existing multiplex screening platforms satisfy market needs?

Extenso. The dairy industry in western countries is generally satisfied with tests like the *Twinsensor*, able to detect beta-lactams and tetracyclines at the same time. In some other countries, there is a need to monitor for chloramphenicol or aflatoxin M1 but it is quite improbable that there is much interest in spending much more in order to cover the risk of antibiotic residues rarely found in milk. The main barriers are the cost of the reader and the need for a trained technician.
However, some big industries are interested in testing for what farmers are using a few times per year. Moreover, when there is evidence that antibiotic residue is present in milk, either because the inhibition test was positive or because fermentation was not occurring properly, the *Extenso* kit could be a useful tool that is simpler, faster, and cheaper than LC-MS/MS.

***Beadyplex* and *Evidence Biochip*.** Both platforms may be good choices for food industries when there is a strong concern over residues. Meat processors are rarely interested in serious multi-analyte screening, particularly when, as in Europe, the percentages of non-compliant meat samples are below 0.5% (EFSA 2019). In the past 30 years, some targets have been closely monitored after scandals (sulphonamides at the end of 1980s, clenbuterol in the nineties, corticosteroids at the end of 1990s and in the early 2000s, etc.). The concern is higher when western food businesses purchase fish, seafood, or honey from developing countries where farmers sometimes do not use antibiotics appropriately. Moreover, in a few cases, retailers have been pushing meat suppliers to conduct the best possible multi-analyte screening. To our knowledge, however, the seafood and fish exporters of India, Vietnam, and Indonesia, etc., are still primarily performing ELISA tests, the honey sector being the only segment where multiplex screening technology is more widely used. Randox has invested quite a lot in this area, their antimicrobial array chips have been validated by ANSES, the France National Agency for Food Safety and European Reference Lab for antibiotic residues (Gaudin et al. 2014, 2016), and several honey producers are using the *Evidence Biochips* to screen for antibiotics in their raw materials.
The main reasons why BeadyPlex and The Randox Evidence are slowly penetrating get market is the relatively complicate procedure and the cost per assay, considering the depreciation of the readers equipment (Flow citometer, luminescent reader).

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Lutz Elflein

Lutz Elflein, PhD, has 20 years professional experience as a certified food chemist, with more than 15 years' experience in bee products. He is currently working at Eurofins in Germany as the Director of Food Authenticity and as the Site Manager of Eurofins Food Integrity Control Services GmbH. Lutz Elflein is Eurofins Germany's designated contact person for expert services regarding honey and other bee products.

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Honey authenticity: scientific, normative, and analytical developments

How new fraud definitions and production guidelines together with state-of-the-art analytical tools help to ensure that honey in the marketplace is authentic and truly labeled.

Food authenticity and food fraud by economically motivated adulteration have been major issues in the food sector since the horse meat scandal in 2013. The EU Commission published a list of the top ten food products subject to food fraud, with honey earning the sixth spot (European Parliament 2013). The EU commission conducted an EU-wide coordinated control plan for honey from 2015 to 2017 to evaluate the current market situation and develop possible action plans for official controls and necessary legislative changes to ensure that consumers receive safe and authentic food. The results revealed that a significant percentage of honey at all stages of the supply chain (production, trade, retail), both from EU and non-EU countries, was found not to be authentic because of mislabeling (botanical/geographical origin) and adulteration with cheaper syrups (European Commission 2017). Thus, while the legislation about honey is very clear in the EU, i.e. a product labelled as "honey" must actually be 100% honey (European Council 2014), inauthentic and adulterated honeys are still present on the market due to the lack of both sufficient (official) controls and the lack

of harmonized and generally accepted guidelines on how to test and assess honey authenticity adequately. This finding applies not only in the EU but also globally. Examples include the 2018 "Honeygate" scandal in Australia with fake honeys occupying up to 27% of the market (ABC News 2018; The Conversation 2018) and the 2018-19 Canadian Food Inspection enhanced honey surveillance which revealed that 22% of honeys imported to Canada were fake (Canadian Food Inspection Agency 2019).
Though the Codex Alimentarius Standard for Honey has served as a global standard for honey for more than three decades (Codex Alimentarius 2019), legally binding requirements (such as the EU Honey Directive) are still lacking in many countries, leading to different production practices, quality levels, and consumer expectations. Therefore, it is still indispensable to improve and harmonize global honey requirements to a uniform level based on the EU and Codex Alimentarius requirements. Recently, two US organizations have launched initiatives to combat widespread honey fraud and unfair competition as national honey regulations are still missing. The U.S. Department

It is still indispensable to improve and harmonize global honey requirements to a uniform level based on the EU and Codex Alimentarius requirements.

of Agriculture (USDA) published a Commercial Item Description (CID) for honey in October 2019 which requires all honeys labeled with the USDA mark to comply with the CID and the United States Pharmacopeia Convention (USP) formed a Honey Expert Panel to develop a Food Chemical Codex (FCC) Identity Standard for Honey (USDA 2019, USP 2019, Lauruick 2019). Moreover, in 2019, Apimondia, the largest beekeeping organization in the world, published its first Statement on Honey Fraud to provide a guidance document for a common understanding about honey purity, authenticity, and the best available and recommended methods to detect fraud (Apimondia 2019). In January 2020, Apimondia updated this statement with further details on appropriate honey production practices, bringing them into accordance with the definitions of the EU Honey Directive and the Codex Alimentarius Standard for Honey (Apimondia 2020). The keynote is that the transformation of nectar into honey must be completely made by bees and that the finished genuine product is matured honey from capped honey combs. During processing and packaging, care must be taken that the honey's essential composition is not changed and/or its quality impaired, that pollen or any other constituent particular to honey is not removed except where this is technically unavoidable in the case of removal of foreign inorganic or organic matter, and that any additions to honey other than honey, including those substances that are naturally contained in honey, are impermissible.
Furthermore, any treatment intended to change honey's essential composition is considered an illicit practice. For the first time, Apimondia clearly stated which practices are considered to be honey fraud, i.e. "methods intended to artificially speed up the natural process of honey production through an undue intervention of man and technology" (Apimondia 2020). Such methods include but are not limited to:

- Harvest of immature honey and dehydration of immature honey with vacuum dryers to produce "matured" honey;
- Sugar feeding during nectar flow or dilution of honey with sugar syrups to increase yield;
- Use of resin technology to remove residues of antibiotics, pesticides, heavy metals, HMF and other contaminants, to lighten the color, or to make any other compositional changes;
- Pollen removal or addition to manipulate the origin determination.

The Apimondia statement thus reifies the definition of honey according to the EU Honey Directive and the Codex Alimentarius Standard of Honey and outlines the appropriate beekeeping and processing practices to produce and sell authentic honey.
Now that the scientific expectations and regulatory requirements for pure and genuine honey have been described, what is the appropriate analytical quality control strategy to verify that the honey is authentic rather than mislabeled or adulterated?
Authenticity assessment of honey includes two main aspects: (a) the verification of the geographical and botanical origin in order to verify accurate labeling and (b) the investigation of possible adulteration with foreign sugars due either to deliberate addition of sugar syrups or to excessive sugar feeding of the bees in order to artificially increase production volume and profits.

Determination of botanical and geographical origin

Microscopic analysis of the pollen spectrum is the reference method for the determination of geographical and botanical origin(s). For this purpose, harmonized and standardized protocols are available (DIN 2002; International Honey Commission 2004). Microscope analysis requires experienced melissopalynology experts with access to information about the natural occurrence of pollen specimens in honeys according to their geographical origin. For a few years, the verification of a declared botanical and/or geographical origin has also been possible through 1H-NMR (Nuclear Magnetic Resonance) profiling which uses large reference databases of authentic honeys. However, the number of statistical models for botanical and geographical origins is still limited to the most common honey varieties and major countries of honey production. Furthermore, it is not currently possible to identify honeys or honey blends of unknown origin by 1H-NMR profiling. This can only be accomplished with high accuracy using microscopic pollen analysis. However, the identification of a geographical origin cannot always be narrowed down to one certain country, e.g. if neighboring countries have similar floral habitats. In these cases, only geographical regions can be stated. As floral habitats change with climate change, the pollen and NMR spectra of monofloral honeys may also change to a certain extent, thus requiring continuous reference database updates to reflect current conditions.
To determine the botanical origin of honey, in addition to employing the pollen spectrum technique, it may further be necessary to examine organoleptic properties (sensory) and physico-chemical parameters (e.g. F/G ratio, electrical conductivity, color, floral marker compounds) to conclude whether a honey can be labelled monofloral or not (e.g. acacia, citrus/orange, rape, sunflower, clover, chestnut, eucalyptus, lavender, etc.). For this purpose, specifications for monofloral honeys in relevant scientific publications, e.g. the Descriptive Sheets of the International Honey Commission (Persano Oddo and Piro 2004), have to be considered. In addition to considering botanical variety, a mismatch between the microscopic pollen spectrum and the sensory or physicochemical properties can also be a first hint of possible illicit manipulation or honey adulteration triggering further investigation with more sophisticated adulteration detection methods like those explained in the next paragraph.

Determination of honey adulteration and illicit processing

To date, practically no officially harmonized and standardized methods exist for honey adulteration detection. The only published harmonized method is the AOAC method 998.12 for the determination of C4 plant sugars in honey (AOAC 2013; Codex Alimentarius 2019). After being in use for almost 40 years, it is fairly obvious that this method cannot cover all of the different types of contemporary honey adulteration that frequently occur in the international market, particularly because the pre-

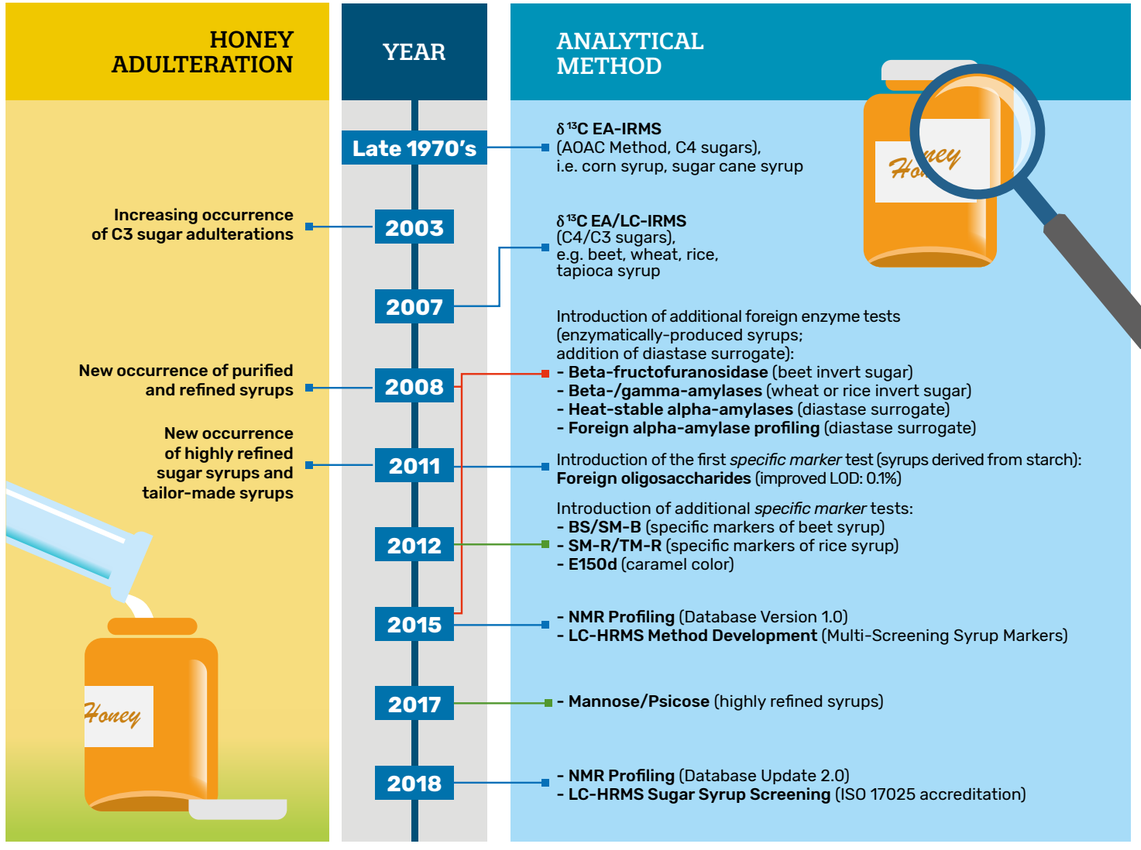

Fig. 1: Historical development of honey adulteration detection methods.

dominant syrup adulterants are currently derived from C3 plant sugars which cannot be detected by the AOAC method. *Fig. 1* provides an overview of the historical development of honey adulteration detection methods over the past years.

Fig. 1 shows the numerous analytical methods available to detect sugar adulterations. Applying all these methods to each honey batch would be very expensive and time-consuming; thus, a risk-based approach based on empirical data from tested honeys and their known origins is necessary in order to set up an adequate quality control plan. This becomes increasingly difficult as the honey trade is further globalized and traceability becomes a greater challenge. Therefore, a more uniform approach to assess honey authenticity regardless of its origin is desirable. Luckily, the more generic isotopic and compound profiling methods for the detection of both known and as-yet unknown adulterations, like 1H-NMR and LC-HRMS, allow for a new state-of-the art approach (*Fig. 2*) (Dong et al. 2018; Dong et al. 2016; Elflein and Raezke 2008; Cabanero et al. 2006; Ulberth 2016; Soares et al. 2017; Siddiqui et al. 2017; Wu et al. 2017; Du et al. 2015; Senyuva et al. 2015; Thomas and Jamin 2016; Spiteri et al. 2015; European Commission 2018).

Based on current scientific and empirical knowledge, the combination of the methods listed in *Fig. 2* provides the highest possible confidence that a honey is pure, authentic, and correctly labeled for its botanical and geographical origin (Eurofins 2019). The methods have their strengths and weaknesses in detecting different types of adulteration so they must be considered complementary methods. The δ^{13}C EA/LC-IRMS, ^{1}H NMR Profiling, and LC-HRMS methods are explained in further detail below.

δ^{13}C EA/LC-IRMS

This test was developed in 2004-2007 as an improved version of the AOAC 998.12 method to detect the presence of added sugars not only from C4 plants (e.g. corn and cane syrup) but also from C3 plants (e.g. rice, wheat, beet syrup) (Elflein and Raezke 2008; Cabanero et al. 2006; Krummen et al. 2004). In addition to determining the δ^{13}C values of protein and bulk honey according to the AOAC procedure (AOAC 2013), the method can also determine the δ^{13}C values of the individual sugars and sugar fractions of honey (i.e. fructose, glucose, disaccharides, trisaccharides) by liquid chromatographic separation and subsequent chemical oxidation into CO_2 gas. In the case of honeys adulterated with starch-based sugar syrups containing small amounts of honey-foreign oligosaccharides as remainders from the starch degradation process, the δ^{13}C value of the oligosaccharides directly indicates the source (C4/C3) of the adulterant. Furthermore, the δ^{13}C isotopic pattern of protein, honey, and the sugar fractions can provide a rough estimate of the nature of the type of adulterant. However, this method, as is the case for all other honey adulteration methods, must be considered a qualitative method. A reliable quantification of the level of adulteration is only possible if the pure honey and the pure adulterant are available as references so that their δ^{13}C values can be determined and used for calculation. The δ^{13}C EA/LC-IRMS method was used by the Joint Research Centre of the European Commission in the EU Coordinated Control Plan 2015-2017 (EU Commission 2016; EU Commission 2017) to check honey products in the European market for adulteration according to the published purity criteria (Elflein and Raezke 2008). From approximately 2300 honeys sampled at all stages of the supply chain, 14% of them were found to be non-compliant. This method is currently the closest there is to a future harmonized standard at the EU level for official controls. Third party proficiency tests have been available since 2019 from FIT-PTS. The δ^{13}C EA/LC-IRMS method has good-to-satisfactory detection capabilities for the major common sugar adulterations (LOD for C4 sugars is approximately 3-5% and approximately 10-30% for C3 sugars, depending on honey type and adulterant). However, the more recent highly refined syrups and the tailor-made syrups which are specifically manufactured to match certain honey types may remain undetected.

TYPE OF ADULTERATION	METHOD DETECTION CAPABILITIES			
	δ^{13}C EA/LC-IRMS	^{1}H NMR Profiling	LC-HRMS	Pollen + Sensory
C4 Plant Sugars	GOOD	SATISFACTORY	VERY GOOD	NOT DETECTABLE only possible in rare cases, if parenchyma cells of sugar cane are present
C3 Plant Sugars	SATISFACTORY	SATISFACTORY	VERY GOOD	NOT DETECTABLE
Tailor-made Syrups	POOR	POOR	VERY GOOD	NOT DETECTABLE
Illegal Processing	NOT DETECTABLE	GOOD	POSSIBLE subject to current R&D activities	POSSIBLE in case of unusual honey sediment
Moisture Reduction Immature Honey	NOT DETECTABLE	POSSIBLE case-dependent	POSSIBLE subject to current R&D activities	POSSIBLE
Geographical and Botanical Origin	NOT DETECTABLE	VERIFICATION ONLY	POSSIBLE subject to current R&D activities	VERY GOOD

Fig. 2: New State-Of-The-Art (SOTA) Approach for the assessment of honey authenticity.

^{1}H-NMR profiling

NMR spectroscopy has been used since the 1970s for quantification and structural analysis and has been used since the late 1980s as a powerful method to test the authenticity of food (e.g. fruit juice, wine, edible oils, and honey). The major commercial application of this method for honey was introduced in 2015 with the Bruker Honey Screener (Bruker 2015; Bruker 2019). A key innovation at that time, ^{1}H NMR profiling is used in a non-target mode for the detection of adulterated honeys by comparing NMR spectra of commercial honeys with those stored in a large reference database of authentic honeys from honey-producing countries worldwide (Spiteri et al. 2015). To date, there is no harmonized and publicly available reference database (though it is under discussion at the EU Joint Research Centre). Laboratories specialized in honey authenticity testing by NMR either use the commercially available Bruker database (license system) or their own proprietary databases. In order to produce identical analytical results and comparable result interpretations, each NMR lab would need to use the same analytical procedure, instrumentation, and reference database. As this is not currently feasible, regular cross-checking and benchmarking is done by the different labs in order to ensure consistent result interpretations as far as possible. The NMR databases must be "living" databases in order to adequately reflect any possible changes in the modes of honey production, changes in climate, and environmental factors affecting the composition (and thus the NMR spectra) of honey as a natural product. Considering that, it is important to be aware that new releases of the NMR database can cause changes in interpretation, i.e. a honey which might have been classified as authentic in the past might now be classified as adulterated or vice versa. This implies that non-compliant results should always be cross-checked with complementary methods (e.g. δ^{13}C EA/LC-IRMS or LC-HRMS) for secondary confirmation and further evaluation of the root cause for failure. The ^{1}H-NMR profiling method is a versatile screening method providing quantitative results for some quality parameters like sugars, HMF, organic acids, and amino acids, as well as verification of botanical/geographical origin and adulteration by statistical models. In addition to the limitation for origin verification already outlined earlier, the adulteration detection in honey is mainly linked to statistical models using compound ratios and a few adulteration markers (mostly indirect detection by mismatching profiles). Therefore, practical experience over the last 5 years shows that NMR is not necessarily the most sensitive method for detecting foreign sugars. Detection limits start at 10-15% of added sugar but can also be much higher, depending on the honey type and adulterant. Therefore, it is generally recommended to combine NMR with the complementary techniques δ^{13}C EA/LC-IRMS and LC-HRMS. In relation to immature honey and honey illicitly processed, e.g. by resin technology, NMR is a good tool to reveal this type of fraud, because the NMR profiles of such honey are significantly different compared to those of authentic and mature honey.

NMR spectroscopy has been used since the late 1980s as a powerful method to test the authenticity of food.

LC-HRMS (High Resolution Mass Spectrometry)

LC-HRMS is the most recent and most powerful analytical tool for honey authenticity assessment (Eurofins 2018). Comparable to NMR, this technique allows a chemical compound profiling of honey. The primary advantages are that (a) the detection limits for the compounds contained in honey (whether natural or artificially added) are at least 10-100 times lower than for NMR, (b) the compounds contained in honey can be separated through liquid chromatography (LC) in different modes (e.g. reversed phase or polar chromatography) for better detection, and (c) the measured compounds can be more easily identified according to their measured exact mass weights (precise determination down to the fifth decimal) by search in publicly or commercially available mass spectral databases. In contrast to NMR, LC-HRMS does not require large databases of authentic honeys to detect adulteration with foreign sugars. Instead, syrup databases are set up. In a first step, a non-target approach is used to screen the adulterants (or known adulterated honeys) for suitable markers which are characteristic of the syrup, but

do not occur naturally in honey. Due to the sensitivity of the method, it is possible to identify not only one single marker but several markers for a certain type of sugar syrup. Once added to the syrup database, the technique can be used in a targeted mode to recognize the specific syrup marker profiles in honeys adulterated with these syrups. The availability of several markers per syrup significantly improves the accuracy of results by minimizing false positive results which may occur with single marker methods and by providing additional information about the type of sugar syrup found. Thus, the differentiation of various syrups by their marker profiles is possible. This information may help trace the source of adulteration and whether it is an actual fraud in the sense of economically motivated adulteration or an accidental adulteration due to bad beekeeping practices (e.g. improper feeding, unsuitable bee feeding products). As the LC-HRMS measurements are always done in full scan mode, data from previously measured honey samples can be re-evaluated retrospectively as soon as new adulterants have been identified and added to the syrup database. As most syrups typically have syrup marker profiles that include both more general marker compounds occurring in many different syrups and syrup-specific marker components, LC-HRMS can also detect as-yet unknown adulterants by the more generic marker components. The sensitivity of LC-HRMS in detecting added foreign sugars is superior to 1H-NMR and δ ^{13}C EA/LC-IRMS. Foreign sugars can be detected at levels as low as <1%. Therefore, a "threshold level" of 5% added foreign sugar is required in order to take into account the technically unavoidable minor traces of sugar syrup found in honey even when good beekeeping practices are followed. The beekeeper can only estimate the required amount of bee feed and will not be able to control the consumption of the feed and the possible income of new nectar flow on a daily basis. Thus, after winter feeding or necessary feeding in between nectar flows, it is almost unavoidable that minor traces of remaining bee feed can be translocated by the bees to the new honey combs. Therefore, the given threshold level can be considered a good separation figure between accidental "contamination" with sugar syrup and deliberate adulteration. Due to the ability to simultaneously screen thousands of compounds in honey by LC-HRMS, it is also possible to use this technique for the determination of the botanical and geographical origin of honey. However, this requires large databases similar to NMR and has so far not evolved much, primarily because of the large effort and high cost required and because of the necessity for a harmonized sampling protocol of authentic reference samples. Therefore, this type of application will surely be subject to further R&D projects in the coming years. A further interesting aspect is that LC-HRMS can also be applied to find specific markers for improper or illicit processing of honey, e.g. excessive heat treatment, chemical or sanitizing treatments, or purifying procedures which remove unwanted substances or added substances (agents) other than syrups which alter the genuine composition of natural honey.

LC-HRMS is the most recent and most powerful analytical tool for honey authenticity assessment.

Conclusion

The state-of-the-art approach (the combination of δ ^{13}C EA/LC-IRMS, LC-HRMS, and ^{1}H-NMR profiling, *Table 2*) presented here provides the current most reliable predictor of honey purity. Adding pollen and sensory analysis for the determination of geographical and botanical origins makes possible a comprehensive and reliable authenticity assessment. Due to the newly available LC-HRMS technique, it is possible to detect both common and highly-sophisticated honey adulteration now occurring in the market (Phipps 2019). Furthermore, LC-HRMS can replace many previously established adulteration detection methods using single marker compounds (e.g. SM-R, TM-R, SM-B, E150d, mannose/psicose, oligosaccharides) and thus makes honey adulteration testing more simple, cost-effective, and reliable.

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Adulterated honey: what does water have to do with it?

Traditional methods for honey authentication become obsolete or demand load of resources. New rapid methods are on the rise.

Balázs Horváth and **David Psomiadis**

***Balázs Horváth, PhD**, is the Technical Director at Imprint Analytics GmbH. He has extensive experience with stable isotope analysis and Isotope Ratio Mass Spectrometry. He uses his expertise to develop new methods and technical capabilities for challenging issues.*

***David Psomiadis, PhD**, is the Head of the Lab and the Business Development Manager at Imprint Analytics GmbH.*
He performed academic research on isotope geochemistry and serves today as the link between isotope analytics and business management at the company.

Raw honey is considered to be a natural energy source with many positive effects on health: antioxidant, antibacterial, and antifungal properties; hay fever prevention; prebiotic; sore throat remedy; etc. On the other hand, if you pay attention to food fraud you will always find honey in the top 10 most often adulterated products, usually surpassed only by olive oil and milk. According to Interpol, 30-35% of all honey currently sold in the world is fraudulent (Save the bees 2019). There is a general consensus that there is no one, single method which can detect adulterated honey. The reason is simply the fact that there are dozens of possible (and profitable) ways to adulterate honey. The circulation of honey fraud news has been identified as a significant factor in consumer doubt related to purchasing behavior (Meerza and Gustafson 2019); it is increasingly difficult for honey manufacturers to claim the premium quality of their products when the whole commodity is affected by frequent fraud cases. The adulteration of honey to market lower-quality honey at normal retail prices can come in a variety of forms. One of them is mislabeling honey concerning geographical or botanical origin. However, the addition of sugar was identified by the Joint Research Centre of EU (JRC) as the most frequently occurring type of fraudulent manipulation (Aries et al. 2016). Exogenous sugar can originate from inappropriate bee-feeding and/or from the direct addition of sugar/syrup to honey.

To prove that sugar/syrups have been added to honey is a challenging task. Honey is a natural product containing large amounts of different sugars like fructose, glucose, and di- and trisaccharides. Their concentration can vary widely. Thus, there is no straightforward way to filter out fraudulent products just by measuring sugar concentration. It has been necessary to find a way to differentiate between authentic honey sugar and external added sugar.

Measuring stable isotope ratios is a known tool for differentiating chemically-identical molecules with different origins/sources (Carter 2017). Although all free-living plants utilize atmospheric carbon dioxide, different photosynthetic pathways result in different ratios between ^{13}C and ^{12}C isotopes (given as $\delta^{13}C$). Thus, if a honey produced by bees from C3 plants is diluted with a C4 sugar syrup (e.g. cane or corn), It is relatively easy to detect. The method is based on the $\delta^{13}C$ measurement of sugars and proteins in honey (AOAC 2013). This test is old, however, and also well-known by dishonest producers.

Blending honey with C3 sugars is much more difficult to discover. Although on a much smaller scale, there is variability in the $^{13}C/^{12}C$ isotope ratio among C3 plants mainly due to species and

There is a general consensus that there is no one, single method which can detect adulterated honey.

THE LAB

Imprint Analytics GmbH is a service provider of analytical solutions to the food and non-food industry. A major part of daily business and R&D is related to food fraud prevention and food authenticity testing. As an accredited laboratory, Imprint Analytics delivers innovative services to the complete spectrum of stakeholders in the food industry.

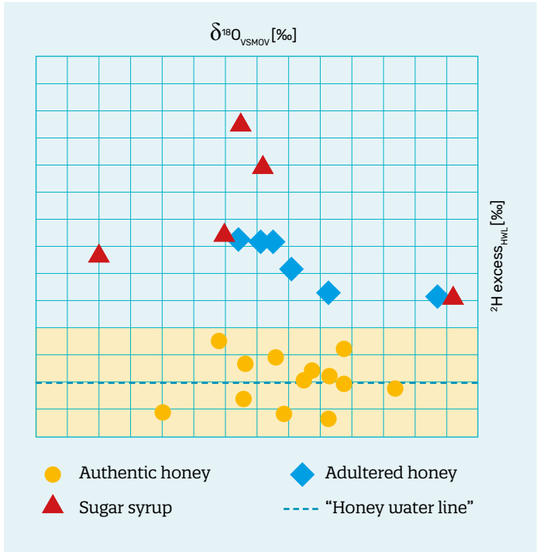

Fig. 1: Isotope composition of water in authentic honey, in some sugar syrups, and in known adulterated honey.

environmental influences. It is possible to separate the different sugar molecules with HPLC and compare the $\delta^{13}C$ values of the different sugar fractions. This method is more complicated and sophisticated than the AOAC 998.12 test. The addition of C3 sugars can be proved down to 10% and C4 sugars down to 1%. (AOAC 2013) In a wide-range survey conducted by JRC in 2016 using this method, 14% of the samples were suspected of containing added sugar (Aries et al. 2016). Nonetheless, if by chance the added sugar has a $\delta^{13}C$ value similar to that of the original honey sugar, the fraud remains undiscovered.

A more recent and more sensitive method is to investigate hydrogen rather than carbon isotope ratios. Deuterium (^{2}H) and hydrogen (^{1}H) ratios are also plant-specific and determined by climate and plant metabolism (Cotte 2007). There is a specific challenge, however: hydrogen bound to oxygen can be exchanged by other hydrogen-bearing molecules in the environment, thus losing the original signal. Only the carbon-bounded hydrogen D/H ratio reveals the original information. This so-called site specific D/H ratio can be measured with SNIF-NMR®. However, this method is requires costly instrumentation and lengthy, elaborate sample preparation as the honey must be fermented to alcohol, making this investigation quite expensive. Another approach is using honey-profiling by NMR (Olawode 2018; Boffo 2012). The concept is based on comparisons of honey profiles with large datasets and the identification of outliers. Although this method is accepted as a powerful screening tool, it still has limitations in its application for verified adulteration indices. These limitations have the consequence that further analysis is requested in order to verify/detect the exact adulteration indicator/parameter. While efforts have been made to measure this site-specific signature in a simpler manner, those methods are still in development.

The scientists at Imprint Analytics GmbH recently tried a different approach. Instead of concentrating on the sugar itself, the water content of the honey was analyzed. The idea is based on the fact that plant water is enriched through evaporation of "heavy" isotopes: specifically, ^{2}H and ^{18}O. Sugar syrups on the other hand are industrially processed with "normal" meteoric water (tap water). As a consequence, plant-authentic honey water and industrially-processed sugar syrup water must be different. The preliminary results show that the water content in most honey is enriched with hydrogen and oxygen isotopes in contrast to sugar syrups. However, there are exceptions identified in both honey and syrups which still make it impossible to establish global threshold values for either oxygen or hydrogen isotope ratios. On the other hand, there was remarkably stable correlation between hydrogen and oxygen isotope ratios. This phenomenon is well known for meteoric waters and it is called the "meteoric water line". Leaning on this example, we defined a "honey water line". Deviation from this line, expressed as "$^{2}H\ excess_{[HWL]}$", made it possible to differentiate between authentic honey, sugar syrups, and honeys with known adulteration (*Fig 1)*. Further investigations shall be conducted in order to prove the reliability of this approach. The expected outcome is a solid, easy, and validated method for detecting sugar additions to honey.

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India's largest meeting point for laboratory technology, analytics, biotechnology, and diagnostics.
www.analyticaindia.com/

10th Annual European Food Sure Summit 2020
April 28 - 29, Milan, Italy
Conference covering all aspects of food quality and food safety.
www.foodsureeurope.com/

8th IDF International Symposium on sheep, goat and other non-cow milk
May 4 - 5, Brussels, Belgium
Conference organized by the International Dairy Federation on the latest scientific advances in the fields of human nutrition and science and technology regarding milk originating from ruminants other than cows.
www.fil-idf.org/sheepandgoat2020/

Food Safety Summit Conference & Expo
May 4 - 7, Rosemont, IL, USA
Conference and expo designed by the Educational Advisory Board (EAB) to meet the educational and informational needs of the entire food industry.
www.foodsafetystrategies.com/food-safety-summit

Food Fraud Prevention and Risk Based Food Allergen Management Workshop Budapest 2020
CANCELLED ~~*May 10, Budapest, Hungary*~~
Workshop on food fraud and allergen management organized by MoniQA Association.
www.moniqa.org/iam/foodfraudallergens-budapest2020

Food Safety Americas 2020
May 12 - 14, San Antonio, TX, USA
Event offering updates designed to improve food safety management in retail, food service, and manufacturing environments.
www.brcgs.com/events/food-safety-americas-2020

EuroResidue IX
May 18 - 20, Egmond aan Zee, The Netherlands
Conference on veterinary drug residue in food, from analytical techniques to regulation.
www.euroresidue.nl/

42nd Mycotoxin Workshop
CANCELLED ~~*May 25 - 27, Brno, Czech Republic*~~
Workshop covering all scientific aspects of mycotoxin research.
www.mycotoxin-workshop.de/

North America Food Safety and Quality NAFS20
June 2 - 3, Chicago, IL, USA
North America's premier food safety event to share best practices, key implementable ideas, and real world strategies to improve both corporate success and public well-being.
foodsafetyna.com/

/technology & company news

Mobile Multianalyte Biosensing

BioMensio will develop and sell a novel biosensor platform for rapid detection of multiple analytes from a small sample volume. The BioMensio mass sensitive micro-array (MSMA) platform is particularly well-suited for a variety of applications due to its ability to detect multiple analytes simultaneously.

BioMensio's core technology is a sensing platform with an array of microscopic weighing scale pixels, called a mass sensitive micro-array (MSMA). The MSMA consists of mass-sensitive transducers based on solidly-mounted resonance (SMR) technology and an application-specific integrated circuit (ASIC) that allows for control of all FBAR and interface to the external read-out electronics. The MSMA chip will be housed in a cartridge containing read-out electronics and microfluidics to form a lab-on-a-chip platform where the end user needs only to introduce the sample and read out the result. Each pixel of a MSMA can be functionalized with a biologically active layer like antibodies or DNA to enable detection of target biomolecules. FBAR functionality has already been demonstrated with bacterial S-layer molecules and DNA samples and recently also for mycotoxin detection. Different biolayers can be applied on different MSMA pixels to allow for detection of multiple analytes (e.g. 36/64 individually detecting pixels) from the same liquid sample.

The advantages of MSMA are: universality, as the detection is based on mass; economies of scale achieved by innovative thin-film microfabrication as the pixels can be functionalized differently in different applications; and multiplexing and miniaturization as microfabrication allows integration of a large number of pixels in a small area. As the MSMA detection mechanism is mass-based, it is inherently suitable for any kind of analyte without time-consuming and costly labelling procedures, enabling development of label-free assays. Potential for lowering costs exists because of the physical and chemical robustness of MSMA chips, which support bio-chemical regeneration mechanisms.

This easy-to-use, sensitive, accurate, and low-cost biosensing device is capable of rapid determination of multiple analytes from a single sample. Furthermore, it can be envisioned as a very suitable detection tool for many applications, such as detection of antibiotic residues in milk, drugs in drivers' saliva samples, production levels of proteins from cell cultures, and infection markers at doctors´ appointments, just to mention a few. BioMensio's goal is to develop a hand-held bioscreening device for multiplexed detection of several biomolecules from a single sample. Time to market for food applications is envisioned to take place within the next two years.

Sanna Auer, Chief Scientific Officer, BioMensio / biomensio.com

LOGIC Multiplex: innovative multiplex screening test for the detection of antibiotics in honey

Honey, like other foods, is prone to various types of contamination and adulteration. Even though the use of antibiotics in beekeeping is generally banned in the European Union, their use is still legal in many other countries around the globe.

According to European Community regulations, there are no MRLs established for antibiotics in honey, except for streptomycin. Because honey with antibiotic residues cannot be sold in the EU, testing for residues is particularly relevant for imported products.

To address these issues, four expert research and commercial partners specialised in multiplex development for the food safety industry (Biorex Food Diagnostics, Wageningen University & Research, Fortress Diagnostics, and Scienion) have collaborated to develop **a** prototype of a novel multiplex screening test for antibiotics in honey and seafood.

The prototype was developed as part of the LOGIC Multiplex project, which received funding from the Eurostars joint programme with co-funding from the European Union Horizon 2020 research and innovation programme. The project, titled "Nano-array lateral flow diagnostics for the rapid detection of antibiotics in food", started in June 2017 with the objective of developing a rapid multiplex prototype test by June 2020. The aim of the project is to offer a novel and unique solution for multiplex screening of antibiotics from 4 drug families (nitrofurans, chloramphenicol, nitroimidazoles, and tetracyclines) in honey, a screening method not currently available on the market anywhere worldwide.

The kit will be ideal for rapid factory floor analysis, providing a fast and simple tool that can be used with minimal expertise and that will offer a time-to-results of less than 10mins/sample. Moreover, the test will offer a new sample preparation using "green" chemistry, so no fume hood or specialist disposal services will be required.

The test device, the Lateral flow Microarray ImmunoAssay (LMIA) is a rapid, multi-analyte test platform that will give signals for up to 8 different analytes, including control spots. Honey sample extracts will be directly added to the LMIA test cartridge, which will be placed in an innovative dedicated reader. This standalone reader analyses the developing spots in real-time and can be controlled wirelessly using a smartphone. If antibiotics are present in the sample, antibiotic-specific spots will appear in the cartridge window, and the results (qualitative/quantitative) will be transferred to dedicated receivers within the honey plant.

logic-multiplex.com

PhasmaFOOD: a portable multi-target device for on-the-spot food quality sensing and shelf-life prediction

PhasmaFOOD is an EU collaborative R&D project funded by the Horizon 2020 Programme that recently concluded in December 2019, with a final review meeting in Brussels where the results were presented to representatives of the EU Commission. The consortium comprised 9 stakeholders from industry and academia (Intrasoft International S.A, Wings ICT Solutions P.C., VizLore Labs Foundation, RIKILT – Wageningen Research, Agricultural University of Athens, Italian National Research Council, Tor Vergata University of Rome, Fraunhofer IPMS, and Freie Universität Berlin). Its main goal was to deliver a miniaturized multi-sensor optical sensing device for the detection of food safety threats such as food spoilage, adulteration, and aflatoxins.

The architecture of the PhasmaFOOD system comprises three main parts: the sensing device, the end user's mobile device with the PhasmaFOOD application installed on it, and the cloud platform and database.

The system integrates heterogeneous visible and near-infrared spectroscopy technologies supported by a custom electronics design featuring embedded memory and processing power and a software architecture that delivers fast characterisation of foods, encompassing an extendable framework for the deployment of smart chemometric algorithms, data fusion strategies, and reference laboratory measurements. The built-in algorithms address data mining and data analysis methods from non-destructive, non-invasive instruments and are independent of the food type and food-tech application.

PhasmaFOOD food quality partners used the PhasmaFOOD prototype in order to collect information and experimental data for data analysis and to complete three diverse use case experiments.

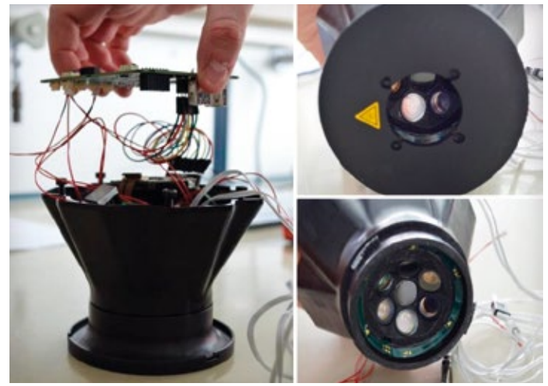
The first case tested for the presence of aflatoxins and (when applicable) deoxynivalenol in maize flour, skimmed milk powder, paprika powder, and tree nuts. Aflatoxin detection accuracy in grained almonds was higher than 70% at all thresholds considered (2-10 ppb), whereas the classification accuracy exceeded 94% with a threshold of 6.4 ng/g[1].

The next case focused on spoilage and shelf-life estimation of fruits, vegetables, meat, and fish.

The last use case involved food fraud and covered skimmed milk powder, meat, olive oils and other edible oils, and alcoholic beverages. The limits of detection for the use cases were generally based on either EU legislation or technical feasibility. Correct classification of adulterated skimmed milk powders was 93%, alcoholic beverage authenticity 96%, adulterated extra virgin olive oils 97%, and adulterated minced meat 98%.

Rapid exploitation opportunities are currently being pursued via pilot applications addressing niche food markets.

Spyros Evangelatos, Senior Research & Innovation Specialist, Intrasoft International S.A / phasmafood.eu

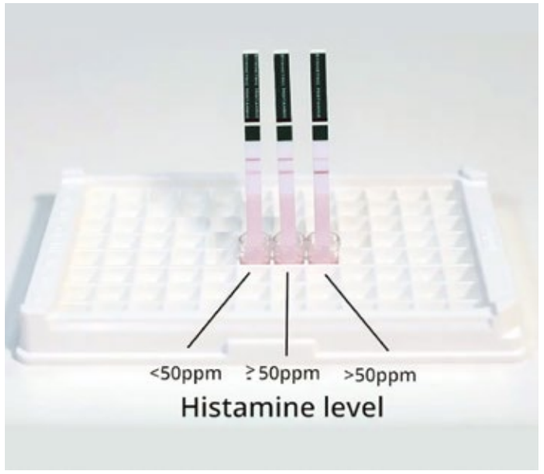


1 - F.R. Bertani, L. Businaro, L. Gambacorta, A. Mencattini, D. Brenda, D. Di Giuseppe, A. De Ninno, M. Solfrizzo, E. Martinelli, A. Gerardino. 2020. Optical detection of aflatoxins B in grained almonds using fluorescence spectroscopy and machine learning algorithms. Food Control. 112, in press.

/product news

Viral Detection is Vital – The VIR*Seek* Solution

Despite the fact that the number of outbreaks linked to noroviruses (NoV) have slightly decreased in recent years, norovirus outbreaks remain one of the most important food safety concerns. NoV are single-stranded RNA (ssRNA) viruses belonging to the family of Caliciviridae with five known genogroups. However, only three (I, II, and IV) are considered relevant to human health. The VIRSeek Solution from **Eurofins GeneScan Technologies** is a comprehensive ISO-compliant workflow for the detection of viruses in food and drinking water. The solution is comprised of five kits, which cover all steps from RNA extraction to real-time RT-PCR detection of hepatitis A virus and norovirus genogroups I & II as well as the process control murine norovirus. The assay time varies based on which matrices are being analysed but it is on the order of 1 hour for sample preparation and 1 hour for PCR.



Symmetric Histamine

Elevated levels of histamine are often found in fish & seafood, posing a serious risk to human health. Developed by **ProGnosis Biotech**, Symmetric Histamine is an innovative lateral flow test that quantifies the levels of histamine in fresh fish, canned fish, frozen fish, and fish meal. The method is the only fully quantitative rapid test that provides a visual detection option for qualitative use in the field. Symmetric Histamine has a simple and fast protocol (just 3 minutes) that does not require special technical or scientific expertise. Moreover, there is no need for acylation.

Romer Labs introduces RapidChek® Campylobacter Test Kit

Due to stricter regulation of Campylobacter in the United States, Romer Labs® has developed the RapidChek® Campylobacter. Following incubation, the test kit detects the three regulated species, *C. jejuni*, *C. coli*, and *C. lari*, in carcass rinses, raw ground chicken, and turkey carcass swabs within 20 minutes. The method couples a sensitive immune-detection strip with an innovative proprietary aerobic all-in-one enrichment media. It requires no specialized equipment and no additional supplements. Kits can be stored at room temperature and they have a long shelf life. Third-party certification is underway to confirm the analytical accuracy and result reliability announced by the company.

Major development in reference materials for allergen quantification

Allergen detection in food and food ingredients is essential to preserve the supply chain, to support businesses, and to guarantee safe food for allergen sufferers. However, a lack of reliable reference materials for analysis threatens proper laboratory testing performance. The **Food Standards Agency (FSA)** tackled this particular issue with a joint project involving the **UK's National Measurement Laboratory (NML)** at **LGC** in collaboration with the **University of Manchester** and **Romer Labs**. The multi-allergen reference material kit developed under this project is a world first. It contains five individual common allergens (milk, egg, almond, hazelnut, walnut) traceable to the SI (International System of Units). This kit will help laboratories and food manufacturers to develop new methods for determining 'true' allergen content and to monitor the performance of these methods on a daily basis.

Aflatoxins and fumonisins in masa flour: a new LFD solution by EnviroLogix

EnviroLogix is committed to providing the agricultural market with innovative solutions. One of its most recent products offers a new, easy, and accurate system for detecting aflatoxins and fumonisins in masa flour. With the QuickTox Flex for masa flour, the corn milling market will benefit from the advantages of the Flex line of mycotoxin assays, such as less hands-on time, temperature and humidity control, and expanded quantification ranges. With the addition of these matrices, aflatoxins and fumonisins can be accurately tested together in 10 minutes using a simple procedure with one extract. The addition of masa flour to the EnviroLogix product portfolio provides corn processors with a screening tool that will improve both operational efficiency and product quality.

Neogen launches first rapid test for Ergot Alkaloids

Neogen has developed a quick and simple lateral flow test for ergot alkaloids, natural toxins produced by a fungus that commonly infects rye and wheat. Neogen's new Reveal® Q+ MAX for Ergot Alkaloids delivers precise quantitative results in the range of 50 – 5,000 ppb only 8 minutes after extraction. This new test is compatible for use with Neogen's Raptor® testing platform, which controls timing, temperature, and reading, as well as integrity and consistency of testing data. Because of the well-established risk to human and animal health posed by ergot alkaloids, EU legislation is expected soon and this new test will help producers meet the new requirements.

food law news

REGULATION (EU) 2018/775

laying down rules for the application of Article 26(3) of Regulation (EU) No 1169/2011 on the provision of food information to consumers, as regards the rules for indicating the country of origin or place of provenance of the primary ingredient of a food.

Regulation (EU) 2018/775 lays down the modalities for the application of Article 26(3) of Regulation (EU) No 1169/2011 where the country of origin or place of provenance of a food is given by any means such as statements, pictorial presentation, symbols or terms, referring to places or geographical areas and it is different from the one of the primary ingredient.
It applies from 1 April 2020. However, foods placed on the market or labelled prior to the date of application of the Regulation may be marketed until the stocks are exhausted.

REGULATION (EU) 2018/848

on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007.

Regulation (EU) 2018/848 establishes the principles of organic production and lays down the rules concerning organic production, related certification and the use of indications referring to organic production in labelling and advertising, as well as rules on controls additional to those laid down in Regulation (EU) 2017/625.
It applies from 1 January 2021. However, products produced in accordance with Regulation (EC) No 834/2007 before 1 January 2021 may be placed on the market after that date until stocks are exhausted.

REGULATION (EU) 2020/16

authorising the placing on the market of nicotinamide riboside chloride as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470.

Nicotinamide riboside chloride as specified in the Annex to this Regulation is included in the Union list of authorised novel foods established in Regulation (EU) 2017/2470. The entry in the Union list referred includes the conditions of use and labelling requirements laid down in the Annex.
The Annex to Regulation (EU) 2017/2470 is amended in accordance with the Annex to this Regulation.

REGULATION (EU) 2020/24

authorising an extension of use of chia seeds (Salvia hispanica) as a novel food and the change of the conditions of use and the specific labelling requirements of chia seeds (Salvia hispanica) under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470.

The entry in the Union list of authorised novel foods as provided for in Article 6 of Regulation (EU) 2015/2283 and included in Regulation (EU) 2017/2470, referring to the novel food chia seeds (*Salvia hispanica*) is amended as specified in the Annex to this Regulation.
The entry in the Union list shall include the conditions of use and labelling requirements laid down in the Annex to this Regulation.
The Annex to Regulation (EU) 2017/2470 is amended in accordance with the Annex to this Regulation.

REGULATIONS (EU) 2020/42 and 2020/43

amending Regulation (EU) No 37/2010 to classify the substance bambermycin and ciclesonide as regards their maximum residue limit.

In Table 1 of the Annex to Regulation (EU) No 37/2010 – which sets out the pharmacologically active substances and their classification regarding MRLs in foodstuffs of animal origin - entries for Bambermycin and Ciclesonide substances are inserted in alphabetical order.

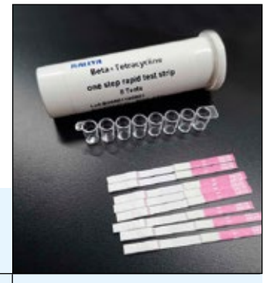
food test compass

Food Test Compass" (FTC) is a growing data-base of commercially available test kits. Other web sites list many test kits but they either primarily cover microbiological testing products or they only present manufacturer specifications with potentially misleading information that prevents serious product comparisons. In some cases, for instance, the company ISO 9001 certification is listed as a product certification. The FTC team is comprised of scientists that have been working in the test kit industry and know what they are managing: test kits are not test tubes or clothes!

Data are from manufacturers web sites / documentation. We invite once again all the companies to provide us with updated correct informations, we will introduce any change needed in the on-line version.

LEGEND **SH** = Shaking / **HE** = Heating

BALLYA



BALLYA INTERNATIONAL
BT Sensor One Steps Test Kit

PRODUCT CODE B20001	
LIMIT OF DETERMIANTIONS 96	**ASSAY TIME** 7'
LOD Beta-lactams: penicillin G 2-4 ppb, ampicillin 3-4 ppb, amoxicillin 3-4 ppb, oxacillin 5-10 ppb, cloxacillin 5-10 ppb, dicloxacillin 5-10 ppb, nafcillin 5-10 ppb, cefquinome 10-15 ppb, cephapirin 5-10 ppb, cefacetrile 100 ppb, cefalonium 10-15 ppb, cefoperazone 5-10 ppb, cefalotin 80 ppb, ceftiofur 80-100 ppb, cefalexin 100 ppb Tetracyclines: tetracycline 100 ppb, oxytetracycline 100 ppb, doxycycline 100 ppb, chlortetracycline 100 ppb	
MATRICES Raw cow milk, pasteurized milk, milk powder	
SAMPLE PREPARATION As it is	**STORAGE TEMPERATURE** 2-8° C
CERTIFICATION /	**VALIDATION** Yes (ILVO)
SHELL LIFE 12 months	**DISPOSABLE** Yes
READER Optional	

BALLYA

BALLYA INTERNATIONAL
BT Sensor Test Kit

PRODUCT CODE A20001	
LIMIT OF DETERMIANTIONS 96	**ASSAY TIME** 10'
LOD Beta-lactams: penicillin G 2-4 ppb, ampicillin 3-4 ppb, amoxicillin 3-4 ppb, oxacillin 5-10 ppb, cloxacillin 5-10 ppb, dicloxacillin 5-10 ppb, nafcillin 5-10 ppb, cefquinome 10-15 ppb, cephapirin 5-10 ppb, cefacetrile 100 ppb, cefalonium 10-15 ppb, cefoperazone 5-10 ppb, cefalotin 80 ppb, ceftiofur 80-100 ppb, cefalexin 100 ppb Tetracyclines: tetracycline 100 ppb, oxytetracycline 100 ppb, doxycycline 100 ppb, chlortetracycline 100 ppb	
MATRICES Raw cow milk, pasteurized milk, milk powder	
SAMPLE PREPARATION SH	**STORAGE TEMPERATURE** 2-8° C
CERTIFICATION /	**VALIDATION** Yes (ILVO)
SHELL LIFE 12 months	**DISPOSABLE** Yes
READER Optional	

Green Spring

SHENZHEN LVSHIYUAN BIOTECHNOLOGIES
Beta-lactams and Tetracyclines combo rapid test strip

PRODUCT CODE LSY-20082	
LIMIT OF DETERMIANTIONS 96	**ASSAY TIME** 6-10'
LOD Beta-lactams: penicillin G 2-4 ppb, ampicillin 2-3 ppb, amoxicillin 2-3 ppb, oxacillin 5-7 ppb, cloxacillin 4-6 ppb, dicloxacillin 6-8 ppb, nafcillin 15-25 ppb, cefquinome 8-15 ppb, cephapirin 8-10 ppb, cefacetrile 20-30 ppb, cefalonium 8-10 ppb, cefoperazone 3-5 ppb, cefalotin 30-40 ppb, ceftiofur 60-100 ppb, cefazolin 40-50 ppb, piperacillin 6-8 ppb Tetracyclines: tetracycline 10-15 ppb, oxytetracycline 5-10 ppb, doxycycline 15-20 ppb, chlortetracycline 5-10 ppb	
MATRICES Milk (from cow, goat and sheep)	
SAMPLE PREPARATION SH, (HE)	**STORAGE TEMPERATURE** 2-8° C
CERTIFICATION /	**VALIDATION** /
SHELL LIFE 12 months	**DISPOSABLE** Yes
READER Optional	

BIOEASY

SHENZHEN BIOEASY BIOTECHNOLOGIES

2IN1 BT (EU) Beta-lactams + Tetracyclines Rapid Test for Milk

PRODUCT CODE YRM1007-40

LIMIT OF DETERMINATIONS 96 | **ASSAY TIME** 8'

LOD Beta-lactams: penicillin G 1.5-2 ppb, ampicillin 3-4 ppb, amoxicillin 3-4 ppb, oxacillin 5-7 ppb, cloxacillin 6-8 ppb, dicloxacillin 10-20 ppb, nafcillin 3 ppb, cefquinome 16 ppb, cephapirin 30 ppb, cefacetrile 50 ppb, cefalonium 14 ppb, cefoperazone 35 ppb, ceftiofur 8 ppb, cefazolin 20 ppb, cefalexin 30 ppb, cefuroxime 8 ppb, desfuroylceftiofur 25 ppb, desacetylcephapirin ≤60 ppb Tetracyclines: tetracycline 16 ppb, oxytetracycline 18 ppb, doxycycline 25 ppb, chlortetracycline 40 ppb

MATRICES Milk (normal, UHT, sterilised, reconstituted milk powder, thawed, skimmed) (from cow, goat, sheep, mare, buffalo, camel)

SAMPLE PREPARATION SH | **STORAGE TEMPERATURE** 2-8° C

CERTIFICATION / | **VALIDATION** Yes (ILVO)

SHELL LIFE / | **DISPOSABLE** /

READER Optional

IDEXX

IDEXX

SNAPduo ST Plus Test

PRODUCT CODE 99-0009837

LIMIT OF DETERMINATIONS 30 | **ASSAY TIME** 6'

LOD Beta-lactams: penicillin G 2 ppb, ampicillin 4 ppb, amoxicillin 3 ppb, oxacillin 3 ppb, cloxacillin 4 ppb, dicloxacillin 4 ppb, nafcillin 3 ppb, cefquinome 16 ppb, cephapirin 30 ppb, cefacetrile 50 ppb, cefalonium 14 ppb, cefoperazone 35 ppb, ceftiofur 8 ppb, cefazolin 20 ppb, cefalexin 30 ppb, cefuroxime 8 ppb, desfuroylceftiofur 25 ppb, desacetylcephapirin ≤60 ppb Tetracyclines: tetracycline 16 ppb, oxytetracycline 18 ppb, doxycycline 25 ppb, chlortetracycline 40 ppb

MATRICES Milk (normal, UHT, sterilised, reconstituted milk powder, thawed, skimmed) (from cow, goat, sheep, mare, buffalo, camel)

SAMPLE PREPARATION SH | **STORAGE TEMPERATURE** 2-8° C

CERTIFICATION / | **VALIDATION** Yes (ILVO)

SHELL LIFE / | **DISPOSABLE** /

READER Optional

BIOEASY

SHENZHEN BIOEASY BIOTECHNOLOGIES

2IN1 BTCef(EU) Beta-lactams + Tetracyclines Rapid Test for Milk

PRODUCT CODE YRM1008-40

LIMIT OF DETERMINATIONS 96 | **ASSAY TIME** 9'

LOD Beta-lactams: penicillin G 1.5-2 ppb, ampicillin 3-4 ppb, amoxicillin 3-4 ppb, oxacillin 5-7 ppb, cloxacillin 6-8 ppb, dicloxacillin 10-20 ppb, nafcillin 20-30 ppb, cefquinome 12-18 ppb, cephapirin 15-18 ppb, cefacetrile 25-30 ppb, cefalonium 6-8 ppb, cefoperazone 4-6 ppb, ceftiofur 80-100 ppb, cefazolin 40-50 ppb, cefalexin 20-30 ppb Tetracyclines: tetracycline 30-50 ppb, oxytetracycline 30-50 ppb, doxycycline 30-50 ppb, chlorotetracycline 30-50 ppb

MATRICES Milk (from cow, buffalo, ewe, goat, mare), pasteurized milk and full cream milk powder

SAMPLE PREPARATION SH, HE | **STORAGE TEMPERATURE** 2-8° C

CERTIFICATION / | **VALIDATION** Yes (ILVO)

SHELL LIFE 18 months | **DISPOSABLE** /

READER Optional

NEOGEN

NEOGEN CORP.

BetaStar® S Combo

PRODUCT CODE BCS002 / BCS014 / BSCR100

LIMIT OF DETERMINATIONS 25 / 250 / 100 | **ASSAY TIME** 5'

LOD Beta-lactams: penicillin G 2 ppb, ampicillin 3 ppb, amoxicillin 2 ppb, oxacillin 6 ppb, cloxacillin 5 ppb, dicloxacillin 4 ppb, nafcillin 20 ppb, cefquinome 16 ppb, cephapirin 20 ppb, cefacetrile 60 ppb, cefalonium 2 ppb, cefoperazone 3 ppb, ceftiofur 30 ppb, cefazolin 90 ppb, cefalexin 3000 ppb, desfuroyl ceftiofur 35 ppb, desacetyl cephapirin 60 ppb Tetracyclines: tetracycline 45 ppb, oxytetracyline 50 ppb, doxycycline 50 ppb, chlortetracycline 80 ppb

MATRICES Raw cow milk

SAMPLE PREPARATION HE | **STORAGE TEMPERATURE** 2-8° C

CERTIFICATION / | **VALIDATION** Yes (ILVO)

SHELL LIFE 12 months | **DISPOSABLE** /

READER Optional

NKBIO

NANKAI BIOTECH

SmarK!T Beta-lactams + Tetracyclines Combo Rapid Test Kit

PRODUCT CODE BT-D204R1

LIMIT OF DETERMINATIONS 50 | **ASSAY TIME** 15'

LOD Beta-lactams: Penicillin G 2-4 ppb, ampicillin 2-3 ppb, amoxicillin 2-3 ppb, oxacillin 3-5 ppb, cloxacillin 5-10 ppb, dicloxacillin 3-5 ppb, nafcillin 12-14 ppb, cefquinome <20 ppb, cefapirin 8-10 ppb, cephacetrile 300 ppb, cefalonium 4 ppb, cefoperazone 5-7 ppb, ceftiofur 50-100 ppb, cefalexin 300 ppb, cefamezin 5-7 ppb Tetracyclines: tetracycline 20-30 ppb, oxytetracycline 30-40 ppb, chlorotetracycline 10-15 ppb, doxycycline 10-15 ppb

MATRICES Milk

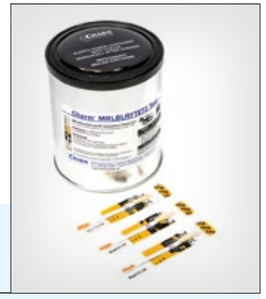
SAMPLE PREPARATION As it is | **STORAGE TEMPERATURE** 2-25° C

CERTIFICATION / | **VALIDATION** /

SHELL LIFE 12 months | **DISPOSABLE** /

READER No

CHARM SCIENCES INC



CHARM SCIENCES INC.

MRL Beta-lactam and Tetracycline 2-Minute Test

PRODUCT CODE MRLBLTET2

LIMIT OF DETERMINATIONS / | **ASSAY TIME** 2-8'

LOD Beta-lactams: penicillin G 2-3 ppb, ampicillin 3-4 ppb, amoxicillin 3-5 ppb, cloxacillin 10-20 ppb, dicloxacillin 10-20 ppb, cefquinome 15-25 ppb, cephapirin 15-25 ppb, cefacetrile 20-40 ppb, cefalonium 10-20 ppb, cefoperazone 1-3 ppb, ceftiofur and metabolites 40-70 ppb, cefazolin 20-40 ppb Tetracyclines: tetracycline 10-30 ppb, oxytetracycline 50-100 ppb, chlortetracycline 50-100 ppb

MATRICES Milk (from cow, goat and sheep)

SAMPLE PREPARATION / | **STORAGE TEMPERATURE** /

CERTIFICATION / | **VALIDATION** Yes (ILVO)

SHELL LIFE / | **DISPOSABLE** /

READER Optional

CHARM SCIENCES INC

CHARM SCIENCES INC.

MRL Beta-lactam and Tetracycline DIP Test

PRODUCT CODE DT-MRLBLTET-20K / DT-MRLBLTET-100K

LIMIT OF DETERMINATIONS 20 / 100 | **ASSAY TIME** 10'

LOD Beta-lactams: penicillin G 2-3 ppb, ampicillin 2-3 ppb, amoxicillin 2-3 ppb, oxacillin 10-15 to ppb, cloxacillin 5-8 ppb, dicloxacillin 4-6 ppb, nafcillin 20-30 to ppb, cefquinome 15-20 ppb, cephapirin 8-10 ppb, cefacetrile 25-50 ppb, cefalonium 4-8 ppb, cefoperazone 1-2 ppb, ceftiofur and metabolites 25-50 ppb, cefazolin 8-10 ppb, cefuroxime 75-100 ppb Tetracyclines: tetracycline 50-75 ppb, oxytetracycline 40-60 ppb, doxycycline 50-75 ppb, chlortetracycline 50-75 ppb

MATRICES Raw milk

SAMPLE PREPARATION HE | **STORAGE TEMPERATURE** /

CERTIFICATION / | **VALIDATION** /

SHELL LIFE / | **DISPOSABLE** /

READER No

美正集团 MEIZHENG GROUP

MEIZHENG GROUP

2 in 1 Beta-lactams & Tetracyclines Combo Test Kit (high sensitive)

PRODUCT CODE JC0209

LIMIT OF DETERMINATIONS 96 | **ASSAY TIME** 10'

LOD Beta-lactams: penicillin G 1-2 ppb, ampicillin 2-3 ppb, amoxicillin 2-3 ppb, oxacillin 5-7 ppb, cloxacillin 3-6 ppb, dicloxacillin 3-6 ppb, nafcillin 7-10 ppb, cefquinome 5-7 ppb, cephapirin 4-8 ppb, cefacetrile 15-20 ppb, cefalonium 3-5 ppb, cefoperazone 3-5 ppb, ceftiofur 70-90 ppb, cefazolin 20-30 ppb, benzathine 3-5 ppb Tetracyclines: tetracycline 7-10 ppb, oxytetracycline 7-10 ppb, doxycycline 7-10 ppb, chlortetracycline 7-10 ppb

MATRICES Raw cow's milk

SAMPLE PREPARATION SH, HE | **STORAGE TEMPERATURE** 2-8° C

CERTIFICATION / | **VALIDATION** /

SHELL LIFE 12 months | **DISPOSABLE** /

READER No

MEIZHENG GROUP
2 in 1 Beta-lactams & Tetracyclines Combo Test Kit (normal)

PRODUCT CODE JC0084

LIMIT OF DETERMINATIONS 96	**ASSAY TIME** 7'

LOD Beta-lactams: penicillin G 2-4 ppb, ampicillin 3-4 ppb, amoxicillin 4-5 ppb, oxacillin 3-6 ppb, cloxacillin 2-5 ppb, dicloxacillin 2-5 ppb, nafcillin 8-12 ppb, cefquinome 10-15 ppb, cephapirin 4-8 ppb, cefacetrile 20-30 ppb, cefalonium 4-8 ppb, cefoperazone 3-6 ppb, ceftiofur 70-90 ppb, cefazolin 40-50 ppb, benzathine 2-4 ppb Tetracyclines: tetracycline 20-35 ppb, oxytetracycline 15-25 ppb, doxycycline 10-18 ppb, chlortetracycline 15-25 ppb

MATRICES Raw cow's milk

SAMPLE PREPARATION SH	**STORAGE TEMPERATURE** 2-8° C
CERTIFICATION /	**VALIDATION** /
SHELL LIFE 12 months	**DISPOSABLE** /

READER No

RING BIOTECHNOLOGY
Beta-lactams+Tetracyclines BT Combo Test Kit

PRODUCT CODE 100002

LIMIT OF DETERMINATIONS 96	**ASSAY TIME** 6-10'

LOD Beta-lactams: penicillin G 2-4 ppb, ampicillin 3-4 ppb, amoxicillin 3-4 ppb, oxacillin 6-8 ppb, cloxacillin 6-8 ppb, dicloxacillin 6-8 ppb, nafcillin 20-30 ppb, cefquinome 15-20 ppb, cephapirin 50-60 ppb, cefacetrile 100 ppb, cefalonium 18-20 ppb, cefoperazone 40-50 ppb, ceftiofur 90-100 ppb, cefazolin 50 ppb Tetracyclines: tetracycline 40-60 ppb, oxytetracycline 80-100 ppb, doxycycline 40-60 ppb, chlortetracycline 100 ppb

MATRICES Milk

SAMPLE PREPARATION SH	**STORAGE TEMPERATURE** 2-8° C
CERTIFICATION /	**VALIDATION** Yes (ILVO)
SHELL LIFE 13 months	**DISPOSABLE** /

READER No

BIOO SCIENTIFIC
PERKIN ELMER
AuroFlow™ BT Combo Strip Test Kit

PRODUCT CODE 1087-01

LIMIT OF DETERMINATIONS 96	**ASSAY TIME** 7'

LOD Beta-lactams: penicillin G 2-3 ppb, ampicillin 3-4 ppb, amoxicillin 3-4 ppb, oxacillin 4-8 ppb, cloxacillin 4-8 ppb, dicloxacillin 5-8 ppb, nafcillin 15-30 ppb, cephquinome 10-20 ppb, ceftiofur 75-100 ppb, cefazolin 35-50 ppb, cephapirin 6-15 ppb, cephalonium 4-8 ppb, cefoperazone 5-20 ppb, cefacetrile 30-50 ppb Tetracyclines: tetracycline 50-100 ppb, oxytetracycline 50-70 ppb, doxycycline 5-20 ppb, chlorotetracycline 15-50 ppb

MATRICES Raw cow milk

SAMPLE PREPARATION SH	**STORAGE TEMPERATURE** /
CERTIFICATION /	**VALIDATION** /
SHELL LIFE 24 months	**DISPOSABLE** /

READER Optional

BIOO SCIENTIFIC
PERKIN ELMER
AuroFlow™ PR1ME™ BT Combo MRL Assay

PRODUCT CODE 1134-02

LIMIT OF DETERMINATIONS 100	**ASSAY TIME** 7'

LOD Beta-lactams: penicillin G 1-2 ppb, penethamate 1-2 ppb, ampicillin 2-4 ppb, amoxicillin 3-4 ppb, cloxacillin 2-4 ppb, oxacillin 2-4 ppb, dicloxacillin 1.5-3 ppb, nafcillin 4-8 ppb, cefacetrile 20-30 ppb, ceftiofur 10-30 ppb, desfuroylceftiofur 20-40 ppb, cephapirin 4-7 ppb, desactylcephapirin 12-24 ppb, cefazolin 40-60 ppb, cefoperazone 3-5 ppb, cephquinome 8-12 ppb, cephalonium 1-2 ppb Tetracyclines: tetracycline 15-25 ppb, oxytetracycline 40-70 ppb, doxycycline 35-55 ppb, chlortetracycline 40-70 ppb

MATRICES Raw cow milk

SAMPLE PREPARATION HE	**STORAGE TEMPERATURE** /
CERTIFICATION /	**VALIDATION** /
SHELL LIFE 12 months	**DISPOSABLE** /

READER Optional

UNISENSOR DIAGNOSTIC ENGINEERING
TwinSensor Milk BT MRL (E.U. Regulation)

PRODUCT CODE KIT020

LIMIT OF DETERMINATIONS 96	**ASSAY TIME** 6'

LOD Beta-lactams: penicillin G 2-3 ppb, ampicillin 3-5 ppb, amoxicillin 3-5 ppb, oxacillin 12-18 ppb, cloxacillin 6-8 ppb, dicloxacillin 6-8 ppb, nafcillin 30-50 ppb, cefquinome 20-30 ppb, cephapirin 6-8 ppb, cefacetrile 30-40 ppb, cefalonium 3-5 ppb, cefoperazone 3-4 ppb, ceftiofur 10-15 ppb, cefazolin 18-22 ppb, cefalexin >750 ppb Tetracyclines: tetracycline 80-100 ppb, oxytetracycline 50-60 ppb, doxycycline 10-15 ppb, chlortetracycline 30-40 ppb

MATRICES Milk

SAMPLE PREPARATION HE	**STORAGE TEMPERATURE** /
CERTIFICATION /	**VALIDATION** Yes (ILVO)
SHELL LIFE 12 months	**DISPOSABLE** /

READER Optional

BEIJING KWINBON BIOTECHNOLOGY
MilkGuard Test Kit for Beta-Lactams & Tetracyclines

PRODUCT CODE KB02114D

LIMIT OF DETERMINATIONS 96	**ASSAY TIME** 10'

LOD Beta-lactams: penicillin G 2-4 ppb, ampicillin 3-4 ppb, amoxicillin 4-5 ppb, oxacillin 6-8 ppb, cloxacillin 6-8 ppb, dicloxacillin 6-8 ppb, nafcillin 20 ppb, cefquinome 20 ppb, cefalonium 10 ppb, cefoperazone 40-50 ppb, ceftiofur 90-100 ppb, ceftriaxone 50 ppb Tetracyclines: tetracycline 40-60 ppb, oxytetracycline 80–100 ppb, doxycycline 40-60 ppb, chlortetracycline 40-60 ppb

MATRICES Raw milk

SAMPLE PREPARATION SH	**STORAGE TEMPERATURE** 2-8° C
CERTIFICATION /	**VALIDATION** /
SHELL LIFE 12 months	**DISPOSABLE** /

READER No

UNISENSOR DIAGNOSTIC ENGINEERING
TwinSensor RT

PRODUCT CODE KIT088

LIMIT OF DETERMINATIONS 96	**ASSAY TIME** 10'

LOD Beta-lactams: penicillin G 1-2 ppb, ampicillin 3-4 ppb, amoxicillin 3-4 ppb, oxacillin 14-20 ppb, cloxacillin 10-14 ppb, dicloxacillin 7-8 ppb, nafcillin >30 ppb, cefquinome 20-30 ppb, cephapirin 2-3 ppb, cefacetrile <125 ppb, cefalonium 1-2 ppb, cefoperazone 0.5-1 ppb, ceftiofur 7-10 ppb, cefazolin 7-10 ppb, cefalexin 850-1100 ppb Tetracyclines: tetracycline 80-100 ppb, oxytetracycline 40-60 ppb, doxycycline 10-15 ppb, chlortetracycline 30-40 ppb

MATRICES Milk

SAMPLE PREPARATION As it is	**STORAGE TEMPERATURE** 2-8° C
CERTIFICATION /	**VALIDATION** /
SHELL LIFE 12 months	**DISPOSABLE** /

READER Optional

KONRUN BIOLOGICAL TECHNOLOGY
Beta-lactamase and tetracycline milk combo test kit

PRODUCT CODE BW1004

LIMIT OF DETERMINATIONS 96	**ASSAY TIME** 10'

LOD Beta-lactams: penicillin G 2-3 ppb, ampicillin 3-4 ppb, amoxicillin 4-6 ppb, oxacillin 6-8 ppb, cloxacillin 6-8 ppb, dicloxacillin 4-8 ppb, nafcillin 20-30 ppb, cefquinome 15-20 ppb, cephapirin 50-60 ppb, cefacetrile 90 ppb, cefalonium 18-20 ppb, cefoperazone 40-60 ppb, cefalotin 80 ppb, ceftiofur 90 ppb Tetracyclines: tetracycline 100 ppb, oxytetracycline 100 ppb, doxycycline 1100 ppb, chlortetracycline 100 ppb

MATRICES Raw milk (from cow and goat), milk powder

SAMPLE PREPARATION /	**STORAGE TEMPERATURE** 2-8° C
CERTIFICATION /	**VALIDATION** /
SHELL LIFE 12 months	**DISPOSABLE** /

READER No

/point of view

Rapid methods: can certification be just as rapid?

I will never forget the face of a lab manager in a tech meeting who reported the very high CV she measured after using a certified ELISA kit. The kit was not certified for the material she had tested. Nor will I ever forget that several feed industries have a museum of LFD readers (each brand requiring its own reader, of course) because they couldn't get good performance for certain raw materials, including mycotoxin screenings.

Test kits and new portable analytical devices are very useful tools for food safety. But they often share a single problem: performance verification. The majority of companies in the food, beverage, and feed industries do not have the time and expertise to validate diagnostic products. Manufacturers conduct their own validations but rarely for all the matrices that end users will test. The most they provide you, if anything, is a "first party" report. Customers, however, need "third party" validation. Laboratories must be in accordance with ISO 17025 and if the kit is not certified by a third party for the test material they need to analyze, internal validation is mandatory. For microbiological tests (pathogens, etc.), there are several certified kits but there are very few for food contaminants and food fraud. The market is too small to allow a profitable return on investment. Because of this, everyone is unhappy. Industries and laboratories are forced to spend a lot of time on home-made verification or real validation and manufacturers can't respond to every market demand. Only the producers of expensive chromatographic instruments are happy with this situation. When biochemical assays are weak, the winner is mass spectrometry or, at least, chromatography. Ah, I forgot, even consumers of food—all of us, that is—should be unhappy. If rapid on-site methods are not available, not used, or used with unreliable results, the risk of contaminated food is higher.

Is it possible to change? Yes, we can. How? With cooperative efforts. We must take advantage of the internet and the attitude of younger generations to share knowledge. What exactly is the idea? We want to create a panel of experts that make a protocol for e-validation of test kits primarily using existing data. Is there more? Yes, of course. IT technologies need to guarantee data protection. The AOAC and AFNOR traditional approach is based on an "aseptic", single independent laboratory verification exercise. The procedure is long (one year or so) and expensive. *Affidia*'s project for "e-coop-validation" has a goal of guaranteeing matrix verification in 6 months and at a more reasonable price. With this, more analyte-matrix combinations will be covered by certification and more test kits will be certified. Is anyone interested in being on our panel of experts for this project? We need representative from kit manufacturers, ISO 17025 certification bodies, and food industries that have experience in validation exercises to design the validation protocol. Contact us if you're interested in being part of this exciting new venture.

Maurizio Paleologo