Modelling the risks and consequences of residues of antimicrobial drugs in the gut and manure of pigs

Maria Eleni Filippitzi (2018)

Ceramic: *Το γουρούνι* (*The pig*). Artist: Yannis Karagkiouzis. Private Collection.

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List of abbreviations

AFSCA-FAVV   Belgian Federal Agency for the Safety of the Food Chain

AM   Antimicrobial

AMR   Antimicrobial Resistance

BelVet-SAC   Belgian Veterinary Surveillance of Antibiotic Consumption

BFA   Belgian Compound Feed Industry Association

CTC   Chlortetracycline

DDDA   Defined Daily Doses Animal

DOX   Doxycycline

EC   European Commission

EC AGRI   Agriculture and rural development Committee of the European Commission

EFSA   European Food Safety Authority

EMA   European Medicines Agency

ERA   Environmental Risk Assessment

ESVAC   European Surveillance of Veterinary Antimicrobial Consumption

FEFAC   European Feed Manufacturers' Federation

GI   Gastro-intestinal

HGT   Horizontal gene transfer

LA-MRSA   Livestock-associated Methicillin-Resistant *Staphylococcus aureus*

MF   Medicated feed

OIE   World Organisation for Animal Health

PCU   Population correction unit

QRA   Quantitative risk assessment

SDZ-TRIM   Sulfadiazine-trimethoprim

TI   Treatment incidence

UDDA   Used Daily Dose Animal

WHO   World Health Organization

CHAPTER 1

General Introduction

1. Antimicrobial use in livestock production

1.1. Livestock production and the issue of antimicrobial resistance

The past few decades, major efforts have been made by the agricultural community to increase livestock production and productivity in order to keep up with the ever-growing demand for food. Figure (Tons) he evolution of the size of the food-producing population in 30 European countries between 2010 and 2015, in population correction unit (PCU). The PCU is estimated for each animal category by multiplying numbers of food-producing animals (e.g. dairy cows, sheep, sows) and slaughtered animals (e.g. pigs, cattle, poultry) by the theoretical weight at the most likely time for treatment (EMA, 2017a). From 2010 to 2015, the total PCU (estimated weight at treatment of livestock and slaughtered animals) was relatively stable for most countries.
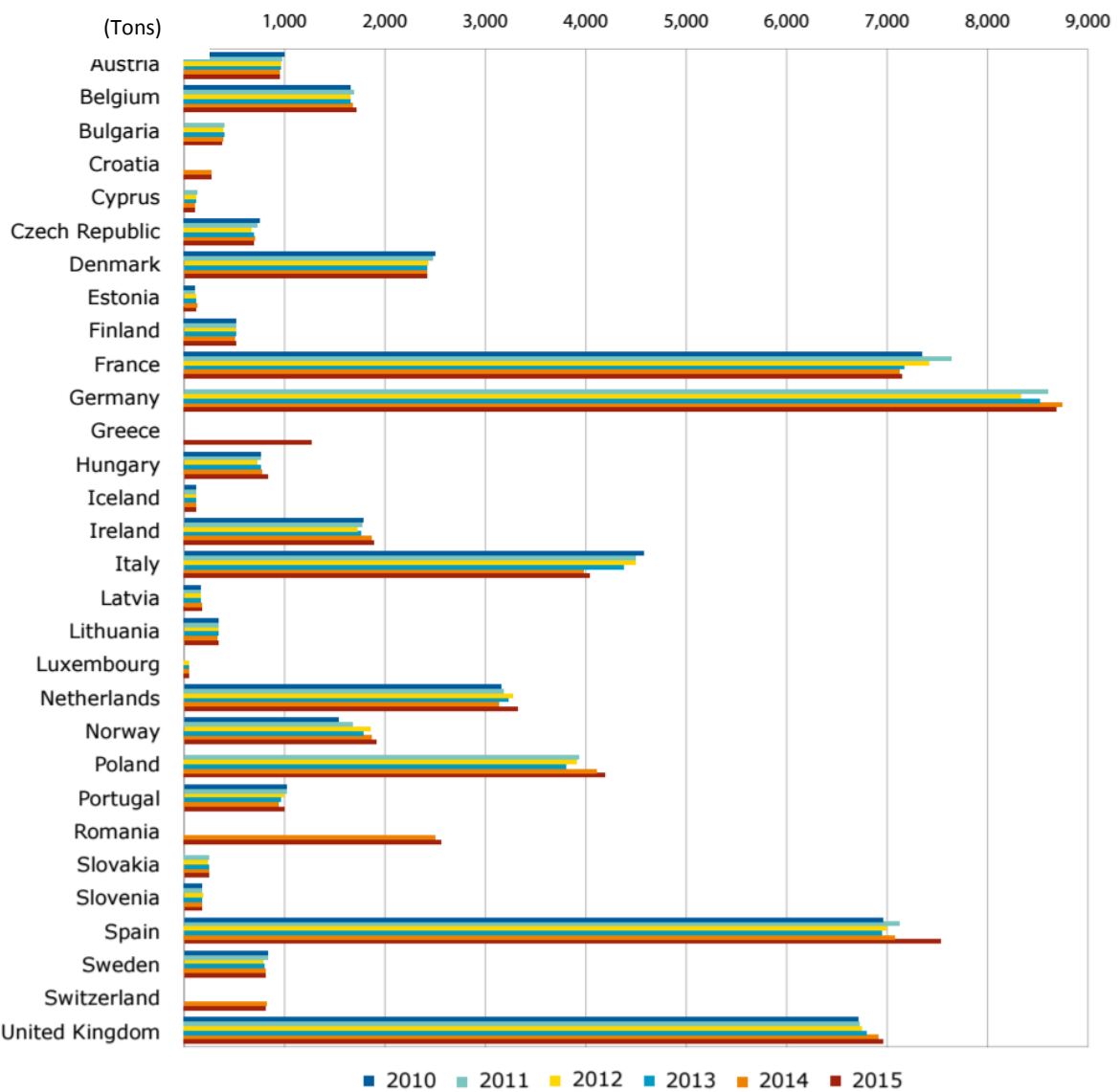

Figure 1. Changes in the PCU for food-producing animals, in 1000 tons, by country, between 2010 and 2015, in 30 European countries. Adopted from EMA (2017a).

The regular use of antimicrobials in animal production has been fundamental for the treatment and prevention of bacterial infections and for the improvement of production efficiency (antimicrobial growth promotion) in food-producing animals. The latter practice is forbidden in the European Union since 2006 (EC, 2003), but it is still commonly performed in countries like the United States and China (OIE, 2017).

Antimicrobials are natural, semi-synthetic or synthetic chemical compounds that, through various mechanisms of action, can inhibit bacterial growth or kill bacteria. Despite their paramount importance in treating bacterial infections, both in humans and animals, the efficacy of these drugs is seriously threatened by the emergence of acquired antimicrobial resistance. Acquired antimicrobial resistance is the ability of a microorganism to multiply or persist in the presence of an antimicrobial agent relative to the susceptible counterpart of the same species (FAO, 2016). In other words, acquired resistance refers to a situation where the microorganism (bacterium) has acquired a characteristic that makes the antimicrobial less or no longer effective. Besides acquired, resistance can also be intrinsic (or natural). Intrinsic resistance is a trait of all bacteria belonging to a specific taxon (e.g. species, genus) and may be due to an inherited or functional characteristic of the bacteria resulting in the inability of an antimicrobial to attack or eliminate the bacterium adequately (Boerlin and White, 2013).

Focusing on acquired resistance, there are three ways it can be acquired. Firstly, it can be acquired through a stepwise mutation of the genes affecting the production of necessary products for physiological cell metabolism. The spectrum of activity of these genes becomes narrower (e.g. leading to the production of less products needed for cell metabolism). A second way for bacteria to acquire resistance is through a mutation or a series of mutations resulting in resistance against inhibitory effects of the antimicrobials. These two ways concern the vertical (chromosomal) transmission of resistance. A third way for bacteria to acquire resistance is by the integration of resistance genes, located on chromosomal DNA, into mobile genetic elements like plasmids or transposons. This is called horizontal gene transfer (HGT) (Schwarz and Chaslus-Dancla, 2001). Antibiotic resistance gene transfer through HGT happens most often through conjugation – the transfer of DNA between bacterial cells through direct contact between donor and recipient cell. HGT is a particularly worrisome phenomenon as it can occur between many different types of bacteria and in nearly all matrices (e.g. soil, water, gut or food). In HGT, genes can be transferred between different bacterial strains or between different species (Rosi et al., 2014).

Antimicrobial resistance may be transferred from animals to humans and vice versa. Livestock-associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) is an example of such a zoonotic bacterium. LA-MRSA is an originally human-associated bacterium which was introduced into the

animal population by humans. Following, the bacterium adapted itself, depending on the animal host (pigs, calves, etc.), and emerged in livestock and human populations (Larsen et al., 2016; Filippitzi et al., 2016). Besides bacteria, antimicrobial resistant genes can also be transmitted between animals and humans (Rolain, 2013; EFSA, 2017a). For instance, a *Salmonella* species that has acquired resistance in the gut of a pig from exposure to antibiotics given to that pig, will not lose its resistant characteristics when transmitted to a human and will remain equally resistant to certain antibiotics in the human gut microbiota (Dewulf, 2018).

An exchange of antimicrobial resistance from animals to humans and vice versa may occur by three different transmission routes (Figure 2) (Marshall and Levy, 2011; FAO, 2016): a. Resistant traits (bacteria or genes) may be transferred from animals to humans and vice versa through direct contact; b. through the consumption by humans of food of animal origin that contains resistant bacteria or genes; c. through the environment (e.g. using manure as a fertiliser, or by irrigating with contaminated water).
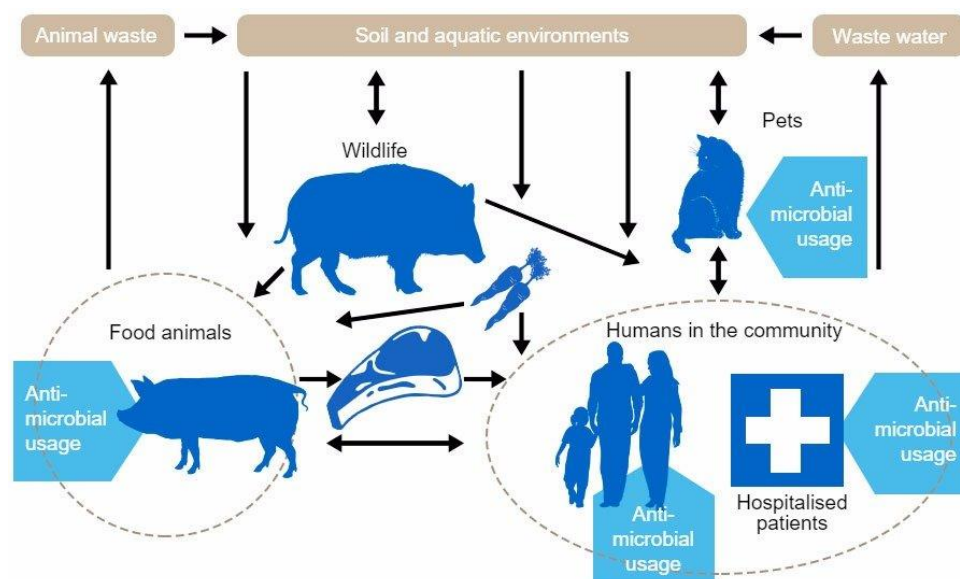
Figure 2. Flow diagram of antibiotic resistance determinants among the different reservoirs. Light blue indicates points of anthropogenic antimicrobial selection pressure (EFFORT, 2018a).

It therefore becomes obvious that all actions to counter the selection and spread of antimicrobial resistance fit in the broader principle of "One Health" (OH) (Robinson et al., 2016). OH is a strategic framework that has been established in 2008 to combat the spread of infectious diseases that emerge (or re-emerge) from the interfaces between animals and humans and the ecosystems in which they live in (FAO, OIE and WHO, 2010). Regarding antimicrobial use and resistance, antimicrobial compounds used to treat or prevent diseases in animals are essentially the same as those in human medicine, even though some antimicrobials are reseved for use in human medicine only. The World

Health Organization (WHO) recommends the restricted use of antimicrobials judged as essential for human medicine (WHO, 2016), while the same accounts for essential antimicrobials for veterinary medicine (e.g. pleuromutilines) (OIE, 2015). In Belgium, starting from 2011, the Belgian centre for Antimicrobial Consumption and Resistance in Animals (AMCRA) has set the goal to achieve a general reduction of antimicrobial use in animals of 50% by 2020, a 75% reduction of critically important antimicrobials for human medicine (e.g. 3rd and 4th generation cephalosporines, quinolones) by 2020 and a 50% reduction of antimicrobial premixes by 2017. Indicatively, the total national consumption of antimicrobial compounds for veterinary use (pharmaceutical and premixes) in Belgium per class of antimicrobials between 2011 and 2016 is shown in Figure 3.
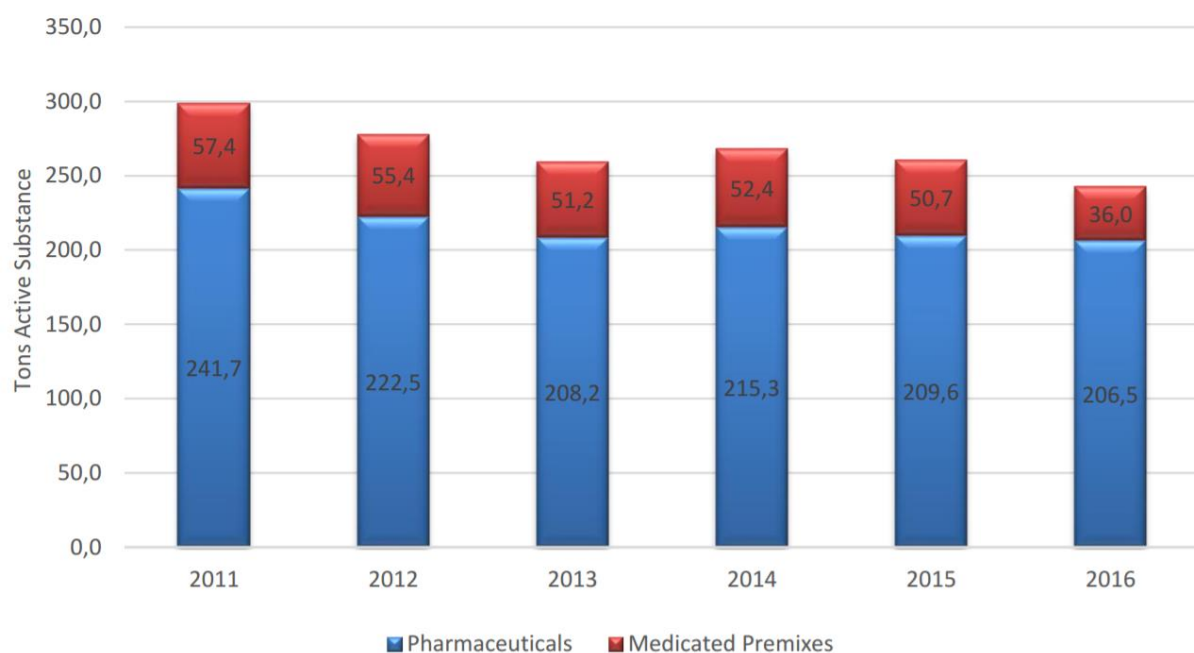
Figure 3. Total national consumption of antibacterial compounds for veterinary use in Belgium for the years 2011-2016 (tons active substance). Adopted from BelVet-SAC (2017).

1.2. Quantification of antimicrobial use in livestock production

The overall use of antimicrobials clearly plays a role in the selection, spread and persistence of antimicrobial resistance and, thus, in the fight against it, avoiding unnecessary antimicrobial use is set as a priority (WHO, 2015). The concerns about the spread of resistance have led to the monitoring of trends in antimicrobial consumption in animals in several European countries through databases on antimicrobials sales such as Denmark, Norway, Sweden and Belgium (DANMAP, 2016; NORM-VET, 2016; Swedres-Svarm, 2016; BelVet-SAC, 2017). The importance of these monitoring procedures is very high, since only on the basis of adequate quantification of antimicrobial use, benchmarking is possible and informed actions can be further taken. Benchmarking refers to the categorization of a

party's (e.g. farm, country) antimicrobial use following comparison with the antimicrobial use of a predefined population of similar parties, with antimicrobial use for all parties being quantified in a similar manner (AACTING, 2018).

At global level, OIE is responsible for data collection on the amount of antimicrobial agents sold or imported for use in animals (in mg/kg biomass) (OIE, 2017). At European Union (EU) level, the European Medicines Agency (EMA) collects national consumption data from EU countries in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project since 2010. For normalization of sales data, the Population Correction Unit (PCU) is used, which takes into account the size of the animal population and the average theoretical weight of the animal species at the time of treatment. The sales data of antimicrobial veterinary medicinal products normalized by the PCU are expressed in mg of active ingredient by PCU (mg/PCU) and 1 PCU equals 1 kg of livestock (EMA, 2017a). In Belgium, the antimicrobial consumption is described annually since 2011 in the BelVet-SAC (Belgian Veterinary Surveillance of Antibiotic Consumption) report (Daeseleire et al., 2016). This report presents national data from food-producing and companion animals, expressed in mg/kg biomass, a comparable measurement to the one used from ESVAC (BelVet-SAC, 2017).

These quantification methods, though, have limitations. A first limitation of these is that the potency of an antimicrobial product is not taken into account (Jensen et al., 2004). Secondly, the estimation of antimicrobials consumption based on sales data can be compromised, since off-label use is a common practice (EMA, 2017b) and the sold antimicrobials may not have been eventually administered to animals. Furthermore, these estimations refer to all animals with no allocation to specific species, as the majority of antimicrobial products are licensed for several species.

Currently, the preferred reference values to compare antimicrobial use between herds, countries and animal species are normalisations with the harmonized Defined Daily Doses Animal (DDDA) and expressed as number of DDDA per defined time period (e.g. 100 days). This quantification system allows also for comparison of use within countries and between herds and prescribers. However, these assigned DDDA values are often a compromise, as doses can differ between countries, indications, and formulations (Postma et al., 2015). Another technical unit of antimicrobial use measurement is the Used Daily Dose Animal (UDDA), which can be used when very detailed information at herd level is available providing information on the exact amount of treatment dose and the exact weight of the animals at the moment of treatment (Timmerman et al., 2006; Callens et al., 2012). This information (i.e. amount of antimicrobials purchased or administered, weight of animals treated) together with information on the number of animals treated and on the period the animals at risk receive an antimicrobial treatment can be used to calculate a treatment incidence (TI) (Timmerman et al., 2006).

A detailed description of indicators to quantify antimicrobial usage based on sales, deliveries or reimbursement data has been provided by Collineau et al. (2017). Currently, there are 29 monitoring systems of antimicrobial use at herd level, developed and used from 16 countries (AACTING, 2018).

Collecting objective data per species, even when they are not yet fully comparable, is a crucial step in the development of targeted reduction strategies. Therefore in most countries, including Belgium, there is a need for species-specific data on antimicrobial use. For this purpose, the ESVAC scientific guidelines paper on the collection of reliable and standardized data on the consumption of antimicrobial agents by animal species describes a methodology to be used to provide estimates on antimicrobial consumption for the actual species at the national level, in tons, per weight group (EMA, 2013). In Belgium, DDDA values have been calculated for pigs (Callens et al., 2012), veal calves (Pardon et al., 2012) and poultry (Persoons et al., 2010) and, therefore, could be used for an extrapolation of the antimicrobial use per species at the national level and a (relative and in absolute values) comparison among them, to target the highest users. Recently, Carmo et al (2017), compared antimicrobial consumption estimates for cattle and pigs (in mg/kg biomass) in Switzerland and Denmark, in order to distinguish species-specific patterns and trends in consumption.

2. Antimicrobial use in pig production

The EU is the world's second biggest producer of pork, after China. Over 257 million pigs were slaughtered in 2016 in the EU and the production of pork amounted to 23.4 million tons (EUROSTAT, 2016). The EU region with the major production, accounting for 30% of EU sows, extends from Germany (Nordrhein-Westfalen) to Belgium (Flanders region). In Belgium, pig production is one of the most important agricultural sectors (Institute for National Accounts, 2015). Belgian farmers housed 6.01 million pigs in 2016, with 95% of total Belgian pig production being located in Flanders (Statistics Belgium, 2017).

The pig production sector is believed to be amongst the highest users of antimicrobials in current animal husbandry production, in absolute values and in terms of treatment incidence (Bos et al., 2013; DANMAP, 2016). In pig production, as well as in broiler and calf production, animals have a short lifetime and younger animals demand relatively more treatment with antimicrobials, as they are more susceptible to diseases (Bondt et al., 2012; Sjölund et al., 2016). This might partially result into a relatively higher use per time period in pigs compared to sectors with longer production cycles, for instance beef cattle.

A detailed description and comparison of the antimicrobials used in pigs, their dosage prescriptions and their most common routes of administration in Belgium, France, Germany and Sweden, has been performed by Postma et al. (2015). Moreover, Callens et al. (2012) collected and quantified herd level

data on the group use of antimicrobial agents in Belgian pig herds. The results of these studies provided a first basis to understand the patterns followed in antimicrobial use in pigs, in terms of substances used, routes of administration, dosage and indications of use, treatment duration.

2.1. Oral group medication of pigs

Antimicrobials can be administered to pigs via different ways, including the oral route (feed or water medication), the topical route (i.e. administration to the site of infection) or the parenteral route. The latter theoretically includes intravenous, intramuscular and subcutaneous injection (EPRUMA, 2014). In pig production, mostly subcutaneous and intramuscular injections are given. The choice of administration route is usually related to the purpose of treatment and location of the infection (Schwarz et al., 2001).

Antimicrobial treatments applied to animals can serve the following purposes: (a) *prophylaxis*, which is applied to groups of animals to prevent bacterial infections and bacterial disease outbreak at the farm; (b) *methaphylaxis*, which is applied to a number of animals in the group to treat (after diagnosis) bacterial infections and to prevent further spread towards the pen-mates; (c) therapy, which mostly involves individual treatment of a limited number of animals; and (d) growth promotion, which is banned in the EU since 2006 (EC, 2003). Callens et al. (2012) observed that prophylactic group treatment, a solely preventive measure, was applied in 93% of all group treatments in the herds they visited in Belgium, whereas metaphylactic or therapeutic treatments were applied in only 7% of the cases. For practical reasons, group treatments are generally achieved through oral administration of the antimicrobial via the feed or drinking water (Schwarz et al., 2001).

When considering the distribution of sales of the various pharmaceutical forms of veterinary antimicrobials for food-producing animals in the EU, oral treatments represent the most common route of antimicrobial administration for livestock species (>90%) (Figure 4). Based on the data collected from nine EU countries, although variation existed between countries, in total 70% of antimicrobial treatments of pigs were administered via the oral route (EFFORT, 2018b). The results from the study by Callens et al. (2012) in Belgian pig farms, show that antimicrobial treatments administered to groups of pigs via the oral route represent the most common way of antimicrobial administration to these species in Belgium, in terms of treatment incidence (TI_{UDDA}, oral = 176.5, while TI_{UDDA}, injectable = 24.2). In the same study, group treatments were primarily administered orally in piglets after weaning via the feed or drinking water, whereas the parenteral route was mostly used in sucking piglets. These antimicrobial treatments administered before the age of ten weeks, correspond to an average of 80% of antimicrobial treatments in pig production (Callens et al., 2012). Postma et al. (2015), having collected data from Belgium, France, Germany and Sweden, identified that most

licensed antimicrobial products for pigs are administered orally, via the feed or water (49.3%), compared to other routes of administration.
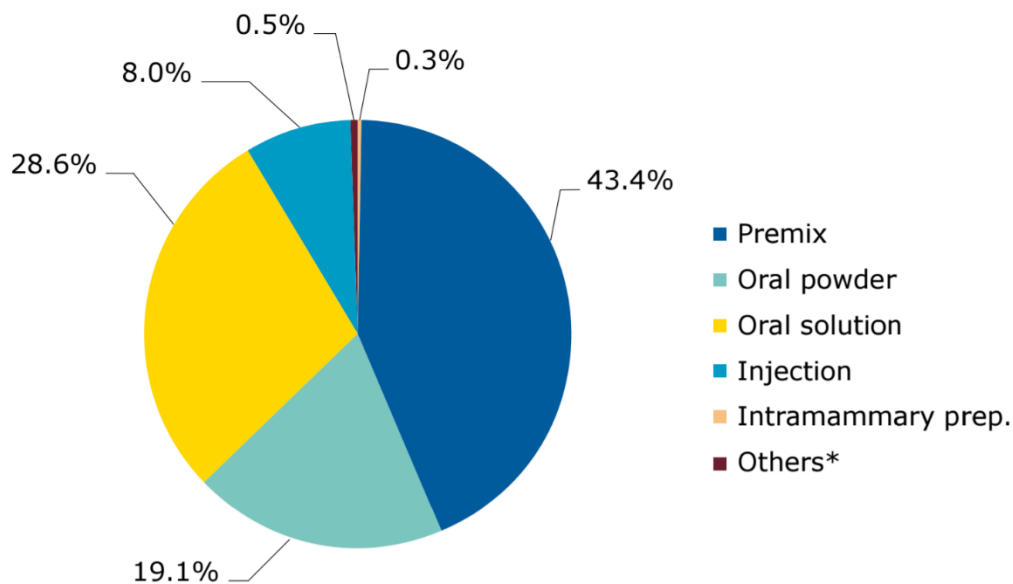
Figure 4. Distribution of sales, in mg/PCU, of the various pharmaceutical forms of veterinary antimicrobial agents for food-producing animals, aggregated by the 30 European countries for 2015. Adopted by the ESVAC Report 2015 (EMA, 2017a). Premixes, oral powders and solutions are the biggest-selling antimicrobial veterinary medicinal products for food producing animals in the EU. This also the case for pigs (Callens et al., 2012; Postma et al., 2015). * Others: Oral pastes, boluses and intrauterine preparations.

However, the oral administration of the antimicrobials via the feed or drinking water depends on the eating and drinking behavior of the animals and, therefore, this route does not always guarantee an accurate dosage and treatment duration, in contrast to the parenteral treatment. These elements (administration route, dosage, treatment interval and treatment duration), together with the type of antimicrobial chosen for treatment and the total amount of antimicrobial used in a population, have an effect on antimicrobial resistance development, selection, spread and persistence of antimicrobial resistance.

2.2. Effect of antimicrobial residues in the gastro-intestinal tract on antimicrobial resistance

Current treatment strategies with antimicrobials exert a selective pressure not only on the pathogen where the treatment was intended for, but also on commensal microbiota, including the intestinal microbiota, for which the treatment was not intended (Devreese et al., 2014). The intestinal microbiota is exposed to residues of antimicrobials due to incomplete absorption of antimicrobials when these are administered orally (i.e. incomplete oral bioavailability) as well as through excretion, via the bile and secretion from blood to gut, of the parent antimicrobial and/or its active metabolites (Riviere and Papich, 2009; De Smet et al., 2017). The extent of oral bioavailability of an antimicrobial is largely

determined by the chemical nature of the drug and the related physicochemical properties, the prandial state of the animal, while interspecies variations can also occur (Martinez and Modric, 2010). In contrast to oral administration, parenteral administration of antimicrobials is assumed to have less impact on the exposure of the intestinal microbiota to residues as there is no effect of incomplete absorption (Baggot and Giguère, 2013).

As already mentioned, antimicrobial compounds of different classes show different pharmacokinetic behavior due to differences in their chemical nature (Baggot and Giguère, 2013). While some antimicrobial agents are almost entirely eliminated by renal excretion (aminoglycoside, most beta-lactam antibiotics), others are eliminated by biliary excretion (e.g. macrolides) whether or not preceded by (hepatic) biotransformation. Some antimicrobials are also metabolised to variable degrees from the parent drug form to inactive metabolites (e.g. sulphonamides) (Prescott and Dowling, 2013). Therefore, the use of different antimicrobials administered leads to variable levels of residues in the GI tract of animals.

The tissue (in this case, the gut) concentration of antimicrobial residues thrives selective pressure on bacteria (Prescott and Dowling, 2013; Baggot and Giguère, 2013). The degree of absorption of antimicrobials in the upper gastrointestinal (GI) tract after oral administration may also influence the selection pressure exerted on the microbiota of the large intestine (Chantziaras et al., 2017a). When an entire animal population is treated, the odds of dilution of resistance selection to occur will be lower than in the case of treatment of a single animal, and a commensal reservoir of resistance genes can be formed (Levy and Marshall, 2004). Moreover, the transfer of resistant bacteria might occur more rapidly to animals being treated, due to disturbance of the commensal microbiota (Barza and Travers, 2002).

To date, only limited specific (e.g. in terms of species, antimicrobials used) research data on the effect of different administration routes on resistance selection and spread are available. For instance, the effect of route of administration of fluoroquinolones on resistance selection in commensal *E.coli* strains in poultry was recently assessed by Chantziaras et al. (2017b) through a series of *in vivo* pharmacodynamic experiments. They concluded that the use of oral treatment seems to select more for fluoroquinolone resistance, compared to the parenteral one. On the other hand, De Smet et al. (2017) observed in pharmacokinetic studies no difference between oral (p.o.) and intramuscular (i.m.) administration of sulfadiazine and trimethoprim on pig gut exposure. In a recent experiment by Burow et al. (2018), it was observed that all untreated contact animals of orally enrofloxacin-treated animals showed enrofloxacin concentrations in their blood serum, while only a part of the contact animals of parenterally treated ones did. They also observed in the orally treated and their contact pigs, an

increase of *E. coli* resistant to antimicrobials other than quinolones (e.g. ampicillin). This evidence points out that effect of administration route on resistance selection is rather compound specific, depending on its physicochemical properties and pharmacokinetic characteristics.

Studies comparing the effect on gut and intestinal exposure of different doses administered also remain scarce. An *in vivo* experiment by Devreese et al. (2014) demonstrated that elevated doses of enrofloxacin, a fluoroquinolone, administered in broilers leads to higher exposure in the gut. However, there was no significant difference in gut concentration between p.o. and i.m. administration of the same elevated dose. According to the previously mentioned *in vivo* study by Chantziaras et al. (2017b), who tested the faecal shedding of *E.coli* strains, no significant effect of enrofloxacin dosing was seen on resistance selection to this antimicrobial, indicating that the same resistance selecting effect is obtained independently of the administered dose (i.e. half, correct, double). This constitutes new additional evidence to the suggestion that an increase or decrease of dosage to some extent selects for resistance. Besides, a dosage is always defined in function of a certain (pathogenic) bacterium in a specific target organ. Consequently, even if the antibiotic treatment is perfectly targeted and kills the pathogenic bacterium, many other (commensal) bacteria are simultaneously influenced, resulting in the formation of a reservoir of resistance, that will interact with the next pathogen introduced (Dewulf, in prep.).

Oral antimicrobial treatments, and especially those administered via feed (in contrast to water administration), are generally expected to be under-dosed due to eating habits of animals (e.g. reduction or loss of appetite of sick pigs or impact of social rank) (van Krimpen, 2007; Soraci et al., 2014). Callens et al. (2012) found at the Belgian pig farms they visited, that oral administrations of antimicrobials were mostly under-dosed (47.3% under-dosed, 23.3% correctly dosed, 29.4% overdosed). The consumption of feed cross-contaminated with antimicrobial residues is also another, relatively neglected case of unintended antimicrobial under-dosing (Peeters et al., 2016). What is worrisome is that, even in exposure of the GI tract of pigs to very low antimicrobial (e.g. doxycycline) concentrations (e.g. 1 mg/L intestinal content), a selection towards antimicrobial resistance has been observed (e.g. in *E.coli*) (Brewer et al., 2013; Peeters et al., 2017, 2018a).

Currently, the level and the conditions of use of feed and water antimicrobial treatments of pigs at the farm level is only anecdotally specified. Acquiring extensive and detailed, including quantitative, information on these treatments would enable us understand the extent of consequences from this use, in terms of pig exposure to antimicrobial residues and resistance selection and spread.

3. **Residues from antimicrobial use in pig production**

Antimicrobial use in pig production may result in the presence of residues (smaller fraction of an initial amount) in meat, in the GI tract of animals and in manure. For the purposes of this thesis, we will focus on the antimicrobial residues in the GI tract and in manure of pigs, as a result of oral treatments applied to this species, including the exposure of pigs to cross-contaminated non-medicated feed.

3.1. Medicated and cross-contaminated feed

Medicated feed is defined as a mixture of one or more veterinary medicinal products or intermediate products with one or more feeds which is ready to be directly fed to animals without further processing. An intermediate product (also called premix) is defined as a mixture of one or more veterinary medicinal products with one or more feedingstuffs, intended to be used for the manufacture of medicated feed (EC, 2014).

Carry-over is, in this context, the transfer of traces of a substance contained in a medicated feed to a non-target feed and results in the cross-contamination of this feed (EC, 2014). Cross-contamination of feed can occur at the feed-mill, in the transport truck delivering the feed to the farm or/and at the farm (AFSCA, 2013). Currently, the level of cross-contamination at these three different levels is limitedly studied, with very few quantitative data on antimicrobial residual concentrations being available in published literature (Putier, 2010; Stolker et al., 2013), while there is no information on the situation at the farm. At farms where antimicrobial medicated feed is used, in the vast majority of cases, the same silo and feeding lines are used for the storage and administration of medicated and non-medicated feed, creating conditions for cross-contamination of the non-medicated feed with antimicrobials retained at different points of equipment used (Figure 5).

The issue is that feeding animals cross-contaminated feed may lead to unintended low dosage medication of the animals with residues of antimicrobials (Mc Evoy, 2002). An estimation of the amount of non-target feed which is cross-contaminated with antimicrobial residues due to antimicrobial medicated feed production in a country would help us estimate the level of exposure of pigs to this cross-contaminated feed. This would be particularly interesting, as these residues cause a public and animal health concern associated with antimicrobial resistance selection and development (AFSCA, 2013; EC, 2016).

Peeters et al. (2016) recently showed that high concentrations of tetracyclines in caecal and colonic content and feces of pigs at steady-state conditions can be reached at low antimicrobial carry-over level (i.e. 3% carry-over) in the feed. *In vitro* experiments with *E. coli* (Peeters et al., 2018a) as well as *ex vivo* experiments simulating the pig caecum (Peeters et al., 2017) showed that intestinal doxycycline concentrations corresponding to approximately 1% and 3% carry-over level in the feed, both select for a *tet*(A) carrying doxycycline resistant *E. coli* strain compared to a blank control. A cross-contamination

level of 1% is proposed by the EU to be established as maximum carry-over level for antimicrobials, while 3% is the proposed maximum carry-over level for other active substances (EC, 2016). To prevent feed cross-contamination at the feed-mills in Belgium, a covenant was made between the Federal Agency for the Safety of the Food Chain (AFSCA-FAVV) and the Belgian Feed Association (BFA) in 2013, according to which the antimicrobial premixes used for the production of medicated feed should no longer be added and mixed in the main mixer, but rather in the dedicated end-of-line mixer, reducing the level and probability of cross-contamination at the feed mill level (BFA, 2013).

Currently, there is no information available regarding the exposure of pigs to cross-contaminated feed with different carry-over levels (e.g. 1%, 3%). This information, expressed in a quantitative manner, combined with estimations on potential for resistance selection pressure, depending on the antimicrobial used, would be very relevant and timely for the discussion over the maximum carry-over level allowed. As previously mentioned, the European Commission (EC) has presented a proposal for a regulation regarding medicated feed, which is not in force yet, suggesting a 1% carry-over limit for antimicrobials in non-target feed (EC, 2014). However, some stakeholders (feed-mill companies, farmers) and the delegated EC AGRI (Agriculture and rural development Committee) have reacted to it, by suggesting that the proposed general limits (1% for antimicrobials and 3% for other active substances) should be amended to 3%, regardless of the active substance. A 1% limit was found to be too stringent and not viable (EC, 2016).

Moreover, there is no information available regarding any differences in the exposure of pigs of different production stages (i.e. piglets, sows and fattening pigs) to cross-contaminated feed. Should any difference between these stages be observed, targeting those with a higher risk of exposure to cross-contaminated feed would be valuable to prevent this phenomenon in an informed way. Finally, there is a lack of a full set of recommendations to prevent carry-over at different levels (i.e. feed-mill, truck, farm), so a comparison of different control options is needed.
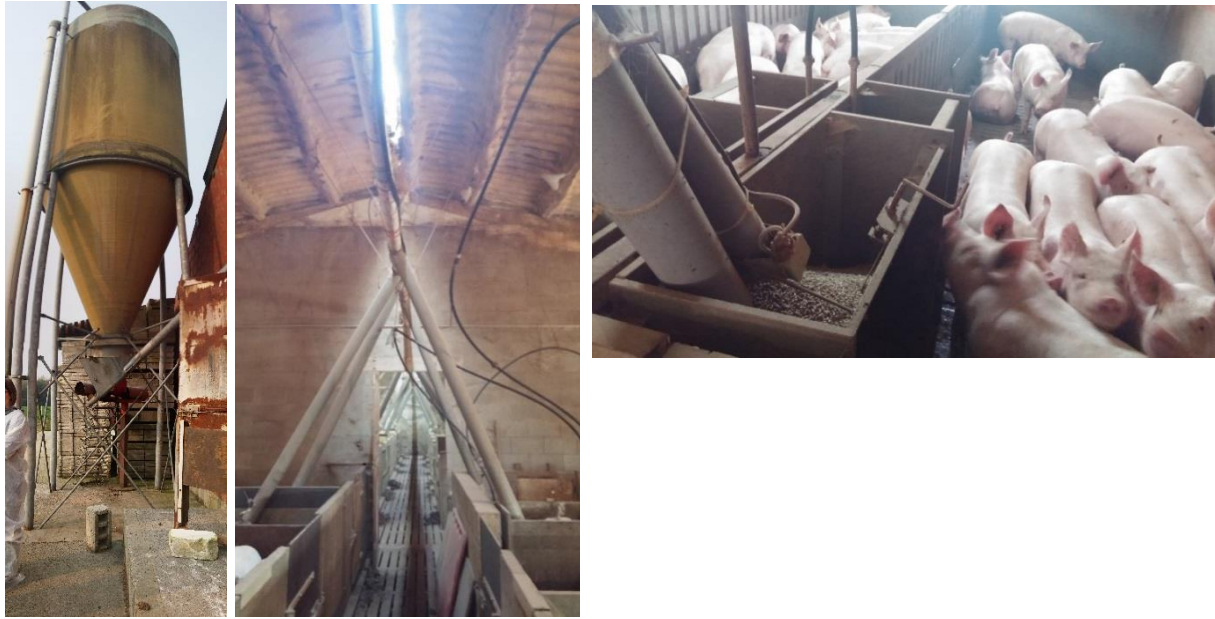
Figure 5. Feed silo and feeding lines used for the administration of feed at pig farms. Source: M. Filippitzi, Groupmedipig project.

3.2. *Medicated drinking water*

Drinking water is another way of animal group treatment with antimicrobials (Rosengren et al., 2008), which has been mainly used and described in poultry industry (de Costa et al., 2008; van der Horst et., 2013). Recently, a trend has been observed in pig industry towards an increased use of water medication over the use of medicated feed (Peeters, 2018b). This can be largely attributed to four important advantages that characterize water medication: the flexibility of therapy start and duration; the possibility to select smaller groups of animals for therapy; the fact that water uptake of sick animals tends to be more stable than feed uptake; and the generally better absorption of antimicrobials from drinking water than from feed (van Krimpen, 2007; AFSCA-FAVV, 2013).

Compared to medicated feed, this method requires some more effort from both the veterinarian and the farmer because several factors need to be taken into consideration to determine the correct dosage. Some of these factors include the average water consumption and physicochemical properties of water and antimicrobials in water. In fact, few antimicrobials are water-soluble, while the pharmaceutical formulation including the excipientia can also play a role in their solubility. Drinking water systems that are not maintained properly can lead to transfer of variable antimicrobial doses of residues between different points in drinking-water pipes (van Krimpen, 2007; Page and Gaultier, 2012). Additionally, different methods of preparing water medication and cleaning the water systems at the farm can have a different effect on the level of removal of residues (Figures 6). Corrosion at many points of this equipment creates possible points of retainment of antimicrobial residues. The reaction of antimicrobials with the wall of metal pipes, for example, can also pose problems (AFSCA-FAVV, 2013). Besides the use of antimicrobials, the source and quality of water used for antimicrobial

administration can also be associated with resistance. A recent study by Dorado-García et al. (2015) observed that pigs that drank water from the public supply instead of from a private source had increased probability for Methicillin-Resistant *Staphylococcus aureus* (MRSA). The quality of water, for example in terms of hardness and pH can affect the solubility of different antimicrobials and can lead to carry-over of residues (De Backer, 2015).

So far very few published studies have focused on the use of water medication in pigs. For instance, the study by Rosengren et al. (2008) provided information on the frequency of administration of antimicrobials via water to pigs, per production stage, in Canada. In the ten farms where antimicrobials were administered by water, six producers reported use in nursery pigs only, two in nursery and grow-finish pigs, one in grow-finish pigs only, and one in sows. In nursery and grow-finish pigs, the most commonly used antimicrobial was penicillin G. Preliminary information is also available from Germany (Burow et al., 2018). Despite the common use in pigs of water medicated with antimicrobials, this information is currently unknown for many countries, including Belgium. Therefore, information regarding the use and preparation of water medication at pig farms in Belgium needs to be collected.
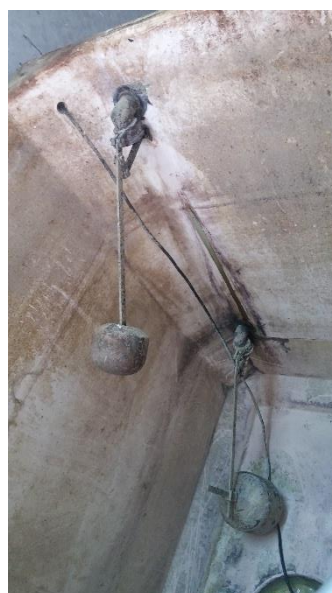
Figure 6. Examples of equipment used for preparation of water medication at Belgian farms visited throughout this study. Source: M. Filippitzi, Groupmedipig project.

3.3. Antimicrobial residues in manure

A large fraction of the overall administered antimicrobials to livestock are excreted unchanged or as (active) metabolite via the feces and urine (Amábile-Cuevas, 2016) and are therefore transferred to the manure pit.

Recently, concerns are growing regarding the level of antimicrobial residues or their active metabolites that end up, via manure application, in soil, and following in groundwater and surface water, and their role in the development and spread of antimicrobial resistance in the environment. Recent research suggest that the antimicrobial residues present in manure may select for resistance in pathogens and commensal bacteria, which may enter the environment after fertilization of soil (Du and Liu, 2012; Rasschaert et al., 2017). Manure may also serve as a reservoir for antimicrobial resistance genes and mobile genetic elements (Binh et al., 2008; Whitehead and Cotta, 2013; Wang et al., 2018). Investigation of the effects of pig manure and sulfadiazine on bacterial communities in soil microcosms using two soil types showed that in both soils manure and sulfadiazine positively affected the quotients of total and sulfadiazine-resistant culturable bacteria (Heuer and Smalla, 2007). The resistant bacteria present in manure may exchange antimicrobial resistance genes (e.g. *sul2*, a sulfadiazine-resistance gene) with soil bacteria, a process even further enhanced by the presence of antimicrobial residues (e.g. sulfadiazine) (Heuer et al., 2008, 2011). However, in a study by Topp et al. (2016), the macrolide antibiotics erythromycin, clarithromycin and azithromycin were dissipated significantly more rapidly in soils with a history of field exposure to 10 mg/kg macrolides, and erythromycin and clarithromycin were also degraded more rapidly in field soil exposed to 0.1 mg/kg macrolides. The authors highlight that the potential accelerated degradation of these drugs in soils amended with manure and sewage sludge, compared to untreated soil, should be investigated further. Figure 7 gives a brief overview of the possible fates of antimicrobial residues and mechanisms of antimicrobial resistance gene acquisition and dissemination by bacteria, beginning with land application of animal manure as the source of entry of medicines, bacteria and resistance genes into the soil environment.

Although research focusing on the spread of antimicrobials in the environment is growing, it is still limited (e.g. Allen et al., 2010; Wellington et al., 2013). For instance, the amount of these residues present in each compartment (i.e. manure, soil, water), which is an important piece of information to assess the risks associated specifically with the use of veterinary antimicrobials and to develop methods to reduce emissions to the environment, has only very limitedly been studied. Recently national projects started attempting to deal with this issue, e.g. in the Netherlands (Zuidema et al.,

2014; RIVM, 2017) and in Belgium (VMM, 2018; Rasschaert et al., 2018). In Belgium, an on-going study attempts to quantify antibiotic residues in pig and veal manure. In 100 samples tested, doxycycline, lincomycin and sulfadiazine were detected in more than 70% of samples (Rasschaert et al., 2018). A study be Berendsen et al. (2018) investigated the fate of a broad scope of antimicrobials during storage of pig, veal and boiler manure. They concluded that oxytetracycline, doxycycline, flumequine and tilmicosin can be expected to end up in environmental compartments. A thorough understanding of the pharmacokinetics in pigs and in the manure of antimicrobials commonly used in pigs is therefore fundamental and would enable us to suggest control options regarding this risk.
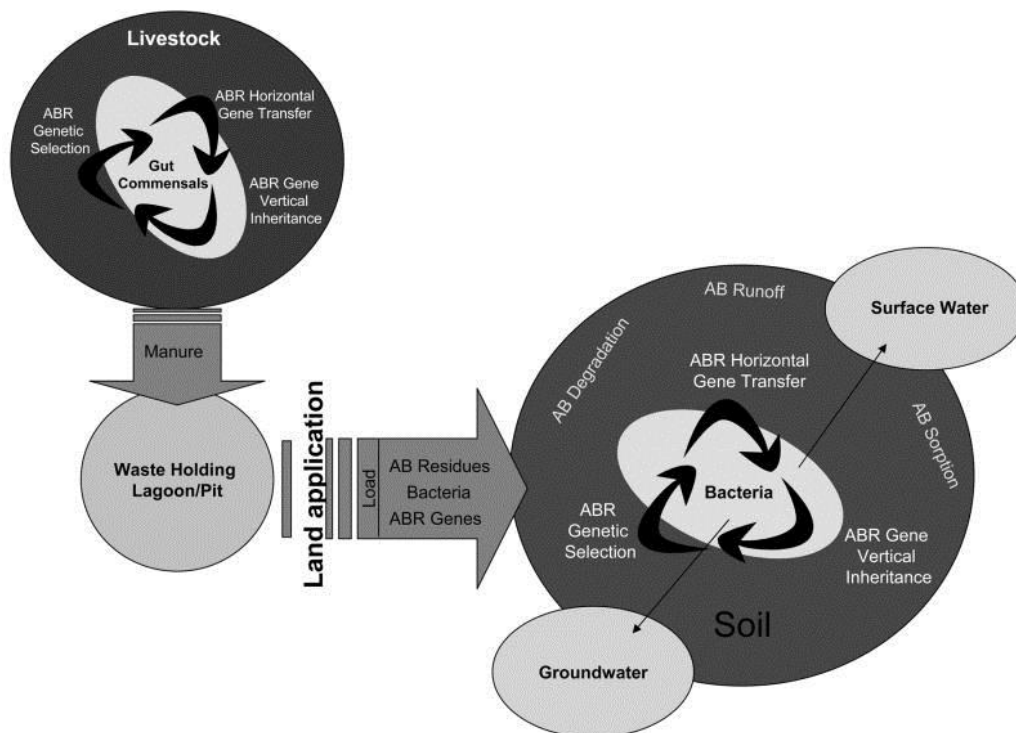
Figure 7. Conceptual view of the possible fates of antimicrobial residues and mechanisms of antimicrobial resistance gene acquisition and dissemination by bacteria, beginning with land application of animal manure as the source of entry of medicines, bacteria and resistance genes into the soil environment. Adopted from Chee-Sanford et al. (2009).*AB= antimicrobial, ABR= antimicrobial resistance.

4. Mathematical modelling for assessment of risks

Mathematical modelling is placed in the core of quantitative risk assessment, which relies on the use of mathematics, especially probability and statistics. 'Risk assessment' refers to a scientifically based methodology that allows to estimate, either qualitatively or quantitatively, the probability and the potential impact of some risk (Vose, 2003). The risk assessment of chemicals, including antimicrobials, in food and feed is a process consisting of four steps: hazard identification, hazard characterization, exposure assessment and risk characterization (EFSA, 2007; CODEX, 2015). The term 'risk analysis' is applied to the whole process from hazard identification, through qualitative and quantitative risk

assessment, to the resultant management decisions and communication to the various stakeholders of the assessment of that risk and the decision that has been or is to be made (EFSA, 2006; CODEX, 2015).

4.1. Usefulness and challenges of mathematical models

Mathematical modelling is a relatively new field that is enjoying a rapid growth in popularity amongst governments and researchers world-wide, due to its usefulness in disease prevention and promotion of public and animal health. Yet, it does not come without some challenges (Basu end Andrews, 2013; Metcalf et al., 2015). A number of common and important strengths and challenges of this method in relation to its role in public and animal health are presented below (Metcalf et al., 2015; Filippitzi, 2017a).

Strengths of mathematical modelling:

- Provides quantitative estimations of the probability of occurrence and/or the magnitude of a risk. It can answer otherwise unanswerable questions and greatly expand our knowledge base from actual study data.

- Can help to assess potential risks and their impacts early in the process, and later allow for interpretation of data from complex and multifactorial systems. As such, these models can be critical tools in guiding public health action and also raise awareness on a new topic.

- Simulates different strategies and predicts the results of each of them. This supports policy makers in informed risk management (EFSA, 2006). It can also play an increasingly important role in helping to guide both the most high impact and cost-effective strategy to prevent or control a health-related risk.

- Separate models, corresponding to smaller parts of a process (e.g. behavior or antimicrobials in different environmental compartments), can be assessed independently (i.e. modular model) and later be combined with other components of the same process for a full assessment of a risk. For this it is important to ensure mathematical continuity in the risk being assessed.

Challenges of mathematical modelling:

- It is important to build simple (but not simplistic) models. Complex models are not necessarily more accurate or reliable simply because they can more easily fit real-world data than simpler models. In complex models, parameter estimation can prove problematic and difficult to be detected.

- All logical and mathematical components of a model must be soundly based on valid research (CODEX, 2015). In absence of certain data, assumptions can be made, given that they are

explicitly described and justified in a transparent manner. Communicating the limits of modelling and communicating how model projections depend on underlying assumptions is essential, as problematic assumptions can lead to flawed public health projections.

- Integrating modellers and model-building into the policy process can often be quite challenging. The creation of an environment where modellers successfully communicate clearly how models can and should be used (e.g. for scenario comparison and not projection; burden estimation, rather than prediction) and policy makers successfully communicate questions they want answers to, is essential (EFSA, 2017b; Filippitzi, 2017b).
- Maintaining the value of models in the face of long term horizons is important. Models that are more flexible in how they are built, following a detailed stepwise approach, can be easily updated using new information.

4.2. Methodological steps

4.2.1. Principles of quantitative risk assessment (QRA) modelling

Mathematical modelling is part of quantitative risk assessment, which is a structured process for evaluating the risk (or risks) resulting from a hazard and involves a series of logical steps. In practice, these steps include: framing the question to be answered, identify all the potential hazards, outline the pathway (sequential steps leading to the risk) or the model framework (sum of components influencing the risk) and collect the information (OIE, 2004). Based on the results of the model developed, the risk is then assessed (Figure 8). In cases where not a full assessment of a risk is required and a specific question is posed to the risk assessors on a certain hazard, then the process can begin with the hazard characterization and the outline of pathways of exposure to this hazard.

Obtaining feedback within the environment of a team of experts and adopting an iterative approach throughout this process are highly recommended (EFSA, 2006). Given the numerical nature of quantitative risk assessment, it usually proves useful to also undertake a sensitivity analysis, which indicates those data inputs with the greatest effect (or impact) on the final output of the model.
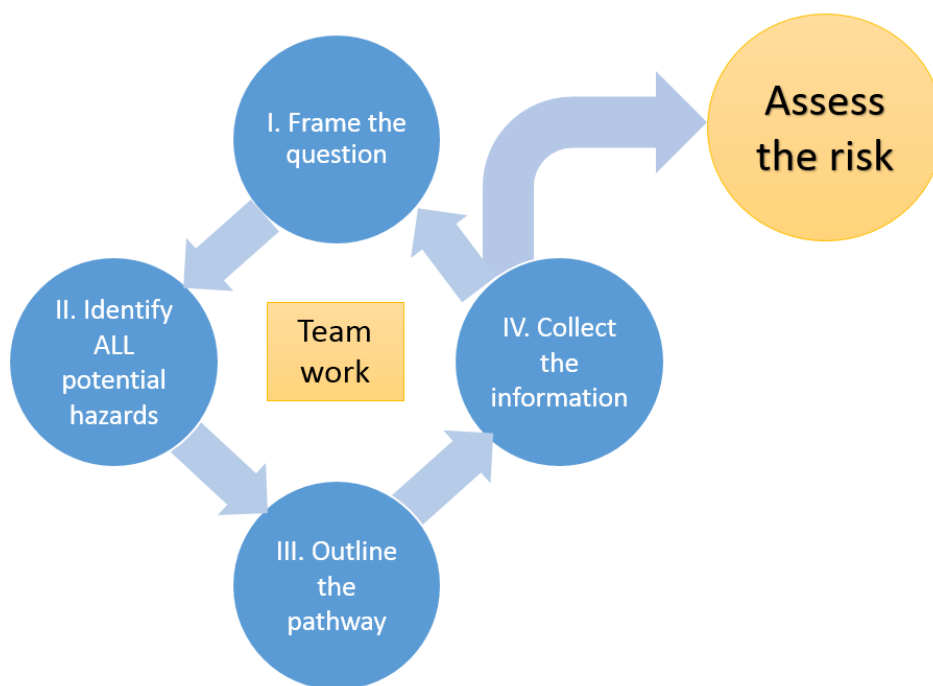
Figure 8. Summary of steps of the risk assessment process. These steps are followed in both quantitative and qualitative risk assessments. Eventually, the risk is assessed based on the results of the model (i.e. mathematical relationship of the inputs) developed.

4.2.2. Concepts of quantitative risk assessment (QRA) modelling

4.2.2.1. Deterministic and probabilistic modelling

All the steps in a pathway or model framework will have a number of input and output variables that must be calculated using available data and appropriate mathematics. There are two approaches that may be used for these calculations, a deterministic and a probabilistic approach. In the deterministic approach, each of the input variables is described by single (point) values. In using point values for the inputs, the output estimate of risk is also a single value. On the other hand, the probabilistic (or stochastic) approach uses probability distributions to describe the input variables. These distributions represent the range of possible values that a variable can take. Using the probabilistic approach means that the output of risk will also be probabilistic (take a range of possible estimates) (OIE, 2004; FAO, 2014).

Since the rapid increase in powerful computer technology, there are a range of commercially available software packages, often based on spread-sheet modeling (e.g. within Microsoft Excel®), to assist in the development of probabilistic quantitative risk assessment (QRA) modelling. The best known examples of these add-on packages are @Risk® (Palisade Corporation) and Crystal Ball® (Oracle). Another example of statistical software package which includes probability distribution functions is R® (The R Foundation). All three packages utilize the Monte-Carlo simulation method. Monte-Carlo

simulation is a technique whereby each input probability distribution is randomly sampled a set number of iterations (times) to produce hundreds (or thousands) of possible scenarios. In simple words, any iteration of a QRA model must be a scenario that could physically occur (Vose, 2003). These scenarios are collected together and built up into an output distribution which can be illustrated graphically and analyzed statistically (e.g. estimate mean value of output). There is a wide range of probability distributions (e.g. Binomial, Beta, Pert) which are fundamental to the development of QRAs and are described elsewhere (e.g. Vose, 2003; OIE, 2004; Rees, 2009). Indicatively, the Beta distribution can be used to model the distribution for the probability of occurrence of some discrete event. The binomial distribution gives the probability distribution $P(n|N)$ of obtaining exactly n successes out of N trials.

4.2.2.2. Uncertainty and variability

As previously mentioned, in the probabilistic modelling approach, inputs are defined using probability distributions to reflect the range of possible values they can take. In this respect, two key concepts need to be defined, uncertainty and variability.

Input uncertainty reflects the degree of knowledge about an input. The more information is available, the smaller the resulting uncertainty. Input uncertainty, therefore, can be reduced by collecting additional information. On the other hand, variability is an inherent characteristic of the model input or output being modelled. Variability therefore cannot be reduced by extra experimentation or data collection since it occurs naturally. The uncertainties and variabilities of the model inputs should be presented and described in detail in the QRA (FAO, 2014).

4.3. Assessment of risks due to residues in feed and animal manure

In Europe, the responsible body for execution of risk assessment in animal health and food and feed safety is the European Food Safety Authority (EFSA) (EC, 2002). The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) in the one carrying out risk assessments (qualitative and quantitative) on contaminants in feed. The Panel assesses if the exposure to a contaminant in feed is likely to be associated with adverse health effects in farm animals, fish and pets in Europe, or to represent a risk to the consumer of foods of animal origin (EFSA, 2012). Although risk assessments on residues of feed contaminants/additives (e.g. coccidiostats used in poultry and rabbits (Dorne et al., 2013)) have been previously performed by EFSA from a toxicological and food safety point of view, only recently a scientific opinion has been published on the risk for the development of antimicrobial resistance (AMR) due to feeding on farm of calves with milk containing residues of antimicrobials (EFSA, 2017c). The approach followed in the latter opinion was qualitative due to the lack of data. Therefore, the development of a quantitative methodology to assess the level of exposure of pigs to feed cross-

contaminated with antimicrobial residues and to assess the possible associated adverse effects related to AMR development, would be very relevant and timely.

Guidelines for environmental risk assessment (ERA) of pharmaceutically active compounds in Europe are available from the European Medicines Agency (EMA, 2004). Assessment of the environmental impact of medicinal products is a legal obligation, and must be performed to evaluate and limit potential adverse effects of medicines on the environment. The ERAs of medicinal, including veterinary, products is to be performed by pharmaceutical companies during the development of new medicines. The outcome of an ERA will serve as the basis for minimising the amount of medicinal product released into the environment by appropriate measures and identifying specific risk-minimisation activities to be taken by the user of the medicine (EMA, 2004). Pharmaceutical companies, in their ERA on veterinary antimicrobials, they assume a total excretion of veterinary antimicrobials administered to animals into the produced manure. This is a safe approach when looking at the ecotoxicity effect only. However, this approach does not take into account the effects of these medicines on environmental pollution from an AMR point of view. So far, little is known regarding these effects of antimicrobial residues and resistant genes on the environment and, especially, by manure fertilization, while the absence of quantitative data is a major gap for the development of risk models as also indicated in literature (Ashbolt et al., 2013; Marti et al., 2013; Wellington et al., 2013; FAO, 2016)). For example, Ashbolt et al. (2013) attempted to perform a human health risk assessment for environmental development and transfer of antibiotic resistance, which was hampered by the lack of knowledge on the exact mechanisms that lead to the development of antimicrobial resistance bacteria in the environment in presence of resistance genes, and their quantitative relationship. Therefore, the estimation of the level of residues of orally administered AM that end up in pig manure and following in soil would be a very important first step for the assessment of the associated risks and of the suggestion of possible control measures.

5. References

(AACTING) Network on quantification of veterinary Antimicrobial usage at herd level and Analysis, CommunicaTion and benchmarkING to improve responsible usage, 2018. Description of existing monitoring systems for collection, analysis, benchmarking and reporting of farm-level veterinary antimicrobial usage. Version 1.2.

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J, 2010. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol. 8(4):251-9.

Amábile-Cuevas C.F., 2016. Antibiotics and Antibiotic Resistance in the Environment. CRC Press/Balkema. Taylor & Francis Group, London, UK.

Ashbolt NJ, Amézquita A, Backhaus T, Borriello P, Brandt KK, Collignon P, Coors A, Finley R, Gaze WH, Heberer T, Lawrence JR, Larsson DG, McEwen SA, Ryan JJ, Schönfeld J, Silley P, Snape JR, Van den Eede C, Topp E, 2013. Human Health Risk Assessment (HHRA) for Environmental Development and Transfer of Antibiotic Resistance. Environ Health Perspect. 121(9):993-1001.

Baggot JD, Giguère S, 2013. Principles of Antimicrobial Drug Bioavailability and Disposition. Antimicrobial Therapy in Veterinary Medicine, Fifth Edition, 41-77. Wiley-Blackwell.

Barza M, Travers K, 2002. Excess infections due to antimicrobial resistance: The "attributable fraction". Clin Infect Dis 34:S126–S130.

Basu S, Andrews J, 2013. Complexity in Mathematical Models of Public Health Policies: A Guide for Consumers of Models. PLoS Med 10(10): e1001540.

(AFSCA-FAVV) Belgian Federal Agency for the Safety of the Food Chain, 2013. Responsible use of antibacterial agents in group treatment of farm animals and the impact on resistance selection (dossier Sci Com 2012/10). p1-56. http://www.afsca.be/

(BelVet-SAC) Belgian Veterinary Surveillance of Antibacterial Consumption., 2017. 2016 National consumption report. p1-44. http://www.belvetsac.ugent.be/

Berendsen BJA, Lahr J, Nibbeling C, Jansen LJM, Bongers IEA, Wipfler EL, van de Schans MGM, 2018. The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. Chemosphere. 204:267-276.

(BFA) Belgian Compound Feed Industry Association, 2013. In Dutch: Convenant betreffende gemedicineerde voeders. p1-14. http://www.favv.be/

Binh CTT, Heuer H, Kaupenjohann M, Smalla K, 2008. Piggery manure used for soil fertilization is a reservoir for transferable antibiotic resistance plasmids. FEMS Microbiology Ecology, 66 (1): 25–37.

Bondt N., Puister L., Ge L., van der Veen H., Bergevoet R., Douma B., van Vliet A., Wehling K, 2012. Trends in veterinary antibiotic use in the Netherlands 2004-2012. LEI of Wageningen UR. The Hague, The Netherlands.

Boerlin P and White DG, 2013. Antimicrobial Resistance and Its Epidemiology. In: Antimicrobial Therapy in Veterinary Medicine, Fifth Edition. John Wiley & Sons, Inc, Hoboken, NJ.

Bos MEH, Taverne FJ, van Geijlswijk IM, Mouton JW, Mevius DJ, Heederik DJJ, et al., 2013. Consumption of Antimicrobials in Pigs, Veal Calves, and Broilers in The Netherlands: Quantitative Results of Nationwide Collection of Data in 2011. PLoS ONE 8(10): e77525.

Brewer MT, Xiong N, Anderson KL, Carlson SA, 2013. Effects of subtherapeutic concentrations of antimicrobials on gene acquisition events in Yersinia, Proteus, Shigella, and Salmonella recipient organisms in isolated ligated intestinal loops of swine. Am J Vet Res. 74(8):1078-83.

Burow E, Tenhagen B-A, Flor M, Käsbohrer A, 2018. Antimicrobial treatments during the life cycle of 408 focal pigs. In proceedings: Quantification, Benchmarking and Stewardship of Veterinary Antimicrobial Usage. First International Conference. Ghent, 27-28 February 2018.

Burow E, Grobbel M , Tenhagen BA, Simoneit C, Ladwig M, Szabó I, Wendt D, Banneke S, Käsbohrer A, 2018. Antimicrobial susceptibility in faecal Escherichia coli from pigs after enrofloxacin administration in an experimental environment. Berliner und Münchener Tierärztliche Wochenschrift. DOI 10.2376/0005-9366-17079.

Callens B, Persoons D, Maes D, Laanen M, Postma M, Boyen F, Haesebrouck F, Butaye P, Catry B, Dewulf J, 2012. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Prev Vet Med. 106 (1): 53 62.

Carmo, L. P., Schuepbach-Regula, G., Muentener, C., Chevance, A., Moulin, G., & Magouras, I., 2017. Approaches for quantifying antimicrobial consumption per animal species based on national sales data: A Swiss example (2006–2013). Eurosurveillance, 22(6), 30458.

Chantziaras I., Callens B., Berge A.K., Boyen F., Haesebrouck F., Dewulf J., 2017a. Antimicrobial resistance selection pressure and spread in food producing animals through factors other than antimicrobial use. Chapter in PhD thesis. Ghent University.

Chantziaras I, Smet A, Haesebrouck F, Boyen F, Dewulf J. 2017b. Studying the effect of administration route and treatment dose on the selection of enrofloxacin resistance in commensal Escherichia coli in broilers. J Antimicrob Chemother.72(7):2142.

Chee-Sanford JC, Mackie RI, Koike S, Krapac IG, Lin YF, Yannarell AC, Maxwell S, Aminov RI, 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J Environ Qual. 38(3):1086-108.

CODEX Alimentarius commission, 2015. Joint FAO/WHO Food Standards Programme. Procedural Manual. Twenty-fourth edition. p1-241. Rome, Italy.

Collineau L, Belloc C, Stärk KD, Hémonic A, Postma M, Dewulf J, Chauvin C, 2017. Guidance on the Selection of Appropriate Indicators for Quantification of Antimicrobial Usage in Humans and Animals. Zoonoses Public Health. 64(3):165-184.

da Costa PM, Bica A, Vaz-Pires P, Bernardo F, 2008. Effects of antimicrobial treatment on selection of resistant Escherichia coli in broiler fecal flora. Microb Drug Resist. 14(4):299-306.

DANMAP 2016. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. Statens Serum Institut, National Veterinary Institute, Technical University of Denmark National Food Institute, Technical University of Denmark. p1-133. https://www.danmap.org/

Daeseleire E, De Graef E, Rasschaert G, De Mulder T, Van den Meersche T, Van Coillie E, Dewulf J, Heyndrick M, 2016. Antibiotic use and resistance in animals: Belgian initiatives. Drug testing and analysis. 8(5-6):549-555.

De Backer P, 2015. Pitfalls of veterinary dosage of veterinary pharmaceuticals in drinking water medication. In: Importance of drinking water in piglets, fattening pigs and sows. Ghent, 5.10.2015.

De Smet J, Croubels S, De Backer P, Devreese M, 2017. Effect of administration route and dose alteration on sulfadiazine-trimethoprim plasma and intestinal concentrations in pigs. Int J Antimicrob Agents. 50:707-714.

Devreese M, Antonissen G, De Baere S, De Backer P, Croubels S, 2014. Effect of administration route and dose escalation on plasma and intestinal concentrations of enrofloxacin and ciprofloxacin in broiler chickens. BMC Vet Res. 10:289.

Dewulf J., 2018. Eight myths on antimicrobial resistance disproved. CID Lines. Ieper, Belgium.

Dorado-García A, Dohmen W, Bos MEH, Verstappen KM, Houben M, Wagenaar JA, Heederik DJJ, 2015. Dose-Response Relationship between Antimicrobial Drugs and Livestock-Associated MRSA in Pig Farming. Emerging Infectious Diseases. 21(6):950-959.

Dorne JL, Fernández-Cruz ML, Bertelsen U, Renshaw DW, Peltonen K, Anadon A, Feil A, Sanders P, Wester P, Fink-Gremmels J., 2013. Risk assessment of coccidostatics during feed cross-contamination: animal and human health aspects. Toxicol Appl Pharmacol. 270(3):196-208.

Du, L.F. and Liu, W.K., 2012. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. Agron. Sustain. Dev. 32, 309–327.

(EFFORT) Ecology from Farm to Fork Of microbial drug Resistance and Transmission project. 2018a. http://www.effort-against-amr.eu/

(EFFORT) Ecology from Farm to Fork Of microbial drug Resistance and Transmission project. 2018b. Analysis of herd level antimicrobial usage in pigs and poultry in 9 European countries based on EFFORT data. Oral presentation at: Quantification, Benchmarking and Stewardship of Veterinary Antimicrobial Usage. First International Conference. Ghent, 27-28 February 2018.

(EPRUMA) European Platform for the Responsible Use of Medicines in Animals, 2014. Administration of veterinary medicines. http://www.epruma.eu/

(EC) European Commission, 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

(EC) European Commission, 2003. Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition.

(EC) European Commission. 2014. Proposal for a regulation of the European Parliament and of the council on the manufacture, placing on the market and use of medicated feed and repealing Council Directive 90/167/EEC.

(EC) European Commission, 2016. Review of medicated feed legislation.

(EFSA) European Food Safety Authority, 2006 (reprinted in 2010). Food Producing Animals. Principles of Risk Assessment of food producing animals: Current and future approaches. EFSA Scientific Colloquium Summary Report.

(EFSA) European Food Safety Authority, 2007 (reprinted in 2010). Risk-Benefit analysis of foods. Methods and approaches. EFSA Scientific Colloquium Summary Report.

(EFSA) European Food Safety Authority, 2012. Special Issue: Risk assessment of contaminants in food and feed. EFSA Journal. 10(10):s1004.

(EFSA) European Food Safety Authority, 2017a. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015.

(EFSA) European Food Safety Authority, 2017b. When Food Is Cooking Up a Storm – Proven Recipes for Risk Communications.

(EFSA) European Food Safety Authority, 2017c. Panel on Biological Hazards (BIOHAZ). Scientific Opinion on the risk for the development of Antimicrobial Resistance (AMR) due to feeding of calves with milk containing residues of antibiotics. EFSA Journal 2017;15(1):4665.

(EMA) European Medicines Agency, 2004. Guideline on environmental impact assessment for veterinary medicinal products Phase II.

(EMA) European Medicines Agency, 2013. Revised ESVAC reflection paper on collecting data on consumption of antimicrobial agents per animal species, on technical units of measurement and indicators for reporting consumption of antimicrobial agents in animals.

(EMA) European Medicines Agency, 2017a. European Surveillance of Veterinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 30 European countries in 2015.

(EMA) European Medicines Agency, 2017b. Reflection paper on off-label use of antimicrobials in veterinary medicine in the European Union (draft).

EUROSTAT 2016. Pork production in the EU. http://ec.europa.eu/eurostat

Filippitzi M. E., Goumperis T., Robinson T., Saegerman C., 2016. Microbiological zoonotic emerging risks, transmitted between livestock animals and humans (2007-2015). Transboundary and Emerging Diseases. 64(4):1059-1070.

Filippitzi M., 2017a. Introduction to quantitative risk assessment modelling. Lecture notes for the ECVPH workshop in Quantitative Risk Assessment, Liège.

Filippitzi M., 2017b. Risk Communication in Food Safety. Lecture notes for the Masters in Public Health course, Athens School of Public Health, Athens.

(FAO) Food & Agriculture Organization of the United Nations, World Organisation for Animal Health (OIE) & World Health Organization (WHO) (2010). – The FAO-OIEWHO Collaboration. Sharing responsibilities and coordinating global activities to address health risks at the animal-humanecosystems interfaces. A Tripartite Concept Note.

(FAO) Food and Agriculture Organization of the United Nations, 2014. Incorporating uncertainty. Chapter 12 in: Risk-based disease surveillance – A manual for veterinarians on the design and analysis of surveillance for demonstration of freedom from disease. FAO Animal Production and Health Manual No. 17. Rome, Italy.

(FAO) Food and Agriculture Organization of the United Nations, 2016. Drivers, dynamics and epidemiology of antimicrobial resistance in animal production.

Heuer H., Focks A., Lamshöft M., Smalla K., Matthies M., Spiteller M., 2008 Fate of sulfadiazine administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in manure and manured soil. Soil Biol. Biochem. 40:1892-1900.

Heuer H, Schmitt H, Smalla K., 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. Curr. Opin. Microbiol. 14, 236–243.

Institute for National Accounts, 2015. Instituut voor de Nationale Rekeningen Actualisatie van de studie over de varkenskolom -Prijzenobservatorium. FOD Economie.

Jensen VF, Jacobsen E, Bager F, 2004. Veterinary antimicrobial-usage statistics based on standardized measures of dosage. Prev Vet Med. 64(2–4):201-215.

Larsen J, Stegger M, Andersen PS, Petersen A, Larsen AR, Westh H, Agersø Y, Fetsch A, Kraushaar B, Käsbohrer A, Feβler AT, Schwarz S, Cuny C, Witte W, Butaye P, Denis O, Haenni M, Madec JY, Jouy E, Laurent F, Battisti A, Franco A, Alba P, Mammina C, Pantosti A, Monaco M, Wagenaar JA, de Boer E, van Duijkeren E, Heck M, Domínguez L, Torres C, Zarazaga M, Price LB, Skov R.L, 2016. Evidence for Human Adaptation and Foodborne Transmission of Livestock-Associated Methicillin-Resistant Staphylococcus aureus. Clin Infect Dis. 63(10):1349-1352.

Levy, SB, Marshall B, 2004. Antibacterial resistance worldwide Causes, challenges and responses. Nat Med. 10:122-129.

Marshall BM, Levy SB, 2011. Food Animals and Antimicrobials: Impacts on Human Health. Clin Microbiol Rev. 24(4):718-733.

Marti R, Scott A, Tien YC, Murray R, Sabourin L, Zhang Y, Topp E, 2013. Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. Appl Environ Microbiol. 79: 5701-5709.

Martinez M, Modric S, 2010. Patient variation in veterinary medicine: part I. Influence of altered physiological states. J Vet Pharmacol Ther. 33(3):213-226.

McEvoy JDG, 2002. Contamination of animal feedingstuffs as a cause of residues in food: a review of regulatory aspects, incidence and control. Anal Chim Acta. 473:3–26.

Metcalf CJ, Edmunds WJ, Lessler J, 2015. Six challenges in modelling for public health policy. Epidemics. 10:93-96.

(NORM-VET) Norwegian Veterinary Institute, 2016. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo. p1-142. https://www.vetinst.no/

(OIE) World organisation for animal health, 2004. Handbook on Import Risk Analysis for Animals and Animal Products. Volume 2. Quantitative risk assessment. http://www.oie.int

(OIE) World organisation for animal health, 2015. OIE list of antimicrobial agents of veterinary importance. p1-9. http://www.oie.int

(OIE) World Organisation for Animal Health, 2017. OIE annual report on antimicrobial agents intended for use in animals. p1-125. http://www.oie.int

Page SW, Gautier P, 2012. Use of antimicrobial agents in livestock. Rev. sci. tech. Off. int. Epiz., 2012, 31 (1), 145-188.

Pardon, B., Catry, B., Dewulf, J., Persoons, D., Hostens, M., De Bleecker, K., Deprez, P., 2012. Prospective study on quantitative and qualitative antimicrobial and anti-inflammatory drug use in white veal calves. The Journal of Antimicrobial Chemotherapy 67. 67:1027-1038.

Peeters LE, Daeseleire E, Devreese M, Rasschaert G, Smet A, Dewulf J, Heyndrickx M, Imberechts H, Haesebrouck F, Butaye P, Croubels S, 2016. Residues of chlortetracycline, doxycycline and sulfadiazine-trimethoprim in intestinal content and feces of pigs due to cross-contamination of feed. BMC Vet Res.12: 209. [doi:10.1186/s12917-016-0803-8].

Peeters LE, De Mulder T, Van Coillie E, Huygens J, Smet A, Daeseleire E, Dewulf J, Imberechts H, Butaye P, Haesebrouck F, Croubels S, Heyndrickx M, Rasschaert G, 2017. Selection and transfer of an IncI1-tet(A) plasmid of Escherichia coli in an ex vivo model of the porcine caecum at doxycycline concentrations caused by cross-contaminated feed. J Appl Microbiol. 123(5):1312-1320.

Peeters LE, Croubels S, Rasschaert G, Imberechts H, Daeseleire E, Dewulf J, Heyndrickx M, Butaye P, Haesebrouck F, Smet A, 2018a. Effect of residual doxycycline concentrations on resistance selection and transfer in porcine commensal Escherichia coli. Int J Antimicrob Agents. 51(1):123-127.

Peeters L., 2018b. Chapter 6. Carry-over of tetracyclines and sulphonamides-trimethoprim in pig feed. PhD thesis. Ghent University.

Persoons D, Dewulf J, Smet A, Herman L, Heyndrickx M, Martel A, Catry B, Butaye P, Haesebrouck F, 2010. Prevalence and persistence of antimicrobial resistance in broiler indicator bacteria. Microb Drug Resist. 16:67-74.

Postma M, Sjölund M, Collineau L, Lösken S, Stärk K, Dewulf J, 2015. MINAPIG Consortium. Assigning defined daily doses animal: a European multi-country experience for antimicrobial products authorized for usage in pigs. J Antimicrob Chemother. 70(1):294-302.

Prescott JF, Dowling PM, 2013. Antimicrobial Therapy in Veterinary Medicine, Fifth Edition. Hoboken, NJ: John Wiley & Sons. Wiley-Blackwell.

Putier F, 2010. Evaluation of cross-contamination in livestock feed delivery trucks. Technics. Kraftfutter/Feed magazine 9-10/2010.

Rasschaert G, Lander C, Daeseleire E, Herman L, Heyndrickx M, 2018. Antibiotic resistant Salmonella and E. coli from pig and veal manure In: Proceedings of the 22th Conference on Food Microbiology. Antibiotic residues in pig and veal manure. Eighth International Symposium on Hormone and Veterinary Drug Residue Analysis. Ghent, 22-25 May 2018.

Rees Michael, 2009. Palisade ® - Selecting the Right Distribution in @RISK. http://go.palisade.com

(RIVM) Rijksinstituut voor Volksgezondheid en Milieu, 2017. Dutch National Institute for Health and Environment. Bronnen van antibioticaresistentie in het milieu en mogelijke maatregelen. Sources of antibiotic resistance in the environment and possible measures. RIVM Rapport 2017-0058. p1-114. https://www.rivm.nl

Riviere JE, Papich MG, 2009. Veterinary Pharmacology and Therapeutics, 9th Edition. Wiley-Blackwell.

Robinson TP, Bu DP, Carrique-Mas J, et al., 2016. Antibiotic resistance is the quintessential One Health issue. Trans R Soc Trop Med Hyg. 110(7):377-380.

Rolain JM, 2013. Food and human gut as reservoirs of transferable antibiotic resistance encoding genes. Front Microbiol. 4:173. doi:10.3389/fmicb.2013.00173.

Rosengren LB, Waldner CL, Reid-Smith RJ, Dowling PM, Harding JC, 2007. Associations between feed and water antimicrobial use in farrow-to-finish swine herds and antimicrobial resistance of fecal Escherichia coli from grow-finish pigs. Microb Drug Resist. 13(4):261-69.

Rosengren LB, Waldner CL, Reid-Smith RJ, Harding JCS, Gow SP, Wilkins WL, 2008. Antimicrobial use through feed, water, and injection in 20 swine farms in Alberta and Saskatchewan. Can J Vet Res. 72(2):143-150.

Rossi F, Rizzotti L, Felis GE, Torriani S, 2014. Horizontal gene transfer among microorganisms in food: current knowledge and future perspectives. Food Microbiol. 42:232-243

Schwarz S, Chaslus-Dancla E, 2001. Use of antimicrobials in veterinary medicine and mechanisms of resistance. Vet Res. 32(3-4):201-25.

Schwarz S, Kehrenberg C, Walsh TR, 2001. Use of antimicrobial agents in veterinary medicine and food animal production. Int J Antimicrob Agents. 17(6):431-437.

Sjölund M, Postma M, Collineau L, Lösken S, Backhans A, Belloc C, Emanuelson U, Beilage EG, Stärk K, Dewulf J, 2016. MINAPIG Consortium. Quantitative and qualitative antimicrobial usage patterns in farrow-to-finish pig herds in Belgium, France, Germany and Sweden. Prev Vet Med. 130:41-50.

Soraci AL, Amanto F, Tapia MO, de la Torre E, Toutain PL, 2014. Exposure variability of fosfomycin administered to pigs in food or water: Impact of social rank. Res Vet Sci. 96:153–159.

Statistics Belgium, 2017. Landbouwgegevens van 2016. FOD Economie. https://lv.vlaanderen.be

Stolker AAM, Manti V, Zuidema T, van Egmond H, Deckers ER, Herbes R, Hooglugt J, Olde Heuvel E, de Jong J, 2013. Carry-over of veterinary drugs from medicated to nonmedicated feeds in commercial feed manufacturing plants. Food Addit Contam Part A. 30:1100–1107.

Swedres-Svarm, 2016. Public Health Agency of Sweden and National Veterinary. Institute Consumption of antibiotics and occurrence of resistance in Sweden. Solna/Uppsala. p1-124. https://www.folkhalsomyndigheten.se

Timmerman T, Dewulf J, Catry B, Feyen B, Opsomer G, de Kruif A, Maes D, 2006. Quantification and evaluation of antimicrobial drug use in group treatments for fattening pigs in Belgium. Prev Vet Med. 74(4):251-263.

Topp E, Renaud J, Sumarah M, Sabourin L, 2016. Reduced persistence of the macrolide antibiotics erythromycin, clarithromycin and azithromycin in agricultural soil following several years of exposure in the field. Sci Total Environ. 562:136-144.

van der Horst MA, Fabri TH, Schuurmans JM, Koenders BB, Brul S, ter Kuile BH, 2013. Effects of therapeutical and reduced levels of antibiotics on the fraction of antibiotic-resistant strains of Escherichia coli in the chicken gut. Foodborne Pathog Dis. 10(1):55-61.

van Krimpen M., 2007. Medicijnverstrekking aan groepen varkens. Animal Science Group, Wageningen UR.

(VMM) Vlaamse Milieumaatschappije, 2018. Flanders Environmental Agency. Emissies naar oppervlakewater van medicijnen uit veterinair gebruik. Emissions to surface water of medicines from veterinary use. VMM-ARDW-DSRW/OL201500029.

Vose D, 2003. Risk Analysis A quantitative guide. Second edition. Wiley. West Sussex England.

Wang M, Liu P, Xiong W, Zhou Q, Wangxiao J, Zeng Z, Sun Y, 2018. Fate of potential indicator antimicrobial resistance genes (ARGs) and bacterial community diversity in simulated manure-soil microcosms. Ecotoxicol Environ Saf. 147:817-823.

Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W, Thomas CM, Williams AP, 2013. The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. Lancet Infect Dis. 13(2):155-165.

Whitehead TR and Cotta MA, 2013. Stored swine manure and swine faeces as reservoirs of antibiotic resistance genes. Lett Appl Microbiol. 56(4):264-267.

(WHO) World Health Organization, 2015. Global action plan on antimicrobial resistance. p1-28. http://www.who.int

(WHO) World Health Organization, 2016. Critically important antimicrobials for human medicine – 5th rev. Geneva. p1-48. http://www.who.int

Zuidema T, Stolker AAM, van Ginkel LA, 2014. Kruiscontaminatie van antibiotica. Onderzoek naar de aanwezigheid van antibioticaresiduen in mest van vleesvarkens en vleeskalveren en op pluimveebedrijven. RIKILT report, Wageningen UR. p1-52.

CHAPTER 2

Scientific aims

In highly productive pig farming, group treatments are often used to administer antimicrobial agents orally, via the feed or drinking water. These treatments can lead to the presence of antimicrobial residues (smaller fraction of an initial amount) in the gastro-intestinal tract (GI) and manure of pigs. Following, antimicrobial residues can spread via manure onto agriculturally used areas.

Practices related to the preparation and administration of oral treatments via medicated feed can further lead to cross-contamination of non-medicated feed with residues of antimicrobials, which also end up in the GI tract of pigs. These practices concern different levels: the feed-mill, the transport truck and the farm.

Antimicrobial residues in different matrices, such as the GI tract of pigs and pig manure, may cause a public and animal health concern associated with the potential for selection and dissemination of antimicrobial resistance.

Currently, in Belgium, there is lack of information regarding the following aspects related to oral group antimicrobial medication of pigs:

(a)   amount of antimicrobials consumed in group, including oral, treatments by the pig population at country level;

(b)   frequency and conditions of use of oral antimicrobial treatments of pigs, via feed and water;

(c)   exposure of Belgian pigs to residues of antimicrobials, due to cross-contaminated feed;

(d)   exposure of Belgian soil amended with pig manure, to residues of antimicrobials administered to pigs via the oral route.

This dissertation aims to fill the aforementioned gaps, by trying to answer and validate the following questions, in a quantitative manner:

1.   What is the estimated amount of antimicrobials consumed in group treatments (oral and/or parenteral) by the national pig population in Belgium per year? How much does it differ from the amount of antimicrobials consumed by other species (i.e. poultry, veal calves)?

2.   How often are antimicrobials administered to groups of pigs via feed compared to water in Belgian farms? Which are the practices and conditions of preparation and administration of antimicrobials via these routes at the farm level?

3.   What is the estimated amount of cross-contaminated pig feed produced in Belgium per year?

4.   How many pigs of different production stages (i.e. piglets, sows and fattening pigs) are estimated to be exposed to cross-contaminated feed in Belgium? What is the extent of the effect of this cross-contaminated pig feed on resistance selection to different antimicrobials?

5.   What is the estimated amount of residues of antimicrobials, administered to pigs in Belgium via the oral route, which is present in manure applied onto Belgian soil?

CHAPTER 3

Antimicrobial use in pigs, broilers and veal calves in Belgium

1. Abstract

Given the risks associated with antimicrobial resistance and its link with antimicrobial use, available data on antimicrobial use in the Belgian pig, broiler and veal calf production were compared. To allow for comparison of the data available from three peer-reviewed scientific articles, the unit of measurement for antimicrobial use was the Treatment Incidence (TI), defined as the number of animals per 1000 treated daily with one 'defined' (DDDA) or 'used daily dose animal' (UDDA). Moreover, extrapolation of farm-level data to national-level data was attempted according to the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) methodology, to estimate the amount of antimicrobials used in Belgium per species. Although, among the three species, the highest TI was observed in veal calves (TI_{DDDA}=414, TI_{UDDA}=379), based on the extrapolation, it was estimated that most antimicrobials were administered to pigs (159.4 tons). Thus, the most rapid decline in the total use could potentially be achieved by targeting the pig sector. During the process of data collection for comparison and calculation, it became obvious that there is a need for harmonized monitoring programs.

2. Introduction

The benefits of antimicrobials hardly need to be reiterated. Since their introduction, they have revolutionized the approach to the treatment, control and prevention of human and animal infections, and have contributed to major advances, ranging from increased disease treatment and life expectancy to intensified modern food production. However, for many years, antimicrobials have been prescribed pointlessly and have often been used with an imprudent prodigality. In animal production, they used to be added to feed for growth promotion (banned in Europe since 2006) (EC, 2005). They have often been administered unnecessarily or overused especially in group treatments, which are generally preferred over individual treatment due to their easier application. Antimicrobials have also been purchased and used as an easier and cheaper solution to prevent or treat conditions, instead of investments in infrastructure or disinfection of the farm. Inevitably, their use has resulted in a decreasing effectiveness in treating common infections and the emergence of multi-resistant strains of bacteria, such as methicillin resistant *Staphylococcus aureus* (MRSA) and extended- spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL) (Anses, 2014).

Evidence is mounting that much of the antimicrobial resistance problem is rooted in the inappropriate and excessive use of antimicrobial agents. This link between antimicrobial use and the emergence of resistance can be demonstrated at different levels, including the in vitro level, at which mutations have been observed to occur in genes of *Salmonella* Typhimurium experimental mutants, leading to an increase of the minimum inhibitory concentration (MIC) of the antimicrobial and confirming resistance (Giraud et al., 1999; Cloeckaert and Chaslus-Dancla, 2001); the individual animal level, at which Berge et al. (2005) observed a shift to increasingly multiple resistant fecal *E. coli* of feedlot steers in response to florfenicol treatment; and the farm, at which higher levels of resistance at group level are related to increased antimicrobial usage (Dewulf et al., 2007; Costa et al., 2008; Persoons et al., 2010). The link between use and resistance can also be shown at the animal sector level, at which Casteleyn et al. (2007) observed that the animal sectors characterized by a high administration of antimicrobials, i.e. poultry and pig sector, demonstrate higher levels of resistant *E. coli* than dairy cattle and wild hares; and the country level, at which Chantziaras et al. (2013) clearly demonstrated that in countries with high antimicrobial usage, higher levels of resistance are also observed in indicator bacteria.

The far-reaching burden of antimicrobial resistance, both from a human health and veterinary perspective, could be summarized as different levels of therapeutic and prophylactic failure, coupled with an increase of treatment costs for resistant infections (Laxminarayan, 2013). Consequently, a global concern for resistance has arisen. The 2013 World Economic Forum highlighted antimicrobial resistance as a major global risk with the ability to destabilize health systems (World economic forum,

2013). Within the same framework, the European Council called upon the member states to strengthen surveillance systems and improve data quality regarding the resistance and use of antimicrobials in human and veterinary medicine (EC, 2008, 2012).

Given the risks associated with antimicrobial resistance, the possible transmission pathways of resistant bacteria between animals and humans, e.g. via direct contact, food or environment, (Wooldridge, 2012) and the overwhelming evidence that antibiotic use has been a powerful selector of resistance (Acar and Moulin 2012; Chantziaras et al., 2013), it is crucial that the levels of antimicrobial consumption in animals are monitored for the animal species and at the national level. Besides, the quantification of antimicrobial use is currently performed at a European level, at which consumption data of antimicrobials in veterinary medicine are collected from the member states by the European Medicines Agency (EMA) in the framework of the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project.

With a view to contributing to the above cause, this study aims to: (1) give an overview of the antimicrobial consumption in veterinary medicine in Belgium in recent years, overall and per sector, i.e. pigs, broilers, veal calves. Additionally, the type of antimicrobial agents used, their administration routes and whether they are over or under-dosed are also in focus; (2) make comparisons regarding antimicrobial consumption among the aforementioned animal sectors and over time, based on the data selected and described to address aim (1); (3) attempt to extrapolate farm-level data to national-level data of antimicrobials consumed by the Belgian pig, broiler and veal calf populations, in order to make estimations of the total use per sector in the country.

3. Materials and Methods

3.1. Selection of scientific data sources

The data to address the objectives of this review were retrieved from the 2012 Belgian Veterinary Surveillance of Antimicrobial Consumption (BelVet-SAC) report (BelVet-SAC, 2012), as well as from the study results of Callens et al. (2012), Pardon et al. (2012) and Persoons et al. (2012) for pigs, veal calves and broilers, respectively. The aforementioned report and scientific articles were considered representative to provide recent and relevant data on antimicrobial consumption in three important sectors of intensive livestock production in Belgium.

In detail, the data provided by the 2012 BelVet SAC report consist of all veterinary antimicrobials sold to pharmacists and veterinarians in Belgium, i.e. antimicrobial pharmaceuticals, and of antimicrobial premixes incorporated in medicated feed, between 2007 and 2012. Likewise to the 2007-2009, 2010 and 2011 reports, in the 2012 BelVet-SAC report, yearly consumption figures of antimicrobials and antimicrobial premixes (mg) are put versus the animal production expressed as produced biomass (kg),

according to the methodology described by Grave et al. (2010). The biomass is calculated as the sum of the amount of pork, beef and poultry meat produced in a year, plus the number of dairy cattle in Belgium, times 500kg of metabolic weight per head. Although horses, small ruminants, companion animals and rabbits (accounting for 6% of the total biomass) are covered in the collected data on antimicrobial use, they are not taken into account when calculating the biomass.

Regarding the specific individual species, i.e. pigs, veal calves, broilers, the articles selected (Callens et al., 2012; Pardon et al., 2012; Persoons et al., 2012) all used similar methodologies and expressed antibiotic use based on the same quantitative measurement (the treatment incidence), which allows to compare results and draw conclusions. The treatment incidence (TI) is defined as the number of animals per 1000 that are treated daily with one defined daily dose animal (DDDA) or used daily dose animal (UDDA). The DDDA corresponds to the average maintenance dose for the main indication in a specified species, while the UDDA reflects the dose actually administered to the animal by the producer or the veterinarian.

The following formulas were used to calculate the TIs:

$$TI_{DDA} = [\text{total amount of drug administered (mg)}] / [\text{DDDA(mg/kg)} \times \text{number of days at risk} \times \text{kg of animal}] \times 1000$$

$$TI_{UDDA} = [\text{total amount of drug administered (mg)}] / [\text{UDDA (mg/kg)} \times \text{number of days at risk} \times \text{kg of animal}] \times 1000 \text{ (Timmerman et al., 2006)}.$$

The data retrieved from Callens et al. (2012) concern the prophylactic, i.e. the administration of an antimicrobial drug to healthy animals known to be at risk, and metaphylactic, i.e. the administration of antimicrobial drugs to clinically healthy animals in contact with animals with detected clinical signs, use of antimicrobials in group treatments between birth and slaughter, i.e. one production cycle, in Belgian pig herds, and were collected retrospectively from 50 closed and semi-closed herds between January and October 2010. Data concerning sows were not included. Persoons et al. (2012) collected antimicrobial consumption records from 32 randomly selected Belgian broiler farms during two non-consecutive production cycles, in 2007 and 2008, while Pardon et al. (2012) collected prospective antimicrobial consumption data from 15 white veal farms in Belgium, between 2007 and 2009, with the complete production cycle as the study period.

3.2. Data used

The data of interest to address the aims of this article retrieved from the studies by Callens et al.

(2012), Pardon et al. (2012), Persoons et al. (2012) and the BelVet-SAC report (2012) include: quantitative information on the consumption of antimicrobials for veterinary use in Belgium, overall and per species, i.e. pigs, veal calves, broilers, expressed in tons of active substance per year, in mg of active substance used per kg of biomass produced or as treatment incidence; quantitative information on the relative use of the different active substances, overall and per species; and qualitative information regarding the administration route and the correctness of dosing, according to the Summary of Product Characteristics (SPC).

3.3. Extrapolation of farm-level data to the national level

Based on the data of Callens et al. (2012), Pardon et al. (2012) and Persoons et al. (2012), an extrapolation to the national level of antimicrobial consumption by the respective animal sectors was attempted. The objective of this extrapolation was to provide estimates on antimicrobial consumption for the actual species at the national level, and was made according to the methodology described at the ESVAC scientific guidelines paper on the collection of reliable and standardized data on the consumption of antimicrobial agents by animal species (EMA, 2013a), which can be summarized as follows:

Amount of antimicrobials administered nationally, per species (tons) = amount of antimicrobials used in the studied population (tons) × [whole national population (number of animals)]/[studied population (number of animals)]

To calculate the total amount of antimicrobials administered in pigs on an annual basis, available data on the total number of pigs slaughtered in Belgium in 2008 were used (Eurostat, 2014a). To calculate the same value for broilers, available data for the total number of broilers slaughtered in Belgium in 2008 were considered (VEPEK, 2012). As for veal calves, the average total number of animals slaughtered in Belgium between 2009-2012 was used (Eurostat, 2014b).

4. Results

4.1. BelVet-SAC national results

According to the BelVet-SAC report, in 2012, the total national consumption of antimicrobial pharmaceuticals for farm and companion animals in Belgium reached 222.5 tons of active substance, while the consumption of antimicrobial premixes incorporated in medicated feed was 55.4 tons. When the above figures were put versus the amount of biomass produced in the same year (calculated as 2.033.855 tons), this gave 109.39 mg of active substance/kg biomass for antimicrobial pharmaceuticals used and 27.22 mg/kg for medicated premixes.

Compared to 2011, a substantial decrease of 7.2% in the total veterinary consumption of antimicrobials was observed in Belgium in 2012 (BelVet-SAC, 2012). During that year, the consumption of antimicrobial pharmaceuticals decreased by 7.9%, while the use of antimicrobial premixes decreased by 3.5%. Looking further backward, a promising decreasing trend of 20.3% in the total consumption can be observed between 2007 and 2012, when the first Bel- Vet-SAC data collection procedures were started (Figure 1).

The antimicrobial compounds most frequently used in 2012 were the combination of sulphonamides and trimethoprim, followed by the antimicrobial classes of penicillins and tetracyclines (Table 1). Compared to 2011, the use of the last two was reduced by 6.1% and 13% respectively, in 2012, whereas the use of sulphonamides and trimethoprimwas increased by 1.6%.

Compared to 2011, the consumption of cephalosporins (especially of the third and fourth generation) and quinolones (especially the fluoroquinolones) was increased in 2012 (2.7% and 3.1%, respectively). Even though this increase was limited in absolute values of active substance, it is of concern since these groups of antimicrobials are listed as critically important for resistance selection in both humans and animals by the World Health Organization (WHO) (WHO, 2011) and the World Organization for Animal Health (FAO/WHO/OIE, 2008). Moreover, this trend has been observed for a second year in a row.

Table 1. Antimicrobial compounds most frequently used in Belgium, overall (BelVet-SAC, 2012) and per livestock species (% in the total of antimicrobials used) (Callens et al., 2012; Pardon et al., 2012; Persoons et al., 2012). For pigs, figures are given for both oral and injectable compounds.

| Pigs		Broilers	White veal calves	Overall
Oral	*Injectable*			
Colistin (30.7%)	Tulathromycin (45%)	Amoxicillin (43%)	Oxytetracycline (23.7%)	Trimethoprim/sulphonamides (31.1%)
Amoxicillin (30%)	Ceftiofur LA[a] (40.1%)	Tylosin (30%)	Amoxicillin (18.5%)	Penicillins (29.7%)
Trimethoprim/sulpho-namides (13.1%)	Amoxicillin (8.4%)	Trimethoprim/sulpho-namides (18%)	Tylosin (17.2%)	Tetracyclines (26.3%)

[a] LA=long acting.
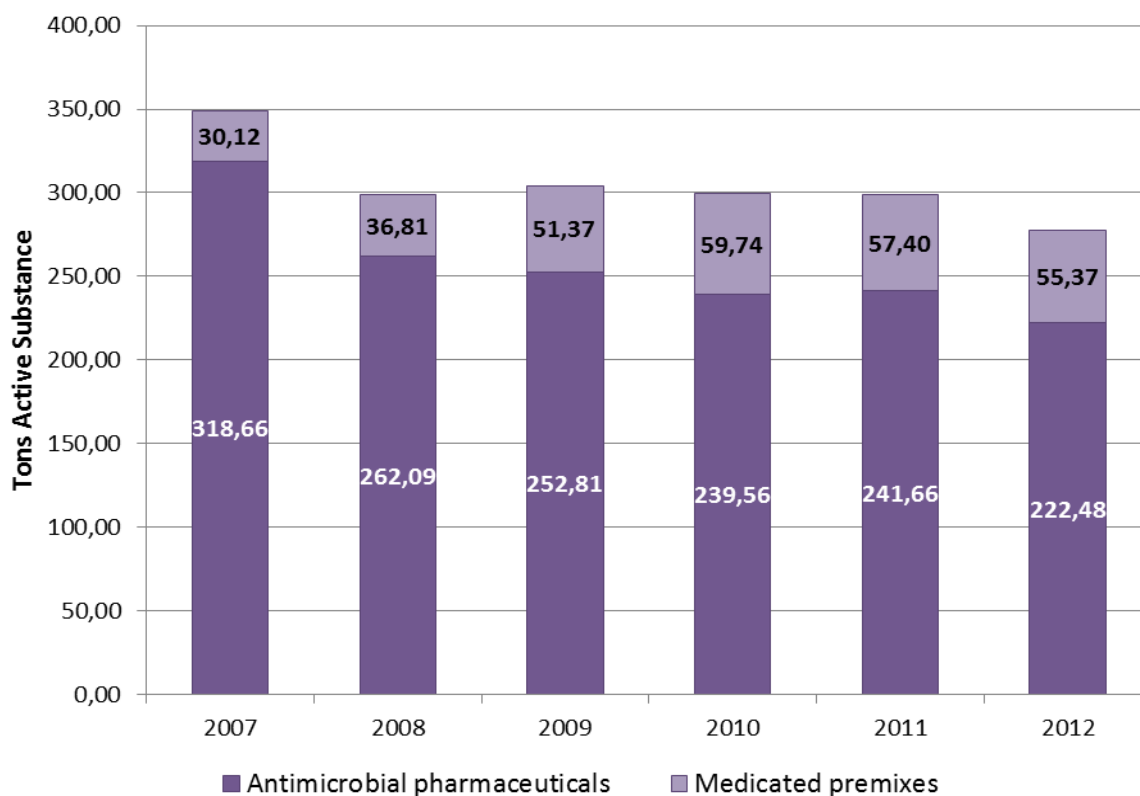
Figure 1. Total national consumption of antimicrobial compounds for veterinary use in Belgium from 2007 till 2012 (tons of active substance) (BelVet-SAC, 2012).

4.2. Antimicrobial use in Belgian pig farms

Callens et al. (2012) quantified the antimicrobial drug consumption in Belgian fattening pig farms expressed in TI. The average values for TI_{DDDA} and TI_{UDDA} were 235.8 and 200.7. This means that on average, 235 pigs were treated with one DDDA and 200 were treated with one UDDA. These values were higher than the values calculated by Timmerman et al. (2006), which were based on data collected in the same way in 2003 (178.1 and 170.3, respectively). In particular, TIUDDA increased by almost 18%. It should also be mentioned that a large variation among the treatment incidences of different herds has been observed (Callens et al., 2012) (Figure 2).

The oral and injectable antimicrobials most frequently used in pig production are shown in Table 1. They are expressed as a percentage of the total amount of antimicrobials used. Callens et al. (2012) found that the majority of group treatments are administered during the farrowing and battery period, while much less treatments are administered during the fattening period. On average, 80% of antimicrobial treatments in pig production are administered before the age of ten weeks. Callens et al. (2012) also concluded that prophylactic antimicrobial group treatment was applied in 93% of all group treatments, whereas metaphylactic or curative treatments were applied in only 7% of the cases.

The main reasons for antimicrobial use include digestive disorders and streptococcal infections in piglets, as well as respiratory problems during the second half of the battery period (Callens et al., 2012). In the study, group treatments were primarily administered via the parenteral route in sucking piglets and orally in piglets after weaning via the feed or drinking water. Generally, oral treatments were more common than injectable ones (TI_{UDDA}, oral =176.5, while TI_{UDDA}, injectable, =24.2). Injectable treatments were frequently overdosed (79.5% overdosed, 8.2% correctly dosed, 12.3% underdosed), while oral administrations were often underdosed (47.3% underdosed, 23.3% correctly dosed, 29.4% overdosed).

Based on the data of Callens et al. (2012), the extrapolated amount of antimicrobials consumed by the national pig population is estimated to be 159.4 tons. It should be noted that this value refers to (prophylactic and metaphylactic) group treatments in fattening pigs only, and does not include therapeutic use nor antimicrobials used in sows.

Figure 2. Treatment incidence based on the "used daily dose animal" (UDDA) and the "defined daily dose animal" (DDDA) in 50 pig farms in Belgium (Callens et al., 2012).

4.3. Antimicrobial use in Belgian broiler farms

Persoons et al. (2012) estimates that the average treatment incidences based on the defined daily dose (TI_{DDDA}) and the actual dose applied (TI_{UDDA}) equal 131.8 and 121.4 daily treated broilers per 1000, respectively. Likewise to pig farms, considerable variations exist in antimicrobial use between farms (Figure 3). Twenty two percent (7/32) of the farms in the study managed to grow broilers without using

antimicrobials in both of the production rounds monitored. In the treated farms, antimicrobials were used in 75% of the production cycles and were almost exclusively administered via drinking water in group treatments. Individual treatment in poultry is practically difficult and therefore not performed.

The antimicrobials most frequently used in broiler production are given in Table 1. In particular, amoxicillin and tylosin were administered in more than half of the treatments examined by Persoons et al. (2012). They are both classified as critically important antimicrobials (class I) for both human and animal health by WHO (WHO, 2011) and OIE (Anonymous, 2008).

The main reasons for antimicrobial administration in broilers are necrotic enteritis and dysbacteriosis, followed by feet disorders, coccidiosis and respiratory problems (Persoons, 2012). In the average flock of the study, most of the antimicrobials administered were usually dosed within the range of correct dosing. However, tylosin was usually underdosed, while amoxicillin and trimethoprim-sulphonamide were slightly overdosed.

According to the ESVAC methodology and based on the data of Persoons et al. (2012), the annual amount of antimicrobials consumed by the Belgian broiler population is estimated to be 26.5 tons.
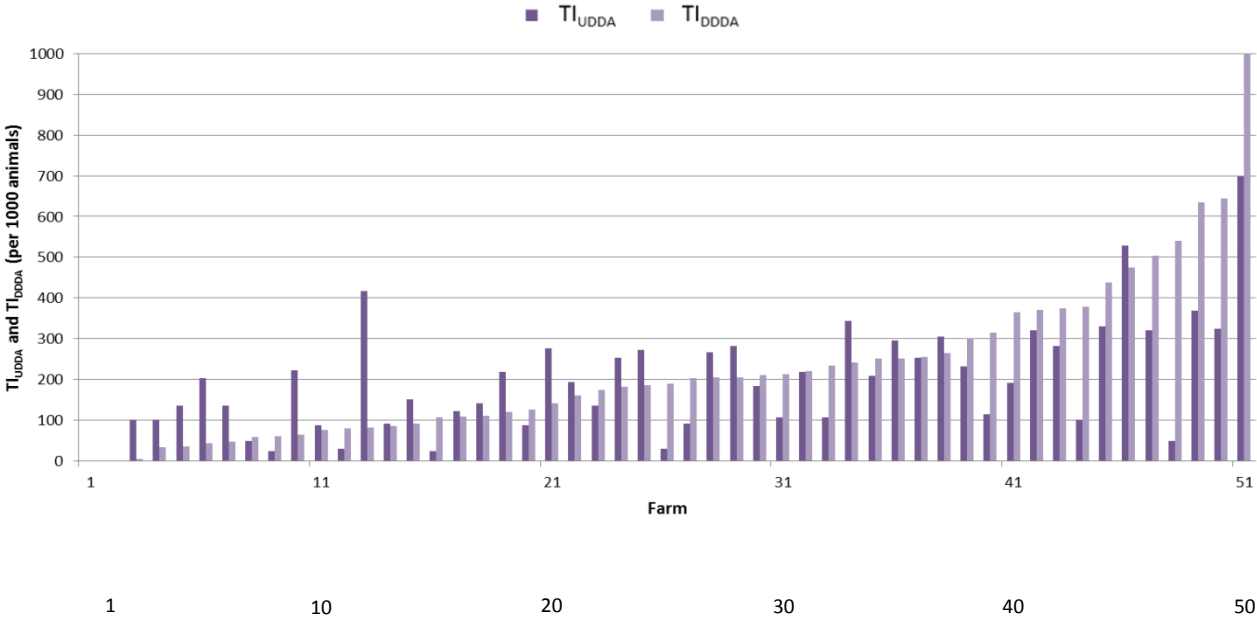

Figure 3. Treatment incidence based on "used daily dose animal" (UDDA) and "defined daily dose animal" (DDDA) in 32 broiler farms in Belgium in two non-consecutive production cycles (Persoons et al., 2012).

4.4. Antimicrobial use in Belgian veal calf farms

Pardon et al. (2012) calculated the TI, based on the DDDA (TI_{DDDA}) and the UDDA (TI_{UDDA}) and considering the actual live weight of the animals, as 414 and 379 calves treated with one daily dose of

antimicrobial agents per 1000 animals, respectively. Also in the veal calf sector, a relatively large variation between farms regarding antimicrobial use was observed (Figure 4).

Table 1 includes the most frequently used antimicrobials for group treatments of white veal calves, which are classified as critically important for public health (Pardon et al., 2012). Thirteen percent of the antimicrobial group treatments were administered prophylactically and 87% metaphylactically. The main indication for administration was respiratory disease (53%). Other common indications were arrival prophylaxis (13%), diarrhea (12%) and dysbacteriosis (12%). Group treatments were used in 97.9% of the cases and were all orally administered in milk.

The antimicrobials were mostly overdosed ($TI_{UDDA} < TI_{DDDA}$). However, likewise to pigs, over 40% of group treatments were underdosed. Especially oxytetracycline and tylosin, when used to treat dysbacteriosis, were systematically and significantly underdosed.

The data of Pardon et al. (2012) were considered representative to be used for extrapolation to the national level, based on which the annual antimicrobial consumption in white veal calves is estimated to be 25.2 tons.
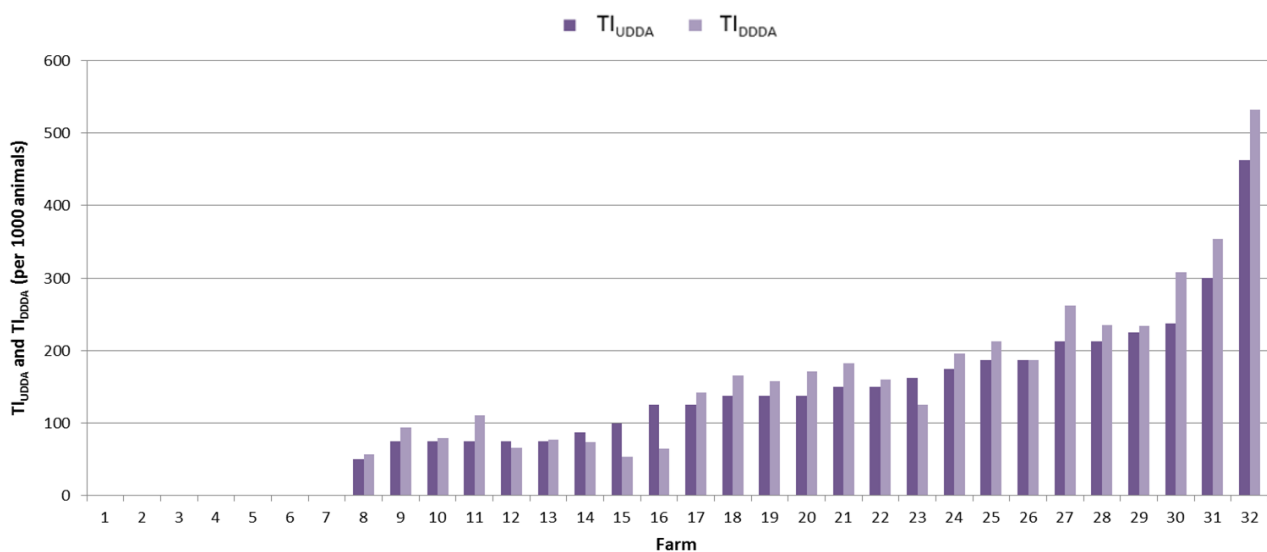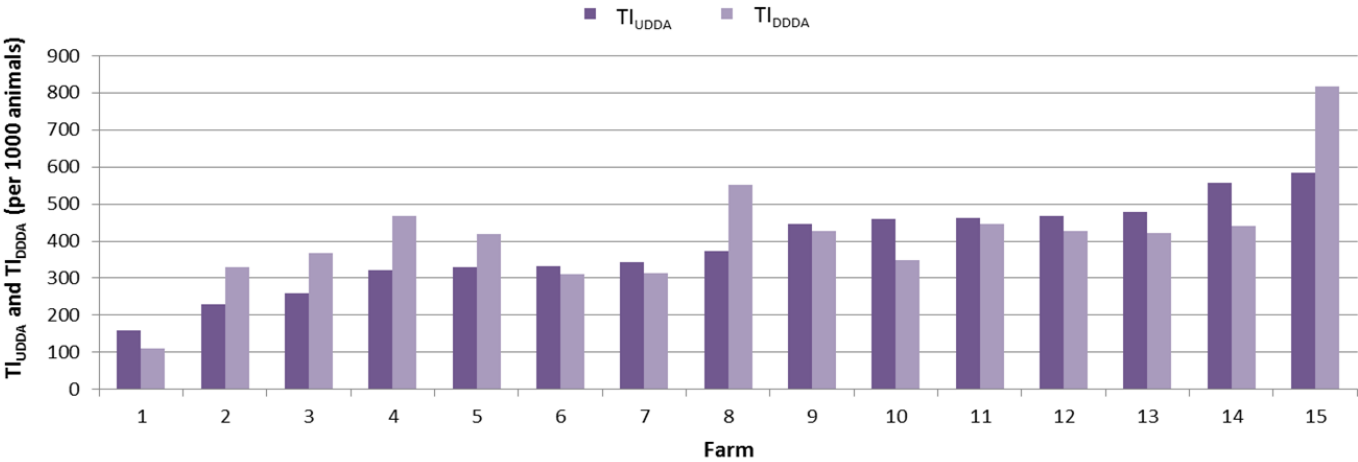

Figure 4. Treatment incidence based on "used daily dose animal" (UDDA) and "defined daily dose animal" (DDDA) in 15 white veal calf farms in Belgium (Pardon et al., 2012).
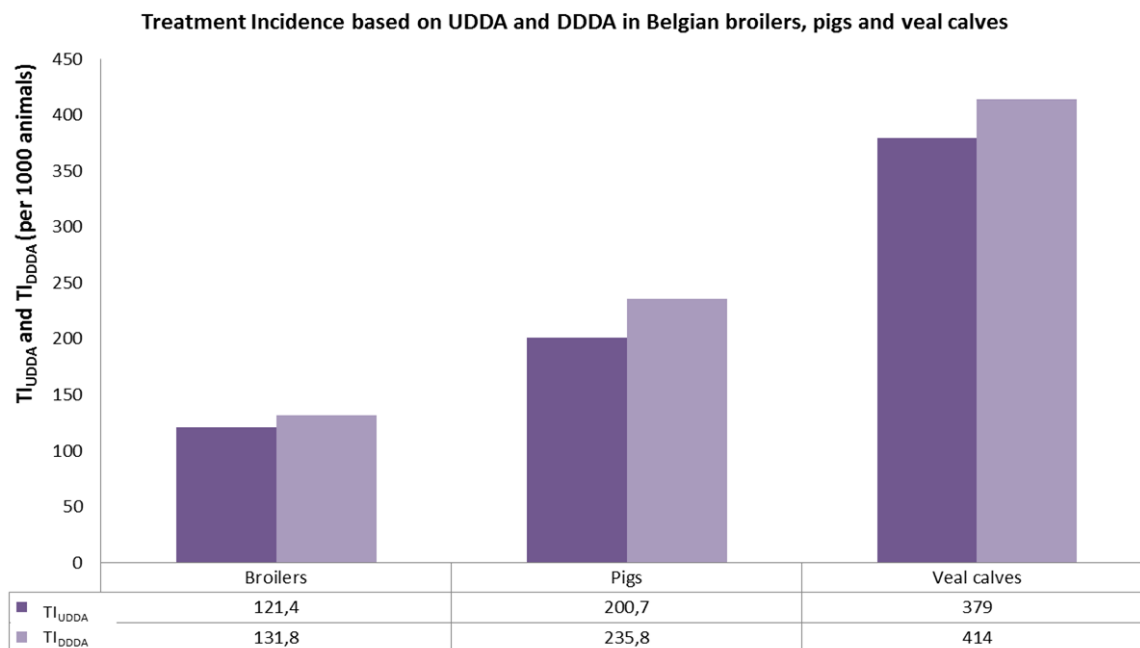
Figure 5. Comparison of the treatment incidences among Belgian broilers, pigs and veal calves.

	Broilers	Pigs	Veal calves
TI_{UDDA}	121,4	200,7	379
TI_{DDDA}	131,8	235,8	414
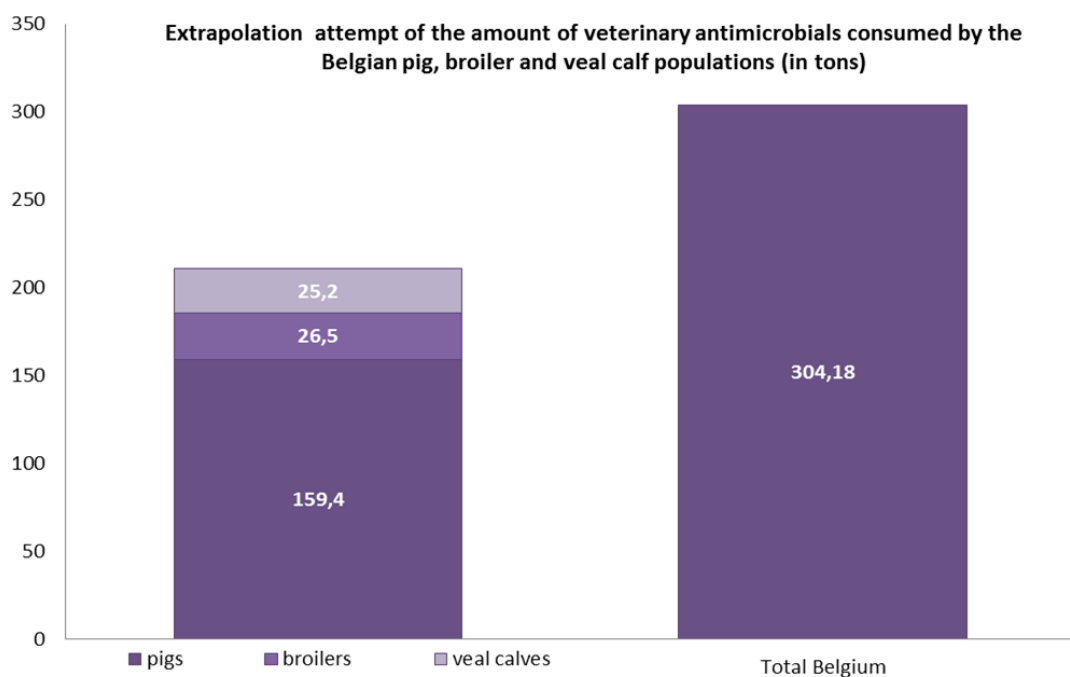
Figure 6. Left column: estimated amount in tons of antimicrobials used annually in Belgian pigs, broilers and veal calves at the national level, after extrapolating farm-level data from selected studies. Right column: total amount in tons of antimicrobials and medicated premixes used in Belgium in 2009 (BelVet-SAC, 2012).

5. Discussion

When it comes to interpreting antimicrobial consumption data, the unit of measurement as well as the use of standard measurement units for time at risk and average weight of animals at time of treatment, are crucial. The scientific data sources selected for this study provide data on the consumption of antimicrobials for veterinary use in Belgium, and are expressed in different ways.

Firstly, the BelVet-SAC report provides overall data for farm and companion animals expressed in tons of active substance per year and in mg of active substance used per kg of biomass produced. This approach is similar to the one used by the European Medicines Agency (EMA) in the ESVAC reports, where consumption data from EU countries are presented in mg of active substance per population correction unit (PCU), a unit of quantification of animal production comparable to the biomass. Although crude and not detailed enough for between-species differentiation, the BelVet-SAC report data provide an interesting insight into the evolution of the total antimicrobial use of the past years.

In contrast to the 2012 BelVet-SAC report, the selected scientific articles provide data per species, i.e. pigs, veal calves, broilers, quantified as treatment incidence (TI) of antimicrobials, at the end-user level. Being based on a sample of herds, these studies do not give a complete overview of the total antimicrobial consumption in Belgium as the BelVet-SAC report does. Additionally, the sampled farms were partially selected according to the willingness of the farmers to cooperate. Hence, there is an inherent risk of bias that should be taken into account. However, the sampled farms provide an insight into the antimicrobial use per species, which is detailed in terms of including data on the microbial agents used, their dosages, indications for administration, and also show a wide, and skewed distribution in use between herds and production types. This signifies that some herds manage to grow their animals without using antimicrobials, while others do not.

Since all three articles used a similar methodology, i.e. TI, the data they provide can be compared, despite referring to different sectors, sample sizes and years of data collection. Based on a general comparison of the TIs estimated for pigs, broilers and veal calves, it was observed that antimicrobial use is more frequent in veal calves (TI_{DDDA}= 414.0, TI_{UDDA}=379.0), followed by pigs (TI_{DDDA}=235.8, TI_{UDDA}=200.7) and broilers (TI_{DDDA}= 131.8, TI_{UDDA}=121.4) (Figure 5). The very high usage in veal calf production is most likely explained by the typical organization of the veal industry and more specifically, by the way veal calves are collected. Furthermore, it is known that the incidence of antimicrobial treatments in veal calves is much higher than in conventional dairy and beef cattle, 6.3 and 5.4 per 1000 cattle, respectively (Pardon et al., 2012).

From a methodological point of view, the use of TI as an indicator of antimicrobial consumption is more relevant in terms of estimating selection pressure for antimicrobial resistance than the total weight of antimicrobials consumed. Moreover, this method overcomes the issue of differences in molecular

weight between different antimicrobial products. If antimicrobial use is systematically quantified in the same way and converted to the same denominator, comparable data may be available and can then be used to follow up the amount of antimicrobials used in different sectors or farms and over time (Timmerman et al., 2006). Besides, in a European framework, an equivalent indicator to the TIs, i.e. the number of defined daily dose animals (DDDA) consumed by weight group/1000 animals produced or livestock/ year, is proposed by the EMA to be used in a standardized manner for reporting the consumption of antimicrobial agents by species (EMA, 2013a). At this point, it should be pointed out that the average animal weight at treatment used in the calculation of TI has a large influence on the obtained results. Therefore, this weight should always be examined with caution to prevent over or underestimation of the TI. Although the use of actual weights of the animals at treatment is laborious, it provides more accurate estimates of TIs.

The difference in methodologies does not allow for immediate comparisons between the BelVet-SAC report and the scientific articles. However, extrapolation from the farm-level data to the national-level data was attempted, based on the methodology described at the ESVAC scientific guidelines paper on the collection of reliable and standardized data on the consumption of antimicrobial agents by animal species (EMA, 2013a).

According to this extrapolation attempt, pigs (sows not included) consume most antimicrobials at the national level in Belgium, i.e. 159.40 tons annually, followed by broilers (26.53 tons) and white veal calves (25.18 tons), which, as already shown, have the highest TI. The relative contribution of the different animal species to the total use is shown in Figure 6, where the results are compared to the total use in 2009, the year considered as the most suitable in accordance with the years of data collections. It should be noted that this value remained almost constant from 2008 till 2011 (Figure 1). According to the gathered data and estimations, the use of antimicrobials in pigs, broilers and veal calves corresponds to 69.4% of the total use in Belgium. Yet, the extrapolation and the relative contribution should be interpreted with caution and only be seen as indicative. Several sources of uncertainty are possible, which are related to the representativeness of the sampled herds and to the fact that treatments in sows and curative treatments were not taken into account at the pig herds. Hence, caution is urged in concluding that the difference between the two columns reflects the antimicrobial use in dairy and beef cattle, small ruminants, horses, layers and companion animals.

Despite these calculations being rough due to the limited data available, it is important to attempt such extrapolations of data to the national level or even to the regional level, depending on the country. The results may prove useful, since they can provide estimates on sales and consumption of antimicrobials at the species level, and possibly per weight category and production type, as well as on

data pools for further (combined) analyses, e.g. ecological or economic analyses. Overall, they could be used to support the targeting of monitoring procedures and efforts to reduce antimicrobial usage. Regarding the antimicrobial compounds, those belonging to the WHO critically important list are frequently administered in all three species (Callens et al., 2012; Pardon et al., 2012; Persoons et al., 2012). Despite the overall decrease in antimicrobial consumption in Belgium in recent years, the use of molecules of critical importance for human medicine has remarkably increased for a second year in a row.

In this study, a number of needs regarding antimicrobial consumption data and related practices were observed. A wide and more detailed data collection covering all production sectors is needed and is important for benchmarking, monitoring and analysis of trends. Additionally, it would be appropriate to use differentiating statistics per animal production sector when comparing different countries, where the type of production system may differ (intensive, extensive). This monitoring work should be repeated every year in a harmonized manner, using the same quantification units. In line with this, scientific articles and reports, in which a more or less uniform methodology is used, would allow broader and more accurate comparisons, e.g. between countries. In addition, it seems that clear guidance on correct dosing needs to be provided on a regular basis to veterinarians, farmers and other stakeholders responsible for antimicrobial administration to animals.

According to the third ESVAC report of EMA for 2011, Belgium holds the sixth position in terms of sales volume of antimicrobials over 25 EU countries (EMA, 2013b). The previous year, Belgium was in third place. Since there was virtually no change in use in Belgium between 2010 and 2011, the change in positioning is solely the result of the addition of new countries with a higher consumption in the dataset, which had not provided data previously. Both places are high. For that reason, the reduction in the antimicrobial use observed by the BelVet-SAC in 2012 is hoped to be maintained (BelVet-SAC, 2012). This reduction in antimicrobial consumption in Belgium (BelVet-SAC, 2012) and also in Europe (EMA, 2013b) over the last few years is a promising trend. However, this reduction should not be taken for granted and efforts should be continued towards this direction. At the Belgian level, the Centre of Knowledge for Antimicrobial Consumption and Resistance in Animals (AMCRA) aims to promote the prudent use of antimicrobials, and has produced a list of guidelines on responsible antimicrobial consumption, where the various antimicrobial classes are differentiated in terms of importance for public and animal health according to the WHO and OIE lists (WHO, 2011; OIE, 2014).

In other words, antimicrobials need to be used sustainably. From a Belgian perspective and given that the use in the three major livestock species accounts for a large proportion of the total use, this could be translated to sector targeting actions. Besides, it has been shown here, that despite of the veal sector demonstrating the highest frequency in use per animal head, the pig sector demonstrates the

highest consumption in absolute values at the national level (Figures 5 and 6). Simultaneously and next to this quantitative approach, it is very crucial that producers of smaller industries, e.g. the veal calf sector, embrace active responsibility in this global health issue, and that all sectors endorse antimicrobial stewardship.

6. References

Acar JF, Moulin G, 2012. Antimicrobial resistance: a complex issue. Rev - Off Int Epizoot. 31 :23-31.

(Anses) French Agency for Food, Environmental and Occupational Health & Safety, 2014. Avis de l'agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail relatif aux Risques d'émergence d'antibiorésistances liés aux modes d'utilisation des antibiotiques dans le domaine de la santé animale (in french). p1-240. http://www.anses.fr/

(BelVet-SAC) Belgian Veterinary Surveillance of Antimicrobial Consumption, 2012. National Consumption Report. http://www.belvetsac.ugent.be

Berge ACB, Epperson WB, Pritchard RH, 2005. Assessing the effect of a single dose florfenicol treatment in feedlot cattle on the antimicrobial resistance patterns in faecal Escherichia coli. Vet Res 36:723-734.

Callens B, Persoons D, Maes D, Laanen M, Postma M, Boyen F, Haesebrouck F, Butaye P, Catry B, Dewulf J, 2012. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Prev Vet Med. 106:53-62.

Casteleyn C, Dewulf J, Catry B, de Kruif A, Maes D, 2007. Antimicrobial resistance in Escherichia coli from livestock, hare, sewage and surface water in Flanders. Vlaams Diergeneeskd Tijdschr. 75:23-30.

Chantziaras I, Boyen F, Callens B, Dewulf J, 2013. Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. J Antimicrob Chemother. 69:827-834.

Cloeckaert A, Chaslus-Dancla E, 2001. Mechanisms of quinolone resistance in Salmonella. Vet Res. 32:291-300.

Costa PMD, Vaz-Pires P, Bernardo F, 2008. Antimicrobial resistance in Escherichia coli isolated in wastewater and sludge from poultry slaughterhouse wastewater plants. J Environ Health. 70:40-53.

Dewulf J, Catry B, Timmermen T, Opsomer G, de Kruif A, Maes D, 2007. Tetracycline-resistance in lactose-positive enteric coliforms originating from Belgian fattening pigs: Degree of resistance, multiple resistance and risk factors. Prev Vet Med 78:339-351.

(EC) Council of the European Union, 2008. Conclusions on Antimicrobial Resistance (AMR). Luxembourg, 10 June 2008. p1-5. http://www.eu2008.si/

(EC) Council of the European Union, 2012. Conclusions on the impact of antimicrobial resistance in the human health sector and in the veterinary sector – a "One Health" perspective. Luxembourg, 22 June 2012. p1-6. http://www.consilium.europa.eu/

(EC) European Comission, 2005. Ban on antibiotics as growth promoters in animal feed enters into effect. Press Release. http://europa.eu/rapid/press-release_ IP-05-1687_en.htm

(EMA) European Medicines Agency, 2013a. Revised ESVAC reflection paper on collecting data on consumption of antimicrobial agents per animal species, on technical units of measurement and indicators for reporting consumption of antimicrobial agents in animals. p1-29. http://www.ema.europa.eu/

(EMA) European Medicines Agency, 2013b. Third ESVAC report on sales of veterinary antimicrobial agents in 25 EU/EEA countries in 2011. p1-98. http://www.ema.europa.eu/

Eurostat, 2014b. Meat production and foreign trade- annual data. http://ec.europa.eu/eurostat

Eurostat, 2014a. Statistic on the pig population, slaughtering and pig meat production. http://ec.europa.eu/eurostat

(FAO/WHO/OIE) 2008. Joint FAO/WHO/OIE Expert Meeting on Critically Important Antimicrobials. FAO, Rome, Italy and WHO, Geneva, Switzerland, 26–30 November 2007. p1-67. http://www.fao.org/home

Giraud E, Brisabois A, Martel JL, Chaslus-Dancla E, 1999. Comparative studies of mutations in animal isolates and experimental in vitro- and in vivo-selected mutants of Salmonella spp. suggest a counterselection of highly fluoroquinoloneresistant strains in the field. Antimicrob Agents Chemother. 43:2131-2137.

Grave K, Torren-Edo J, Mackay D, 2010. Comparison of the sales of veterinary antibacterial agents between 10 European countries. J Antimicrob Chemother. 65:2037-2040.

Heuer H, Smalla K, 2007. Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. Environ Microbiol. 9:657–66.

Laxminarayan R, Duse A, Wattal C, Zaid, A, Wertheim H, Sumpradit N, 2013. Antibiotic resistance -the need for global solutions. The Lancet Infectious Diseases Commission, Version 1.

(OIE) World Organisation for Animal Health, 2014. List of antimicrobial agents of veterinary importance. p1-9. http://www.oie.int

Pardon B, Catry B, Dewulf J, Persoons D, Hostens M, De Bleecker K, Deprez P, 2012. Prospective study on quantitative and qualitative antimicrobial and anti-inflammatory drug use in white veal calves. The J Antimicrob Chemother. 67:1027-1038.

Persoons D, Dewulf J, Smet A, Herman L, Heyndrickx M, Martel A, Catry B., Butaye P, Haesebrouck F, 2010. Prevalence and persistence of antimicrobial resistance in broiler indicator bacteria. Microb Drug Resist. 16:67-74.

Persoons D, Dewulf J, Smet A, Herman L, Heyndrickx M, Martel A, Catry B, Butaye P, Haesebrouck F, 2012. Antimicrobial use in Belgian broiler production. Prev Vet Med 105:320-325.

Timmerman T, Dewulf J, Catry B, Feyen B, Opsomer G, de Kruif A Maes D, 2006. Quantification and evaluation of antimicrobial drug use in group treatments for fattening pigs in Belgium. Prev Vet Med 74:251-263.

(VEPEK) Verboond voor pluimvee, eieren en konijnen, 2012. Overzicht van de Belgische pluimvee en konijnenhouderijen 2008. p1-109. http://www.agripress.be

(WHO) World Health Organization, 2011. Critically important antimicrobials for human medicine. p1-48. http://www.who.int

Wooldridge M, 2012. Evidence for the circulation of antimicrobialresistant strains and genes in nature and especially between humans and animals. Rev - Off Int Epizoot. 31:231-247.

World economic forum, 2013. Global risks 2013. An initiative of the risk response network. 8th Report, Cologny/Geneva. p1-80. http://www3.weforum.org/

CHAPTER 4

Patterns of antimicrobial use in pigs via feed and water: A field-study in pig farms in Belgium

1. Abstract

In intensive pig farming, group treatments are often used to administer antimicrobial agents via the feed or drinking water. Unfortunately, problems can arise when administrating medication via the oral route, such as the cross-contamination of compound feed and water due to carry-over of antimicrobial residues. To date, no data are available regarding the frequency and level of this problem (and other, e.g. homogeneity) occurring at the farm level. Therefore, a field study was performed in 52 pig farms in Belgium, with the aim of examining the level of use and the conditions of preparation and administration of antimicrobials via the feed and water. In the farms visited, water medication was used more (n=47 farms) than in-feed medication (n=36). Per production stage, water medication was used more in piglets (87.5%) and fattening pigs (91%), while top dressing was mainly used in sows (55%). More differences between production stages were observed in relation to the administration of medicated feed (e.g. type of feed), rather than the administration of water medication. Although the preparation of water medication is labor intensive and specific concerns are present (e.g. precipitation), it can be considered as a better choice for antimicrobial administration, compared to in-feed medication, with regard to less carry-over and more animals receiving a correct dose of medication are expected when using water medication. However, technical issues (e.g. possibility of blockage of the water pipe due to insufficient cleaning) should be prevented.

2. Introduction

In intensive pig farming, group treatments are often used to administer antimicrobial agents via the feed or drinking water (BelVet-SAC, 2017). Also deworming agents are often administered via this route. In terms of treatment incidences (TI) of antimicrobial compounds, expressed as the daily number of treated pigs per 1000 animals with a used daily dose animal (TI_{UDDA}), a TI_{UDDA} of 176.5 for oral treatment, compared to a TI_{UDDA} of 24.2 for parenteral treatment of pigs in Belgium, was estimated by Callens et al. (2012), indicating that oral therapy is by far more commonly used than parental therapy. Based on the data of Callens et al. (2012) and according to an extrapolation exercise (Filippitzi et al., 2014), the amount of antimicrobials consumed by the national pig population of fattening pigs in prophylactic and metaphylactic group treatments was estimated to be 159.4 tons, representing 52.4% of the total antimicrobial use in Belgium. Even though a downward trend in terms of antimicrobial use, including premixes, has been observed in Belgium the past years (BelVet-SAC, 2017), oral group treatments of pigs remain the most common route of antimicrobial administration to this species (Postma et al., 2015). The most commonly used antimicrobial classes administered orally to pigs in Belgium are penicillins, tetracyclines, sulphonamides and macrolides (BelVet-SAC, 2017).

There are five ways for administering medication, including antimicrobials, to groups of animals, including pigs, via the oral route. The first is the mixing of medicated premixes in compound feed by approved manufacturers of medicated animal feed (1), after the prescription of a veterinarian. In Belgium, since 1/1/2014, the mixing of antimicrobials by the manufacturer only takes place in an end-of-line mixer or via a fine dosing system at the moment of delivery to the farmer (BFA, 2013). The second and third possibilities for oral treatment include topdressing (2) and the use of an automatic in feed dosing system (3), respectively. When topdressing is used, the pig farmers receive the required amount of medication according the veterinarian's prescription and, then, they add the desired amount of medication with, for instance, a measuring cup over the feed. If necessary, they also mix the medication as good as possible in the feed. When an automatic dosing system is used, the medication is added into the feed at the feed line. Finally, treatment of groups of animals can also be performed with the use of drinking water medication, via a water tank (4) or a dosing pump at the waterline (5). Currently, there are no data available on the distribution and frequency of use of these methods in Belgium.

Unfortunately, problems can arise in relation to the administration of antimicrobials via any of these aforementioned routes. One of these problems is the cross-contamination of compound feed due to carry-over of remaining residues (Borràs et al., 2011), which is associated with the potential for antimicrobial resistance selection (Peeters et al., 2017, 2018). Other problems concern the potential carry-over of residues in water, the insufficient homogeneity and stability of medication in the feed or water (Stolker et al., 2013), the segregation of antimicrobial (in the feeders/water pipes), and issues of solubility of the drug in the drinking water (AFSCA/FAVV, 2013). To be able to identify the level of occurrence of these problems at pig farms in Belgium, there is first a need for scientific research with the aim of examining the level of use and the conditions of preparation and administration of antimicrobials via the feed and water.

The aim of this study was to perform a field study at pig farms in Belgium, in which different types of available systems for oral group treatment with antimicrobials would be identified and studied, providing a solid base for further research into the homogeneity, stability and carry-over of medicated feed and medicated drinking water. At each of the participating farms visited, the process of mixing and storage of medicated feed and water are described in detail, while the group size of the treatment group and the frequency of oral administration of antimicrobials are also recorded. Moreover, this study offers a unique insight into the experiences of pig farmers with the use of medicated feed and water and reveals which practical problems often occur, which are the preferred oral treatment options and how effective these treatments are.

3. Materials and methods

3.1. The questionnaire

A survey, containing both open and closed-ended questions, was conducted in collaboration with pig veterinarians. The questionnaire was subdivided into 6 sections: general information about the farm, construction of drinking water/feeding system, cleaning and disinfection, group treatment via feed and drinking water, preparation of feed and water medication and finally personal experience with drinking water/feed medication. In total, the survey contained 46 questions, of which the answers to 21 of these questions are presented and analyzed, of which 7 were open and 14 were closed. The full questionnaire is available in Annex in dutch and french. A pre-test was conducted regarding content and consistency of the questions.

3.2. Farm visits

Herd veterinarians were found on the internet, using contact details of the Belgian branch of International Pig Veterinary Society (IPVS). First, an e-mail was sent, followed by a phone call to explain the study objectives and to verify the veterinarian's willingness to participate. The farms were visited, accompanying the veterinarian when he or she went on a visit to the farm. The purpose of the study was explained to the pig farmer and it was emphasized to the farmers that questionnaires would be processed anonymously. A face-to face questionnaire interview was combined with a visit at the farm site. All visits and interviews were conducted by the first author between February and October 2017.

3.3. Data processing and analysis

All information from the survey, coded numerically to assist analysis, was entered into Microsoft Excel, 2010 and recoded into categorical data. Descriptive statistics were performed in SPSS v.24.0 (SPSS Incl., Chicago, IL).

4. Results

4.1. General information about the farms

Fifty two (52) farms participated in the field study. Table 1 shows the average size of the participating farms (i.e. total number of sows, piglets and fattening pigs and boars present at the farm). A detailed description of all practices and personal experiences regarding feed and water administration of antimicrobials is provided in the following section. The results for feed and water antimicrobial administration are presented together, one after the other, per question.

Table 1. Descriptive statistics for the pig farms participating in the field study.

Pig production stage kept at farm	Number of farms keeping pigs in each production stage	Number of pigs in a production stage in all types of farms keeping pigs in this production stage			
		Mean	Median	Minimum	Maximum
Sows	20	315	200	80	757
Piglets	16	1,156	725	248	4,000
Fattening pigs	44	1,866	1,275	250	10,000
Total number of farms	52[*]				

Notes: For presentation purposes, the mean values of the number of pigs are rounded. * Eleven (11) of these farms are closed farms keeping pigs of all three production stages.

4.2. Practices related to administration of antimicrobials via feed and water

Frequency of use of feed/water medication

Antimicrobials were administered to pigs via the feed in 69% of the visited farms (n=36). In this dataset, in-feed medication was most often used in piglets (75%; n=12), followed by sows (65%; n=13) and fattening pigs (59%; n=26).

Antimicrobials were administered to pigs via water in 81% of the visited farms (n=42). This method was very often used in piglets (81%; n=13) and fattening pigs (84; n=37). In this dataset, it was not used much in sows (70% of farms with sows did not use this method; n=14).

Construction of feeding/water system

Type of feed for sows, piglets and fattening pigs

In the 20 farms with sows, the most common type of feed administrated to sows was pellets (75%), followed by meal (35%) and wet/dry feed (15%). In 5 farms with sows, more than one type of feed was used.

In the 16 farms with piglets, the most common type of feed administered was meal (76%), while grains/pellets (2 farms) and crumb (2 farms) were less used. One of these farms used two types of feed for piglets (meal and pellets).

In the 44 farms with fattening pigs, meal and grain/pellet feed were equally used (43% each), followed by wet/dry feed (6 farms) and wet/mush feed (1 farm). Ten of the 44 farms used two types of feed and in all cases these were meal and pellets.

Drinking water for sows, piglets and fattening pigs

In 90% (n=18) of farms with sows, well water is used. In very few farms, tap (n=2) or drainage water (n=1) is used. In one farm, both well and tap water is used. Surface and rain water was not used in any of the visited farms.

In 94% (n=15) of farms with piglets, well water is used. In one farm, tap and drainage water is used. In two farms using well water, tap water is also used in one of them, and drainage water and rain water in the other. Surface water was not used in any of the visited farms.

The vast majority of farms with fattening pigs also used well water for fatteners (89%; n=39). Four farms used tap water, one of which also used drainage water. Few of the 39 farms using well water, also used tap, drainage, rain or surface water (total number of these uses=5).

Oldest and newest part of feed/water installation

The oldest and newest parts of the installation for feed supply have been present at the farms visited for, in average, 29.31 years (SD=0.19) and for 10.1 years (SD=7.6), respectively. Regarding the installation for water supply, the oldest and the newest parts of the installation have been present for, in average, 25 years (SD=0.4) and for 8 years (SD=5.2), respectively.

Cleaning and disinfection (cleaning and disinfection)

Cleaning and disinfection frequency of different parts

Silo: 65% of farms never clean or disinfect the silo. Fewer farms do so once each or every two years (19%), twice a year (14%) or more than twice a year (2%).

Feed tank: In 51 out of 52 farms, there was no feed tank present. The one farm that had one, cleans it once a month.

Feeding troughs: 54% of farms clean and disinfect the feeding troughs after the end of a production cycle. Some farms clean and disinfect at weaning (these are farms with sows) (10%), once a year (4%) or 3-6 times a year (2%). Two farms report that they clean and disinfect with a variable frequency. A percentage of 27% of farms never clean or disinfect the feeding troughs.

Water lines: 62% of farms clean and disinfect the water lines. Most farms of these farms do so when there are visible problems or dirt (35%) or once/twice a year (29%). In a few farms, cleaning and disinfection is performed permanently (n=4), 3-4 times a year (n=2), at the end of round, vacancy or before new round (n=2), after therapy (n=2), once a month (n=1). The rest 38% of farms never clean and disinfect the water lines.

Cleaning piping before and after feed/water medication

The vast majority of farms (94%; n= 33) using feed with antimicrobials did not clean or disinfect the pipelines before the use of this feed. The same pattern was seen for cleaning and disinfection of pipelines after the use of feed with antimicrobials (97%; n=34 do not clean or disinfect). Most farms using water medication, although in a smaller percentage than the farms using feed medication (79%; n=33), never clean the piping system before administering antimicrobials in water; some always clean it (19%; n=8) and one farm sometimes does so. Most of the same farms (55%; n=23) never clean the piping system after administering water medication, a number of them always clean it (31%; n=13) and fewer farms sometimes do so (n=6).

Cleaning of tools used for feed and water medication

In 71% (n=10) of farms preparing medicated feed at the farm, the tools used to make this feed are never cleaned afterwards. The practice of cleaning these tools is rare (two farms always clean them, two farms sometimes do). On the contrary, at the farms where water medication is used, the used tools are always cleaned in 69% of cases (n=29), while these tools are sometimes cleaned in 19% (n=8) and never cleaned in 12% (n=5) of them.

Group treatment via feed/ drinking water

Combination of antimicrobials in feed/water

In 3 farms, combinations of antimicrobials in feed (i.e. doxycycline-trimethoprim-sulphonamides) were used.

In farms using water medication, no combination of compounds was used in the majority of cases (51%). In some cases (21%), a combination of amoxicillin and acetylic acid was used. In the few times that combinations of antimicrobials were used, these included combinations of amoxicillin, trimethoprim and sulphonamides (n=2), amoxicillin and colistin (n=2), doxycycline and tylosin (n=1), amoxicillin and doxycycline (n=1).

Smallest group of sows, piglets and fatteners for feed/water medication

In the majority of the farms administering medication to sows via feed, the sows received the feed individually (46%; n=6). Other farms administered this feed at pen (23%; n=3) or stable level (n=3). In one of these farms, the medicated feed was administered per sow compartment (n=1). There was a large variation in the number of sows per group at the farms visited. On the other hand, in the majority of the farms administering medication to piglets via feed, the piglets received the feed at stable level (50%; n=6) and less commonly at compartment (n=3) or pen level (n=1). Medication via feed was

administered to fattening pigs at stable level in 81% of farms with fattening pigs (n=21), whereas in fewer cases, it was administered at compartment (n=5 times) or pen level (n=2 times). An general indication of the group size for piglets and fattening pigs at the visited farms is 300 animals per stable, 150 per compartment and 15 per pen.

In the farms using water medication in sows, water medication was administered at the stable level in three farms, at the compartment level in two farms and at the pen level in one farm. According to the farms using water medication in piglets, water medication was administered at the compartment level in 62% of farms (n=8) and at the stable level in 39% of farms (n=5). In the farms using water medication in fatteners, water medication was administered at the stable (54%; n=20) or the compartment (46%) level.

Separate pipeline for feed/water

None of the farms administering medication via feed (n=36) had a separate pipeline for medicated and non-medicated feed. As for farms using water medication, the majority of them do not have a separate piping (72%), while the rest do.

Preparation of feed/water medication

Method/device used for preparation of medicated feed/water

At the farms using medicated feed, medicated feed was purchased from the feed manufacturer in 69% of cases (n=30). Top dressing was used in 25% of cases (n=11; farms with sows), while in a few cases (7%; n=3) a dosing device on the feed line was used at the farm. Some farms used more than one method.

At farms using water medication, the device used the most to prepare it was a mechanical dosing pump (54%; n=26), followed by an electric dosing pump (25%). In 19% of cases, drinking water medication was prepared by mixing a premix in a small amount of water (the pre-solution), which was then added to a drinking water reservoir. Some farms used more than one devices to prepare water medication.

Frequency of preparing medication and daily administration pattern

When medicated feed is prepared at the farm (based on 7 answers received from 14 farms preparing it at the farm), it is prepared once a day in most of these farms (86%; n=6) or when the stock solution is finished (n=1). The feed with medication, when used, is mainly administered during the whole day (76%; n=28). In fewer cases (24%) it is administered for a few hours a day.

In the farms using water medication, water medication is mostly prepared once a day (60%; n=25). In other farms, it is prepared two times a day when amoxicillin is used (n=9), or when the pre-solution is

finished (n=4), twice a day (n=2), once every 14 days (n=1) or every 4-6 hours, depending on the need (n=1). In the majority of cases (72%; n=31), water medication is administered throughout the day, while in some cases (26%) for several hours during the day.

4.3. Farmers' experience with administration of antimicrobials via feed and water

Ease of use

The majority of the farmers (92%; n=33) reported no problems regarding the ease of use of medicated feed.). The few issues that were reported are heavy workload due to the individual animal treatment with medicated feed (n=3), and the limited amount of registered products available for preparation of this feed (n=1).

No problems were reported from 77% of farmers (n=36) regarding the ease of use of water medication. In some cases, issues mainly related to the workload of preparing water medication were reported: in six farms medicated feed is preferred and is considered easier to use while requires less work; in one farm it is said that stirring requires more work; in another farm the rinsing of the pump is necessary and therefore requires more work; in a last farm, it is reported that a lot of work is required, when piglets and fattening pigs need to be treated with water medication at the same time.

Practical problems

Twenty-two out of the 30 farmers (73%) reported to encounter no practical problems during the execution of the therapy. The problems that were reported are that sick pigs stop eating (n=4), that medicated feed is less tasteful (n=2), that the timing of feed administration is not flexible (n=1) and that sometimes the day of treatment can be forgotten (n=1).

No practical problems were reported from 19 farmers (40%) regarding the execution of therapy when using water medication. Eleven farmers (23%) reported the issue of precipitation of antimicrobials. Other issues were also sometimes raised, including solubility problems (n=6), the reduction of the amount of water consumed by ill pigs (n=5) and clogged nipples (n=2). Further issues involve the responsible person forgetting to switch off the water disinfection before administration of water medication (n=2), forgetting to switch off the faucets from normal to medicated water (n=1) or accidentally adding medication in water (n=1). The necessary rinsing of the pump (n=2), the fact that the medicated water is less tasteful (n=1), the difficulty of cleaning a large reservoir (n=1) and the lack of infrastructure allowing the treatment of more than one stable at once (n=1) were also reported as practical issues during the execution of therapy with water medication.

Effectiveness

Most farmers who have been using antimicrobial medicated feed regularly (n =18), find the treatment to be usually effective (61%; n= 11), or reasonably effective (n=5), in contrast to few of them reporting regular relapse of animals (n=2). Likewise, most farmers using antimicrobials in water report that the treatment administered via this route is mostly effective (56%; n=23). Thirteen of them suggest that the treatment is moderately effective but there are often relapses, while five of them suggest that the treatment is reasonably effective but sometimes the treated animals relapse.

5. Discussion

5.1. Limitations

Fifty two (52) farms agreed to participate in this study. This is a limited number of pig farms, but they were selected to include farms using all different ways of administration of in-feed and water medication in Belgium. However, any extrapolation attempts based on this data should be cautiously made. In some cases, a certain level of information bias might be included, as the interviewed person could not provide an answer (e.g. the responsible person was not present at the time of visit, or has changed).

5.2. Comparison between feed and water medication

In the total 52 farms visited, antimicrobials were administered in more farms via water (n=42) than via feed (n=36), although both methods of administering medication were widely used. Per production stage, water medication was used more in piglets and fattening pigs, while top dressing was mainly used in sows. In terms of differences between production stages, more differences were observed in relation to the administration of medicated feed, rather than the administration of water medication. In the latter case, the patterns in the practices observed were generally applied per farm as a whole rather than per production stage.

The type of feed and the quality of water used for the administration of antimicrobials affect the carry-over level of residues, the stability and homogeneity of both the feed and water medication. As expected, piglets are usually fed with meal, which is generally the first type of feed used. In terms of carry-over, the powder form of meal makes it more difficult to purge the equipment at the feed-mill, the truck and the farm, between batches (Strauch and Freil, 2008). Meal is also highly used to feed fattening pigs. Moreover, it is more difficult to add antimicrobials in pellets, as the feed is heated before being pressed into pellets and this damages the antimicrobilas.

In case water medication is used, the drinking water quality is very important in keeping the drinking water medication stable and homogeneously mixed, as well as ensuring sufficient water uptake. Drinking water quality depends on various reasons, including the source, the filtration and treatment

(e.g. acidification) of the water, and the condition of the water pipelines, as sludge and residues from previous treatments could result in clogging and contamination of the waterlines and nipples. A sample to determine the drinking water quality should be taken at least once a year, which is not the case at the moment. If the water quality is good, in terms of hardness, pH, level of coliforms (De Backer, 2015), and the antimicrobial compounds used have good solubility in water, less carry-over and better homogeneity would be expected from the use of water medication, compared to feed. However, precipitation of antimicrobials in water is often reported. Results from sample testing of feed and water, would allow us to compare in an informed way the levels of carry-over, the stability and the homogeneity between feed and water medication.

Regarding the frequency of preparing the medicated feed/water at the farm, this is usually done once a day both for feed and water medication, when administered. However, all farmers (n=42) using antimicrobial medication in water prepare it themselves at the farms, whereas fewer farmers (n=14) prepare medicated feed at the farm. When used, in the majority of cases, feed and water medication is administered to animals throughout the day.

Comparing the frequency of cleaning and disinfection in relation to the preparation and administration of in-feed and water medication, it was mainly observed that cleaning and disinfection of the feed silo and feeding troughs was not a common practice in the majority of the farms visited. Exception to this was the cleaning and disinfection of feeding troughs after the end of a production cycle (54%). On the contrary, cleaning and disinfection of waterlines, which improves water quality and prevents carry-over, was practiced more often (62% of farms).

Focusing on the personal experience of the farmers with each of these methods, most of them find it easier to use in-feed medication, rather than water medication, due to the workload involved in the preparation of the latter method. However, from the point of view of antimicrobial use, the ease of use is contra-indicated as it results in over use of antimicrobials. Therefore, a more laborious preparation and use of medication would normally result in a more cautious use of antimicrobials. Regarding the execution of treatment when using water medication, the farmers report more problems, with precipitation being the most common problem stated. The farmers report a similar level of effectiveness for both methods, however few farmers using medicated feed provided an answer to the respective question. Regular relapse is reported, although limitedly (n=2), only in relation to the use of in-feed medication.

5.3. Conclusion

In all farms of this dataset, medication (mainly antimicrobials) is administered to pigs via the oral route. Water medication was used in more farms than in-feed medication. Although the preparation of water

medication is labor intensive and specific concerns are present, it can be considered as a better choice for antimicrobial administration, compared to in-feed medication, with regard to carry-over and dosing. In case of using water medication, less carry-over can be expected. Moreover, more animals are expected to receive a correct dose of medication (e.g. sick pigs drink water, adjustment of dosage is possible). However, extra attention must also be paid to the possibility of blockage of the water pipe and/or nipples, so thorough checking and cleaning of these parts is a must. More research is required to estimate the carry-over levels at the farm with sample testing, and to assess other important issues, such as the homogeneity and stability of antimicrobials in feed and water.

6. Acknowledgements

7. References

(AFSCA-FAVV) Belgian Federal Agency for the Safety of the Food Chain, 2013. Responsible use of antibacterial agents in group treatment of farm animals and the impact on resistance selection (dossier Sci Com 2012/10). p1-56. http://www.afsca.be/

(BelVet-SAC) Belgian Veterinary Surveillance of Antibacterial Consumption, 2017. 2016 National consumption report. p1-44. http://www.belvetsac.ugent.be/

(BFA) Belgian Compound Feed Industry Association, 2013. In Dutch: Convenant betreffende gemedicineerde voeders. p1-14. http://www.favv.be/

Borràs S, Companyó R, Granados M, Guiteras J, Pérez- Vendrell AM, Brufau J, Medina M, Bosch J, 2011. Analysis of antimicrobial agents in animal feeds. Trends Analyt Chem. 30:1042–1064.

Callens B, Persoons D, Maes D, Laanen M, Postma M, Boyen F, Haesebrouck F, Butaye P, Catry B, Dewulf J, 2012. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Prev Vet Med. 106(1):53 62.

De Backer P, 2015. Pitfalls of veterinary dosage of veterinary pharmaceuticals in drinking water medication. In: Importance of drinking water in piglets, fattening pigs and sows. Ghent, 5 October 2015.

Filippitzi ME, Callens B, Pardon B, Persoons D, Dewulf J, 2014. Antimicrobial use in pigs, broilers and veal calves in Belgium. Vlaams Diergeneeskd Tijdschr. 83(5):215.

Peeters LE, De Mulder T, Van Coillie E, Huygens J, Smet A, Daeseleire E, Dewulf J, Imberechts H, Butaye P, Haesebrouck F, Croubels S, Heyndrickx M, Rasschaert G, 2017. Selection and transfer of an IncI1-tet(A) plasmid of Escherichia coli in an ex vivo model of the porcine caecum at doxycycline concentrations caused by cross-contaminated feed. J Appl Microbiol. 123(5):1312-1320.

Peeters LE, Croubels S, Rasschaert G, Imberechts H, Daeseleire E, Dewulf J, Heyndrickx M, Butaye P, Haesebrouck F, Smet A, 2018. Effect of residual doxycycline concentrations on resistance selection and transfer in porcine commensal Escherichia coli. Int J Antimicrob Agents. 51(1):123-127.

Postma M, Sjölund M, Collineau L, Lösken S, Stärk K, Dewulf J, 2015. MINAPIG Consortium. Assigning defined daily doses animal: a European multi-country experience for antimicrobial products authorized for usage in pigs. J Antimicrob Chemother. 70(1):294-302.

Stolker AAM, Manti V, Zuidema T, van Egmond H, Deckers ER, Herbes R, Hooglugt J, Olde Heuvel E, de Jong J, 2013. Carry-over of veterinary drugs from medicated to nonmedicated feeds in commercial feed manufacturing plants. Food Addit Contam Part A. 30:1100–1107.

Strauch W and Freil A, 2008. Avoiding cross-contamination (Part 1). Technics. Kraftfutter / Feed magazine 7-8/2008.

CHAPTER 5

Risk of cross-contamination due to the use of antimicrobial medicated feed throughout the trail of feed from the feed mill to the farm

1. Abstract

The cross-contamination of non-medicated feed with residues of antimicrobials causes an animal and public health concern associated with the potential for selection and dissemination of resistance in commensal bacteria and potentially zoonotic bacteria. To identify the extent of this situation, we built a risk model that provides a way to estimate the percentage of cross-contaminated feed in total and at different levels where cross-contamination may occur (i.e. the feed mill, the transport truck, the farm), depending on the level of antimicrobial medicated feed produced in a country per year. The model, estimated that, when antimicrobial medicated feed represents a hypothetical x_i=2% of the total feed produced in a country per year, then 5.5% (95%CI 3.4% ; 11.4%) of the total feed produced in a year could be cross-contaminated with different levels of antimicrobials due to practices related to medicated feed. In detail, 1.80% (95%CI 0.2% ; 7.7%) of the total feed produced in such a country would be cross-contaminated due to antimicrobial carry-over occurring at the feed mill level, 1.83% (95%CI 1.3% ; 2.0%) at the transport truck level and 1.84% (95%CI 1.2% ; 2.0%) at the farm level. The model also demonstrated that, even in cases where antimicrobial medicated feed would be produced in end-of-line mixers or fine dosing system on trucks, the risk of cross-contamination would not be negligible; the percentage of cross-contaminated feed produced in a country (where x_i=2%) per year would be 3.7% (95%CI 2.9% ; 4.0%) and 2.4% (95%CI 1.6% ; 2.7%), respectively. Therefore, the risk, being the result of factors occurring at different levels, is hard to be reduced to zero and the use of antimicrobial medicated feed should be avoided as much as possible to reduce selection pressure.

2. Introduction

The transfer of traces of an active substance contained in a medicated feed (e.g. antimicrobials or anti-parasites) to a non-medicated feed is referred to as "carry-over". The carry-over of any unintended substance in feed can result in its "cross-contamination" (European Commission 2014a). The batches usually cross-contaminated are the ones obtained just after production of a medicated feed that rightfully contains the substance (Borras et al. 2011). Contamination may occur in one piece of equipment, or it may result from a combination of residues throughout the entire system of mixing, manipulation and storage operations at the feed mill. As a consequence, cross-contamination is not a risk concerning the feed production solely, since it can also occur during the transport and delivery of the feed and its storage and distribution at the farm.

The occurrence and levels of cross-contamination can be affected by various factors including human error, production practices (e.g. under-filling of the mixer), handling procedures and properties of the compounds (e.g. adhesive strength, particle size, electrostatic properties) (McEvoy et al. 2002; Strauch & Freil 2006a, 2006b, 2008a, 2008b). For instance, antimicrobials in powder form make it more difficult to purge the equipment at the feed mill, the truck and the farm, between batches. Fast production lines, deficiencies in plant layout and worn equipment parts (especially lifting screws and elevators) may also cause residual quantities of medicated feed to be retained at various points along the production line (Vukmirovic et al. 2010; European Commission 2014a).

The consequent consumption of cross-contaminated batches may lead to presence of residues in the animals fed (Segato et al. 2011), which can possibly be transferred to animal products (Leeman et al. 2007). In Belgium 99.6% of antimicrobial premixes are produced for pigs (BelVet-SAC 2014). Smaller percentages of antimicrobial medicated feed are also administered to poultry and rabbits (e.g. 0.4% in Belgium (BelVet-SAC 2014)), depending on the country (European Commission 2010),. Zuidema et al. (2012) estimated that once a year 11% of piglets, 38% of pigs (<50kg) and 100% of lactating sows used to be exposed to cross-contaminated flushing batches in the Netherlands, before the voluntary

cessation of medicated feed production in 2011. These percentages represent a minimum estimation, since they only consider the cross-contamination occurring during manufacturing of the feed.

The cross-contamination of supposedly non-medicated feed with residues of antimicrobial compounds causes an animal and public health concern associated with the potential for selection and dissemination of resistance in commensal bacteria (e.g. *Enterobacteriaceae*, *Enterococcus spp*) and potentially zoonotic bacteria (e.g*. Campylobacter spp*) (Berends et al. 2001; MARAN 2010; European Commission 2014a). The antimicrobial compounds mostly used for the preparation of medicated feed in the European Union (EU) belong to the group of sulphonamides, penicillins and tetracyclines, which are considered essential against specific infections, with no sufficient therapeutic alternatives and that are listed as veterinary critically important antimicrobial agents (VCIA) by the OIE (OIE 2014). Tetracyclines are further listed as highly important antimicrobials for human medicine by the WHO (WHO 2011). Besides resistance selection, cross-contamination may also cause the presence of antimicrobial residues in the meat. If present in the meat and following processing, cooking and digestion, antibacterial residues are in general expected to be at a too low concentration for posing adverse effects to human health (Baptista et al. 2010). However, few reported cases indicate that highly sensitive individuals might present allergic reactions to penicillin residues in meat (Dewdney et al. 1991).

Taking these risks into consideration as well as the omissions of the current Council Directive (90/167/EEC) on manufacture, commercialization and use of medicated feed, the European Commission (EC) has recently proposed its revision. With the proposed Regulation, EU wide residue limits for veterinary medicines in ordinary feed will be established at a limit to avoid the development of antimicrobial resistance (European Commission 2014a, 2004b). Where no specific carry-over limits have been set for an antimicrobial active substance, 1% of the active substance is suggested as the maximum limit allowed in the last batch of medicated feed or of intermediate product produced before the production of non-target feed (European Commission 2014a). It is stated that the new

Regulation shall also apply to the transport of feed, with no further details provided, while no mention has been made of the cross-contamination occurring at the farm level.

The need for modernization of medicated feed production and related practices (e.g. manufacture quality control, storage, transport) is further emphasized by the high sales of antimicrobial premixes in the EU (EMA 2014; JIACRA 2015). According to the most recent European Surveillance of Veterinary Antimicrobial Consumption report (EMA 2014), antimicrobial premixes were the biggest-selling antimicrobial veterinary medicinal product (VMP) for food-producing animals in the EU, accounting for 35.5% of the overall sales (mg/PCU) in 2012. Yet, the situation varies substantially between countries. In Belgium for example, antimicrobial premixes represented 19.6% of the overall sales (mg/kg of biomass) of antimicrobial VMP in 2014, after a reduction of 8.7% had occurred since 2011 (BelVet-SAC 2015).

There is very limited to no quantitative information available in the literature on the estimated impact of the production, delivery and storage of antimicrobial medicated feed on cross-contamination. Therefore, and given the risks associated with antimicrobial residues in animal feed (Borras et al. 2011; European Commission 2014a), there is a need to quantitatively assess this impact. Aiming at this, the study examined all possible pathways of exposure of animals to feed which is cross-contaminated with traces of antimicrobials from previously produced, transported and stored medicated feed, in order to estimate the percentage of cross-contaminated feed produced per year. Based on the Belgian case and focusing on pig feed, the study attempted to build an approach applicable to a broader extent of cases (e.g. other countries, other species).

3. Materials and methods

3.1. Hazard identification

Traces of antimicrobials may be incorporated into batches of non-medicated feed during their production, transport, unloading and/or storage at the farms, before being fed to animals. Therefore, these potentially cross-contaminated batches of non-medicated feed, which are produced, delivered

and/or stored directly after batches of medicated feed, were considered as the hazard in this study. The model attempted to estimate the percentage of this cross-contaminated feed in the form of batches, in the total feed produced in a country per year.

3.2. Model description

The potential carry-over of antimicrobial traces from the firstly produced medicated feed to the following produced non-medicated feed was assessed along the complete production chain from the feed mill over the transport truck, and the storage and distribution at the farm level (Figure 1). The model was therefore subdivided in three modules, one for each level (i.e. the feed mill, the transport truck and the farm) and five exposure pathways were considered: the pathway 1, referring to cross-contamination occurring at the feed mill; pathways 2 and 3, referring to cross-contamination related to the transportation and unloading of the feed; and pathways 4 and 5, describing the potential cross-contamination in the farm silo and during the distribution of the feed, respectively (Figure 1).

We assumed that the antimicrobial medicated feed represents a hypothetical level of 40% of the medicated feed produced (60% antiparasitic) and 2% of the total animal feed produced in a country per year. Regarding the antimicrobials used as medicated feed premixes, it was assumed that a 50% of their total amount belongs to the sulfonamides, 25% to the penicillins, 15% to the tetracyclines and 10% to other groups (e.g. macrolides, polymyxines). These numbers are roughly based on the Belgian reality in 2014 (BFA pers.comm.; BelVetSac 2014). The model provides a way to estimate the percentage of cross-contaminated feed in total and per module, for different levels of antimicrobial medicated feed produced in a country per year.
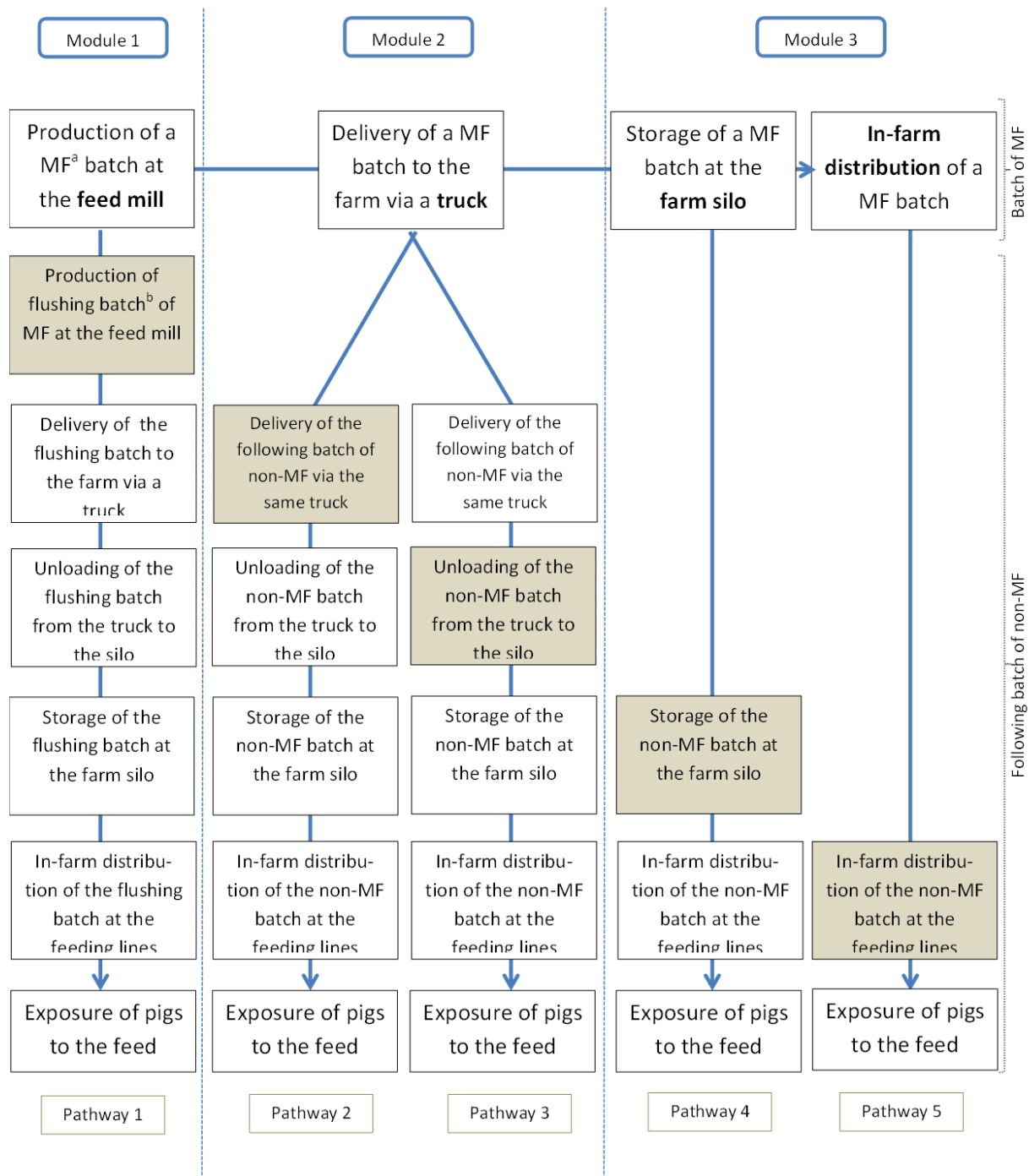
Figure 1. Flowchart of the model: (a) MF stands for medicated feed; (b) although usually three flushing batches are produced after MF, the model took into consideration only the first flushing batches, since they can contain considerably higher levels of antimicrobial residues than the second and the third. The grey blocks indicate the risky point of cross-contamination at each pathway separately. Of course, cross-contamination of a feed batch can occur at more than one points.

3.1. Cross-contamination at the feed mill

Within a feed mill, different compound feeds can be manufactured in the same production line and pass through the main mixer. In these cases, the potential cross-contamination can occur at one or more different points throughout the production line, such as the main mixer, the surge bin, the bucket elevator, the holding bins, the pellet mill, the pellet cooler and the holding bins before loading onto the delivery trucks. In order to clean out the mixing and conveying systems, batches of plain feed (usually three) are produced directly after the production of medicated feed (Stolker et al. 2013). These so-called flushing batches, which may be cross-contaminated to a certain level, are then transported to farms, stored and fed to pigs (BFA 2013) (pathway 1).

In this first module, the weight of cross-contaminated feed to which pigs could be exposed to in a year through pathway 1 (feed mill) was estimated using data published by Stolker et al. (2013), for a 2% level of antimicrobial medicated feed produced in a country per year (Equation 2). Stolker et al. (2013) have published data on the occurrence and the levels of antimicrobial carry-over in flushing batches. The data was retrieved from 21 feed mills visited, after the collection and analysis of samples from 140 first-flushing feed batches and 29 second- and third- flushing batches. The model presented here took into account only the first flushing batches, since, according to the study, their cross-contamination was much more frequent and high in antimicrobial (AB) levels. The mean probability of cross-contamination of a flushing batch at the feed mill (p_1), assumed to equal that of a first flushing batch, was estimated as 0.86 (95% CI 0.73; 0.95), with the AB concentrations ranging from 0.1 to 154 mg/kg (Table 1).

Assuming that one flushing batch is produced after each batch of medicated feed (MF), the number of cross-contaminated flushing batches (i.e non-MF batches) produced in a country i per year via pathway 1 (N_{1i}) was estimated as:

$$N_{1i} = \text{RiskBin} (N_{ai}, p_1), \quad \text{Equation (1)}$$

where N_{ai} is the number of flushing batches produced in a country i per year and p_1 is the probability of cross-contamination of a flushing batch at the feed mill.

The weight of cross-contaminated flushing batches (i.e. non-MF batches) produced in a country i per year (W_{li}) was estimated using the following formula:

$$W_{li} = N_{li} * w_{fl}, \text{Equation (2)}$$

where and w_{fl} is the weight of one flushing batch.

Table 1. Detailed summary of the first module (pathway 1) of the exposure model: variables, probability calculation of the input parameters, equations and main sources.

Process	Variable	Description	Probability calculation	Reference
Feed mill: production of a flushing batch[a] (i.e. non-AB MF[b] batch) after the production of an AB MF batch	p_1	Probability of cross-contamination of a flushing batch at the feed mill	~beta [d]	Stolker et al. 2013
		Weight of one flushing batch (tn[c])	~discrete [e] (0.5, 2, 4)	BFA (pers.comm)
		Weight of one batch of AB MF (tn)	~discrete [f] (1, 4)	BFA (pers.comm.)
		Weight of the total feed produced in a country per year (tn)	e.g. 6,500,000 tn for BE [g]	BFA (pers.comm.)
		Level of AB MF produced in a country per year (%)	e.g. 2% for BE	BFA (pers.comm.)
	N_{ai}	Number of flushing batches produced in a country per year [h]		
	N_{li}	Number of cross-contaminated flushing batches produced in a country per year	~binomial [i] (N_{ai}, p_1)	
	W_{li}	Weight of cross-contaminated flushing batches produced in a country per year (tn)	= N_{li} *	

Note: a. AB MF stands for antimicrobial medicated feed; b. the model considered only first flushing batches of feed produced; c. 1tn= 1,000 kg ; d. the beta distribution describes the uncertainty about p1, given a number of first flushing batches being tested with a number of them found cross-contaminated; e. the discrete distribution describes wfl that can take one of

several explicit discrete values (most likely the value of 2tn); f. the discrete distribution describes wmf that can take one of several explicit discrete values (most likely the value of 4tn); g. BE stands for Belgium; h. assumed to equal the number of MF batches produced in a country per year; i. the binomial distribution shows the number of cross-contaminated flushing batches produced (N_{li}) where there is a probability p1 of cross-contamination of a flushing batch.

3.3. Cross-contamination during the transport and unloading of the feed

It is generally accepted that this type of carry-over only concerns the first batch transported immediately after medicated feed and may occur following two distinguished pathways (Putier (Tecaliman) pers. comm.). Cross-contamination may take place in the bin of the delivery truck during successive loading of feed into the same bin (intra-bin contamination) (pathway 2). It may also take place in the transfer system, when traces of previously transported medicated feed remain in the conveyor screws and cross-contaminate the non-medicated feed (non-MF) subsequently delivered to a farm (inter-bin contamination) (pathway 3) (Putier 2010).

In this second module, data described by Putier (2010) were used. In the study, batches of non-medicated feed transported right after the transport of medicated feed were tested for antimicrobial residues under worst-case test conditions (e.g. longest transfer circuit in the truck, other bins empty to reduce effectiveness of the air blow cleaning systems). Samples were taken from nine different types of delivery trucks and showed that the levels of carry-over were lower in shorter and modern trucks, compared to longer and older ones. It is suggested that the intra-bin contamination (pathway 2) concerns the first 25-50 kg of non-medicated feed placed in the truck (Putier (Tecaliman) pers. comm.). It is also claimed that it is usually 20-30 kg of feed (Putier 2010)) that remain in the conveyor screws of the trucks and persist during the subsequent delivery posing a risk for potential inter-bin contamination (pathway 3).

According to Putier' s (2010) experiment, traces of antimicrobials were detected in almost all trucks tested. The mean probability of cross-contamination of a non-MF batch was estimated as $p_2 = 0.83$ (95% CI 0.25 ; 0.99) via pathway 2, and $p_3 = 0.91$ (95% CI 0.4 ; 1) via pathway 3. In terms of antimicrobial concentrations, inter-bin contamination (i.e. between bins) appears to be higher (levels of

contaminations ranged between 0.04% and 1.41%) than intra-bin contamination (i.e. inside the bins) (levels of contaminations ranged between 0.00% and 0.44%).

For this model exercise it was hypothesized that after each batch of medicated feed a batch of non-medicated feed is transported via the same truck. Therefore, the number of cross-contaminated non-MF batches produced in a country i per year via pathway 2 (N_{2i}) was calculated as:

$$N_{2i} = \text{RiskBin} (N_{bi}, p_2), \text{Equation (3)}$$

where N_{bi} is the number of non-MF batches produced and delivered directly after a MF batch in a country i per year, assumed to equal to the number of flushing batches produced per year (N_{ai}); p_2 is the probability of cross-contamination of a non-MF batch via pathway 2 (Table 2).

The number of cross-contaminated non-MF batches produced in a country i per year via pathway 3 (N_{3i}) was estimated as:

$$N_{3i} = \text{RiskBin} (N_{bi}, p_3), \text{Equation (4)}$$

where N_{bi} is the number of non-MF batches produced and unloaded directly after a MF batch in a country i per year, assumed to equal to the number of flushing batches produced per year (N_{ai}); p_3 is the probability of cross-contamination of a non-MF batch via pathway 3 (Table 2).

The weight of cross-contaminated feed that pigs could be exposed to in a year via pathways 2 (W_{2i}) and 3 (W_{3i}) was calculated based on the following formulas (Equations 5, 6):

$$W_{2i} = N_{2i} * w_{n\text{-}mf}, \text{Equation (5)}$$

$$W_{3i} = N_{3i} * w_{n\text{-}mf}, \text{Equation (6)}$$

where $w_{n\text{-}mf}$ is the weight of a non-MF batch, assumed to equal the weight of a MF batch (w_{mf}) (Table 2).

Given that pathways 2 and 3 may occur simultaneously, the following assumed probabilities of occurrence were considered in order to estimate the weight of cross-contaminated batches via module

2 (W_{IIi}) as a whole: the probability of only intra-bin cross-contamination occurring (p_I), where $p_I = 0.30$; the probability of only inter-bin cross-contamination occurring (p_{II}), where $p_{II} = 0.30$; and the probability of both intra- and inter-bin cross-contamination occurring simultaneously (p_{III}), where $p_{III} = 0.40$. Consequently, W_{IIi} was calculated as follows:

$$W_{IIi} = (W_{2i} * p_I) + (W_{3i} * p_{II}) + (W_{(2,3)i} * p_{III}), \text{ Equation (7)}$$

The weight of cross-contaminated batches of non-MF produced in a country i per year when intra- and inter- bin contaminations occur simultaneously ($W_{(2,3)i}$) was estimated as:

$$W_{(2,3)i} = \text{RiskBin}(N_{bi}, p_2 * p_{3|2}) * w_{n-mf}, \text{ Equation (8)}$$

where $p_{3|2}$ is the conditional probability of cross-contamination of a batch of n-AB MF due to pathway 3 given that pathway 2 is occurring (Table 2).

Table 2. Detailed summary of the second module (pathways 2 and 3) of the exposure model: variables, probability calculation of the input parameters, equations and main sources.

Process	Variable	Description	Probability calculation	Reference
Transport truck: delivery of batches of n-AB MF[a]) directly after the delivery of AB MF in the same truck	p_2	Probability of cross-contamination of a batch of n-AB MF, due to feed remaining in the bin of the truck	~beta	Putier 2010
	p_3	Probability of cross-contamination of a batch of n-AB MF, due to feed remaining in the conveyor screws of the truck	~beta	Putier 2010
	$p_{3|2}$	Probability of cross-contamination of a batch of n-AB MF due to pathway 3 given that pathway 2 is occurring	~beta	Assumed
	w_{n-mf} [b]	Weight of a batch of n-AB MF (tn)	$= w_{mf}$	Table 1
	N_{bi}	Number of batches of n-AB MF delivered directly after a batch of AB MF	$= N_{ai}$	Table 1
	N_{2i}	Number of intra-bin cross-contaminated batches of n-AB MF produced in a country per year	~binomial (N_{bi}, p_2)	
	N_{3i}	Number of inter-bin cross-contaminated batches of n-AB MF produced in a country per year	~binomial (N_{bi}, p_3)	

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$N_{(2,3)i}$	Number of cross-contaminated batches of n-AB MF produced in a country per year, when pathways 2 and 3 occur simultaneously	~binomial $(N_{bi}, p_2 * p_{3|2})$
W_{2i}	Weight of intra-bin cross-contaminated batches of n-AB MF produced in a country per year due to feed delivery (pathway 2)(tn)	$= N_{2i} * w_{n\text{-}mf}$
W_{3i}	Weight of inter-bin cross-contaminated batches of n-AB MF produced in BE per year due to feed delivery (pathway 3)(tn)	$= N_{3i} * w_{n\text{-}mf}$
$W_{(2,3)i}$	Weight of cross-contaminated batches of n-AB MF produced in a country per year due to feed delivery, when intra- and inter- bin contamination occur simultaneously (tn)	$= N_{(2,3)i} * w_{n\text{-}mf}$
W_{IIi}	Total weight of cross-contaminated batches of n-AB MF produced in BE per year due to feed delivery (module 2)(tn)	$= (W_{2i} * 0.3) +$ $+(W_{3i} * 0.3) +$ $+(W_{(2,3)i} * 0.4)^d$

Note: a. n-AB MF stands for non-antimicrobial medicated feed; b. assumed to equal the weight of a MF batch (w_{mf}); c. 1tn= 1,000 kg; d. assumed p_I= 0.30 for only intra-bin contamination occurring, p_{II}= 0.30 for only inter-bin contamination occurring, p_{III}= 0.40 for both types of contamination occurring simultaneously.

3.4. Cross-contamination at the farm

Cross-contamination is also possible at the farm level, where 'carry-over' of antimicrobial traces can occur at the farm silo, when non-medicated feed is stored subsequently to medicated feed (pathway 4). Cross-contamination may also occur during the distribution of the feed in the farm (pathway 5).

Since no data were found on the carry-over potentially occurring at the storage facilities or means of distribution used for feed at the farm, it was assumed to approach pathway 4 (storage at the farm silo) likewise to pathway 2, and pathway 5 (in-farm distribution of feed) to pathway 3.

Similarly to module 2, it was assumed for the purposes of this model that a batch of non-medicated feed is stored and in-farm distributed after each batch of medicated feed in the same silo and using

the same equipment, respectively. The number of cross-contaminated non-MF batches produced in a country i per year via pathway 4 (N_{4i}) was calculated as:

$$N_{4i} = \text{RiskBin} (N_{ci}, p_4), \text{Equation (9)}$$

where N_{ci} is the number of non-MF batches produced and stored at the farms after a batch of MF in a country i per year, assumed to equal N_{ai}; p_4 is the probability of cross-contamination of a non-MF batch via pathway 4 (Table 3).

The number of cross-contaminated non-MF batches produced in a country i per year via pathway 5 (N_{5i}) can be estimated as follows:

$$N_{5i} = \text{RiskBin} (N_{ci}, p_5), \text{Equation (10)}$$

where N_{ci} is the number of non-MF batches produced and distributed within the herd after a batch of MF in a country i per year, assumed to equal N_{ai}; p_5 is the probability of cross-contamination of a non-MF batch via pathway 5 (Table 3).

The weight of cross-contaminated feed that pigs could be exposed to in a year via pathways 4 (W_{4i}) and 5 (W_{5i}) was calculated based on the following formulas (Equations 11, 12):

$$W_{4i} = N_{4i} * w_{n\text{-}mf}, \text{Equation (11)}$$

$$W_{5i} = N_{5i} * w_{n\text{-}mf}, \text{Equation (12)}$$

where $w_{n\text{-}mf}$ is the weight of a non-MF batch, assumed to equal the weight of a MF batch (w_{mf}) (Table 3).

At the farm level (module 3 as a whole), it is logical to expect that, in the majority of cases, the cross-contamination potentially occurring during the distribution of feed concerns the same batches previously stored. Since this is not always the case though – as the pathways concern different equipment- the following assumed probabilities of occurrence were considered: the probability of cross-contamination occurring only during storage at the farm silo (p_a), where $p_a = 0.25$; the probability

of cross-contamination occurring only during the feed distribution within the farm (p_b), where $p_b = 0.25$; and the probability of both types of cross-contamination occurring simultaneously (p_c), where $p_c = 0.5$. Consequently, W_{IIIi} was calculated as follows:

$$W_{IIIi} = (W_{4i} * p_a) + (W_{5i} * p_b) + (W_{(4,5)i} * p_c), \text{ Equation (13)}$$

The weight of cross-contaminated batches of non-MF produced in a country i per year when both types of cross-contamination at the farm level occur simultaneously ($W_{(4,5)i}$) was estimated as:

$$W_{(4,5)i} = \text{RiskBin} (N_{ci}, p_4 * p_{5|4}) * w_{n\text{-}mf}, \text{ Equation (14)}$$

where $p_{5|4}$ is the conditional probability of cross-contamination of a batch of n-AB MF due to pathway 5 given that pathway 4 is occurring (Table 3).

Table 3. Detailed summary of the third module (pathways 4 and 5) of the exposure model: variables, probability calculation of the input parameters, equations and main sources.

Process	Variable	Description	Probability calculation	Reference
Farm: storage and in-farm distribution of batches of n-AB MF[a]) directly after the delivery of AB MF, in the same silo and using the same equip-ment	p_4 [b]	Probability of cross-contamination of a batch of n-AB MF, due to its storage in the farm silo directly after a AB MF batch	~beta	Table 2
	p_5 [b]	Probability of cross-contamination of a batch of n-AB MF, due to its in-farm distribution directly after a AB MF batch	~beta	Table 2
	$p_{5|4}$	Probability of cross-contamination of a batch of n-AB MF due to pathway 5 given that pathway 4 is occurring	~beta	Assumed
	$w_{n\text{-}mf}$	Weight of a batch of n-MF (tn[c])	$= w_{mf}$	Table 1
	N_{ci}	Number of batches of n-AB MF stored at the farm, and later distributed, directly after a batch of AB MF	$= N_{ai}$	Table 1
	N_{4i}	Number of cross-contaminated batches of n-AB MF produced in a country per year due to storage in the farm silo	~binomial (N_{ci}, p_4)	
	N_{5i}	Number of cross-contaminated batches of n-AB MF produced in a country per year due to their in-farm distribution	~binomial (N_{ci}, p_5)	

$N_{(4,5)i}$	Number of cross-contaminated batches of n-AB MF produced in a country per year, when pathways 4 and 5 occur simultaneously	~binomial $(N_{ci}, p_4 * p_{5\|4})$
W_{4i}	Weight of cross-contaminated batches of n-AB MF produced in a country per year due to pathway 4 (tn)	$= N_{4i} * w_{n\text{-}mf}$
W_{5i}	Weight of inter-bin cross-contaminated batches of n-AB MF produced in BE per year due to pathway 5 (tn)	$= N_{5i} * w_{n\text{-}mf}$
$W_{(4,5)i}$	Weight of cross-contaminated batches of n-AB MF produced in a country per year at the farm level, when pathways 4 and 5 occur simultaneously (tn)	$= N_{(4,5)i} * w_{n\text{-}mf}$
W_{IIIi}	Total weight of cross-contaminated batches of n-AB MF produced in BE per year at the farm level (module 3) (tn)	$= (W_{4i} * 0.25) +$ $+(W_{5i} * 0.25) +$ $+(W_{(4,5)i} * 0.5)^d$

Note: a. n-AB MF= non-antimicrobial medicated feed; b. in the absence of data concerning the cross-contamination occurring at the farm, it was assumed that $p_4 = p_2$ and $p_5 = p_3$ (Table 2); c. 1tn= 1,000 kg; d. assumed $p_a = 0.25$ of only pathway 4 occurring, $p_b = 0.25$ for pathway 5 occurring, $p_c = 0.5$ for both types of contamination occurring simultaneously.

3.5. Model implementation

The model was built using @Risk® software (Palisade Corporation®) and was run at 10,000 iterations per simulation. The weight of cross-contaminated batches of feed that pigs are exposed to in a year in a country i (W_{Ti}), under the described circumstances, was calculated in total, based on the estimations for each module:

$$W_{Ti} = W_{Ii} + W_{IIi} + W_{IIIi}, \text{ Equation (15)}$$

Where W_{Ii} the weight of cross-contaminated batches produced in a country i per year at the feed mill level, W_{IIi} the weight of cross-contaminated batches produced in a country i per year at the truck level and W_{IIIi} the weight of cross-contaminated batches produced in a country i per year at the farm level.

Based on our initial assumptions, 50% * W_{Ti} of the total feed produced in a country is expected to contain residues of sulfonamides, 25% * W_{Ti} penicillins and 15% * W_{Ti} tetracyclines.

Subsequently, the percentage of cross-contaminated feed produced in a country i per year was calculated as follows:

$$C_{1i} = W_{Ti} / T_i * 100, \text{ Equation (16)}$$

Where T_i is the weight of the total feed produced in a country per year.

The same formula was also used to estimate the percentage of cross-contaminated feed due to each module separately, by replacing W_{Ti} with W_{Ii}, W_{IIi} or W_{IIIi}.

The relative percentages of cross-contaminated feed attributed to each module y were also estimated using the following formula:

$$C_{yi} = W_{yi} / W_{Ti} * 100, \text{ Equation (17)}$$

Along with a 'worst-case' scenario, comprising the occurrence of all possible pathways, two 'what-if' scenarios were considered. They were generated based on the fact that in some cases (e.g. in Belgium) the antimicrobial premixes used for the production of medicated feed are no longer added and mixed in the main mixer, but rather in the end-of-line mixer or a mobile mixer fixed on the truck (i.e. fine dosing system (FDS)) (BFA 2013).

The first scenario considered the occurrence of cross-contamination through the transport-related and farm storage- and distribution- related pathways, when medicated feed is only produced in a dedicated end-of-line mixer, and by doing so cross-contamination at the feed mill is avoided. In this case, the following formula was used to estimate the percentage of cross-contamination of batches of feed in the total weight of feed produced per year (C_{2i}):

$$C_{2i} = (W_{IIi} + W_{IIIi})/T_i * 100, \text{ Equation (18)}$$

The second scenario explored the effect of using FDS trucks on cross-contamination levels. Using the FDS trucks, the cross-contamination at the feed mill (pathway 1) and inside the bin of the truck

(pathway 2) is averted and the following formula could be then used to estimate the percentage of cross-contamination of batches of feed in the total weight of feed produced per year (C_3):

$$C_{3i} = [(W_{3i} * p_{II}) + W_{IIIi}]/T_i * 100, \text{ Equation (19)}$$

where W_{3i} is the weight of cross-contaminated batches produced in a country i per year via pathway 3 (inter-bin cross-contamination) and p_{II} is the probability of only pathway 3 occurring ($p_{II} = 0.30$). The aforementioned variables and equations are summarized in Table 4.

Table 4. Detailed summary of the variables and equations considered, by taking into account all modules and different scenarios.

Variable	Description	Formula[a]
W_{Ti}	Total weight of cross-contaminated batches of non-medicated feed produced in a country i per year (tn[b])	$= W_{Ii} + W_{IIi} + W_{IIIi}$
W_{ABi}	Total weight of batches of non-medicated feed produced in a country i per year, cross-contaminated with:	
	Sulfonamides (tn)	$= 50\% * W_{Ti}$
	Penicillins (tn)	$= 25\% * W_{Ti}$
	Tetracyclines (tn)	$= 15\% * W_{Ti}$
C_{1i}	Percentage of cross-contaminated feed in the total of feed produced in a country i per year [c]	$= (W_{Ti}/T_i)*100$ [d]
C_y	Percentage of cross-contaminated feed in the total of cross-contaminated feed produced in a country i per year, per module y	$= (W_y/W_{Ti})*100$
C_{2i}	Percentage of cross-contaminated feed in the total of feed produced in a country i per year for Scenario 2 (end-of-line mixer)	$= (W_{IIi}+W_{IIIi})/T_i*100$
C_{3i}	Percentage of cross-contaminated feed in the total of feed produced in a country i per year for Scenario 3 (FDS truck)	$= [(W_{3i}*p_{II})+W_{IIIi}]/T_i*100$

Note: a. For details on the variables of the formulas see previous tables; b. 1tn= 1,000 kg; c. feed in the form of batches; d. the same formula can be used to estimate this percentage per module, by replacing W_{Ti} with the respective weight.

4. Results

Figure 2 gives an overview of the estimated percentage s of cross-contaminated feed produced in a country i per year, in total and per module, against the total feed and the total cross-contaminated feed produced. This model estimated that, given our assumptions, when x_i=2% of the feed produced in a country per year is antimicrobial medicated feed, C_{1i}= 5.5% (95%CI 3.4% ; 11.4%) of the total feed produced in a year (T_{1i}) is cross-contaminated with different levels of antimicrobials due to practices related to medicated feed, with a high uncertainty. In detail, 1.80% (95%CI 0.2%; 7.7%) of T_{1i} in such a country would be due to module 1, 1.83% (95% CI 1.3%; 2.0%) due to module 2 and 1.84% (95% CI1.2%; 2.0%) due to module 3.

The percentage of cross-contaminated feed produced in a country per year when x_i=2% was found to have a mean value of C_{2i}= 3.7% (95% CI2.9%; 4.0%) of T_{1i} when medicated feed is produced in end-of-line mixers (scenario 1) and C_{3i}= 2.4% (95% CI 1.6%; 2.7%) of T_{1i} when FDS trucks are used (scenario 2). These figures showed a 29.6% reduction in percentages of cross-contamination due to scenario 1 and a 53.9% reduction due to scenario 2, from the baseline scenario considering all pathways.

In order to indicatively provide figures on the total weight of cross-contaminated batches in total (W_{Ti}) and per module (W_{Ii}, W_{IIi}, W_{IIIi}), we considered T_{1i}= 6,500,000 tons, which equals the total feed produced in a year in Belgium. The distributions of W_{Ti}, W_{Ii}, W_{IIi} and W_{IIIi} along with their mean values are shown in figure 3.
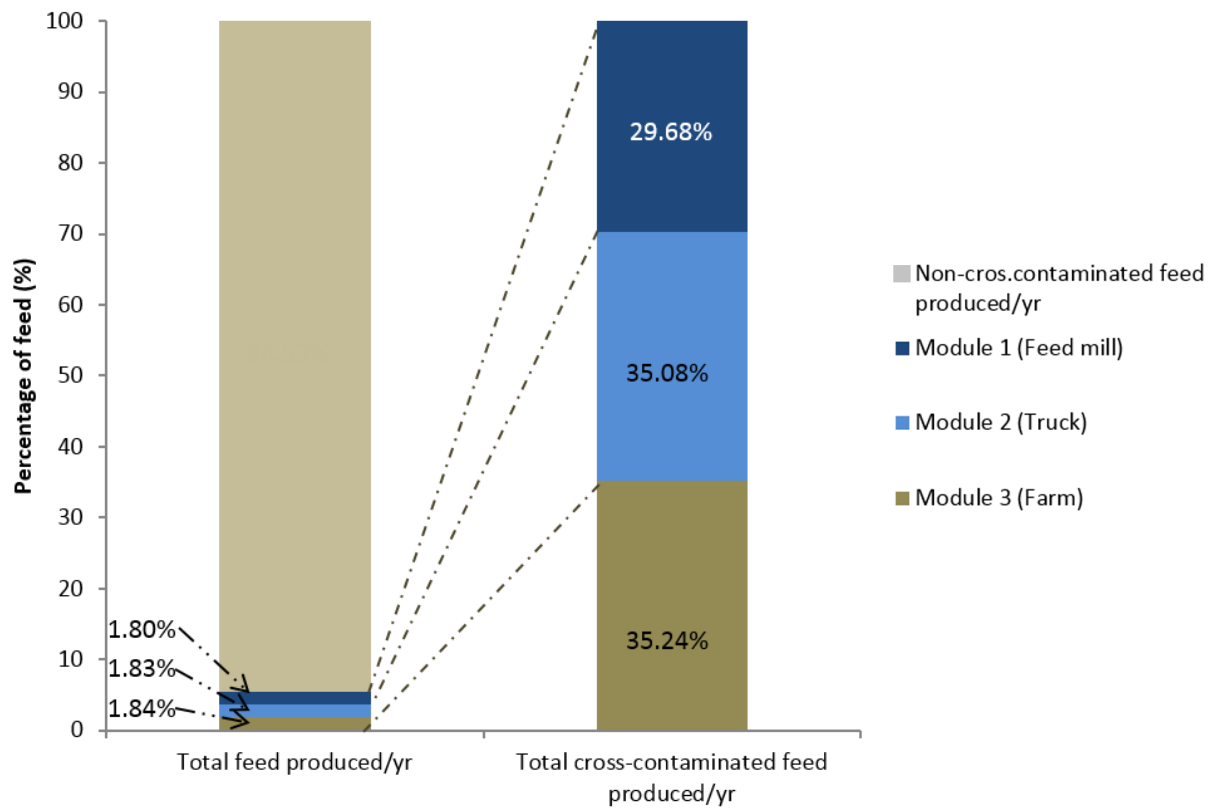
Figure 2. Percentage (%) of cross-contaminated feed in the total feed produced (Ti) produced in a country i per year and in the total of cross-contaminated feed produced in a country i per year, per module (C$_y$). Note: The mean values are rounded for presentation purposes.
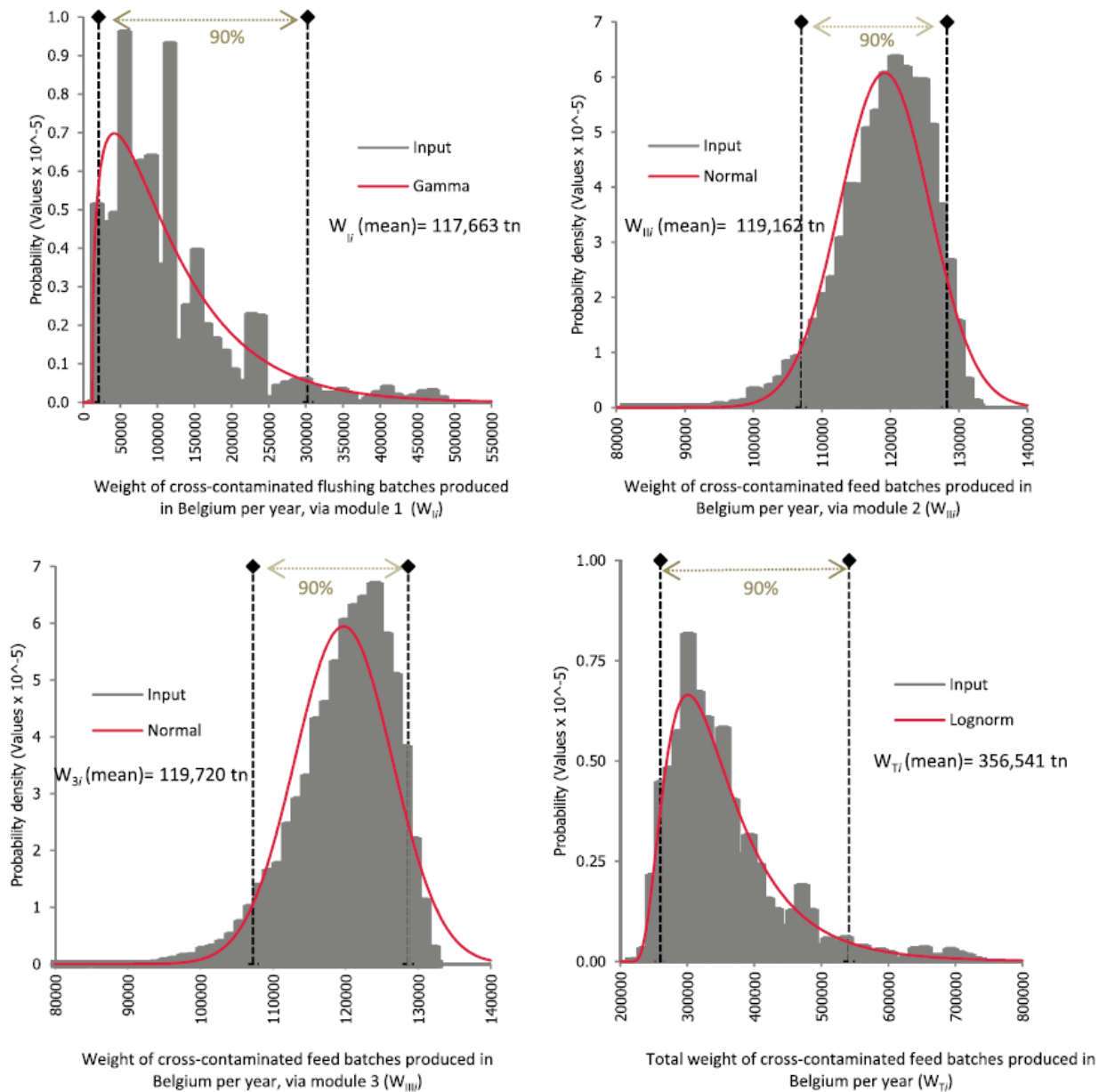
Figure 3. Distributions of the weight of cross-contaminated feed batches produced in a country i per year via module 1 (feed mill) (W_{Ii}), module 2 (W_{IIi}), module 3 (W_{IIIi}) and in total (W_{Ti}) (tons). Note: The calculations were performed using Belgian data for a level of antimicrobial medicated feed produced x_i= 2% and total feed produced in a year T_i= 6,500,000 tn.

5. Discussion

In spite of the many unknowns that did not allow the risk estimation in a more detailed way than the worst-case approach, the model showed a real risk of cross-contamination of feed due to production, distribution and storage of medicated feed. The risk, being the result of factors occurring at different

levels, is hard to be fully excluded and thus, the use of medicated feed should be avoided as much as possible. The model can be used to provide estimations for various situations (i.e. for different levels of AB MF, for different policies applied) and should then be informed with data after carefully considering the specific situation. It should be emphasized though that a high degree of uncertainty is included in the model's estimations and its results should be interpreted with caution. The main reason for the high level of uncertainty is the very limited amount of quantitative data available, since only a minimal number of published studies investigated the issue of cross-contamination of feed with antimicrobials. The studies by Stolker et al. (2013) and Putier (2010) for Tecaliman (i.e. Technical centre by and at the service of the French Feed Industry) were the only sources of data for the respective subjects, while a total absence of publications was observed on the carry-over occurring at the farms.

The model was therefore built upon a number of assumptions. The assumptions on the level of AB MF produced (i.e. 2% represents the situation in Belgium) and the levels of antimicrobials used as premixes were driven by data provided by the Belgian Compound Feed Industry Association (BFA, pers. comm.) and the European Commission (European Commission 2010). The latter assumption, concerning antimicrobials as premixes, does not represent all countries. It is the case for Belgium for instance, where approximately 50% of the antimicrobial premixes used contain sulfonamides, but at EU level the most-selling premixes contain tetracyclines. Focusing on the feed mill pathway, only the first flushing batches were considered, as they contain considerably higher levels of antimicrobial residues than the second and third. Due to lack of detailed data on the feed delivery rates, it was hypothesized that each time a batch of medicated feed is delivered to a farm, a batch of non-medicated feed is delivered right after by the same truck. Therefore, the weight of cross-contaminated batches through the delivery-related pathways might be slightly overestimated. The major assumption of the model concerns the farm-related pathways, which were likewise of the delivery-related ones (i.e. considering the same probabilities of cross-contamination), given the absence of published research. As for the probabilities of occurrence assumed to estimate the total weight of cross-contaminated batches of n-AB MF produced in Belgium per year due to feed delivery (W_{IIi})(module 2) and at the farm level (module

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3)(W_{IIIi}), they were considered to avoid an overestimation of the number of cross-contaminated batches, since at the truck and farm levels the cross-contamination via the different pathways concerns in most cases the same batches of feed. The probability of concurrent occurrence of pathways was assumed to be higher at the truck level, compared to the farm level, as in the first case, the possible contamination is related to one piece of equipment (i.e. the truck as a whole).

Another important piece of information missing, in association with the link to resistance, concerned the destination of feed. Thus it was not possible at this stage to model the species and age categories exposed to the cross-contaminated feed. Regarding feed delivery, there is no regulation in place defining the destination of batches transported right after medicated feed. Consequently, these batches can reach finisher pigs for instance. The lack of detailed data on the antimicrobial (AB) concentrations detected did not allow us to estimate the weight of cross-contaminated batches for different concentration levels (e.g. AB concentration >1%; >2.5%), and as a consequence we combined all cases with an assumed level of cross-contamination above zero. If more data become available , as part of the iterative nature of risk assessment, the respective variables of the model could be re-estimated and the result could be refined.

The model also demonstrates that a considerable risk of cross-contamination can be avoided when medicated feed is not produced in the main mixing line of feed mills, as it is the case for Belgium. An even higher reduction of risk can be achieved with the use of FDS trucks, a so far limited use due to their cost. Nevertheless, even in cases where the aforementioned scenarios would be actually implemented, the risk would not be completely removed given that sources of cross-contamination also still exist at the truck and farm levels and hence should not be overlooked.

All in all, carry-over of antimicrobials even at detection limit seems unavoidable and a rather challenging concept with regard to the technical and economic possibilities the feed industry has today. Thus, compliance with the rules of good manufacturing practice (GMP) is of outmost importance to control the phenomenon. At the feed mill, the carry-over can be reduced in different

ways targeting, for instance, the practices (i.e. flushing after medicated feed production) (Strauch & Freil 2008), the process (i.e. reducing the process length), the feed (i.e. sequencing medicated feed production) and/or the active product used (i.e. choosing non dusty products). To minimize the risk during feed delivery, the use of new trucks, the use of back bins to reduce the length of the circuit and mainly the careful purging after delivery are crucial preventive measures. Raising the awareness of the farmers and their personnel on the matter and the importance of purging is also essential to avoid any cross-contamination occurring at the farm. The use of a separate silo for medicated feed would be optimal. Yet, the ultimate and most efficacious way to avoid cross-contamination is through the reduction of the production and use of medicated feed. The recent EC proposal (2014) on medicated feed provides a prohibition on the preventive use of antimicrobials included in medicated feed, which is the main reason for group treatments. Therefore, although medicated feed has the main advantage of ensuring higher homogeneity and stability of the antimicrobial in the feed, alternative solutions (i.e. water medication and top dressing/mixing of ready-to-use veterinary medicines into feed) seem to gain ground (European Commission 2010).

Topics for further research should include the levels of cross-contamination occurring at the farm level, the potential consequences of antimicrobial carry-over in animals, as well as the possible risks of the alternatives of medicated feed.

6. Acknowledgements

7. References

Baptista FM, Alban L, Olsen AM, Petersen JP. Danish Agricultural & Food Council Copenhagen, Denmark, 2010. Epidemiological Evaluation of the residue surveillance program in Danish pigs. https://agricultureandfood.dk/.

Borràs S, Companyó R, Granados M, Guiteras J, Pérez-Vendrell AM, Brufau J, Medina M, Bosch J, 2011. Analysis of antimicrobial agents in animal feeds. Trends Analyt Chem. 30:1042–1064.

(BelVet-SAC) Belgian Veterinary Surveillance of Antibacterial Consumption, 2014. 2015 National consumption report p1-40. http://www.belvetsac.ugent.be/

(BFA) Belgian Compound Feed Industry Association, 2013. In Dutch: Convenant betreffende gemedicineerde voeders. p1-14. http://www.favv.be/

Berends BR, Van den Bogaard AEJM, van Knapen F, Snijders JMA, 2001. Human health hazards associated with the administration of antimicrobials to slaughter animals. Vet Q. 23:10–21.

Dewdney JM, Maes L, Raynaud JP, Blanc F, Scheid JP, 1991. Risk assessment of antibiotic residues of beta-lactams and macrolides in food products with regard to their immuno-allergic potential. Fd. Chem. Toxic. 29, 477-483.

(EC) European Commission, 1990. Council Directive of 26 March 1990 laying down the conditions governing the preparation, placing on the market and use of medicated feeding-stuffs in the Community (90/167/EEC). http://eur-lex.europa.eu/

(EC) European Commission, 2010. Evaluation of the EU Legislative Framework in the Field of Medicated Feed. http://ec.europa.eu/

(EC) European Commission, 2014a. Proposal for a regulation of the European Parliament and of the council on the manufacture, placing on the market and use of medicated feed and repealing Council Directive 90/167/EEC. http://ec.europa.eu/

(EC) European Commission, 2014b. Commission staff working document Executive summary of the impact assessment. Accompanying the document Proposal for a Regulation of the European Parliament and of the council on the manufacture, placing on the market and use of medicated feed and repealing Council Directive 90/167/EEC. http://eur-lex.europa.eu/

(EMA) European Medicines Agency, 2014. Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2012. Fourth ESVAC report. p1-128 http://www.ema.europa.eu/

(JIACRA) Joint Interagency Antimicrobial Consumption and Resistance Analysis Report, 2015. ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. Stockholm/Parma/London: ECDC/EFSA/EMA. EFSA Journal. 13(1):4006, 114 pp.

(MARAN) Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2008. p1-85. https://www.wur.nl

McEvoy JDG, 2002. Contamination of animal feedingstuffs as a cause of residues in food: a review of regulatory aspects, incidence and control. Anal. Chim. Acta. 473:3–26.

Leeman WR, Van den Berg KJ, Houben GF, 2007. Transfer of chemicals from feed to animal products: The use of transfer factors in risk assessment. Food Add. Cont. Part A 24:1–13.

(OIE) World Organisation for Animal Health, 2014. OIE List of antimicrobial agents of veterinary importance. p1-9. http://www.oie.int/

Putier F, 2010. Evaluation of cross-contamination in livestock feed delivery trucks. Technics. Kraftfutter / Feed magazine 9-10/2010.

Segato G, Benetti C, Angeletti R, Montesissa C, Biancotto G, 2011. Doxycycline and sulfadimethoxine transfer from cross-contaminated feed to chicken tissues. Food Add. & Cont. Part A 28:860-868.

Stolker AAM, Manti V, Zuidema T, van Egmond H, Deckers ER, Herbes R, Hooglugt J, Olde Heuvel E, de Jong J, 2013. Carry-over of veterinary drugs from medicated to non-medicated feeds in commercial feed manufacturing plants. Food Add. & Cont. Part A 30:1100-1107.

Strauch W and Freil A, 2006a. Avoiding carry-over (Part 1). Additives/ Feed magazine 4/2006.

Strauch W and Freil A, 2006b. Avoiding carry-over (Part 2). Additives. Kraftfutter / Feed magazine 5/2006.

Strauch W and Freil A, 2008a. Avoiding cross-contamination (Part 1). Technics. Kraftfutter / Feed magazine 7-8/2008.

Strauch W and Freil A, 2008b. Avoiding cross-contamination (Part 2). Technics. Kraftfutter / Feed magazine 9-10/2008.

Vukmirović D, Duragić O, Sredanović S, Lević J, Čolović R, Ivanov D, Kokić B, 2010. Determination of working accuracy as an important step for implementation of corrective measures in feed plant. Food Feed Res. 37: 7-12.

(WHO) World Health Organization, 2011. Critically Important Antimicrobials for Human Medicine. 3rd Edition. p1-38. http://www.who.int

Zuidema T, van Holthoon FL, van Egmond HJ, Hooglugt J, Bikker P, Aarts H, Olde Heuvel E, 2010. In Dutch: "Omvang en implicaties van antibiotica-versleping in mengvoeders voor varkens". RIKILT report no. 2010.005, Wageningen UR. p1-71.

CHAPTER 6

Probabilistic risk model to assess the potential for resistance selection following the use of antimicrobial medicated feed in pigs

1. Abstract

The cross-contamination of non-medicated feed with residues of antimicrobials (AM) causes a public and animal health concern associated with the potential for selection and dissemination of resistance. To analyze the associated risks, a probabilistic model was built using @Risk® (Palisade Corporation®) to show the potential extent of the effect of cross-contaminated pig feed on resistance selection. The results of the model include estimations of the proportion of pigs per production stage with residues of doxycycline, chlortetracycline, sulfadiazine and trimethoprim in their intestinal contents from caecum and colon, as a result of exposure to cross-contaminated feed with different carry-over levels, in Belgium. By using a semi-quantitative approach, these estimations were combined with experimental data on AM concentrations associated with potential for resistance selection pressure. Based on this model it is estimated that 7.76% (min=1.67; max=36.94) of sows, 4.23% (min=1.01; max=18.78) of piglets and 2.8% (min=0.51; max=14.9) of fatteners in Belgium have residues of doxycycline in their intestinal tract due to consumption of feed with at least 1% carry-over. These values were estimated to be almost triple for sulfadiazine, but substantially lower for chlortetracycline and trimethoprim. Doxycycline concentrations as low as 1 mg/L (corresponding to consumed feed with at least 1% carry-over) can select for resistant porcine commensal *E. coli in vitro* and *in vivo*. Conclusions on this risk could not be drawn for the other AM at this stage, due to lack of literature data on concentrations associated with resistance development. However, since the possibility of resistance mechanisms (*e.g.* co-selection) occurring cannot be excluded, the results of this model highlight that the use of AM medicated feed should be minimized where possible. In case of medicated feed production, good practices should be thoroughly followed at all levels of production, distribution, storage and administration, with a special focus on the feed distributed to piglets and sows.

2. Introduction

The use of antimicrobial (AM) medicated feed (MF) can lead to cross-contamination of non-medicated feed produced, transported and in-farm distributed directly after MF (Putier et al. 2010; Stolker et al. 2012). A previously developed exposure model (Filippitzi et al. 2016) estimated that, in a country, where MF represents 2% of the total feed produced in a year, a non-negligible 5.5% (95% CI = 3.4%; 11.4%) of the total feed produced in a year can contain residues of antimicrobials due to practices related to the production of medicated pig feed. This level corresponds, in the case of Belgium, to a mean weight of 356.541 tons of batches of pig feed cross-contaminated with various levels of antimicrobials. In the case of consumption of this cross-contaminated feed by pigs, their intestinal microbiota is exposed to low, sub-clinical concentration levels of antimicrobials (*e.g.* tetracycline, tylosin, sulfamethazine), which can induce selection of resistant bacteria as demonstrated *in vitro* (Gullberg et al. 2011, 2014) and *in vivo* (Brewer et al. 2013).

The levels of AM contained in the cross-contaminated batches of feed vary considerably (Zuidema et al. 2010; Putier et al. 2010; Stolker et al. 2012). According to the recent proposal for a European Commission Regulation on medicated feed, 1% of an active AM compound of the last produced batch of MF is suggested as the maximum carry-over limit allowed in the next batch produced (EC, 2014). So far, the Member States have followed different approaches. Some have no official values for tolerated carry-over, while others apply the zero tolerance principle (BFA 2013; EC 2016).

With a view to determine the intestinal fecal concentrations of chlortetracycline (CTC), doxycycline (DOX) and sulfadiazine-trimethoprim (SDZ-TRIM) after administration of cross-contaminated feed to pigs, Peeters et al. (2016) set up an *in vivo* experiment, showing that the mean concentrations of 10mg/L CTC and 4 mg/L DOX reached in the feces were higher than concentrations that have been shown to cause resistance selection. Specifically for DOX, a 3% carry-over level in the feed resulted in porcine fecal concentrations of approximately 4 mg/L. Two studies that followed from the same research group (Peeters et al. 2017, 2018) aimed to determine the effect of these residual DOX

concentrations. The conclusion was that DOX intestinal concentrations of 1 and 4 mg/L, caused by feed cross-contamination levels of approximately 1% and 3% respectively, can select for resistant porcine commensal *Escherichia coli* (*E. coli*) *in vitro* as well as *in vivo.* Even much lower concentrations of tetracycline (*i.e.* 15 ng/ml and 45 ng/ml) have been shown to select for resistance *in vitro*, based on competition experiments between resistant and susceptible strains (Gullberg et al. 2011, 2014). But also *in vivo*, Brewer et al. (2013) found that concentrations of tetracycline and sulfamethazine as low as 1 µg/mL in intestinal contents increased the frequency of transfer of resistance genes in pigs. This suggests that unintended low antimicrobial concentrations can possibly exert selective pressure on resistant bacteria present and may co-select for other resistance genes carried by these bacteria, and consequently poses a serious concern for animal and public health and calls for targeted actions.

Therefore, the first objective of this study was to build a probabilistic model that could be used to compare the extent of the exposure of piglets, sows and fattening pigs to cross-contaminated feed considering different antimicrobials and different carry-over levels (*e.g.* 1%, 3%). The second objective was to combine these with estimations on potential for resistance selection pressure, depending on the substance used. For illustration purposes, doxycycline was the substance used as example. The model was parametrized using data available in the literature and national data on feed production and antimicrobial use in Belgium.

3. Materials and Methods

3.1. Hazard identification

Traces of antimicrobials may be incorporated into batches of non-medicated feed during their production, transport, unloading and/or storage and distribution at the farms. These cross-contaminated batches of non-medicated feed are fed to pigs. The consumption of feed containing certain carry-over levels of some antimicrobials has been shown to result in residual antimicrobial concentrations in the intestinal content from caecum and colon and feces of pigs (Peeters et al. 2016), which can potentially select for resistance *in vitro* (Gullberg et al. 2011, 2014; Peeters et al. 2018) and

in vivo (Brewer et al. 2013; Peeters et al. 2017). Therefore, as the hazard in this study, were considered the antimicrobial residues contained in intestinal content of pigs which have consumed feed cross-contaminated due to practices related to the use of medicated feed.

3.2. Model design

Figure 1 shows the model framework designed in order to estimate the number and proportion of pigs per production stage, which consume cross-contaminated feed and end up containing antimicrobial residues in their intestinal contents, in a country. If the concentration of these residues is equal or higher than concentrations associated with resistance selection pressure, the model concludes that there is potential for resistance selection. Tables 1 and 2 describe the different variables of the model and the respective distributions, formulas or parameters used to estimate them. These tables represent a scenario that considers the presence of an antimicrobial in cross-contaminated batches in any possible concentration higher than zero (AM > 0; scenario A). The scenarios of 1% (scenario B) and 3% (scenario C) AM carry-over levels were also considered (Tables 3 and 4, respectively), as they lead to different concentration of residues in pigs and, therefore, to different potentials for resistance selection pressure.
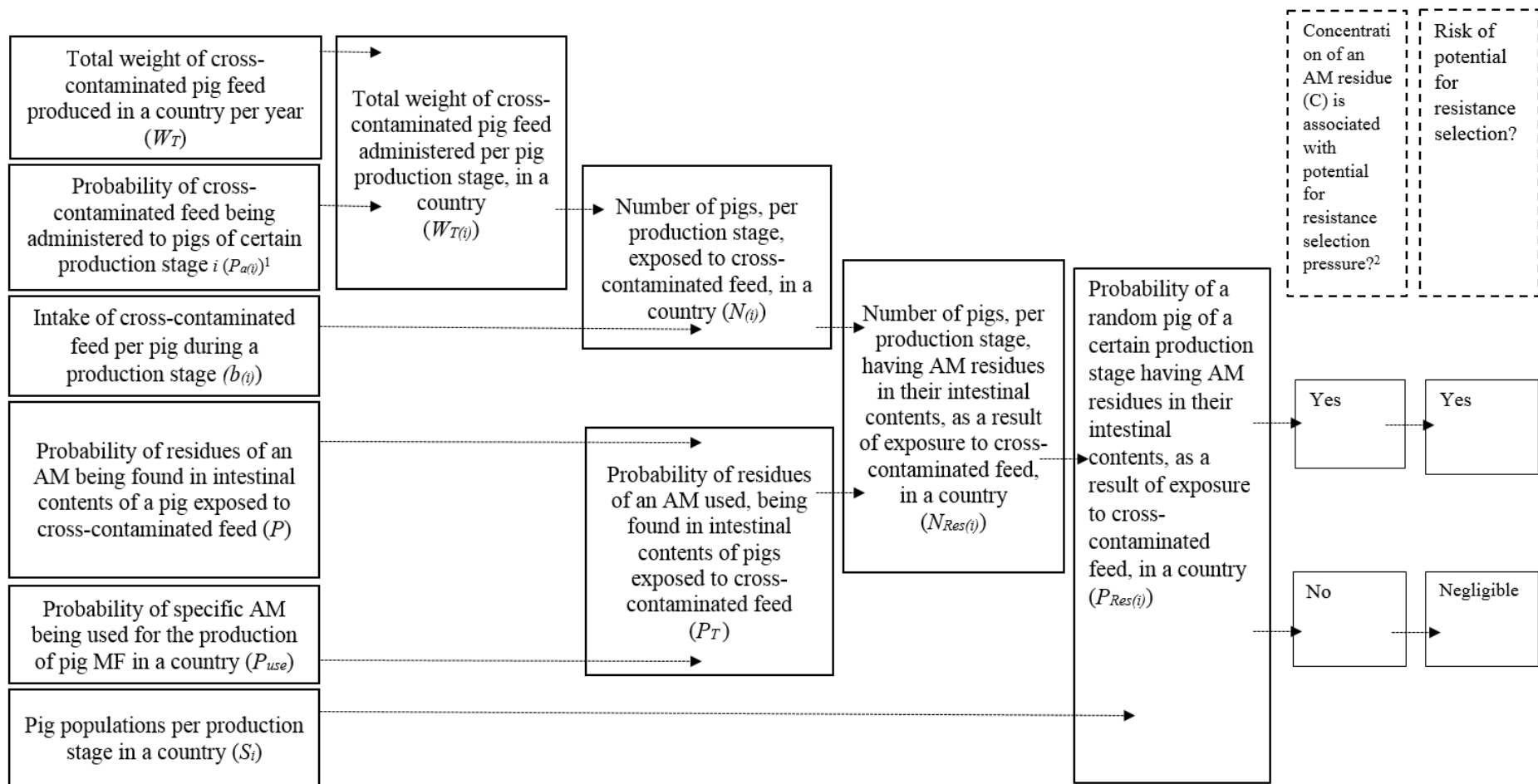
Figure 1. Model framework designed to estimate the number of pigs per production stage, which consume cross-contaminated feed and end up containing antimicrobial residues in their intestinal contents, in a country, and to whether there is potential for resistance selection. Notes: [1] For piglets, sows and fattening pigs; [2] if the concentration of these residues is equal or higher than minimum known concentrations associated with resistance selection pressure, the model concludes that there is potential for resistance selection (see Table 1).

Number of pigs, per production stage, exposed to cross-contaminated feed, in a country (N_i)

The number of pigs, per production stage, exposed to cross-contaminated feed, in a country (N_i) was estimated using the following equation (1):

$$N_{(i)} = W_{T'(i)} / b_{(i)} , \quad (1)$$

where $W_{T'(i)}$ is the total weight of cross-contaminated pig feed produced in a country *i* per year, reaching the pigs of different production stages, and $b_{(i)}$ is the intake of cross-contaminated feed per pig during a production stage *i* (Table 1).

To estimate $W_{T'(i)}$, the equation (2) that follows was used:

$$W_{T'(i)} = (W_T * P_{a(i)}) / y, \quad (2)$$

where W_T is the total weight of cross-contaminated pig feed produced in a country per year, $P_{a(i)}$ is the probability of the cross-contaminated feed W_T being administered to pigs of certain production stage *i* (*i.e.* piglets until 20kg, sows, fattening pigs) (Table 2) and *y* is the number of production rounds per production stage in a random pig farm in a country, per year.

To estimate the intake of cross-contaminated feed per pig during a certain production stage ($b_{(i)}$), the following equation was used:

$$b_{(i)} = b_d * d_a * n = (w_b * n) / n_b , \quad (3)$$

where b_d refers to the daily feed intake of a pig at different production stages, d_a refers to the days of administration of a cross-contaminated feed batch, per production stage and equals $d_a = w_b / (b_d * n_b)$, *n* is the number of deliveries of cross-contaminated feed batches to a random farm, per production stage, w_b is the weight of a cross-contaminated feed batch and n_b is the number of pigs in a batch, per production stage in a farm, in a country.

Table 2 presents in detail the estimation of $P_{a(i)}$ (*i.e.* the probability of the cross-contaminated feed W_T being administered to pigs of certain production stage *i*), which is based on the equation:

$$P_{a(i)} = W_{T(i)} / W_T , \quad (4)$$

where $W_{T(i)}$ is the total weight of cross-contaminated pig feed administered to pigs of a certain production stage i, per year and W_T is the total weight of cross-contaminated pig feed produced in a country per year. The total weight of cross-contaminated pig feed administered to pigs of a certain production stage i ($W_{T(i)}$) was calculated as:

$$W_{T(i)} = y * n * w_{(b)} * k, \qquad (5)$$

with y as the number of production rounds in a random pig farm in a country, per year, n as the number of deliveries of cross-contaminated feed batches to a random farm, per production stage, $w_{(b)}$ as the weight of a cross-contaminated feed batch and k as the number of farms with pigs of certain production stages in a country. The latter variable k was estimated based on the level of each pig farm production type in a country (*e.g.* percentage of single site farrow-to-finish farms, which have piglets, sows and fattening pigs; percentage of fattening pig farms, which grow only fattening pigs; *etc*).

For the parametrization of this model regarding W_T, we used the estimations of a previously developed risk model (Filippitzi et al. 2016), which concerned a country where antimicrobial medicated feed production represents 2% of the total feed produced per year. The results of this model were based on available data regarding the feed production in Belgium. This is also the case for the current model, which is parametrized based on: available, published (i.e. weight of total and medicated feed produced in Belgium, averages suggested by BFA (e.g. BFA, 2014) and unpublished (i.e. weight of a medicated and non-medicated feed batch) data from the Belgian Feed Association (BFA); available data from two on-going research projects on Belgian pig farms focusing on the distribution of pig production (i.e. data on number of production rounds per production stage, number of pigs per production stage) and on the use of antimicrobial treatments of pigs via feed (i.e. frequency of feed deliveries, method of oral administration of antimicrobials); and expert opinion (three researchers with expertise in porcine health management and veterinary epidemiology and two swine practitioners, based in Belgium).

Probability of residues of an antimicrobial used for pig medicated feed production, being found in intestinal contents of pigs exposed to cross-contaminated feed (P_T)

With a view to estimate the probability of residues of an antimicrobial (AM) used, to be found in intestinal contents of pigs exposed to cross-contaminated feed (P_T), the following mathematical expression was employed:

$$P_T = P * P_{use}, \quad (6)$$

where P is the probability of residues of an AM being found in intestinal contents of a pig exposed to cross-contaminated feed, while P_{use} is the probability of this AM being used for the production of pig medicated feed in a country (Table 1).

For this model, we estimated P by replacing in a Beta distribution (Table 1), data from Peeters et al. (2016) regarding the concentrations of chlortetracycline (CTC), doxycycline (DOX) and sulfadiazine-trimethoprim (SDZ-TRIM) found in feces, caecum and intestinal contents of pigs, after administration of feed containing a 3% carry-over level of these antimicrobials. In absence of other similar published experimental data and by considering prior research on bioavailabilty of antimicrobials in pigs (Riviere and Papich 2009), we assumed the same P for any other carry-over level, for the purposes of this model. This is supported by the fact that these AM undergo linear kinetics.

For the calculation of P_{use} for the aforementioned antimicrobials, we fitted a Beta distribution (Table 1) based on the Belgian national data on antimicrobials premixes used for the production of medicated pig feed, from the 2016 Belgian Veterinary Surveillance of Antibacterial Consumption report (BelVet-SAC 2017). The AM premixes used for pigs represent the 99.3% of the total amount of AM premixes used in Belgium.

Number of pigs, per production stage, having residues of an AM in their intestinal contents, as a result of exposure to cross-contaminated feed, in a country (N_{Res})

The number of pigs, per production stage, with residues of an AM being found in their intestinal contents as a result of exposure to cross-contaminated feed, in a country ($N_{Res(i)}$) can be estimated as follows:

$$N_{Res(i)} = N_{(i)} * P_T, \quad (7)$$

with $N_{(i)}$ denoting the number of pigs, per production stage i, exposed to cross-contaminated feed, in a country i and P_T denoting the probability of residues of an antimicrobial used for pigs medicated feed production, being found in intestinal contents of pigs exposed to cross-contaminated feed (Table 1).

This model estimated the $N_{Res(i)}$ of piglets, fattening pigs and sows using Belgian data, per antimicrobial (*i.e.* CTC, DOX, SDZ, TRIM). Subsequently, the uncertainty over the probability of a pig of a certain production stage having residues of an AM, as a result of exposure to cross-contaminated feed, in a country ($P_{Res(i)}$) was estimated using the beta distribution:

$$P_{Res(i)} \sim RiskBeta (N_{Res(i)}+1, S_{(i)} - N_{Res(i)} +1), (8)$$

with $S_{(i)}$ denoting the pig populations per production stage i in a country (in one production round) (Statistics Belgium 2017).

The proportion of pigs of a certain production stage i having residues of an AM in their intestinal contents, as a result of exposure to cross-contaminated feed, in a country ($L_{(i)}$) was then estimated as:

$$L_{(i)} = P_{Res(i)} * 100 (9)$$

3.3. Model implementation

The quantitative part of model, with $P_{Res(i)}$ as its final estimate, was built using @Risk® software (Palisade Corporation®) and was run at 10 000 iterations per simulation. Following a semi-quantitative approach, the model reaches a binary outcome over the potential for resistance selection (i.e. risk yes/negligible). According to it, there is a potential for resistance selection (RS_1), if:

$$P_{Res(i)} > 0 \text{ and } CC_1, \qquad (10)$$

where C is the concentration of residues of an AM found in intestinal contents of pigs, as a result of exposure to cross-contaminated feed, C_1 is the minimum known concentration of an AM associated with potential for resistance selection pressure, while P_{Res} is estimated as shown in equation (8). We refer to a potential, as more factors seem to play a role in resistance development.

On the other hand, there is negligible potential for resistance selection (RS_2), if:

$$P_{Res(i)} > 0 \text{ and } C<C1, \text{ OR } P_{Res(i)} = 0 \qquad (11)$$

Thanks to availability of experimental data (Peeters et al., 2017, 2018), doxycycline was the substance used as the example to discuss over the potential for resistance selection (equations 10, 11).

In the case of the scenarios of 1% (scenario B) and 3% (scenario C) AM carry-over levels, the estimation of the probabilities of a pig of a certain production stage having residues of an AM in its intestinal contents, as a result of exposure to cross-contaminated feed with at least 1% ($P_{Res\,i(1)}$) or 3% ($P_{Res\,i(3)}$) AM carry-over level in a country, followed the same logic as the estimation of P_{Res} (Table 1). The only difference concerned the estimation of the total weights of cross-contaminated pig feed produced per year, with at least 1% ($W_{T(1)}$) or 3% ($W_{T(3)}$) AM carry-over level. In case of scenario B (Table 3):

$$W_{T(1)} = W_{I} * p_1 + W_{II} * p_2 + W_{III} * p_3, \qquad (12)$$

where W_I, W_{II} and W_{III} are the weights of cross-contaminated batches of non-antimicrobial medicated feed (non-AM MF) produced in a country per year at the feed-mill, the transport truck and the farm level, respectively, as described in Filippitzi et al. (2016); p_1, p_2 and p_3 are the probabilities of a feed batch being cross-contaminated with at least 1% AM carry-over level at the feed mill, the transport truck and the farm level, respectively. Beta distributions were used to estimate p_1 and p_2, based on data from available literature (Putier et al. 2010; Stolker et al. 2012), while due to absence of data, p_3 was assumed to equal p_2. In case AM MF represents 2% of the total feed produced in a country, as it is the case for Belgium (Filippitzi et al. 2016), then equation (12) can be replaced by the formula:

$$W_{T(1)} = 0.3*W_T*p_1 + 0.35*W_T*p_2 + 0.35*W_T*p_3, \quad (13)$$

where W_T is the total weight of cross-contaminated pig feed produced in a country per year.

In case of scenario C (Table 4), the total weight of cross-contaminated pig feed produced per year, with at least 3% AM carry-over level ($W_{T(3)}$) is calculated as:

$$W_{T(3)} = W_I*p_4, \quad (14)$$

with W_l being the weight of cross-contaminated batches of non-AM MF produced in a country per year at the feed-mill level and p_4 being the probability of a feed batch being cross-contaminated at the feed mill with at least 3% AM carry-over level. For this scenario, only the feed-mill level is taken into consideration, as AM carry-over levels of over 3% have not been observed at the transport or farm levels. For the estimation of p_4 a beta distribution is used, based on data regarding the cross-contamination of the first batches produced directly after batches of AM MF (Stolker et al. 2012). These so-called first flushing batches, which are produced to clean the production lines, can contain the higher AM concentrations compared to the second or third. As explained for equation (12), in case AM MF represents 2% of the total feed produced in a country, then $W_{T(3)}$ equals:

$$W_{T(3)} = 0.3*W_T*p_4, \qquad (15)$$

where W_T is the total weight of cross-contaminated pig feed produced in a country per year.

Moreover, in order to study the impact of the different model variables on $N_{Res(i)}$ (i.e. number of pigs, per production stage, with residues of an AM being found in their intestinal contents as a result of exposure to cross-contaminated feed, in a country; equation 7) sensitivity analyses were performed.

Table 1. Detailed summary of distributions and formulas used to estimate the model variables, considering the presence of an antimicrobial in cross-contaminated batches in any possible concentration higher than zero (AM > 0) (scenario A)

Variable	Description	Distribution / Formula / Parameter estimate	Reference
W_T	Total weight of cross-contaminated pig feed produced in a country per year	= 5.5%*T [1] For BE: ~RiskLognorm(146586,6;86253; RiskShift(209139))	Filippitzi et al., 2016
$P_{a(i)}$	Probability of the cross-contaminated feed W_T being administered to pigs of certain production stage i [2]	$P_{a(pigl)}$= 0.782 (mean value) $P_{a(sow)}$= 0.015 (mean value) $P_{a(fatt)}$= 0.201(mean value)	See Table 2
$W_{T'(i)}$	Total weight of cross-contaminated pig feed, administered per pig production stage i, in one production round	= $(W_T * P_{a(i)})$/ y	
y	Number of production rounds per production stage in a random pig farm in a country, per year	$y_{(pigl)}$ = 7.2 $y_{(sow)}$ = 1 $y_{(fatt)}$ = 2.5	Unpublished data from Belgian project; expert opinion
$b_{(i)}$	Intake of cross-contaminated feed per pig during a production stage i	= b_d*d_a*n = $(w_b*n) / n_b$	
b_d	Feed intake per pig at different production stages, per day		
d_a	Days of administration of a cross-contaminated feed batch[3], per production stage	= $w_b / (b_d * n_b)$	
w_b	Weight of a cross-contaminated feed batch (tn)	~RiskDiscrete(1, 4) [4]	BFA (pers.comm.)
n	Number of deliveries of cross-contaminated feed batches to a random farm, per production stage	$n_{(pigl)}$~RiskPert(1;1;2) $n_{(sow)}$~RiskPert(0;0;1) $n_{(fatt)}$ = 1	Unpublished data from Belgian project; expert opinion
n_b	Number of pigs in a batch, per production stage in a farm, in a country	= n_f/ z	
n_f	Number of pigs per production stage in a farm, in a country	$n_{f(pigl)}$~RiskPert(200;800;950) $n_{f(sow)}$~RiskPert(200;400;600) $n_{f(fatt)}$~RiskPert(200;750;900)	Unpublished data from Belgian project [5]; expert opinion
z	Number of batches of pigs per production stage in a farm, in a country[6]	$z_{(pigl)}$~RiskPert(1;2;2) $z_{(sow)}$=3 $z_{(fatt)}$~RiskPert(1;1;2)	Unpublished data from Belgian project; expert opinion

$N_{(i)}$	Number of pigs, per production stage i, exposed to cross-contaminated feed[7]	$= W_{T'(i)} / b_{(i)}$	
$S_{(i)}$	Pig populations per production stage i in a country (in one production round)	$S_{(pigl)} = 1\ 533\ 564$ $S_{(sow)} = 416\ 877$ $S_{(fatt)} = 4\ 061\ 013$	Landbouwgeg evens, 2017
P	Probability of residues of an AM being found in intestinal contents of a pig exposed to cross-contaminated feed	~RiskBeta $(c+1, d-c+1)$	
c	Number of samples[8] from pigs receiving cross-contaminated feed, found with residues of an AM	$c_{(CTC)} = 54$; $c_{(DOX)} = 54$; $c_{(SDZ)} = 54$; $c_{(TRIM)} = 2$ [9]	Peeters et al., 2016
d	Total number of samples[8] from pigs receiving cross-contaminated feed, tested for residues of an AM	$d_{(CTC)} = 54$; $d_{(DOX)} = 54$; $d_{(SDZ)} = 54$; $d_{(TRIM)} = 54$	Peeters et al., 2016
P_{use}	Probability of an AM being used for the production of pig MF in a country	~RiskBeta$(e+1, f-e+1)$	
e	Amount of an AM used as premix for medicated pig feed (kg)	$e_{(CTC)} = 36$ kg; $e_{(DOX)} = 4\ 975$ kg; $e_{(SDZ)} = 14\ 181$ kg; $e_{(TRIM)} = 2\ 836$ kg	BelVet Sac data, 2017
f	Total amount of all AM used as premixes for medicated pig feed (kg)	$35\ 698$ kg	BelVet Sac data, 2017
P_T	Probability of residues of an AM used for pig MF production, being found in intestinal contents of pigs exposed to cross-contaminated feed	$= P * P_{use}$	
$N_{Res\ (i)}$	Number of pigs per production stage i having residues of an AM in their intestinal contents, as a result of exposure to cross-contaminated feed, in a country[7]	$= N_{(i)} * P_T$	
$P_{Res(i)}$	Probability of a pig of a certain production stage having residues of an AM in its intestinal contents, as a result of exposure to cross-contaminated feed, in a country	~RiskBeta $(N_{Res(i)}+1, S_{(i)} - N_{Res(i)} +1)$	
$L_{(i)}$	Level of pigs of a certain production stage i having residues of an AM in their intestinal contents, as a result of exposure to cross-contaminated feed, in a country	$= P_{Res(i)} * 100$	
C_1	Concentration of residues of an AM is associated with potential for resistance selection pressure (minimum known concentration)	e.g. for DOX, 1 mg/L	Peeters et al., 2017
RS_1	Risk of potential for resistance selection[10]	If $P_{Res} > 0$ and $C \geq C_1$, where C is the concentration of residues of an AM found in intestinal contents of pigs, as a result of exposure to cross-contaminated feed	
RS_2	Negligible risk of potential for resistance selection[10]	If $P_{Res} > 0$ and $C < C_1$, OR If $P_{Res} = 0$	

Notes: [1]T is the total weight of feed produced or used (if feed is imported/exported) in a country where 2% of T is AM medicated feed and 5.5 is the mean value of this level; [2]piglets, sows and fattening pigs; [3]assumed to equal the days of administration of a feed batch, per production stage; [4]the discrete distribution describes w_b that can take one of several explicit discrete values (most likely the value of 4tn); [5]max values were lowered to avoid overestimation of n_f; [6]corresponds to the number of pig batches fed from the same mixing line and silo; [7]for sows, that live over a year, this estimation is per year; [8] samples of caecum, intestinal content and feces; [9]CTC= chlortetracycline, DOX=doxycycline, SDZ=sulfadiazine, TRIM=trimethoprim; [10]we refer to a potential, as more factors seem to play a role in resistance development.

Table 2. Summary of the distributions and formulas used to estimate the probability of cross-contaminated feed being administered to pigs of certain production stage (Pa(i))

Variable	Description	Distribution / Formula	Reference
$W_{T(i)}$	Total weight of cross-contaminated pig feed administered to pigs of a certain production stage i, per year	$W_{T(i)} = y*n* w_{(b)}*k$	
y	Number of production rounds in a random pig farm in a country, per year		See Table 1
n	Number of deliveries of cross-contaminated feed batches to a random farm, per production stage		See Table 1
$w_{(b)}$	Weight of a cross-contaminated feed batch		See Table 1
k	Number of farms with pigs of certain production stages in a country	$k_{(pigl)} = 70\%*m$ $k_{(sow)} = 70\%*m$ $k_{(fatt)} = 60\%*m$, where m is the total number of pig farms in the country	Expert opinion[1]
W_T	Total weight of cross-contaminated pig feed produced in a country per year	$W_T = W_{T(pigl)}+ W_{T(sow)}+W_{T(fatt)}$[2]	
$P_{a(i)}$	Probability of cross-contaminated feed being administered to pigs of certain production stage	$= W_{T(i)} / W_T$[3]	

Notes: [1]This is based on the type of production in Belgian pig farms (*e.g.* percentage of single site farrow-to-finish farms, of fattening pig farms *etc*); [2]assumed that W_T is administered to piglets, sows

and fattening pigs. The weight distributed to pigs of each production stage is estimated using the formula for $W_{T(i)}$; [3]after replacing all variables in the equation, the specification of *m* is not needed.

Table 3. Detailed summary of distributions and formulas used to estimate the model variables in case of scenario B (i.e. AM carry-over level of at least 1%)

Variable	Description	Distribution / Formula	Reference
$W_{T(1)}$	Total weight of cross-contaminated pig feed produced per year, with at least 1% AM carry-over level	$= W_I {*}p_1 + W_{II} {*}p_2 + W_{III} {*}p_3$ [1]= $= 0.3{*}W_T{*}p_1 + 0.35{*}W_T{*}p_2 + 0.35{*}W_T{*}p_3$ [2, 3]	Filippitzi et al., 2016
p_1	Probability of a first flushing batch being cross-contaminated at the feed mill level with at least 1% AM carry-over level	~beta(69+1, 140-69+1)	Zuidema et al., 2010 Stolker et al., 2013
p_2	Probability of a non-AM medicated feed batch being cross-contaminated at the transport truck level with at least 1% AM carry-over level	~beta(1+1, 20-1+1)	Putier et al., 2010
p_3	Probability of a non-AM medicated feed batch being cross-contaminated at the farm level with at least 1% AM carry-over level	~beta(1+1, 20-1+1)	Assumed
$W_{T'(1)}$	Total weight of the cross-contaminated pig feed produced per year, with at least 1% AM carry-over level, administered to pigs of a certain production stage *i*	$= W_{T(1)} {*} P_{a(i)}$	See Table 2
$N_{i(1)}$	Number of pigs, per production stage, exposed to cross-contaminated feed with at least 1% AM carry-over level, in a country	$= W_{T'(1)} / b_{(i)}$	See Table 1
$N_{Res\,i(1)}$	Number of pigs per production stage *i* having residues of an AM in their intestinal contents, as a result of exposure to cross-contaminated feed, with at least 1% AM carry-over level, in a country	$= N_{i(1)} {*} P_T$	See Table 1
$P_{Res\,i(1)}$	Probability of a pig of a certain production stage having residues of an AM in its	~RiskBeta($N_{Res\,i(1)}$ +1,$S_{(i)}$ - $N_{Res\,i(1)}$ +1)	See Table 1

	intestinal contents, as a result of exposure to cross-contaminated feed, with at least 1% AM carry-over level, in a country		

Notes: [1] These are the weights of cross-contaminated batches of non-AM MF produced in a country per year (where AM MF is 2% of total feed produced) at the feed-mill level (W_I), the transport truck level (W_{II}) and the farm level (W_{III}); [2] rounded values; [3] W_T is the total weight of cross-contaminated feed produced in a country per year.

Table 4. Detailed summary of distributions and formulas used to estimate the model variables in case of scenario C (i.e. AM carry-over level of at least 3%)

Variable	Description	Distribution / Formula	Reference
$W_{T(3)}$	Total weight of cross-contaminated pig feed produced per year, with at least 3% AM carry-over level	$= W_I{}^1 * p_4 = 0.3 * W_T * p_4{}^2$	Filippitzi et al., 2016
p_4	Probability of a feed batch[3] being cross-contaminated at the feed mill with at least 3% AM carry-over level	~Riskbeta(28+1, 140-28+1)	Zuidema et al., 2010; Stolker et al., 2013
$W_{T'(3)}$	Total weight of the cross-contaminated pig feed produced per year, with at least 3% AM carry-over level, administered to pigs of a certain production stage i	$= W_{T(3)} * P_{a(i)}$	See Table 2
$N_{i(3)}$	Number of pigs, per production stage, exposed to cross-contaminated feed with at least 3% AM carry-over level, in a country	$= W_{T'(3)} / b_{(i)}$	See Table 1
$N_{Res\ i(3)}$	Number of pigs per production stage i having residues of an AM in their intestinal contents, as a result of exposure to cross-contaminated feed, with at least 3% AM carry-over level, in a country	$= N_{i(3)} * P_T$	See Table 1
$P_{Res\ i(3)}$	Probability of a pig of a certain production stage having residues of an AM in its intestinal contents , as a result of exposure to cross-contaminated feed, with at least 3% AM carry-over level, in a country	~RiskBeta($N_{Res\ i(3)}$ +1, $S_{(i)}$ - $N_{Res\ i(3)}$ +1)	See Table 1

Notes: [1] This is the weight of cross-contaminated batches of non-MF produced in a country per year (where MF is 2% of total feed produced) at the feed-mill level; [2] rounded value; [3] corresponds to first flushing batches of feed, produced directly after batches of medicated feed, to clean the production lines.

4. Results

Tables 5 and 6 show the number (N_{Res}) and the proportion (%) ($L_{(i)}$) of piglets, sows and fattening pigs in Belgium having residues of chlortetracycline (CTC), doxycycline (DOX), sulfadiazine (SDZ) and trimethoprim (TRIM) in their intestinal content (from caecum and colon), as a result of exposure to cross-contaminated feed, estimated using respectively equations 7 and 9. This is estimated for the different scenarios A, B and C. Indicatively, the distributions of $N_{Res(piglets,1)}$ and $L_{(piglets,1)}$ with at least 1% carry-over level of DOX are shown in Figure 2.

For each of the three scenarios, it was estimated that the majority of pigs with residues in their intestinal content due to cross-contaminated feed (N_{Res}) contain residues of SDZ, followed by DOX. Much fewer pigs were estimated to contain residues of CTC and TRIM. For all scenarios and AM, the highest proportion of pigs with residues ($L_{(i)}$) was estimated for sows, followed by piglets and fattening pigs, while in absolute values the order was reversed.

Using equation 10 for the case of doxycycline (DOX) and given that: (a) the probability of a piglet, sow or fattening pig having residues of DOX, as a result of exposure to cross-contaminated feed ($P_{Res(i)}$) in Belgium has a positive value (Table 5); and that (b) the concentration of DOX residues found in intestinal contents of these pigs as a result of exposure to cross-contaminated feed (C) in case of scenario B is estimated to be higher than the minimum known concentration of DOX associated with potential for resistance selection pressure ($C_1 = 1$ mg/L) (Peeters et al. 2017, 2018), it is concluded that there is a risk of potential for DOX resistance selection in Belgium (RS_1: yes), considering the assumptions of the model.

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The results of the sensitivity analysis performed for the number of piglets with residues of DOX being found in their intestinal contents as a result of exposure to cross-contaminated feed with at least 1% carry-over level, in Belgium ($N_{Res(piglets,1)}$) are shown in Figure 3. Similar pattern in terms of variables and ranking were also observed in the case of other production stages and AM.

Table 5. Min, mode, mean and max values of the number of pigs per production stage having residues of chlortetracycline (CTC), doxycycline (DOX), sulfadiazine and trimethoprim (SDZ-TRIM) in their intestinal contents, as a result of exposure to cross-contaminated feed, in Belgium, for different scenarios (i.e. AM>0 (scenario A), at least 1% (scenario B) and 3% AM carry-over levels (scenario C)).

| AM/Scenarios	$N_{Res(piglets)}$				$N_{Res(sows)}$				$N_{Res(fatteners)}$			
	min	mode	mean	max	min	mode	mean	max	min	mode	mean	max
CTC												
Scenario A	461	1 786	2 341	15 820	223	624	1 182	8 930	997	2 404	4 101	21 925
Scenario B	867	204	499	4 073	39	149	248	1 468	155	571	860	6 037
Scenario C	31	70	144	760	15	37	72	347	50	148	249	1 861
DOX												
Scenario A	76 346	169 007	310 674	2 013 987	35 295	91 595	157 109	736 312	116 196	320 425	536 733	2 943 168
Scenario B	13 548	45 195	65 468	444 481	5 805	18 086	33 404	273 115	21 185	61 977	113 086	1 126 081
Scenario C	3 892	8 804	18 897	132 342	1 767	4 372	9 552	42 915	7 052	18 509	33 176	161 028
SDZ												
Scenario A	224 425	519 542	881 644	4 477 727	104 619	242 285	448 736	2 518 046	344 660	786 368	1 538 395	8 245 206
Scenario B	41 322	79 556	184 343	972 388	16 407	53 199	93 272	540 265	52 316	178 628	325 431	1 764 878
Scenario C	13 156	32 287	53 947	354 529	4 826	16 253	27 523	143 193	20 010	57 137	94 325	490 433
TRIM												
Scenario A	235	5 849	9 564	66 119	106	1 735	4 907	38 116	738	4 222	16 820	101 521
Scenario B	41	860	2 058	14 863	2	174	1 025	12 006	46	1 723	3 530	28 161
Scenario C	14	250	586	5 093	6	120	298	1 912	25	561	1 043	11 793

Notes: Most values are rounded. The values for the number of sows with residues ($N_{Res(sows)}$) are estimated per year and should be interpreted as "at least" values.

Table 6. Min, mode, mean and max values of the level (%) of pigs per production stage having residues of chlortetracycline (CTC), doxycycline (DOX), sulfadiazine and trimethoprim (SDZ-TRIM) in their intestinal contents, as a result of exposure to cross-contaminated feed, in Belgium, for different scenarios (i.e. AM>0 (scenario A), at least 1% (scenario B) and 3% AM carry-over levels (scenario C)).

AM/Scenarios	$L_{(piglets)}$				$L_{(sows)}$				$L_{(fatteners)}$			
	min	mode	mean	max	min	mode	mean	max	min	mode	mean	max
CTC												
Scenario A	0.03	0.07	0.15	0.82	0.05	0.19	0.29	1.56	0.02	0.06	0.1	0.55
Scenario B	<0.01	0.02	0.03	0.19	<0.01	0.03	0.06	0.32	<0.01	0.01	0.02	0.13
Scenario C	<0.01	<0.01	<0.01	0.05	<0.01	<0.01	0.02	0.12	<0.01	<0.01	<0.01	0.04
DOX												
Scenario A	5.33	10.57	20.13	94.39	9.24	20.38	34.75	99.74	3.45	9.21	13.17	49.26
Scenario B	1.01	2.79	4.23	18.78	1.67	4.57	7.76	36.94	0.51	1.29	2.8	14.9
Scenario C	0.27	0.87	1.23	5.47	0.41	1.31	2.3	12.1	0.19	0.53	0.81	3.93
SDZ												
Scenario A	15.71	28.94	46.15	99.99	28.39	54.83	64.49	99.69	8.28	17.91	35.27	99.67
Scenario B	2.42	5.91	12.01	57.73	4.28	10.19	22.4	98.90	1.81	6.42	7.99	38.95
Scenario C	0.74	2.76	3.52	19.64	1.28	4.22	6.55	40.17	0.37	1.09	2.32	10.74
TRIM												
Scenario A	0.03	0.41	0.63	4.91	0.07	0.41	1.15	13.51	<0.01	0.19	0.41	6.38
Scenario B	<0.01	0.05	0.13	1.25	<0.01	0.08	0.25	2.73	<0.01	0.06	0.09	0.56
Scenario C	<0.01	0.02	0.03	0.34	<0.01	0.04	0.07	0.58	<0.01	0.01	0.02	0.21

Notes: The values of $L_{(sows)}$ are estimated per year.
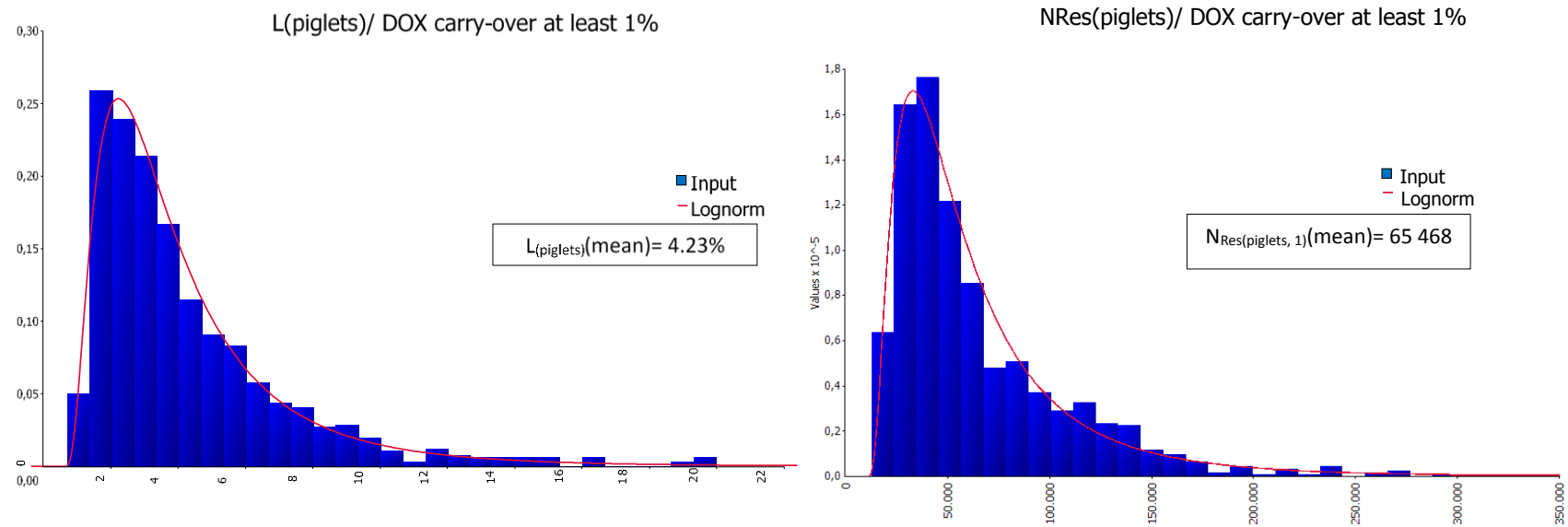
Figure 2. Distribution of the number ($N_{Res(piglets)}$) and level (%) of piglets ($L_{(piglets)}$) in Belgium having residues of doxycycline (DOX) in their intestinal contents, as a result of exposure to cross-contaminated feed with at least 1% carry-over level.
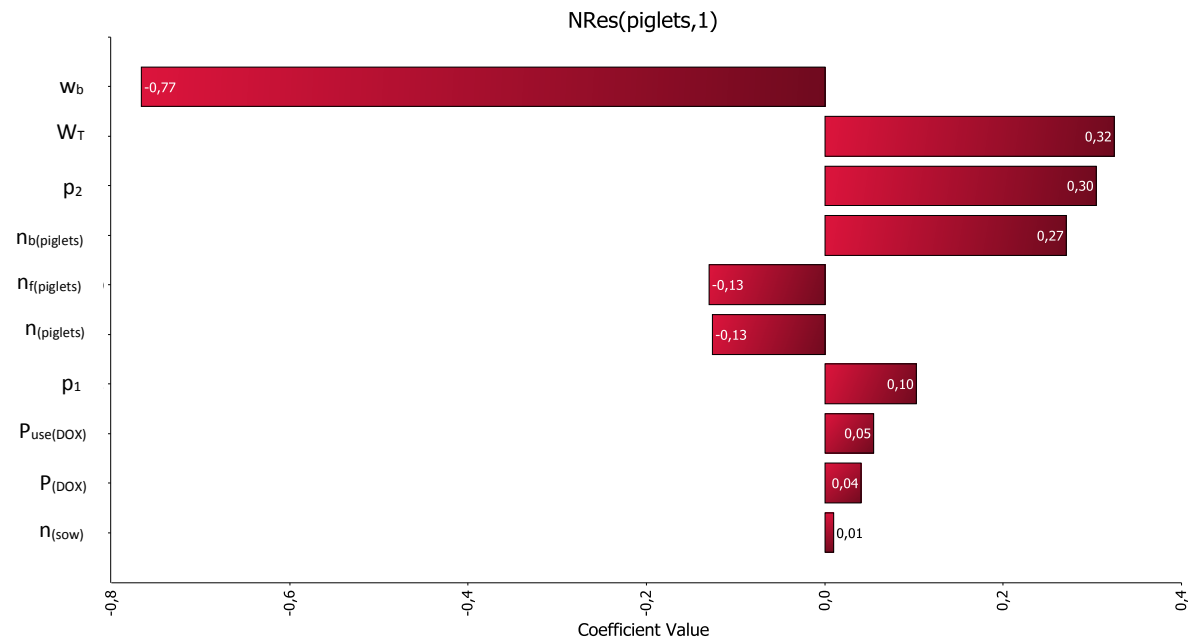
Figure 3. Sensitivity analysis performed for the number of piglets with residues of doxycycline (DOX) being found in their intestinal contents as a result of exposure to cross-contaminated feed with at least 1% carry-over level, in Belgium ($N_{Res(piglets,1)}$). Notes: The details of each variable are presented in Tables 1, 2 and 3: w_b stands for the weight of a cross-contaminated feed batch, W_T is the total weight of cross-contaminated pig feed produced in a country per year, p_2 is the probability of a non-AM MF batch being cross-contaminated at the transport truck level with at least 1% AM carry-over level, $n_{b(piglets)}$ is the number of piglets in a batch in a farm, $n_{f(piglets)}$ is the number of piglets in a farm, $n_{(piglets)}$ is the number of deliveries of cross-contaminated feed batches to piglets at a farm, p_1 is the probability of a first flushing batch being cross-contaminated at the feed mill level with at least 1% AM carry-over level, $P_{use(DOX)}$ is the probability of doxycycline being used for the production of pig MF in a country, $P_{(DOX)}$ is the probability of residues of doxycycline being found in intestinal contents of a pig exposed to cross-contaminated feed.

5. Discussion

The model estimated that mean proportions ($L_{(i)}$) of 7.7% of sows, 4.2% of piglets and 2.8% of fattening pigs in Belgium have residues of DOX in their intestinal contents (caecum and colon), due to consumption of feed cross-contaminated with at least 1% carry-over. Almost triple proportions of pigs were estimated to have residues of SDZ, for the same scenario (Table 6). On the contrary, the estimated proportions of pigs with residues of chlortetracycline (CTC) and trimethoprim (TRIM) were negligible (mean values of $L_{(i)}$ <1) in all production stages and for all scenarios. In Belgium, CTC is the AM used the least as premix, while SDZ is the AM used the most (BelVet-SAC 2017). SDZ and TRIM, generally administrated in combination in a ratio TRIM:SDZ = 1:5, are both AM with high bioavailability (Nielsen et al. 1994; Baert et al. 2001). However, expected lower residual concentrations in intestinal content and feces have only been observed for TRIM (Peeters et al. 2018), as it has been shown that in the case of SDZ an excretion of the AM from blood back to the gut takes place (De Smet et al. 2017).

The proportion of animals having residues of the different antimicrobials due to consumption of cross-contaminated feed was estimated to be higher for sows and piglets. This observation for sows, compared to piglets and fattening pigs, can be attributed largely to the lower population of sows in a production round ($S_{(i)}$). To a less extent it can be attributed to the higher number of batches of sows in a farm (z) which are linked to different feed silos, usually filled in at different feed deliveries (Table 1). Focusing only on carry-over at the farm level, the latter comment mainly concerns farms that use medicated feed in sows and not top dressing, which is another method often used for sow treatments. The higher proportion observed for piglets compared to fattening pigs can be mainly attributed to the estimation of the probability of the cross-contaminated feed being administered to pigs of each production stage ($P_{a(i)}$). In detail, the number of production rounds per year (y) and the number of farms with pigs of a certain production stage in Belgium (k) were higher for piglets and contributed to a higher estimation of P_a for this production stage (Table 2). These observations suggest that an initial targeting of sows and especially piglets, in terms of prevention of AM resistance related to cross-

contaminated feed, is required. In absolute values, the reversed order compared to proportions (*i.e.* higher number of pigs exposed to cross-contaminated feed estimated for fattening pigs, followed by piglets and sows) is mainly due to the differences in the size of the population of each production stage. The sensitivity analysis revealed that, for the same scenario and antimicrobial, the number of pigs in a batch, per production stage in a farm (n_b) was the variable affecting the outcome N_{Res} the most. Fortunately, this is a variable that was relatively easy to estimate and therefore with a relatively high accuracy.

The semi-quantitative approach followed allowed us to conclude that, for the case of doxycycline, there is a potential for resistance selection (*RS*) in Belgium, since the concentration of DOX residues estimated to be found in pigs that have consumed cross-contaminated feed with at least 1% carry-over level (C, is associated with potential for resistance selection pressure (Peeters et al. 2017, 2018). This is of course only one item playing a role in the development of resistance selection, as other factors have been suggested to also affect this complex and multifactorial process (*e.g.* the environment of the porcine intestinal microbiota, the dosage, frequency or route of administration, biosecurity-related factors) (Burow and Käsbohrer 2017). When experimental results allowing to specify *P* and C_1 become available, conclusions over *RS* could be also drawn for other commonly used antimicrobials (*e.g.* penicillins) and/or other antimicrobials resistance to which may be transmitted from pigs to humans (*e.g.* pleuromutilins (Alban et al. 2017)).

The quantitative part of the model is built in a way that allows its applicability to different countries. Therefore, in case of application, the legal framework in a country should be considered, given the current absence of a uniform European approach. For instance, since for the purposes of this paper, the model is parametrized with Belgian data, there is a possible overestimation of N_{Res} for fatteners, as in Belgium, the administration of the first and second flushing batches of feed produced at the feed mill directly after medicated feed to pigs > 80kg is forbidden (BFA 2013). Additionally, the estimation of N_{Res} for sows is possibly underestimated and should be interpreted as "at least", as in most cases

sows live longer than a year, while the value of W_T used (i.e. total weight of cross-contaminated pig feed produced in a country) is estimated per year. Finally, to estimate W_T in another country it is recommended to consult the previous model (Filippitzi et al., 2016) which allows to take into account any differences among countries (e.g. level of medicated feed produced compared to total feed produced, level of import/export of total feed produced if it occurs, etc).

The fact that low cross-contamination levels have the potential for resistance selection to certain AM, poses a further risk of group level resistance selection (*e.g.* co-selection of resistance genes conferring resistance to additional agents due to genetic linkages or other adaptations). And even though the presence of residues of other AM in intestinal contents of pigs is lower (*e.g.* TRIM) (De Smet et al. 2017) the possibility of co-resistance or cross-resistance, mechanisms that affect different AM classes or different AM of the same class, respectively, cannot be excluded. Very low antibiotic levels (*e.g.* of tetracycline) found in animals might in fact also be sufficiently high to maintain multiresistance plasmids, while they can result in resistance selection in combination with other compounds (*i.e.* other AM or metals) present in feed (Baker-Austin et al. 2006; Gullberg et al. 2014; Pal et al. 2015). The presence of AM residues in intestinal contents and feces of pigs due to consumption of cross-contaminated feed, adds up to the amount of AM residues present in pig manure, which may select for antibiotic resistance in pathogens and commensal bacteria that may enter the environment after fertilization. The cross-contaminated batches can practically reach any pig, even in farms that do not use medicated feed, causing a risk of wider concern. This means that the use of AM medicated feed should be avoided as much as possible, especially for preventive use. The use of water medication could be an alternative, as with this method, less carry-over and more animals receiving a correct dose of medication are expected. In case of medicated feed production, good practices (e.g. regular cleaning of equipment) should be thoroughly followed at the feed-mill, during transport and unloading, as well as storage and distribution at the farm. The use of an end-of-line mixer for the production of medicated feed at the feed-mill, a fine-dosing-truck and the use of a separate silo for medicated and non-medicated feed are also ways to reduce cross-contamination at the different levels.

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7. References

Alban A, Ellis-Iversen J, Andreasen M, Dahl J and Sönksen UW, 2017. Assessment of the risk to public health due to use of antimicrobials in pigs—an example of pleuromutilins in Denmark Front Vet Sci. 4: 1-16.

Baert K, De Baere S, Croubels S, Gasthuys F, De Backer P, 2001. Pharmacokinetics and bioavailability of sulfadiazine and trimethoprim (trimazin 30%) after oral administration in non-fasted young pigs. J Vet Pharmacol Ther. 24:295-298.

Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV, 2006. Co-selection of antibiotic and metal resistance. Trends Microbiol. 14:176-182.

(BelVet-SAC) Belgian Veterinary Surveillance of Antibacterial Consumption, 2017. National consumption report 2016. p1-44. http://www.belvetsac.ugent.be/

(BFA) Belgian Compound Feed Industry Association, 2013. In Dutch: Convenant betreffende gemedicineerde voeders. p1-14. http://www.favv.be/

(BFA) Belgian Compound Feed Industry Association, 2014. Statistical Report 2014. p http://www.favv.be/

Brewer MT, Xiong N, Anderson KL, Carlson SA, 2013. Effects of subtherapeutic concentrations of antimicrobials on gene acquisition events in Yersinia, Proteus, Shigella, and Salmonella recipient organisms in isolated ligated intestinal loops of swine. Am J Vet Res. 74(8):1078-83.

Burow E, Käsbohrer A, 2017. Risk factors for antimicrobial resistance in Escherichia coli in pigs receiving oral antimicrobial treatment: a systematic review. Microb Drug Resist. 23(2):194-205.

De Smet J, Croubels S, De Backer P, Devreese M, 2017. Effect of administration route and dose alteration on sulfadiazine-trimethoprim plasma and intestinal concentrations in pigs. Int J Antimicrob Agents. 50:707-714.

(EC) European Commission, 2014. Proposal for a regulation of the European Parliament and of the council on the manufacture, placing on the market and use of medicated feed and repealing Council Directive 90/167/EEC. http://eur-lex.europa.eu/

(EC) European Commission, 2016. Review of medicated feed legislation. http://www.europarl.europa.eu/portal/en

Filippitzi ME, Sarrazin S, Imberechts H, Smet A, Dewulf J, 2016. Risk of cross-contamination due to the use of antimicrobial medicated feed throughout the trail of feed from the feed mill to the farm. Food Addit Contam Part A. 33(4):644-55.

Gullberg E, Cao S, Berg OG, Ilback C, Sandegren L, Hughes D, Andersson DI, 2011. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. Plos Pathog. 7(7). doi:10.1371/journal.ppat.1002158.

Gullberg E, Albrecht LM, Karlsson C, Sandegren L, Andersson DI, 2014. Selection of a Multidrug Resistance Plasmid by Sublethal Levels of Antibiotics and Heavy Metals. 5(5). doi:10.1128/mBio.01918-14.

Nielsen P, Gyrd–Hansen N, 1994. Oral bioavailability of sulfadiazine and trimethoprim in fed and fasted pigs. Res Vet Sci. 56:48–52.

Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DG, 2015. Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. BMC Genomics. 16:964. doi:10.1186/s12864-015-2153-5.

Peeters LE, Daeseleire E, Devreese M, Rasschaert G, Smet A, Dewulf J, Heyndrickx M, Imberechts H, Haesebrouck F, Butaye P, Croubels S, 2016. Residues of chlortetracycline, doxycycline and sulfadiazine-trimethoprim in intestinal content and feces of pigs due to cross-contamination of feed. BMC Vet Res.12: 209. doi:10.1186/s12917-016-0803-8.

Peeters LE, De Mulder T, Van Coillie E, Huygens J, Smet A, Daeseleire E, Dewulf J, Imberechts H, Butaye P, Haesebrouck F, Croubels S, Heyndrickx M, Rasschaert G, 2017. Selection and transfer of an IncI1-tet(A) plasmid of Escherichia coli in an ex vivo model of the porcine caecum at doxycycline concentrations caused by cross-contaminated feed. J Appl Microbiol. 123(5):1312-1320.

Peeters LE, Croubels S, Rasschaert G, Imberechts H, Daeseleire E, Dewulf J, Heyndrickx M, Butaye P, Haesebrouck F, Smet A, 2018. Effect of residual doxycycline concentrations on resistance selection and transfer in porcine commensal Escherichia coli. Int J Antimicrob Agents. 51(1):123-127.

Putier F, 2010. Evaluation of cross-contamination in livestock feed delivery trucks. Technics. Kraftfutter/Feed magazine 9-10/2010.

Riviere JE, Papich MG, 2009. Veterinary Pharmacology and Therapeutics, 9th Edition. Wiley-Blackwell.

Statistics Belgium, 2017. Landbouwgegevens. https://varkensbedrijf.be/

Stolker AAM, Manti V, Zuidema T, van Egmond H, Deckers ER, Herbes R, Hooglugt J, Olde Heuvel E, de Jong J, 2013. Carry-over of veterinary drugs from medicated to nonmedicated feeds in commercial feed manufacturing plants. Food Addit Contam Part A. 30:1100–1107.

Zuidema T, van Holthoon FL, van Egmond HJ, Hooglugt J, Bikker P, Aarts H, Olde Heuvel E, 2010. In Dutch: "Omvang en implicaties van antibiotica-versleping in mengvoeders voor varkens". RIKILT report no. 2010.005, Wageningen UR. p 1-71.

CHAPTER 7

Quantitative risk model to estimate the level of antimicrobial residues that can be transferred to soil via manure, due to oral treatments of pigs

1. Abstract

Veterinary antimicrobials can spread via manure onto agricultural fields, representing an emission of these products or their active metabolites into the environment. This causes concerns regarding the role of antimicrobial residues in the development, selection and spread of resistance. Aiming to approach this issue quantitatively, first a literature review was performed on the bioavailability and extent of *in vivo* biotransformation of twelve antimicrobials commonly administered orally in pigs, and on the level of their persistence in manure. This information was then used in a model estimating the level of each of these administered antimicrobials that is present in manure at the end of common storage durations in pits and, thus, readily applied onto soil. The model estimated that at least 18.42% of the total amount of these orally administered antimicrobials is present in manure which is readily applied onto soil. Based on sales of antimicrobials, it was estimated that this percentage corresponds to over 25, 000 kg of antimicrobial residues in pig manure in Belgium. If export and processing are considered, the aforementioned percentage is reduced to at least 9.67%. From the studied antimicrobials, the highest level of residues in stored manure was estimated for doxycycline, as a combining result of its high use in pigs, low bioavailability and high stability in manure. However, even for antimicrobials not expected to be found in manure at the end of maximum storage, certain levels of these can be present in fractions of manure stored for shorter duration. Other antimicrobials are readily degraded and therefore pose less threat. The results of this study highlight the importance of rational antimicrobial use and of further research on biodegradation kinetics of antimicrobials and their degraded products in different environmental compartments, to efficiently control the spread of residues and/or resistance genes from manure to these matrices.

2. Introduction

Antimicrobials (AM) are widely used in livestock farming for the prevention or treatment of diseases. According to recent attempts to estimate AM use at species level, one of the highest uses of AM in livestock concerns treatments administered to pigs (Filippitzi et al., 2014; Carmo et al., 2017) via the oral route (Callens et al., 2012; Postma et al., 2015). It has been estimated that a large fraction of the overall administered AM are excreted unchanged via the feces and urine (Amábile-Cuevas, 2016) and are, thus, present in the animal manure produced. Indicatively in Flanders (Dutch-speaking part of Belgium, representing 95% of the pig production in the country), 39.5 million kg nitrogen (N) of pig manure was produced in 2016, 52.4% of which was brought onto Flemish agricultural land without prior processing (VLM, 2017). The AM residues present in manure may select for antimicrobial resistance in pathogens and commensal bacteria (Rasschaert et al., 2017) which may enter the environment after fertilization of soil (Du and Liu, 2012). Manure may also serve as a reservoir for AM resistance genes and mobile genetic elements (Binh et al., 2008; Whitehead and Cotta, 2013). Further, the resistant bacteria present in manure may exchange antimicrobial resistance genes with soil bacteria, a process even further enhanced by the presence of AM residues (Heuer et al., 2008, 2011). Therefore, concerns are growing regarding the level of AM residues or their active metabolites that end up in soil, and following in groundwater and surface water, and their role in the development and spread of AM resistance. This risk of AM resistance development and spread due to the presence of AM residues from veterinary use in the environment, could have serious implications for both animal and public health, given that many AM are used in veterinary as well as in human medicine and co-selection may occur.

However, research focusing on the spread of AM for veterinary use in the environment is still in its infancy. To which amount and extent these residues are present in each compartment (i.e. manure, soil, water) is only limitedly studied although it is an important piece of information to assess the risks associated with the use of veterinary AM and to develop methods to reduce emissions to the

environment. This study focuses on the first compartment of the fate of veterinary AM in the environment, the manure, and the behavior in this matrix of some commonly used AM administered orally to pigs. By developing a quantitative risk model, this study aims to estimate the level of these residues which are present in liquid pig manure that is readily available to be applied onto land (*i.e.* at the end of its storage) and at the time of its disposal on soil. Given the availability of data on the amount of veterinary AM used in Belgium (BelVet-SAC, 2017), this country was used as an example to illustrate the results of the model.

3. Materials and Methods

To ultimately estimate the level of AM residues from oral use in pigs which are present in pig manure at the end of storage (E) and the level of residues in pig manure that is applied onto soil (E_S) compared to the initial amounts of AM administered, a modular approach was followed. Based on this approach, the level of residues of each AM being excreted in freshly produced manure (X) (module 1, "animal level") was first estimated. Following, the level of residues of each AM being still present in manure after common storage duration was estimated (Z) (module 2, "stored manure level"). Finally, the level of residues of each AM which are present in manure applied on soil, considering also manure processing, was estimated (E_P) (module 3, "applied manure level"). Figure 1 shows the model framework designed to estimate E and E_S. A detailed summary of the variables and equations of the model developed is presented in Table 1.

To investigate the availability of information that would allow us to run such a model, literature was reviewed on the behavior of commonly used AM within the pig after oral administration (pharmacokinetics) and in the manure produced, stored and processed. The focus was on the AM used the most in pigs *per os* (p.o.) in Belgium. The initial list of nine AM was formulated based on data from the Belgian Veterinary Surveillance of Antibacterial Consumption (Bel-Vet SAC) database for 2016 (results based on this data can be found in BelVet-SAC (2017)) and a table by Postma et al. (2016) and included: oxytetracycline, doxycycline, amoxicillin, sulfadiazine, trimethoprim, lincomycin, tylosin,

colistin and spectinomycin. It was supplemented by three more AM, i.e. tilmicosin, flumequine, tiamulin, residues of which have also been found in manure samples tested during an experiment conducted in Belgium (Rasschaert et al., 2018; manuscript in preparation). The use of these twelve AM corresponds to around 62% of the total amount of AM used in Belgium in 2016.
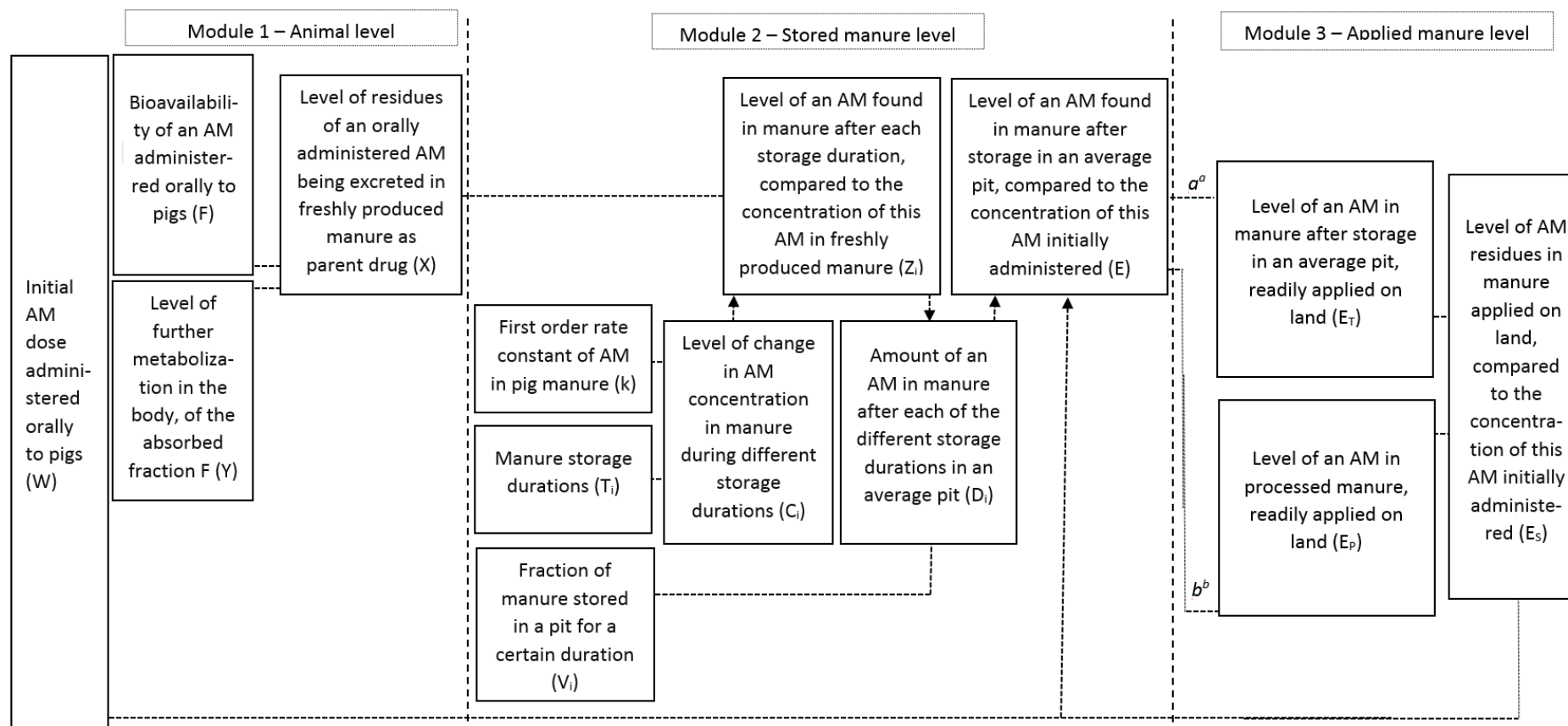
Figure 1. Model framework designed to estimate the level of residues of an antimicrobial (AM) from oral use in pigs which are present in pig manure that is readily available to be applied onto land (E) / **Notes:** The same framework is used for both the individual pit and the country level; [a] a is percentage of the total manure produced that is directly applied on land after storage; [b] b is the percentage of the total manure produced that is processed before being applied on land.

Table 1. Summary of the variables and formulas of the model.

Variable	Description	Formula	Reference
W	Amount of an antimicrobial (AM) administered orally to the pigs (kg)[a]		
W'	Total amount of an AM administered orally to the pigs in a country (kg)		For Belgium: 2016 BelVet-SAC database; see section "Amounts of AM used orally in pigs"
Module 1 – animal level			
F	Bioavailability of an AM administered to pigs orally		Multiple, per AM; see Table2
Y	Level of further metabolization in the body, of the absorbed fraction F		
X	Level of residues of an orally administered AM being excreted in freshly produced manure as parent drug	$X = (1-F) + F*(1-Y)$	
B	Total amount of each AM being excreted in freshly produced manure as parent drug, in a country (kg)	$B = W' * X$	
Module 2 – stored manure level			
$A_{(0)}$	Concentration of an AM in freshly produced manure		
A_i	AM concentration in stored manure after a time period	$A_i = A_{(0)} * e^{(-k*T_i)}$	Assuming first-order kinetics (Riviere and Papich, 2009)
k	First order rate constant of an AM in manure	$k = \ln(2)/DT_{50}$	Assuming first-order kinetics (Riviere and Papich, 2009)
T_i	Manure storage duration (days)	e.g. $T_1 \leq 30$ d 30 d $< T_2 \leq 120$d 120 d $< T_3 \leq 180$ d	See section "Estimation per manure pit"
C_i	Level of change in AM concentration during a storage duration[b]	$C_i = = e^{(-k*T_i)} -1$	
Z_i	Level of an AM found in manure at the end of a storage duration (e.g. after 2 months), compared to the concentration of this AM in freshly produced manure	$Z_i = 1 - C_i$	
$Z_{i,\Delta t}$	Level of an AM present in manure stored for a certain duration (e.g. between 2 – 4 months), compared to the concentration of this AM in freshly produced manure	~RiskUniform(min, max) min= $Z_{i(fin)}$; max= $Z_{i(beg)}$; OR ~RiskPert(min, most likely, max) min= most likely= $Z_{i(fin)}$; max= $Z_{i(beg)}$;	See section "Estimation per manure pit"
DT_{50}	50% disappearance time of an AM in pig manure (days)		Multiple, per AM; see Table3
DT_{90}	90% disappearance time of an AM in pig manure (days)	$DT_{90} = \ln(10)/k$	Assuming first-order kinetics (Riviere and Papich, 2009)

V_i	Fraction of manure stored for each of the different durations	$V_i =$	See section "Estimation per manure pit"
D_i	Amount of an AM in manure at the end of a storage duration in an average pit (kg)	$D_i = V_i * W * X * Z_i$	
$D_{i,\Delta t}$	Amount of an AM present in manure stored for a certain duration in an average pit (kg)	$D_{i,\Delta t} = V_i * W * X * Z_{i,\Delta t}$	See section "Estimation per manure pit"
D_T	Total amount of an AM present in manure after storage in an average ready-to-be-emptied pit	$D_T =$	
E	Level of an AM found in manure after storage, compared to the concentration of this AM initially administered orally to pigs producing the manure	$E = D_T / W$	
	Total amount of each AM administered orally to pigs that is present in manure at the end of storage, at country level	$= V_i * B * Z_{i,\Delta t}$	
E'	Level of an AM found in manure after storage, compared to the total amount of this AM administered orally to pigs, at country level	E' / W' , with =	
Module 3 - applied manure level			
E_T	Level of an AM initially administered orally that is present in pig manure after storage, readily applied onto land	$E_T = a * E'$	
a	Percentage of the total manure produced that is applied on land after storage without prior processing	For Belgium (BE), a=0.525	Flemish Land Agency (VLM, 2017)
E_P	Level of an AM initially administered orally that is present in processed pig manure	$E_P = b *c * d * E'$	
b	Percentage of the total manure produced that is processed before being applied on land	For BE, b=0.47	Flemish Land Agency (VLM, 2017)
c	Percentage of pig manure which is processed using a specific method	For BE, c=0.845 (biological treatment)	Flemish Coordination Centre for Manure Processing (VCM, 2016)
d	Level of an AM present in manure after processing depending on the method used	e.g. d_{SDZ}=0.43; d_{DOX}=0.08	Van den Meersche et al. (2017) (for biological treatment)
E_S	Level of residues of an AM in manure applied on land, compared to the total amount of this AM administered orally to pigs, at country level	$E_S = E_T + E_P$	

Notes: [a] Refers to pigs producing manure that is stored in the same pit; [b] corresponds to a concentration reduction.

3.1. Literature review

To collect information to be used in the model, a structured literature review was performed in PubMed using, first, the following search string: (antibiotic or antimicrobial) AND (bioavailability OR degradability OR persistence OR transformation OR processing) AND (manure OR feces) AND (pig OR swine). The 118 publications retrieved using the search string were submitted to a first manual screening for their relevance on title and abstract. Those considered relevant were subject to second screening eligibility on the full text. The references of the retrieved publications were also checked and reviewed when found to be relevant. Regarding the bioavailability and the extent of *in vivo* biotransformation of the veterinary AM, the book Veterinary Pharmacology and Therapeutics (Wiley-Blackwell® 2009) was also used as reference, as well as the Summary of Product Characteristics (SPC) per AM. Following, PubMed was screened using the same search string structure as previously mentioned, by replacing (antibiotic or antimicrobial) with the name of each AM from the list of compounds of interest.

3.2. Amounts of AM used orally in pigs

The BelVet-SAC database for 2016 provides detailed information on veterinary AM use in Belgium. In terms of amounts of AM used, the database does not include exact information on use per species, but it includes, for each compound, the species it is administered to. Therefore, in case a compound was used for pigs, cattle and poultry, it was assumed that 50% of the total amount used would be administered to pigs. In case a compound was used for pigs and bovine or pigs and poultry, it was assumed that 70% or 64% of the total amount used would be administered to pigs, respectively. These percentages were calculated based on the amounts of antimicrobials per class per kg biomass for bovine, pigs and poultry in Belgium, also available from BelVet-SAC. This approach was followed for AM compounds administered orally excluding premixes (e.g. tablets, oral solutions, powders). In the

case of AM compounds used orally as premixes, the 99.3% of their total amounts used were considered to be administered to pigs.

3.3. Model description

3.3.1. Fraction of antimicrobials excreted in manure (module 1, "animal level")

The extent of systemic exposure after administration of a drug is defined as bioavailability or absolute availability, usually denoted as F. The intravenous injection is the only route of administration that guarantees that 100% of the dose is systemically available ($F = 1.0$). In the case of oral drug administration, a fraction of the administrated drug is absorbed ($F \leq 1.0$) and the non-absorbed fraction (1-F) of the initial dose will remain in the gut and will be excreted in the manure assuming no gastrointestinal degradation. Of the absorbed fraction F, a proportion will be metabolized in the body (Y) whereas the non-metabolized fraction of the parent drug (1-Y) will be excreted again (e.g. renal excretion, biliary excretion) and will therefore also end up in the manure. To provide an estimate of the level of residues of an orally administered AM being excreted in freshly produced manure as parent drug (X) the following equation was used:

$$X = (1-F) + F*(1- Y), \qquad \text{Eq. (1)}$$

where F is the oral bioavailability of the specific drug and Y is the extent of drug metabolization in the body. The specification of F and Y for each AM of interest to this study was based on data available in the literature. In case more than one estimations of F or Y for an AM were found, their average value was used in the above equation.

For the use of equation (1), no degradation of the compound and no binding in the gastrointestinal tract are assumed. Moreover, it is assumed that its metabolites are microbiologically inactive. First-order pharmacokinetics are also assumed, meaning that the fractional rate of drug excretion is constant.

3.3.1.1. Estimation at country level

Given the availability of data allowing the estimation of the amount of each AM administered orally to pigs in Belgium, the total amount of each AM being excreted in freshly produced manure in this country as parent drug can be estimated. The formula used was:

$$B = W' * X, \qquad \text{Eq. (2)}$$

where W' is the total amount of an AM administered orally to the pigs in Belgium (kg) and X is the fraction of this AM being excreted in freshly produced manure as parent drug, estimated as shown in equation 1.

3.3.2. Fraction of antimicrobials present in stored manure (module 2, "stored manure level")

3.3.2.1. Estimation per common storage durations

In conventional manure management, pig manure is long-term stored in pits under anaerobic conditions until field application. The average storage duration of manure before being applied on soil is estimated by expert judgement to be on average four to six months (max = 6 months), especially during winter (VLM, 2014). During summer, some pits are partly emptied every few weeks (e.g. manure stored up to one month). Since fresh manure is added to the pit and is mixed with previously stored material, it can be expected that fractions of the manure are usually stored for different durations; for instance, for up to one month, from one up to four months, and from four up to six months. During storage, the AM residues present in fresh manure are generally degraded at different levels in the course of time, depending on various factors (*e.g.* characteristics of the AM itself, conditions of oxygen present or temperature, animal source of manure). A commonly used measure to specify the level and time of degradation of a compound (*e.g.* an AM) in a matrix (*e.g.* manure) is the 50% (DT_{50}) disappearance time, that is in this case the time needed for the degradation of 50% of the initial concentration of an AM in the manure (Riviere and Papich, 2009). When information was not available for certain compounds, but was available for the group they belong to, the latter was used for the estimations of this model as AM belonging to the same group are expected to behave in a similar way in the majority of cases.

To estimate the level of change in the concentration of an AM during different storage durations T_i (*i.e.* up to one month, from one up to four months, and from four up to six months) (C_i), the AM concentration in stored manure after each period (A_i) was estimated first, assuming first-order kinetics:

$$A_i = A_{(0)} * e^{(-k*T_i)}, \quad \text{Eq. (3)}$$

where $A_{(0)}$ is the concentration of an AM in freshly produced manure and k is the first order rate constant estimated as $k = \ln(2)/DT_{50}$ for each AM (Riviere and Papich, 2009). Each of the different manure storage durations is denoted by T_i.

Therefore, the level of change in AM concentration during different storage durations (C_i), corresponding to a concentration reduction, can be estimated as:

$$C_i = = e^{(-k*T_i)} - 1, \quad \text{Eq. (4)}$$

The level of an AM found in manure after (each) storage duration, compared to the concentration of this AM in freshly produced manure (Z_i), can then be calculated as:

$$Z_i = 1 - C_i, \quad \text{Eq. (5)}$$

To perform the above calculations, we used DT_{50} values per AM found in literature, in order to estimate k for each AM, as previously described. In case more than one estimation of DT_{50} for an AM was found, their average value was used to estimate k and replace it in the above equations.

In order to provide also an estimation of the time period that residues of each AM of interest could still be found in stored manure, the DT_{90} (90% disappearance time) values for each AM in manure were calculated as follows:

$$DT_{90} = \ln(10)/k, \quad \text{Eq. (6)}$$

where the k first order rate constant, estimated as previously described (Riviere and Papich, 2009).

3.3.2.2. Estimation of residues in manure stored in an average pit

Assuming that an average pit is filled with manure in a constant rate until the pit is full and it is emptied at once after 6 months, then this ready-to-be-emptied pit contains different fractions of manure stored for different durations (V_i): 16.7% of manure stored up to 1 month, 50% of manure stored >1 up to 4 months and 33.3% of manure stored >4 to 6 months. Therefore, the amount of each AM in manure at the end of the different storage time periods (*e.g.* after one month) (kg) in an average pit (D_i) can be estimated as:

$$D_i = V_i * W * X * Z_i, \qquad Eq.(7)$$

where V_i is the fractions of manure stored in the pit for a certain duration i; W is the amount of the AM administered orally to the pigs producing this manure (kg); X is the level the orally administered AM being excreted in freshly produced manure as parent drug, estimated using equation 1; Z_i is the level of the AM found in manure at the end of a storage duration, compared to the concentration of this AM in freshly produced manure, estimated using equation 5.

Since, as previously described, different fractions of the manure in an average pit can be stored for different durations (*e.g.* between two to four months), in such a case the range of values of the amount of an AM present in manure stored for this duration (kg) ($D_{i,\Delta t}$) can be estimated as:

$$D_{i,\Delta t} = V_i * W * X * Z_{i,\Delta t}, \quad Eq. (8)$$

where $Z_{i,\Delta t}$ corresponds to the range of values of the level of this AM present in manure stored for the same duration, compared to the concentration of this AM in freshly produced manure. A Uniform distribution can be employed to estimate this variable more representatively:

$$Z_{i,\Delta t} \sim RiskUniform(min, max), \quad Eq. (9)$$

with min= $Z_{i(fin)}$ and max= $Z_{i(beg)}$, estimated using equation 5 and considering the end and the beginning of this certain duration period, respectively.

To avoid an overestimation of $Z_{i,\Delta t}$ in the case of antimicrobials which are fast degraded in manure (*i.e.* DT_{90} <30 days), a Pert distribution can be employed instead:

$$Z_{i,\Delta t} \sim RiskPert(min, most\ likely, max), \quad Eq. (10)$$

with most likely= min = $Z_{i(fin)}$ estimated using equation 5 and considering the end and the beginning of this certain duration period, respectively.

The total amount of an AM present in manure after storage in an average ready-to-be-emptied pit (D_T), given the previously described considerations, is estimated as:

$$D_T = = D_1 + D_2 + D_3, \qquad \text{Eq.(11)}$$

where D_1, D_2 and D_3 are the amounts of an AM that can be present in manure for each of the three storage time periods T_i considered (kg), estimated using equation 8 (Table 1).

Consequently, the level of an AM found in manure after storage and thus readily available to be applied onto soil, compared to the concentration of this AM initially administered orally to pigs producing the manure (E) is calculated as:

$$E = D_T / W, \qquad \text{Eq. (12)}$$

where W is the amount of the AM administered orally to the pigs producing this manure (kg).

3.3.2.3. Estimation at country level

Assuming that all pig manure in Belgium is treated in a similar way as described in previous sections per pit (*e.g.* storage in pits, for up to six months, anaerobic conditions), it is then possible to estimate the total amount of each AM administered orally to pigs that is present in manure at the end of storage at country level (D_T'), based on equation 11 and by estimating first $D_{i,\Delta t}$ (equation 8) at country level as:

$$= V_i * W' * X * Z_{i,\Delta t}, \qquad \text{Eq. (13)}$$

where W' is the total amount of the AM administered orally to the pigs in the country (kg) and the rest variables are the same as for the estimation per pit (equation 8).

Finally, the level of an AM found in manure after storage, compared to the total amount of this AM administered orally to pigs at country level (E'), according to equation 11, can be:

$$E' = D_T'/ W', \qquad \text{Eq. (14)}$$

3.3.3. Fraction of antimicrobials present in manure readily available to be applied onto soil (module 3, "applied manure level")

Different fractions of the total, including pig, manure produced in a country, are either directly applied on soil after storage, or directly exported, or processed (treated) after storage and afterwards applied on soil or exported (VLM, 2017). Depending on the country, different methods of manure processing can be employed, including a biological treatment which follows denitrification (anaerobic) and nitrification (aerobic) processes (Smet et al., 2003), biothermal drying (also called composting) (Massé et al., 2014; Youngquist et al., 2016) or anaerobic digestation (Weiland, 2010; Widyasari-Mehta et al., 2016).

Therefore, the level of an AM in the total pig manure after storage in average pits, that is applied on soil without prior processing (E_T) is estimated as:

$$E_T = a * E', \qquad \text{Eq. (15),}$$

where a is the percentage of the total pig manure produced that is applied on soil without processing after storage.

The level of an AM in processed pig manure (E_P) is estimated as:

$$E_P = b*c*d*E', \qquad \text{Eq. (16),}$$

where b is the percentage of the total pig manure produced that is processed before being applied on soil, c is the percentage of pig manure which is processed using a specific method, d is the level of the AM present in manure after processing depending on the method used, and E' is the fraction of an AM found in manure after storage, compared to the amount of this AM administered orally to pigs (equation 14).

Finally, the level of AM residues in manure applied on soil (E_S), compared to the amount of this AM administered orally to pigs, can be estimated as:

$$E_S = E_T + E_P, \qquad \text{Eq. (17).}$$

Focusing on Belgium and assuming that available data from Flanders apply in the whole country, a percentage a of total pig manure produced in this country is put on soil without prior processing, a percentage b of it is processed, while the rest is exported as raw pig manure (VLM, 2017). The manure that is to be processed is first separated with centrifugation in a solid and a liquid fraction (Van den Meersche et al., 2017). The solid fraction is mainly processed by biothermal drying and is following exported (VCM, 2016), while the liquid fraction is mainly processed in biological nitrification-denitrification plants (Smet et al., 2003; VLM, 2017). This latter biological treatment is the most common processing technique for pig manure in Flanders and Belgium (VCM, 2016). The result of this treatment, the effluent, is then stored in big open air lagoons and can be used on agricultural fields (Smet et al., 2003). Since the processing of liquid pig manure corresponds to a percentage c of the total processed pig manure (VCM, 2016), it was assumed that an equal percentage of liquid pig manure (c) is processed with biological treatment.

4. Results

4.1. Literature review

From the literature review, published information on the bioavailability (F) and metabolization (Y) inside the pig was retrieved for all selected, orally distributed antimicrobials (Table 1). For amoxicillin (experiments in fasted pigs), sulfadiazine (SDZ) and trimethoprim (TRIM) (experiments in fasted and non-fasted pigs), more than one F value (max 15% difference between them) was found and therefore their average was used in the model. For lincomycin, two distant F values were found (40% in non-fasted, 70% in fasted pigs), of which the F value estimated in non-fasted pigs was used, assuming to be more representative of the average situation at a farm. For the other AM, only one F value was found and used.

Information on persistence (DT_{50}) in pig manure under anaerobic conditions was found for five out of these twelve AM (Table 2), namely oxytetracycline, doxycycline, lincomycin, tylosin and tiamulin. Two DT_{50} values were found for oxytetracycline and doxycycline and therefore their average was used for the model estimations. The DT_{50} value for lincomycin was calculated based on equation (3), as information on the reduction of AM concentration in stored manure was available (Kuchta and Cessna, 2009). The DT_{50} values for amoxicillin, spectinomycin, flumequine were not available in literature and therefore available DT_{50} values estimated for the group they belong to (i.e. DT_{50} for penicillins, aminoglycosides, fluoroquinolones, respectively) were used for the model. For tilmicosine, no significant degradation is observed over 73 days under anaerobic conditions (SPC, 2010), so this period was used as a reasonable estimation of its non-specified DT_{50} in manure. The DT_{50} value for sulfadiazine, although estimated in bovine manure, was considered relevant based on further information on persistence of this AM in pig manure (Heuer et al., 2008; Massé et al., 2014). No information regarding persistence in pig manure was available for trimethoprim and colistin.

Information regarding the percentages of the total pig manure produced in Belgium that is put on soil without prior processing (*a*), the percentage of it that is processed (*b*) (VLM, 2017) and the percentage of pig liquid manure which is processed with biological treatment (*c*) (VCM, 2016) was available (Table 1). Data regarding the level of residues in manure after processing with biological treatment (*d*) were only available by Van den Meersche et al. (2017). By testing samples taken at different stages of manure treatment in two farms where pigs were administered doxycycline (DOX), sulfadiazine (SDZ), trimethoprim (TPM) and tylosin (TYL), they observed no residues of TMP or TYL in the liquid fraction of manure or in the storage lagoon, while they also observed a 56.7% (d_{SDZ}=0.43) and 91.4% (d_{DOX}=0.08) reduction of SDZ and DOX, respectively, during this treatment of liquid manure. Results were expressed on the matrix itself, being solid material or liquid lagoon. Recalculation to dry matter of the different matrices could place the results in another perspective (e.g. it could be in some cases that no reduction of certain antimicrobials has taken place).

4.2. Model results

4.2.1. Module 1 ("animal level")

For each of the twelve selected AM, the total amount of each AM administered orally to the pigs in Belgium (kg) (W') and the estimations of the model which are respective to module 1 ("animal level") are summarized in Table 2. The total amount of the initially administered dose (X=1) of doxycycline and colistin was estimated to be found in freshly produced manure. High levels of spectinomycin (X=0.99), oxytetracycline (X=0.98), tilmicosin (X=0.97) and amoxicillin (X=0.93) were also estimated. The lowest levels of AM ending up in fresh manure were predicted for flumequine (X=0.05) and tiamulin (X=0.10). Generally, low bioavailability (F) and/or low metabolization (Y) of an AM result in a high level of residues of this AM being excreted in freshly produced manure (X), and vice versa.

Given their low bioavailability and low metabolization, resulting in a high level of residues in freshly produced manure (X), combined with their high use in pigs in Belgium (W'), the highest amount of residues ending up in freshly produced pig manure was estimated for amoxicillin (B=46,273 kg) and doxycycline (B=26,868 kg). High levels of residues in fresh manure were also estimated for the very commonly used sulfadiazine (B=23,360 kg), an AM predicted to be excreted in manure in lower levels than the aforementioned AM. These results show that both pharmacokinetics (X) and usage amounts (W') affect the total amount residues in freshly produced manure (B).

Table 2. Module 1 ("animal level"): summary for each of the twelve selected antimicrobials (AM), of the pig production stage it is mostly administered to, of the information retrieved from the literature review (i.e. F, Y), of the total amount of each AM administered orally to the pigs in Belgium (kg) (W') and of the estimations of the model (i.e. X, B)

AM class	AM compound administered to pigs orally	Bioavailability of AM (F)	Level of metabolisa-tion of AM (Y)	Level of AM in fresh manure (X)	Amount of AM used orally in pigs (BE) (kg) (W')[e]	Amount of AM excreted in fresh manure (kg) (B)	All references per AM
Tetracyclines	Oxytetracycline	0.05	0.50	0.98	5 224.52	5 120.03	Nielsen and Gyrd-Hansen (1996), Riviere and Papich (2009)
Tetracyclines	Doxycyline	0.21	0	1	26 867.82	26 867.82	Riond and Riviere (1990), Riviere and Papich (2009)
Penicillins	Amoxicillin	0.25 [a]	0.30	0.93	49 755.55	46 272.66	Agersø et al. (1998), Reyns et al. (2007), Ramos et al. (2012)
Sulfonamides	Sulfadiazine	0.85 [a]	0.56	0.52	44 922.17	23 359.53	Lamshoft et al. (2007), Riviere and Papich (2009)
	Trimethoprim	0.92 [a]	0.50	0.54	9 194.47	4 965.01	Mengelers et al. (2001), Riviere and Papich (2009)
Lincosamides	Lincomycin	0.40 [b]	0.78	0.69	2 991.28	2 063.98	Hornish et al. (1987), Nielsen and Gyrd-Hansen (1998), Rostel et al. (2000)
Macrolides	Tylosin	0.225[c]	0.60	0.87	2 293.98	1 995.76	Lewicki et al. (1991), JEFCA (2009)
Macrolides	Tilmicosin	0.20	0.15	0.97	1 523.06	1 477.37	MacNeil (1995), SPC (2010)
Polymyxins	Colistin	0	NA[d]	1	2 738.59	2 738.59	Riviere and Papich (2009), EMA (2009)
Aminocyclitols	Spectinomycin	0.10	0.10	0.99	3 988.38	3 948.50	Bhavsar and Thacker (2012)
Fluoroquinolo-nes	Flumequine	1	0.95	0.05	194.79	9.74	Mevius et al. (1990)
Pleuromutilins	Tiamulin	0.90	1	0.10	594.33	59.43	Almeida et al. (2014), SPC (2011)
Total of these compounds					150 288.94	118 741.34	

Notes: [a] Average of close available F values (max 15% difference between them); [b] F value estimated in non-fasted pigs; [c] for tylosin A; [d] NA=not applicable (since oral bioavailability is 0); [e] based on 2016 Belgian data from BelVet-SAC.

4.2.2. Module 2 ("stored manure level")

Table 3 presents the results of the literature review (i.e. DT_{50} values, estimations of DT_{90} and k) and the first part of the model estimations which correspond to module 2 "stored manure level", while Table 4 summarizes the second part of module 2 estimations. The separation in first and second parts is only due to presentation purposes. Comparing the ten AM for which information was available (corresponding to 57% of total amount of AM used in pigs), tiamulin, flumequine, lincomycin and doxycycline were found to be the most persistent in pig manure after common storage durations [i.e. higher levels of these AM present in stored manure (Z_i, $Z_{i,\Delta t}$) estimated], as the highest DT_{90} values were estimated for them. The opposite was estimated for tylosin, amoxicillin and sulfadiazine (Table 3).

Comparing again the AM for which information was available, the highest levels of an AM found in manure after storage, compared to the amount of this AM administered orally to pigs (%) (E') were estimated for doxycycline (E'=55%), followed by tilmicosine, lincomycin and oxytetracycline, while the lowest E' were estimated for flumequine (E'=2.93%), followed by tylosin, tiamulin and amoxicillin (Table 4). These are combined results of the estimated level of each AM being excreted in fresh manure (X) and the level of each AM being present in manure after storage ($Z_{i,\Delta t}$). For instance, doxycycline and oxytetracycline are AM with estimated high excretion level in fresh manure and high persistence in stored manure. On the contrary, tylosin and amoxicillin, although highly excreted in fresh manure, they are fast degraded in this matrix, while flumequine and tiamulin, although highly persistent in stored manure, they are limitedly excreted in fresh manure. The model estimated that a mean value of E'=18.42% of the total amount of the ten AM initially administered, for which information was available, is present unmetabolised in manure after storage.

Focusing on Belgium and taking also into account the total amounts of each AM used (W'), it is estimated that in this country the highest total amount of residues in stored manure (D'_T) are residues of doxycycline, followed by considerably lower amounts of amoxicillin and sulfadiazine. The lowest D'_T

were estimated for flumequine, tiamulin and tylosin. The model estimated that a mean value of $D'_T =$ 25,493 kg of residues of these ten AM are still present in manure after storage in average ready-to-be-emptied pits in Belgium.

Table 3. Summary of the results of the literature review (i.e. DT_{50} values, estimations of DT_{90} and k) and of the first part of the model estimations which correspond to module 2 ("stored manure") (i.e. C_i, Z_i, $Z_{i,\Delta t}$, $D'_{i,\Delta t}$)

AM class	AM compound	DT50 (d) (min - max)	DT90 (d) (min - max)	k	Level of reduction in AM concentration during a storage duration (%) (C_i)			Level of an AM found in manure at the end of a storage duration, compared to the concentration of this AM in fresh manure (%) (Z_i)			Mean value of the level of an AM present in manure stored for a certain duration, compared to the concentration of this AM in fresh manure (%) ($Z_{i,\Delta t}$)			Mean amount of an AM present in manure stored for a certain duration in an average pit in Belgium (kg) ($D'_{i,\Delta t}$)			All references per AM
					After 1 month (m)	After 4 m	After 6 m	After 1 m	After 4 m	After 6 m	$T_1 \le 1m$	$1m < T_2 \le 4m$	$4m < T_3 \le 6m$	$V_1{}^h$= 16,67%	V_2= 50,00%	V_3= 33,33%	
Tetracyclines	Oxytetracycline	12-79 [a]	40-262	0.0152	37	84	94	63	16	6	81.5	39.5	11	695.47	1011.21	187.73	Massé et al. (2014), Wohde et al. (2016), Blackwell et al. (2015)
Tetracyclines	Doxycyline	53-120 [a]	176-399	0.008	21	62	76	79	38	24	89.5	58.5	31	4 007.78	7858.84	2776.34	Massé et al. (2014), Wohde et al. (2016), Widyasari-Mehta et al. (2016)
Penicillins	Amoxicillin	5 [b]	17	0.1386	98	100	100	2	0	0	51	1	0	1 388.18	231.36	0	Massé et al. (2014)
Sulfonamides	Sulfadiazine	17 [c]	56	0.0408	71	99	100	29	1	0	64.5	15	0.5	2 511.15	1751.96	38.93	Kreuzig and Höltge (2005), Lamshoft et al. (2007)
	Trimetho-prim	NF [d,e]	NF	NF	NE[i]	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NF
Lincosamides	Lincomycin	91	302	0.0075	20	59	74	80	41	26	90	60.5	33.5	309.60	624.35	230.48	Kuchta and Cessna (2009)
Macrolides	Tylosin	<2 [f]	<7	0.3466	100	100	100	0	0	0	50	0	0	55.45	0	0	Massé et al. (2014), Wohde et al. (2016), Loke et al. (2000), Kolz et al. (2005)
Macrolides	Tilmicosin	73	243	0.0095	25	68	82	75	32	18	87.5	53.5	25	195.46	358.53	111.69	SPC (2010)
Polymyxins	Colistin	NF	NF	NF	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NF
Aminocyclitols	Spectinomycin	30 [b]	100	0.0231	50	94	98	50	6	2	75	28	4	493.56	552.79	52.65	Massé et al. (2014), Kuchta and Cessna (2009)
Fluoroquinolones	Flumequine	100 [b]	332	0.0069	19	56	71	81	44	29	90.5	62.5	36.5	1.47	3.04	1.19	Massé et al. (2014)
Pleuromutilin	Tiamulin	>200 [g]	>664	0.0035	10	34	47	90	66	53	95	78	59.5	9.41	23.18	11.79	Schlüsener et al. (2006)

Notes: [a] The average of these DT_{50} values was used for the estimation of k; [b] these values correspond to group level estimations (Massé et al., 2014); [c] reasonable estimation, based on experimental data from bovine manure; [d] NF=quantitative estimate not found; [e] a likely short degradation period is expected (Haller et al., 2002); [f] for tylosin A; [g] the model estimations for this AM present minimum values; [h] the fractions of manure stored for different durations are denoted as V; [i] NE= not estimated.

Table 4. Summary of the second part of model estimations respective to module 2 ("stored manure") (i.e. mean values of D'_T, E') and of estimations respective to module 3 ("applied manure") (i.e. mean value of E_T)

AM class	AM compound	Total amount of an AM present in manure after storage in average ready-to-be-emptied pits, at country (Belgian) level (kg) (D'_T) (mean value)	Level of an AM found in manure after storage, compared to the amount of this AM administered orally to pigs (%) (E') (mean)[d]	Fraction of the amount of an AM initially administered orally that is present in pig manure which is applied onto Belgian soil without prior processing (%) (E_T) (mean)[e]
Tetracyclines	Oxytetracycline	1 894.41	36	19
Tetracyclines	Doxycycline	14 642.96	55	29
Penicillins	Amoxicillin	1 619.54	3	2
Sulfonamides	Sulfadiazine	4 302.05	10	5
	Trimethoprim	NE[b]	NE	NE
Lincosamides	Lincomycin	1 164.43	39	20.44
Macrolides	Tylosin	55.45	2	1.27
Macrolides	Tilmicosin	665.68	44	23
Polymyxins	Colistin	NE	NE	NE
Aminocyclitols	Spectinomycin	1 099.00	28	14
Fluoroquinolones	Flumequine	5.70	2.93	1.54
Pleuromutilin	Tiamulin	44.37[c]	7	3.92
Total of these compounds[a]		25 493.59	18.42	9.67

Notes: [a] total from AM compounds for which quantitative info was available; [b]NE= not estimated; [c] to be considered as minimum estimation; [d] E' is estimated for each AM and thus is applicable both at pit and country level; [e] for Belgium, $E_T=0.525*E'$.

4.2.3. Module 3 ("applied manure level")

In a country where 52.5% of the total produced pig manure is applied on soil unprocessed, like in Belgium (VLM, 2017), the level of each AM initially administered orally to pigs that is present in this stored but unprocessed manure applied on soil (E_T) is assumed to correspond respectively to the 52.5% of the level of each AM present in manure at the end of storage (E') (Table 4). Therefore, it is estimated that in Belgium, $E_T=9.67\%$ of the total amount of the ten AM is present unmetabolised in unprocessed manure at the moment of deposition on the soil.

In the same country, 40% of the total produced pig manure is processed with biological treatment, in which processed manure certain AM residues can also be present. Given the availability of data regarding the level of residues in manure after processing (d) with biological treatment for doxycycline (DOX) and sulfadiazine (SDZ), it was possible to estimate for these AM, their levels in the total processed pig manure, compared to their level in fresh manure ($E_{P(DOX)}$= 2%; $E_{P(SDZ)}$= 1%) and their total level in manure applied on soil, compared to the total initial amount of these AM administered to pigs ($E_{S(DOX)}$= 30%; $E_{S(SDZ)}$= 6%). The rest of manure (estimated 7.5%) is eventually exported and, therefore, not applied on Belgian soil.

5. Discussion

This model estimated that at least 18% of the total amount of antimicrobials (AM) administered to pigs orally are still present in manure readily applied onto soil (E'), in a country where the ten studied AM are used (Table 4). In Belgium, this percentage corresponds to over 25,000 kg of AM residues present in manure at the end of storage due to oral group treatments of pigs (D'_T). If manure processing and export are taken into account, the level of AM residues that can be transferred to soil via manure application is reduced to at least E_T=9.67% for this country. From the selected AM, the use of doxycycline (E'=55%), a tetracycline, is estimated to cause the highest level of residues that can end up in soil via this pathway. This is a combining result of its low bioavailability (Table 2) and high stability in manure (Table 3). In Belgium, where doxycycline is also highly used for pigs orally (*p.o.*) (Table 2), the highest total amount of residues present in manure after common storage durations and conditions (D'_T) concerns this AM. However, amoxicillin, despite being the AM mostly used in pigs *p.o.* in Belgium, is predicted to be found in comparatively small amounts in stored manure. In conclusion, besides usage amounts, the pharmacokinetic behavior of different AM is a decisive factor in the occurrence of AM ending up in the environment.

Along with doxycycline and oxytetracycline, the model estimated that high levels of residues of tiamulin, flumequine, lincomycin and tilmicosin, compared to their initial concentration in fresh

manure, can still be found after six months of manure storage in pits (Z_i), while a lower level is also estimated for spectinomycin. On the contrary, residues of amoxicillin, as a β-lactam model compound (Berendsen et al., 2015), and tylosin (AM with high levels in fresh manure, but not very stable in it), are not estimated to be found in manure stored for six months (Z_i). As for sulfadiazine, it has been observed that metabolites of the AM have been reversibly converted to sulfadiazine in manure during storage (Lamshöft et al., 2010), and therefore more residues of this AM than the estimated level would be expected to be present at the end of common manure storage durations.

The results of the literature search and this model regarding the AM that can be found in manure after common storage durations are in accordance with the manure test results from the Belgian experiment (Rasschaert et al., 2018; manuscript in preparation), with two exceptions: spectinomycin, which was not detected in the tested samples, although it was predicted to be according to this study; and tilmicosine, which was rarely found in the tested samples, in contrast to the estimations of this model. For spectinomycin, this might be due to the fact that our model estimations were based on one available reference presenting the results of a simulation experiment (Kuchta and Cessna, 2009), so the different conditions could have had a different effect on residues. Another possible explanation is that the limit of detection (LOD) of the method used for sample testing was rather high. When a more sensitive method is validated, samples could be re-analysed to check if residues of spectinomycin can be detected. For tilmicosince, this difference could be attributed to the fact that a reasonable estimation of DT_{50} was used in the model, as described in the Methods. Trimethoprim, for which no information on persistence was found in literature, was also rarely found in tested samples in the experiment and in low concentrations. In two studies performed in Spain (Carballo et al., 2016) and the Netherlands (Zuidema et al., 2014), doxycycline and oxytetracycline were the AM residues mostly found in samples of stored pig manure. According to this model, residues of oxytetracycline are predicted to be found less commonly, possibly because this AM is used less commonly in Belgium and/or because the average of two distant DT_{90} values available was used. The maximum DT_{90} value found for oxytetracycline is quite high ($DT_{90(max)}$=262 days), compared to the minimum one ($DT_{90(min)}$=40

days) (Wohde et al., 2016), pointing out a potential underestimation of the stability of this AM in stored manure.

To our knowledge, this is the first attempt to model together the mechanisms of metabolisation inside the animal body and of degradability in unprocessed and processed manure of AM commonly used orally in pigs and to provide estimates of the level of the administered AM that end up in manure readily applied onto soil (E' and E_S). These estimates can be further used in combined models assessing the fate of these AM in other environmental compartments, such as soil and water. Besides, the AM we focused on for the purposes of this manuscript are commonly used in many countries (EMA, 2017) and the results of the review and the model are relevant to them. Another strength of this model is that, although it followed a deterministic approach in a large part (equations 1-8), it can easily be transformed into a merely probabilistic one, upon availability of more data on bioavailability (F), metabolisation (Y), DT_{50} and degradability due to processing (d) depending on the method used.

This model assumed first-order kinetics for the selected AM inside the pig and in manure. In absence of published quantitative data regarding the time until total degradation of each AM (parent drug) in manure, this assumption allowed us to perform our calculations by estimating first the DT_{90} (time until degradation of 90% of AM in this matrix) values per AM, based on DT_{50} values retrieved. That is because the DT_{90} values, which are the closest estimates to the time until total AM degradation, are rarely specified in publications. Therefore, the exact estimations of this model need to be perceived as indicative. Moreover, although the application of manure to agricultural fields also likely introduces breakdown products (metabolites) into the environment along with the parent compound (Wohde et al., 2016), persistence data for degradation products were not found in the reviewed literature. It was therefore assumed that these products are inactive and were not included in the estimations of this study.

The amounts of AM residues present in manure after passive storage due to oral pig treatments in Belgium (D'_T) which are estimated according to this model, represent a proportion of the total amount

of AM residues that can end up in soil from veterinary use (*e.g.* other AM used in pigs, residues from consumption of cross-contaminated feed, other routes of administration, use in other species are not considered). To reduce the amounts of AM residues in manure, processing of animal waste with different methods prior to soil disposal is suggested as an effective approach (*e.g.* Varel et al., 2012; Chai et al., 2016; Youngquist et al., 2016) and should therefore be performed as much as possible. As shown in this study, biological treatment, which is the main method used for processing of pig manure in Belgium, reduced the amount of residues of doxycycline and sulfadiazine in this matrix. This method may as well be a tool to considerably reduce residues from other AM, antibiotic resistance genes and zoonotic pathogens present in the manure (Van den Meersche et al., 2017). To control the release of AM residues from manure to soil, the implementation of regulations dealing with fertilization of soil with manure at EU (EC, 1991) and country level and the specification of safety limits for pharmaceuticals in manure (currently unavailable) to regulate its applicability as fertilizer are also very crucial. Moreover, it would be interesting to investigate the effect of U.V. light on the degradation of antimicrobials in manure. For example, the results of Gómez-Pacheco et al. (2012) showed that medium-pressure mercury lamp radiation is effective for the photooxidation of tetracyclines in aqueous phase, mainly when these are at low concentrations. On the other hand, tetracycline-HCl in dry state is stable when stored at room temperature, regardless of exposure to light (Wu et al. 2005).

The results of this study highlight the importance of using veterinary antimicrobials rationally as their residues may spread in the environment and play a role in the selection, development and spread of antimicrobial resistance in different environmental compartments, for instance through the introduction of antimicrobial residues and resistance genes (e.g. *sul2*, a sulfadiazine-resistance gene) (Heuer et al., 2008), first, in soil. This research also highlights the class-specific behavior of different AMs in the majority of cases, which can be further used to advise on use of the different classes on a (supra)national level. As revealed from this study, the physical-chemical properties of tetracyclines (especially doxycycline) place them to a position of interest in this respect, as they can persist in high levels in manure stored for common storage durations, while on top of this, they are known to strongly

sorb (Kemper, 2008) and are therefore expected to remain in the soil or to be transported into surface waters via particles. Additionally, it is clear that more research is required to understand kinetics of biodegradation and potencies of degraded products of various antimicrobials in different environments (manure, soil, waste water).

6. Acknowledgements

7. **References**

Agersø H, Friis C, 1998. Bioavailability of amoxycillin in pigs. J Vet Pharmacol Ther. 21(1):41-46.

Almeida A, Duarte S, Nunes R, Rocha H, Pena A, Meisel L, 2014. Human and Veterinary Antibiotics Used in Portugal-A Ranking for Ecosurveillance. Toxics. 2:188-225.

Amábile-Cuevas CF, 2016. Antibiotics and Antibiotic Resistance in the Environment. CRC Press/Balkema. Taylor & Francis Group, London, UK.

(BelVet-SAC) Belgian Veterinary Surveillance of Antibacterial Consumption, 2017. 2016 National consumption report. p1-44. http://www.belvetsac.ugent.be/

Berendsen BJ, Wegh RS, Memelink J, Zuidema T, Stolker LA, 2015. The analysis of animal faeces as a tool to monitor antibiotic usage. Talanta. 132:258-268.

Bhavsar SK, Thacker AM, 2012. Pharmacokinetics of Antimicrobials in Food Producing Animals. Readings in Advanced Pharmacokinetics - Theory, Methods and Applications, 157-178. London, United Kingdom.

Binh CTT, Heuer H, Kaupenjohann M, Smalla K, 2008. Piggery manure used for soil fertilization is a reservoir for transferable antibiotic resistance plasmids. FEMS Microbiol Ecol. 66(1):25–37.

Blackwell P, Boxall A, Kay P, Noble H, 2005. Evaluation of a Lower Tier Exposure Assessment Model for Veterinary Medicines J. Agric Food Chem. 53:2192–2201.

Callens B, Persoons D, Maes D, Laanen M, Postma M, Boyen F, Haesebrouck F, Butaye P, Catry B, Dewulf J, 2012. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Prev Vet Med. 106 (1):53 62.

Carballo M, Sonia Aguayo S, Gonzalez M, Esperon F, Torre ADL, 2016. Environmental assessment of tetracycline's residues detected in pig slurry and poultry manure. J Environ Prot. 7:82-92.

Carmo LP, Schuepbach-Regula G, Muentener C, Chevance A, Moulin G, Magouras I, 2017. Approaches for quantifying antimicrobial consumption per animal species based on national sales data: A Swiss example (2006–2013). Eurosurveillance. 22(6):30458.

Chai R, Huang L, Li L, Gielen G, Wang H, Zhang Y, 2016. Degradation of Tetracyclines in Pig Manure by Composting with Rice Straw. Int J Environ Res Public Health. 13(3):254.

Du LF, Liu WK, 2012. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. Agron Sustain Dev. 32:309–327.

(EC) European Commission, 1991. Council Directive of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources (91 / 676 /EEC). Official Journal. L 375 / 1, p. 1-8.

(EMA) European Medicines Agency, 2009. Colistin. Summary Report of the European Agency for the Evaluation of veterinary medicinal products. p http://www.ema.europa.eu/

(EMA) European Medicines Agency, 2017. European Surveillance of Veterinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 30 European countries in 2015. p http://www.ema.europa.eu/

Filippitzi ME, Callens B, Pardon B, Persoons D, Dewulf J, 2014. Antimicrobial use in pigs, broilers and veal calves in Belgium. Vlaams Diergeneeskd Tijdschr. 83(5):215.

Gomez-Pacheco CV, Sanchez-Polo M, Rivera-Utrilla J, Lopez-Penalver JJ, 2012. Tetracycline degradation in aqueous phase by ultraviolet radiation. Chemical Engineering Journal. 187. p.89(7).

(VCM) Flemish Coordination Centre for Manure Processing, 2016. VCM-enquete operationele stand van zaken mestverwerking in Vlaanderen. p1-35 https://www.vcm-mestverwerking.be

Heuer H, Focks A, Lamshöft M, Smalla K, Matthies M, Spiteller M, 2008. Fate of sulfadiazine administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in manure and manured soil. Soil Biol Biochem. 40:1892-1900.

Heuer H, Schmitt H, Smalla K, 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. Curr Opin Microbiol. 14:236–243.

Hornish RE, Gosline RE, Nappier JM, 1987. Comparative metabolism of lincomycin in the swine, chicken, and rat. Drug Metab Rev. 18(2-3):177-214.

(JEFCA) Joint FAO/WHO Expert Committee on Food Additives, 2009. Toxicological evaluation of certain veterinary drug residues. WHO Food Additives Series 61. p1-240. http://www.who.int/

Kemper N, 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecol Indic. 8(1):1-13.

Kolz AC, Moorman TB, Ong SK, Scoggin KD, Douglass EA, 2005. Degradation and metabolite production of tylosin in anaerobic and aerobic swine-manure lagoons. Water Environ Res. 77(1):49-56.

Kreuzig R, Höltg S, 2005. Investigations on the fate of sulfadiazine in manured soil: Laboratory experiments and test plot studies. Environl Toxicol Chem. 24:771–776.

Kuchta SL, Cessna AJ, 2009. Lincomycin and spectinomycin concentrations in liquid swine manure and their persistence during simulated manure storage. Arch Environ Contam Toxicol. 57(1):1-10.

Lamshöft M, Sukul P, Zühlke S, Spiteller M, 2007. Metabolism of 14C-labelled and non-labelled sulfadiazine after administration to pigs. Anal Bioanal Chem. 388(8):1733-1745.

Lamshöft M, Sukul P, Zühlke S, Spiteller M, 2010. Behaviour of (14)C-sulfadiazine and (14)C-difloxacin during manure storage. Sci Total Environ. 408(7):1563-1568.

Lewicki J, Reeves P, Swan G, 1991. Tylosin. Addendum to the monograph prepared by the 38th Meeting of the Committee. FAO Food and Nutrition Paper 41/4.

Loke ML, Ingerslev F, Halling-Sørensen B, Tjørnelund J, 2000. Stability of Tylosin A in manure containing test systems determined by high performance liquid chromatography. Chemosphere. 40(7):759-765.

MacNeil JD, 1995. Joint FAO/WHO Expert Committee on Food Additives. 45th Meeting. Tilmicosin. In: Residues of some veterinary drugs in animals and foods. Food and Agriculture Organization of the United Nations. http://www.fao.org/home

Massé DI, Saady NMC, Gilbert Y, 2014. Potential of Biological Processes to Eliminate Antibiotics in Livestock Manure: An Overview. Animals. 4(2):146–163.

Mengelers MJ, van Gogh E, Huveneers MB, Hougee PE, Kuiper HA, Pijpers A, Verheijden JH, van Miert AS, 2001. Pharmacokinetics of sulfadimethoxine and sulfamethoxazole in combination with trimethoprim after oral single- and multiple-dose administration to healthy pigs. Vet Res Commun. 25(6):461-481.

Mevius DJ, Breukink HJ, Guelen PJM, Jansen T, de Grkve B, 1990. Pharrnacokinetics, metabolism and renal clearance of flumequine in veal calves. J Vet Pharmacol Ther. 13(2):159-169.

Nielsen P, Gyrd-Hansen N, 1996. Bioavailability of oxytetracycline, tetracycline and chlortetracycline after oral administration to fed and fasted pigs. J Vet Pharmacol Ther. 19(4):305-311.

Nielsen P, Gyrd-Hansen N, 1998. Bioavailability of spiramycin and lincomycin after oral administration to fed and fasted pigs. J Vet Pharmacol Ther. 21(4):251-256.

Postma M, Sjölund M, Collineau L, Lösken S, Stärk K, Dewulf J, 2015. MINAPIG Consortium. Assigning defined daily doses animal: a European multi-country experience for antimicrobial products authorized for usage in pigs. J Antimicrob Chemother. 70(1):294-302.

Postma M, 2016. Antimicrobial usage in pig production: check, improve and reduce in Belgium and the EU. PhD Thesis. Ghent University.

Ramos F, Boison F, Friedlander LG, 2012. Amoxicillin. Food and Agriculture Organization of the United Nations. http://www.fao.org/home

Rasschaert G, Lander C, Daeseleire E, Herman L, Heyndrickx M, 2017. Antibiotic resistant Salmonella and E. coli from pig and veal manure In: Proceedings of the 22th Conference on Food Microbiology. Brussels, 21 September 2017.

Rasschaert G, Daeseleire E, Meirlaen J, Huygens J, Herman L, Heyndrickx M, 2018. Antibiotic residues in pig and veal manure. Eighth International Symposium on Hormone and Veterinary Drug Residue Analysis. Ghent, 22-25 May 2018. (Accepted for oral presentation)

Reyns T, De Boever S, Baert K, Croubels S, Schauvliege S, Gasthuys F, De Backer P, 2007. Disposition and oral bioavailability of amoxicillin and clavulanic acid in pigs. J Vet Pharmacol Ther. 30(6):550-555.

Riond JL, Riviere JE, 1990. Pharmacokinetics and metabolic inertness of doxycycline in young pigs. Am J Vet Res. 51(8):1271-1275.

Riviere JE, Papich MG, 2009. Veterinary Pharmacology and Therapeutics, 9th Edition. Wiley-Blackwell.

Rostel B, Zmudzki J, MacNeil JD, 2000. Lincomycin, in Residues of Some Veterinary Drugs in Foods, FAO Food and Nutrition Paper 41/13. 59–74.

Schlüsener MP, von Arb MA, Bester K, 2006. Elimination of macrolides, tiamulin, and salinomycin during manure storage. Arch Environ Contam Toxicol. 51(1):21-28.

Smet E, Debruyne J, Deckx J, Deboosere S, 2003. Manure treatment according to the Trevi-concept. Commun Agric Appl Biol Sci. 68(2 Pt A):125-131.

Summary of Product Characteristics (SPC), 2010. Pulmotil® medicated premix (Elanco®).

Summary of Product Characteristics (SPC), 2011. Denagard® medicated premix. UK DEFRA (UKPAR_327974).

Van den Meersche T, Rasschaert G, Haesebrouck F, Van Coillie E, Daeseleire E, Heyndrickx M, 2017. The Effect of Biological Treatment of Manure on the Presence of Antibiotic Residues, Antibiotic Resistance Genes, and Zoonotic Pathogens. Book of abstracts IAFP's European symposium on food safety, International Association for Food Protection, European symposium on food Safety. Brussels, 29 March 2017.

Varel VH, Wells JE, Shelver WL, Rice CP, Armstrong DL, Parker DB, 2012. Effect of anaerobic digestion temperature on odour, coliforms and chlortetracycline in swine manure or monensin in cattle manure. J Appl Microbiol. 112(4):705–715.

(VLM) Vlaamse Landmaatschappij, 2014. In Dutch: Opslag van dierlijke mest. Storage of animal manure. https://www.vlm.be/nl

(VLM) Vlaamse Landmaatschappij, 2017. Mestrapport 2016. p1-195. https://www.vlm.be/nl

Weiland P, 2010. Biogas production: current state and perspectives. Appl Microbiol Biotechnol. 85:849.

Whitehead TR, Cotta MA, 2013. Stored swine manure and swine faeces as reservoirs of antibiotic resistance genes. Lett Appl Microbiol. 56(4):264-267.

Widyasari-Mehta A, Suwito HR, Kreuzig R, 2016. Laboratory testing on the removal of the veterinary antibiotic doxycycline during long-term liquid pig manure and digestate storage. Chemosphere. 149:154-160.

Widyasari-Mehta A, Hartung S, Kreuzig R, 2016. From the application of antibiotics to antibiotic residues in liquid manures and digestates: A screening study in one European center of conventional pig husbandry. J Environ Manage. 177:129–137.

Wohde M, Berkner S, Junker T, Konradi S, Schwarz L, Düring RA, 2016. Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. Environ Sci Eur. 28(1):23.

Wu Y and Fassihi R, 2005. Stability of metronidazole, tetracycline HCl and famotidine alone and in combination. Int. Pharm. 290: 1-13.

Youngquist CP, Mitchell SM, Cogger CG, 2016. Fate of Antibiotics and Antibiotic Resistance during Digestion and Composting: A Review. J Environ Qual. 45(2):537-545.

Zuidema T, Stolker AAM, van Ginkel LA, 2014. Kruiscontaminatie van antibiotica. Onderzoek naar de aanwezigheid van antibioticaresiduen in mest van vleesvarkens en vleeskalveren en op pluimveebedrijven. RIKILT report, Wageningen UR. p1-52.

CHAPTER 8

General Discussion

1. Residues of antimicrobials in non-target pig feed: how big is the problem in relation to antimicrobial resistance?

The production and use of antimicrobial medicated feed results in cross-contamination of non-target feed with residues of antimicrobials. Since this cross-contamination can occur through different pathways, at different levels (i.e. feed mill, transport truck, farm) (Figure 1) and depends on various factors (e.g. properties of the compound, fast production lines, age of transport trucks), it is generally considered unavoidable to a certain extent. On the other hand, keeping the level of antimicrobial carry-over to the proposed maximum limit of 1% (EC, 2014) is very demanding from an operational point of view (e.g. requires thorough sampling and control of flushing batches) and probably too expensive to keep it profitable. This is the reason why the Association of Dutch Feed Produces (NEVEDI) voluntarily decided to cease the production of medicated feed in feed mills in 2011. In Belgium, to reduce the risk of cross-contamination during the production of medicated feed at the feed-mill, the antimicrobial premix can only be mixed in the feed at a dedicated end-of-line mixer, since 2013 (BFA, 2013).

The biggest consumer of antimicrobial medicated feed is the pig population (e.g. 99.3%, in Belgium (BelVet-SAC, 2017)). To assess the level of exposure of the pig population to feed accidentally cross-contaminated with antimicrobial residues and the consequent potential for resistance selection, two models were developed (Chapters 4, 5). Even though the estimations of the models are largely based on Belgian data, important conclusions can be drawn to a wider scale.

First, this cross-contamination can occur at one or more of the following levels: the feed mill, the transport truck as well as the farm. This means that the risk needs to be controlled at three different levels, largely independent from each other, and therefore it is very hard to be fully removed. This was confirmed when two what-if scenarios were tested (Chapter 4), which concerned the use of an end-of-line mixer at the feed-mill (scenario 1) and the use of a fine-dosing system truck (scenario 2). Both of these scenarios exclude the cross-contamination at the feed-mill, where the highest level of carry-over has been observed (>3%) (Stolker et al., 2013). The second model also excludes the cross-contamination in the bin of the transport truck (1% carry-over possible in the truck bin during feed transport) (Putier, 2010). Even though a significant reduction of the risk was observed in these scenarios (30% for scenario 1; 54% for scenario 2), carry-over could still occur albeit at a lower level (1% carry-over possible during feed unloading (Putier, 2010); predicted 1% carry-over at the farm). Further research currently executed at the faculty of Veterinary Medicine of Ghent University aims to estimate the level of antimicrobial carry-over at the farm level.

Secondly, the production at the feed-mill and the use of antimicrobial medicated feed results in the cross-contamination of batches of non-target feed which can generally reach any pig, often

irrespectively of whether medicated feed is used at a farm. For instance, in Belgium, flushing batches of feed are fed to piglets, sows and fattening pigs <80kg. Likewise, a batch of non-target feed can get cross-contaminated in the truck, if transported directly after a medicated feed batch, and then be fed to animals of the farm it is delivered to. According to the second model developed (Chapter 5), the risk of exposure to feed cross-contaminated with antimicrobial residues is higher for piglets and sows, and lower for fattening pigs, in absolute and in relative values. The most important reason for the higher exposure of sows is the fact that, since sows are not expected to be slaughtered soon, the risk of residues in their meat due to cross-contaminated feed is low and, therefore, the production of sow feed is preferred after the production of a batch of medicated feed. Another reason for the higher exposure of sows in relative values [e.g. 7.7% and 22.4% of sows in Belgium exposed to feed with 1% carry-over of doxycycline (DOX) and sulfadiazine (SDZ), respectively], is the fact that different feed is given to sows depending on their stage (i.e. lactation, gestation), which is often transported or stored at different times, representing more opportunities for cross-contamination. Other reasons include the smaller population and longer duration of this production stage compared to the other two stages. In terms of exposure to these residues, the piglets follow after sows (e.g. 4.2% and 12% of piglets in Belgium exposed to feed with 1% carry-over of DOX and SDZ, respectively) and precede the fattening pigs in relative values. As for the sows, feed for piglets is also produced after medicated feed, since piglets are further away from the time of slaughter than fattening pigs and the risk for residues in meat is low. The level of exposure of piglets is higher compared to fattening pigs also due to the distribution of pig production (e.g. number of production rounds per year is higher for piglets). In response to in-feed antimicrobials, including residues in cross-contaminated feed, younger pigs appear to exhibit more changes in their gut microbiota compared to older swine (Holman and Chénier, 2015). As a consequence, feeding potentially cross-contaminated feed to young piglets is likely more detrimental.

Thirdly, the exposure of pigs to residual concentrations of different antimicrobials depends on the pharmacokinetic properties of each compound and the level of use of each compound for the production of medicated feed. Given the important role of pharmacokinetics, the general proposed limit of 1% carry-over can result in completely different intestinal concentrations for different antimicrobial compounds. For instance, Peeters et al. (2016) found a correlation between the oral bioavailability and the intestinal residual concentrations of doxycycline (DOX), chlortetracycline (CTC) and sulphadizine-trimethoprim (SDZ-TRIM): the transfer of the antimicrobial from feed to gut was high to very high in the case of tetracyclines, which have generally low oral bioavailability in pigs (DOX 21-50%, CTC 6-18%). On the other hand, SDZ and TRIM, compounds with high oral bioavailability (SDZ 85%, TRIM 92%), showed a low transfer from feed to gut. This correlation of intestinal concentrations should be interpreted carefully, as other (unknown) pharmacokinetic properties might also influence

these concentrations. For example, tetracyclines, undrego enterohepatic circulation (del Castillo et al., 2013). Moreover, these results should not be extrapolated to other antimicrobials as each one has its specific properties. At country level, the level of use of different antimicrobials also affects the exposure of pigs to different residues. For instance, in Belgium, SDZ is the antimicrobial used the most as premix (always in combination with TRIM), and therefore residues from exposure to feed cross-contaminated with SDZ are estimated to be found in many pigs (Chapter 5), although at a lower concentration in the gut than antimicrobials with lower bioavailability.

Fourthly, cross-contamination of feed with antimicrobials, including doxycycline (DOX), likely promotes the spread of antimicrobial resistance. A study by Peeters et al. (2018) showed that residual concentrations of DOX as low as 1 and 4 mg/L likely to be found in the porcine caecum and colon of pigs, due to cross-contaminated feed with 1% and 3% carry-over level respectively, have the potential to enrich tetracycline resistant commensal *E. coli in vitro*. Using also an *ex vivo* model simulating the porcine caecum, Peeters et al. (2017) observed that residual intestinal DOX concentrations, corresponding to a carry-over level equal and higher than 1%, have the potential to select for DOX resistant *E. coli* and to promote conjugation. Therefore, the presence of residual DOX concentrations in the porcine microbiota also forms a threat to the health of other species, including humans, in particular because of co-transfer of important resistance genes together with the DOX resistance genes. Yet, the type of antimicrobial and the associated resistance mechanisms may strongly influence the extent to which selection of resistant bacteria occurs. Consequently, no conclusions can be drawn on the selective pressure of residual concentrations of other antimicrobials in absence of experimental data available in literature.

At this stage, it is rather challenging to specify the contribution of (pig) feed cross-contamination with antimicrobial residues to the problem of antimicrobial resistance in animals. A rough estimation would suggest that it is likely small, for instance compared to the intended use of antimicrobials to animals (pigs). However, given the four aforementioned points (occurrence at different levels, wide exposure of pigs, role of pharmacokinetics, role of residues in selection pressure), this issue should be controlled. Recommendations to control this issue are described in the respective sections (7.3.1, 7.3.2).
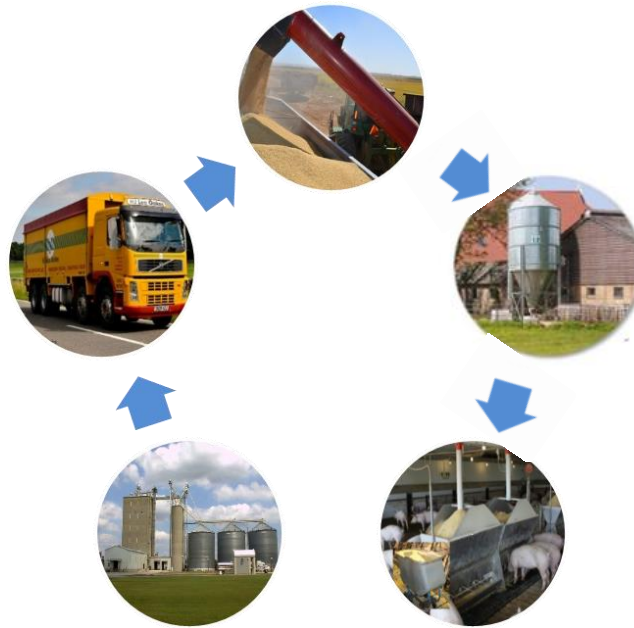
Figure 1. Five processes related to medicated feed use: production at the feed-mill, transport and unloading from truck, storage and distribution at the farm.

2. Residues of in-feed and in-water antimicrobials in pig manure: how big is the problem in relation to antimicrobial resistance?

Residues of antimicrobials administered to pigs in feed or water end up in manure which, processed or unprocessed, is applied on soil as fertilizer and soil amendment. Safety limits for antimicrobials in manure to regulate its applicability as fertilizer are currently unavailable. The release of antimicrobial residues from manure to soil is unintendedly controlled to a certain (unknown) extent by the implementation of regulations dealing with fertilization of soil with manure at the European Union (EU) (EC, 1991). These regulations mainly focus on controlling and limiting the release of nitrogen and phosphorus in soil.

In the EU, oral treatments represent the most common route of antimicrobial administration for livestock species (>90%) (EMA, 2017). In Belgium, oral is also the most common route of administration of antimicrobials to (groups of) pigs, which are the highest consumers of antimicrobials in the country in absolute terms, compared to other important agricultural sectors (Chapter 2). To estimate the level of antimicrobials administered to pigs via feed and water that end up in soil via manure application, a model was developed in Chapter 6. Although the estimations of the model are based on Belgian data

in terms of amounts (i.e. estimated kg of residues in manure), important conclusions can be drawn to a bigger scale.

Firstly, residues of many antimicrobials are still present (as parent compound) in pig manure after storage of even 6 months, which is a common maximum storage duration. This is predicted to be the case for 8 out of 10 antimicrobials commonly used in pigs, namely: doxycycline, oxytetracycline, sulfadiazine, tilmicosin, lincomycin, spectinomycin, flumequine and tiamulin (Chapter 7). This is a combining result of the pharmacokinetics and the stability in pig manure of each compound. Regarding pharmacokinetics, a general observation was that low (oral) bioavailability and/or low metabolisation of an antimicrobial result in a high level of residues of this antimicrobial being excreted as parent compound in freshly produced manure, and vice versa. Based on the model, the highest level of residues in stored manure concerns two tetracyclines (doxycycline and oxytetracycline), the macrolide tilmicosin and the lincosamide lincomycin, from the ten antimicrobials here studied. In particular, the highest level of residues is estimated for doxycycline (DOX) (i.e. 55% of DOX in manure after storage for six months, compared to amount of DOX initially administered to pigs orally), which is one of the most commonly used oral (p.o.) antimicrobials (BelVet-SAC, 2017; EMA, 2017). On the contrary, amoxicillin, despite being the antimicrobial mostly used in pigs p.o. in Belgium, is predicted to be found in comparatively small amounts in stored manure, as it is not stable in this matrix. As for sulfadiazine, which is also highly used in pigs p.o., it has been observed that metabolites of the antimicrobial have been reversibly converted to sulfadiazine in manure during storage (Lamshöft et al., 2010), and therefore more residues of it than the estimated (low) level would be expected to be present at the end of manure storage. Persistence data for antimicrobial metabolites were not available and therefore not included in the estimations of the model.

Secondly, land application of manure containing active antimicrobial residues enables the spread of antimicrobial resistance. Antimicrobial residues in manure may select for resistance in pathogens and commensal bacteria, that may be introduced to soil after fertilization (Du and Liu, 2012; Rasschaert et al., 2017). Manure may also serve as a reservoir for antimicrobial resistance genes and mobile genetic elements (Binh et al., 2008; Whitehead and Cotta, 2013; Wang et al., 2018). Residual antimicrobials in the soil may possibly assist in developing antibiotic resistant microbial populations (Morris and Masterton, 2002), microbial activity (Jjemba, 2002), and alter soil microbial constitution and functions. The occurrence of antimicrobial resistance genes in various soil bacteria has been detected (Heuer et al., 2008, 2011) and their abundance in soils has increased significantly in time (Knapp et al. 2010). Furthermore, it has been suggested that the concentrations of antimicrobial residues found in agricultural soils (i.e. even 100 times lower than the minimum inhibitory concentrations (MIC) values) could be high enough to enrich these environments with resistant bacteria (Liu et al., 2011; Sandegren,

2014; Carballo et al., 2016). Moreover, antimicrobial residues could be absorbed by and accumulated in edible parts of vegetables, such as lettuce (Cui et al., 2008), potato (Dolliver et al., 2007), and leek (Wang and Han, 2008). The uptake of antimicrobials by vegetables differs between antimicrobials and vegetable species (Boxall et al., 2006). The consumption of (raw) vegetables poses a threat to human and animal health in terms of transmission of antimicrobial resistance via the food-borne route. Leaching of antibiotics into deeper soil or into groundwater is also possible (Joy et al., 2013). Yet, the sparsely available existing information needs to be expanded in order to fully appreciate the relationship between the presence of residual concentrations of antibiotics in manure and soil and the development and spread of antimicrobial resistance in the environment.

Thirdly, the treatment (processing) of pig manure before application reduces the amount of antimicrobial residues introduced in soil via manure application. The level of reduction is different depending on the method used. Generally, there are three methods to treat manure: biological treatment which follows denitrification (anaerobic) and nitrification (aerobic) processes (Smet et al., 2003), biothermal drying (also called composting) (Massé et al., 2014; Youngquist et al., 2016) or anaerobic digestation (Weiland P., 2010; Widyasari-Mehta et al., 2016). In Belgium, the liquid fraction of pig manure is mainly processed with biological treatment, while the solid fraction is mainly processed by biothermal drying (composting) and is, after drying, exported (VCM, 2016). So far, there is no published information on the effectiveness of the biological manure treatment regarding the level of reduction of antimicrobial residues. However, preliminary results from a Belgian project examining this (Van den Meersche et al., 2017) showed that biological manure treatment in one farm substantially reduced the amount of residues of doxycycline (estimated 92% reduction) and sulfadiazine (estimated 57% reduction) in pig manure. In a follow-up study on another farm, this reduction was not observed, so more results are needed to conclude if and at what extent biological manure treatment can cause reduction in residues. Composting is also considered as an effective method to remove antimicrobial residues. For instance, doxycycline was removed up to 70% as parent compound after composting of pig feces for 112 days under laboratory conditions (Szatmári et al., 2011). Indicatively, oxytetracycline was reduced by 85-99% after composting of calf manure (Arikan et al., 2007), likely due to additional aerobic bioactivity (Massé et al., 2014). Anaerobic digestion can also degrade antimicrobials to various extents depending on certain factors (e.g. class of antimicrobial, bioreactor operating conditions) (Varel et al., 2012). Despite the documented effectiveness of these methods in reducing the level of antimicrobial residues in manure, manure is very often applied on soil without prior processing. In Belgium, 52.5% of the total produced pig manure is estimated to be applied on soil unprocessed (VLM, 2017). Even if the reduction of residues is not full during treatment and the results seem to vary between different compounds and methods, manure treatment is an

effective approach to reduce the amount of antimicrobial residues spread to soil. However, no conclusions can be drawn yet regarding the effectiveness of these methods in completely removing antimicrobial resistance genes and bacteria from different factions of manure (e.g. effluent, sludge) or other manure products (i.e. compost, digestates). Research currently executed at the Flanders research institute for agriculture, fisheries and food (ILVO) aims to fill this latter knowledge gap.

It is very challenging to specify the level of contribution of oral pig antimicrobial treatments to the development and spread of antimicrobial resistance in soil (and the environment). The main reasons for this are the limited information available regarding the behavior of many antimicrobials in manure of pigs (even more limited for other species, e.g. cattle), and the limited information regarding the role of different antimicrobials in the development and spread of resistance in manure and soil. However, if we focus on the contribution of oral pig antimicrobial treatments to the amount of residues ending up in soil due to agricultural practices, the contribution could be assumed to be quite high. This can be assumed as: first, pigs are among the highest livestock consumers of antimicrobials and specifically in Belgium, are estimated to be the highest, compared to other important sectors such as poultry (Persoons et al., 2010), veal calves (Pardon et al., 2012) (Chapter 2) and cattle (Stevens et al., 2016). Second, the oral administration of antimicrobials is generally assumed to result in the excretion of more residues in fresh pig manure, compared to the parenteral, due to incomplete absorption of antimicrobials. Third, the exposure of pigs to cross-contaminated feed (Chapter 5) and water also adds up to the amount of residues in manure from oral use of antimicrobials. Consequently, controlling the issue of antimicrobial residues in manure and especially those resulting from oral treatments in pigs, is a necessity. Recommendations to control this issue are described in the respective section (7.3).

3. Antimicrobial treatment in pigs through feed and water: The way forward?

In this section, recommendations regarding the oral use of antimicrobials to pigs are provided. With a focus on the subjects of this thesis (Figure 2), these recommendations cover four general axes: practices, evidence, awareness and policy. These are also the broader focus areas of the FAO Action Plan on antimicrobial resistance (Figure 3) (FAO, 2016).

3.1. Reduce oral antimicrobial treatments of pigs

Reducing the amount and frequency of oral antimicrobial treatments of pigs is a logic and straightforward way to achieve a reduction in the total use of antimicrobials in pigs (and livestock). This is important regarding antimicrobial resistance, since it has been shown that antimicrobials use is

the strongest driver for antimicrobial resistance (e.g. Chantziaras et al., 2014). A reduction of antimicrobial treatments can be achieved by the following ways:

- Increased focus on disease prevention through improvement of herd management, vaccination schemes, biosecurity, water and feed quality. Postma et al. (2015) showed that improved herd management and biosecurity resulted in a reduction of antimicrobial use of 52% in finisher pigs (from birth to slaughter) and 32% in breeding animals. They also observed improved production results (e.g in daily weight gain, number of piglets/sow/year) and thereby an economic profit (Collineau et al., 2017). Alternatives to antimicrobial use also include the use of probiotics, prebiotics and competitive exclusion products (Chantziaras et al., 2017), while the shift from conventional to organic farming is generally linked to less antimicrobial use and resistance (Österberg et al., 2016).

- Prohibition of prophylactic antimicrobial treatments. This includes a reduction in the production of medicated feed, as this type of administration is very often prophylactic. A strong reduction of the total antimicrobial use and of medicated feed is already observed in Belgium during the past years (e.g. 31% decrease in use of medicated feed between 2014-2016) (BelVet-SAC, 2017). Therefore, the results presented in Chapter 3 are believed to have been also reduced in absolute values. The prohibition of prophylactic antimicrobial treatments would also result in a reduction of the administration of in-water medication, which should only be used for metaphylactic or therapeutic purposes when necessary. Moreover, from a global perspective, the use of growth promoters (use of lower than therapeutic doses of antimicrobials) should also be prohibited (outside the EU and the USA).

- Only treat the animals which should be treated, e.g. via parenteral injection. In this respect, it is also important to keep the number of animals that need to be treated limited, through early detection of infectious diseases (e.g. using biomarkers), separation of the early detected infected animals and individual treatment of them. This in accordance with the most recent WHO guidelines on the use of important antimicrobials in food-producing animals, urging to stop using antimicrobials in healthy animals (WHO, 2017a).

- Choice of antimicrobial, based on correct laboratory identification of the bacterium and an accurate assessment of its *in vitro* antimicrobial susceptibility. In practice, this laboratory testing is rather expensive and time demanding and, therefore, the choice of antimicrobial is based on the clinical diagnosis and experience the veterinarian. Still, regular susceptibility testing should be promoted, even with late reception of results, to increase the veterinarian's experience and to keep him/her better informed of the next disease outbreak in the same epidemiological unit.

- Based on the results of this thesis, it is recommended to use doxycycline (DOX) only after laboratory testing, due to the pharmacokinetic properties (low absorption), high excretion in manure and association with resistance selection in the pig gut. Moreover, DOX is stable in manure and soil, posing also an environmental risk. Besides, it is a highly important antimicrobial for human health, according to WHO (2017b). Targeting specific compounds should always be approached very cautiously, as the decrease in use of one antimicrobial (or a class) can potentially result in the increase of another antimicrobial (or class). For instance, this was recently observed in Denmark, where the reduction of the use of tetracyclines, within the framework of the so-called Yellow Card Initiative, resulted in a considerable increase of the use of macrolides (Okholm Nielsen et al., 2018). The role of this change with regard to antimicrobial resistance is currently unknown.

3.2. Reduce cross-contamination of feed and water

Reducing antimicrobial cross-contamination following oral treatments, albeit challenging, is crucial to reduce the potential for resistance selection in the gastro-intestinal tract and to reduce the level of antimicrobial residues in manure. Besides the reduction of oral antimicrobial treatments in pigs, including reduction of the production of medicated feed, a number of other measures can be taken to reduce carry-over in non-target feed and water.

Reduce carry-over in feed

- At the feed-mill. The risk of carry-over can be avoided considerably if the premix is added in the feed through a dedicated end-of-line mixer, instead of the main mixing line. This is the case for Belgium, since 2013. If the same mixing line is used for the production of medicated and non-medicated feed, then it is crucial to target practices (production of flushing feed), the process (reduction of process length) and the feed (choice of less and non-powder products) that reduce the risk of cross-contamination. Cross-contamination at the feed-mill is also avoided when a fine dosing system is used. This is a recent practice which involves the mixing of the antimicrobial with feed at the time of delivery at the farm. Even though this is a more efficient method in terms of reducing the risk of carry-over, it is (still) expensive and therefore the use of these trucks is limited.
- During transport and unloading of feed. Cross-contamination during transport (and also at the feed-mill) is minimized when a fine dosing system is used. When normal trucks are used to transport medicated feed, carry-over can be reduced by the use of new trucks (in years of use) and the use of the back bins to reduce the length of the circuit. The purging of trucks after each delivery is a responsibility of the drivers and, therefore, raising their awareness is of big importance. A professional cleaning of the trucks in regular intervals by dedicated companies would also be

beneficial to avoid persisting points of residue retainment, but this should act in a complementary way to the purging by the drivers.

- At the farm. Carry-over at the farm depends on the practices of the farmer. Cleaning and disinfection of the silo, the pipelines for feed and the feeding troughs is rarely performed (Chapter 3). Therefore, raising the awareness of farmers and the personnel on the importance of regular and thorough purging is essential. To avoid cross-contamination during storage, the use of a separate silo for medicated feed would be optimal. The use of top dressing as an alternative method for antimicrobial administration (often used in sows), although less risky in terms of carry-over, it is not recommended due to possible inhomogeneity of the feed and consequent improper dosage.

Reduce carry-over in water

Besides medicated feed, antimicrobials are often administered orally via water. Based on the field-study (Chapter 3), in-water medication was the most common method of oral administration of antimicrobials to piglets and fattening pigs. Compared to medicated feed, it is assumed that in-water medication is a less risky practice in terms of carry-over, since it can occur only at one level, the farm. This means that to control this issue, it is important to focus on the farmer and the personnel and raise their awareness on the importance of:

- Cleaning and disinfection. Cleaning and disinfection of waterlines, which improves water quality, removes the biofilm (group of microorganisms in which cells stick to each other and often also to a surface) and prevents carry-over, is practiced in the majority of the farms visited in Belgium (62%), but an increase of this practice should be stressed, especially after the administration of in-water antimicrobials. Cleaning of the tools used for the preparation of the medication is also a good practice. The use of a separate pipeline for the administration of water medication (e.g. in only 8% of visited farms in Belgium) would also reduce considerably the risk of carry-over, while the type of pipelines and of water tank and the use of pipelines of good quality is also important.
- Use of good quality water. Insufficient water quality, among other things (e.g. homogeneity and stability problems), likely worsens the condition of the pipelines, where points of residue retainment are created. Additionally, the hardness and pH of the water can affect the solubility of different antimicrobials. For example, low pH is needed to dissolve tetracyclines, otherwise the drug sinks to the bottom and can cause the presence of residues. A sample to determine the drinking water quality should be taken regularly, which is not the case at the moment (Filippitzi et al., 2017).

Furthermore, appropriate pharmaceutical formulations for use in water should be developed and used, especially for use with dosing pumps.

3.3. Policy measures

Establishing new policy and regulations is a key step in the efforts towards the reduction of antimicrobial use and the battle against antimicrobial resistance. From a worldwide perspective, a ban of the use of subtherapeutic antimicrobials as growth promoters should be extended outside Europe and the USA. Fortunately, an important (46%) decrease in the percentage of countries authorising use of antimicrobials as growth promoters has been observed in the past few years (between 2012-2015), according to the most recent OIE report on antimicrobial use in animals (OIE, 2016). Another important measure would be the prohibition of prophylactic treatments. Antimicrobial stewardship (a systematic approach to optimising use of antimicrobials, to reduce inappropriate use, and reduce adverse consequences of antimicrobials, including antimicrobial resistance) has been touted as one of the key strategies required in tackling worldwide escalation of antibiotic resistance (Tiong et al., 2016). However, due to economic (and political) interests (e.g. rising economies in Asia or Latin America shifting to large-scale intensive farming), it would be challenging, yet not impossible, that these measures could be implemented at a full global scale. From a European Union (EU) perspective, it is necessary to establish a uniform policy to control the issues of concern of this thesis: the carry-over of antimicrobials residues in feed and the transfer of antimicrobials residues to soil via manure. Recommendations at policy level regarding these issues could be:

Control of carry-over in feed

Establishing legal limits for carry-over of antimicrobials should have a positive effect in preventing antimicrobial resistance selection and reducing the amount of residues in the environment. According to the last proposal for a regulation adopted by the European Commission (EC, 2014), it is proposed to introduce a general limit for carry-over of antimicrobials of 1% of the active substance of the last produced batch of medicated feed at the feed-mill. However, achieving the 1% limit at the feed-mill is rather challenging from a practical and economical point of view (e.g. regular and thorough testing and sampling of often inhomogeneous flushing batches, or purchase of additional mixers). This is the reason why this proposal has caused the reaction of several agencies (e.g. Committee on Agriculture and Rural Development (AGRI), European Feed Manufacturers' Federation (FEFAC)), who claimed that the proposed 1% limit is not feasible in practice (EC, 2016).

However, besides the practical concerns that arise regarding the 1% limit, the concerns regarding the potential for antimicrobial resistance development remain. It has been recently shown by Peeters et al. (2017, 2018) that doxycycline concentrations corresponding to 1% carry-over, select for resistance

to *E. coli*. They also observed that a 1% carry-over level of doxycycline possibly does not reduce the risk on resistance selection substantially compared to a 3% carry-over level. This 3% carry-over is the current limit used for feed additives other than antimicrobials, and is suggested by AGRI to be applied also on antimicrobials. Moreover, further studies point out that substantially lower antimicrobial concentrations (e.g. 100-fold lower) can still select for resistance bacteria (Gullberg et al., 2011). The selective pressure, presumably, depends on the type of antimicrobial and the bacteria they are exposed to. Therefore, establishing general carry-over limits might not be as relevant as focusing on specific antimicrobials (or classes). However, more research is still needed to identify these antimicrobials and their minimum concentrations in feed that can select for resistance.

At this stage, it would be acceptable to stick to the maximum 1% for antimicrobials with a possibility of refinement (reduction) of this 1% maximum level in light of new research on specific antimicrobials. Reducing the use of antimicrobials in medicated feed substantially would be a more straightforward first approach to tackle (also) the cross-contamination issue. Besides, a number of countries (e.g. the Netherlands, Denmark) have shown that the use of antimicrobial premixes in feed is not needed, as they have banned this practice without additional difficulties (e.g. production loss).

Control of antimicrobial residues in manure

Establishing safety limits for antimicrobials in manure to regulate its applicability as fertilizer would be beneficial in reducing the amount of residues in the environment and preventing the development and spread of antimicrobial resistance in the environment. As described for the limits for in-feed antimicrobials, the limits for antimicrobials in manure should also likely be compound or class specific, if we consider the variation in the behavior of different antimicrobials in different matrices. More research is needed to identify these antimicrobials and their minimum concentrations in manure that can select for resistance in manure and/or soil. Alternatively, focusing on increasing the levels of manure processing, which is suggested as an effective approach to reduce antimicrobial residues in manure, could also be an approach. In fact, this falls within the scope of the European, including Belgian (Flemish) agenda, on top of which stands the evolution towards a circular economy. This evolution also influences the manure processing sector, where nutrient recuperation and production of other products from manure grow in importance (VCM, 2018). Finally, setting national level surveillance programs to measure antimicrobial residues in manure are equally important to other surveillance programs currently in place (e.g. for antibiotic-resistant bacteria in animals, food, and humans (EFSA, 2018)), especially because land application of manure represents a major avenue of antimicrobial introduction to environmental systems and water supplies.

Figure 2. Four focus areas of the FAO Action Plan on antimicrobial resistance. The recommendations provided for the subjects of this thesis (see figure 3) cover these four areas with a focus on these subjects. Governance stands for political commitment, appropriate policy and a relevant regulatory or legislative framework (FAO, 2016).
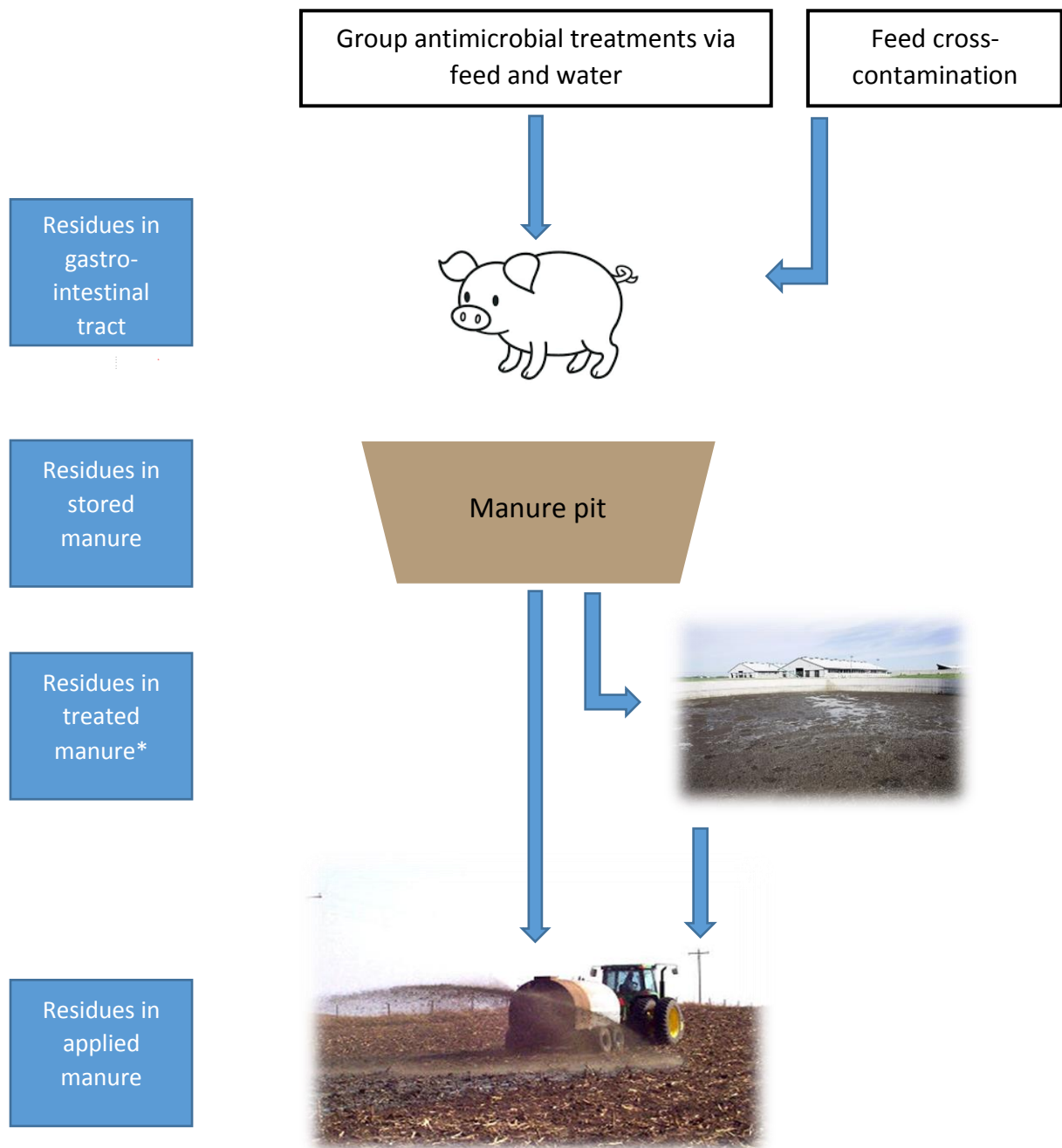
Figure 3. Schematic overview of the subjects of interest in this doctoral thesis. * Image of manure treated with biological treatment.

4. Future research

This thesis provides a detailed insight into the administration of antimicrobials to pigs via the oral route, as well as the risks and consequences stemming from this use, with a focus on antimicrobial resistance. However, many questions remain and new questions have emerged during this study. Future research on these subjects could help increase our knowledge regarding the impact of mass antimicrobial treatments of pigs on antimicrobial resistance selection.

- There is a need for more data on intestinal antimicrobial concentrations after oral administration of residues due to different carry-over levels, including the 1% level. Additionally, more data is needed on the association between these intestinal antimicrobial concentrations and the potential for resistance selection to bacteria. The focus should first be on antimicrobials typically used in feed (and water) medication (e.g. amoxicillin, sulphonamides).

- More information is needed with respect to the use of water medication, as an alternative to medicated feed. With respect to carry-over, there is a lack of data on the extent of cross-contamination of feed and water occurring at the (pig) farm after administration of antimicrobials. Moreover, an informed comparison of the two methods is needed focusing on other critical factors that affect drug delivery in pigs (e.g. homogeneity and stability of antimicrobials in feed and water).

- There is a lack of data on the amount of antimicrobial residues in manure and on the efficiency of different manure treatments in reducing these residues, inactivating resistant bacteria and destroying resistance genes in manure. The identification of degradation products (biologically active metabolites) in agricultural waste management is another primary research gap. Moreover, there is lack of compound-specific data on stability of most antimicrobials in manure. The focus should again be on commonly used antimicrobials which are also known or predicted to be rather stable in the manure matrix (e.g. tetracyclines, macrolides, sulfadiazine).

- Another important remark is to start adding in environmental risk assessments of antimicrobials in the environment (which mainly focus on toxicity (EMA 2000, 2016)), elements related to antimicrobial resistance, even if the body of knowledge is still limited. These elements might represent the increase in the abundance of antimicrobial-resistant bacteria and resistance genes, which can be caused by the application of resistant bacteria to the environment (e.g. via manure), the acquisition of resistance by environmental bacteria (e.g. by horizontal gene transfer), and the proliferation of indigenous resistant bacteria. For instance, an integration of a genomics-based approach (e.g. whole genome sequencing) into microbial quantitative risk assessment could improve the understanding of how livestock production (e.g. oral pig treatments) contributes to the development of antimicrobial resistance of human health concern, and explore strategies for reducing antimicrobial resistance in food production systems.

5. References

Arikan OA, Sikora LJ, Mulbry W, Khan SU, Foster GD, 2007. Composting rapidly reduces levels of extractable oxytetracycline in manure from therapeutically treated beef calves. Bioresour Technol. 98(1):169-176.

(BelVet-SAC) Belgian Veterinary Surveillance of Antibacterial Consumption, 2017. 2016 National consumption report. p1-44. http://www.belvetsac.ugent.be/

(BFA) Belgian Compound Feed Industry Association, 2013. In Dutch: Convenant betreffende gemedicineerde voeders. p1-14. http://www.favv.be/

Binh CTT, Heuer H, Kaupenjohann M, Smalla K, 2008. Piggery manure used for soil fertilization is a reservoir for transferable antibiotic resistance plasmids. FEMS Microbiol Ecol. 66(1):25–37.

Boxall ABA, Johnson P, Smith EJ, Sinclair CJ, Stutt E, Levy L, 2006. Uptake of veterinary medicines from soils into plants. J Agric Food Chem. 163(3):2288–2297

Carballo M, Sonia Aguayo S, Gonzalez M, Esperon F, Torre ADL, 2016. Environmental assessment of tetracycline's residues detected in pig slurry and poultry manure. J Environ Prot. 7:82-92.

Chantziaras I, Boyen F, Callens B, Dewulf J, 2014. Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: a report on seven countries. J Antimicrob Chemother. 69(3):827-834.

Chantziaras I., Callens B., Berge A.K., Boyen F., Haesebrouck F., Dewulf J., 2017a. Antimicrobial resistance selection pressure and spread in food producing animals through factors other than antimicrobial use. Chapter in PhD thesis. Ghent University.

Collineau L, Rojo-Gimeno C, Léger A, Backhans A, Loesken S, Nielsen EO, Postma M, Emanuelson U, Beilage EG, Sjölund M, Wauters E, Stärk KDC, Dewulf J, Belloc C, Krebs S, 2017. Herd-specific interventions to reduce antimicrobial usage in pig production without jeopardising technical and economic performance. Prev Vet Med. 1;144:167-178.

Cui X, Qiao XL, Han CW, Wang Z, 2008. Uptake of oxytetracycline and its phytotoxicity to lettuce. J Agro-Environ Sci. 27(3):1038–1042.

del Castillo JRE, 2013. Tetracyclines. In: Antimicrobial Therapy in Veterinary Medicine, Fifth Edition. Eds Prescott JF, Dowling PM. Hoboken, NJ: John Wiley & Sons. Wiley-Blackwell.

Dolliver H, Kumar K, Gupta S,. 2007. Sulfamethazine uptake by plants from manure-amended soil. J Environ Qual. 36:1224–1230.

Du LF, Liu WK, 2012. Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. Agron Sustain Dev. 32:309–327.

(EC) European Commission, 1991. Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources.

(EC) European Commission, 2014. Proposal for a regulation of the European Parliament and of the council on the manufacture, placing on the market and use of medicated feed and repealing Council Directive 90/167/EEC.

(EC) European Commission, 2016. Review of medicated feed legislation.

(EFSA and ECDC) European Food Safety Authority and European Centre for Disease Prevention and Control, 2018. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. EFSA Journal 16(2):5182-5270.

(EMA) European Medicines Agency, 2000. Guideline on environmental risk assessment (EIAS) for veterinary medicinal products (phase 1). CVMP/VICH/592/98.

(EMA) European Medicines Agency, 2016. Guideline on environmental impact assessment for veterinary medicinal products in support of the VICH guidelines GL6 and GL38. EMA/CVMP/ERA/418282/2005.

(EMA) European Medicines Agency, 2017. European Surveillance of Veterinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 30 European countries in 2015. p http://www.ema.europa.eu/

(FAO) Food and Agriculture Organization of the United Nations, 2016. The FAO Action Plan on Antimicrobial Resistance 2016-2020. http://www.fao.org/home

Filippitzi ME, Brinch Kruse A, Postma M, Sarrazin S, Maes D, Alban L, Nielsen LR, Dewulf J,. 2017. Review of transmission routes of 24 infectious diseases preventable by biosecurity measures and comparison of the implementation of these measures in pig herds in six European countries. Transbound Emerg Dis. 65(2):381-398.

(VCM) Flemish Coordination Centre for Manure Processing, 2016. VCM-enquete operationele stand van zaken mestverwerking in Vlaanderen. p1-35 https://www.vcm-mestverwerking.be

Gullberg E, Cao S, Berg OG, Ilback C, Sandegren L, Hughes D, Andersson DI, 2011. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. Plos Pathog. 7(7). doi:10.1371/journal.ppat.1002158.

Heuer H, Focks A, Lamshöft M, Smalla K, Matthies M, Spiteller M, 2008. Fate of sulfadiazine administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in manure and manured soil. Soil Biol Biochem. 40:1892-1900.

Heuer, H, Schmitt H, Smalla K, 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. Curr Opin Microbiol 14:236–243.

Holman D, Chénier MR, 2015. Antimicrobial use in swine production and its effect on the swine gut microbiota and antimicrobial resistance. Can J Microbiol. 61(11):785-798.

Jjemba PK, 2002. The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: a review. Agric Ecosyst Environ. 93:267–278.

Joy SR, Bartelt-Hunt SL, Snow DD, Gilley JE, Woodbury BL, Parker DB, Marx DB, Li X, 2013. Fate and transport of antimicrobials and antimicrobial resistance genes in soil and runoff following land application of swine manure slurry. Environ Sci Technol. 47(21):12081-12088.

Knapp CW, Dolfing J, Ehlert PAI, Graham DW, 2010. Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. Environ Sci Technol 44:580–587

Lamshöft M, Sukul P, Zühlke S, Spiteller M, 2010. Behaviour of (14)C-sulfadiazine and (14)C-difloxacin during manure storage. Sci Total Environ. 408(7):1563-1568.

Liu A, Fong A, Becket E, Yuang, J, Tamae C, Medarno L, Maiz M, Wahba C, Lee C, Lee K, Tran KP, Yang H, Hoffman RM, Salih A, Miller JH, 2011. Selective Advantage of Resistant Strains at Trace Levels of Antibiotics: A Simple and Ultrasensitive Color Test for Detection of Antibiotics and Genotoxic Agents. Antimicrob Agents Chemother. 55:1204-1210.

Massé DI, Saady NMC, Gilbert Y, 2014. Potential of Biological Processes to Eliminate Antibiotics in Livestock Manure: An Overview. Animals. 4(2):146–163.

Morris AK, Masterton RG, 2002. Antibiotic resistance surveillance: action for international studies. J Antimicrob Chemother 49(1):7–10.

(OIE) World Organisation for Animal Health, 2016. OIE Annual report on the use of antimicrobial agents in animals. p1-67. http://www.oie.int/

Okholm Nielsen E, Kirkeskov Sie M, Kjær Nielsen H, 2018. Enhancing Awareness of Antimicrobial Use in Danish Pig Farmers through the Yellow Card Initiative at AACTING conference. Ghent, 27-28 February 2018. (Oral presentation)

Österberg J, Wingstrand A, Nygaard Jensen A, Kerouanton A, Cibin V, Barco L, Denis M, Aabo S, Bengtsson B, 2016. Antibiotic Resistance in Escherichia coli from Pigs in Organic and Conventional Farming in Four European Countries. PLoS One. 11(6):e0157049.

Pardon B, Catry B, Dewulf J, Persoons D, Hosten M, De Bleecker K, Deprez P, 2012. Prospective study on quantitative and qualitative antimicrobial and anti-inflammatory drug use in white veal calves. J Antimicrob Chemother 67:1027-1038.

Peeters LE, Daeseleire E, Devreese M, Rasschaert G, Smet A, Dewulf J, Heyndrickx M, Imberechts H, Haesebrouck F, Butaye P, Croubels S, 2016. Residues of chlortetracycline, doxycycline and sulfadiazine-trimethoprim in intestinal content and feces of pigs due to cross-contamination of feed. BMC Vet Res. 12:209. doi:10.1186/s12917-016-0803-8.

Peeters LE, De Mulder T, Van Coillie E, Huygens J, Smet A, Daeseleire E, Dewulf J, Imberechts H, Butaye P, Haesebrouck F, Croubels S, Heyndrickx M, Rasschaert G, 2017. Selection and transfer of an IncI1-tet(A) plasmid of Escherichia coli in an ex vivo model of the porcine caecum at doxycycline concentrations caused by cross-contaminated feed. J Appl Microbiol. 123(5):1312-1320.

Peeters LE, Croubels S, Rasschaert G, Imberechts H, Daeseleire E, Dewulf J, Heyndrickx M, Butaye P, Haesebrouck F, Smet A, 2018. Effect of residual doxycycline concentrations on resistance selection and transfer in porcine commensal Escherichia coli. Int J Antimicrob Agents. 51(1):123-127.

Persoons D, Dewulf J, Smet A, Herman L, Heyndrickx M, Martel A, Catry B, Butaye P, Haesebrouck F, 2010. Prevalence and persistence of antimicrobial resistance in broiler indicator bacteria. Microb Drug Resist 16:67-74.

Postma M, Stärk KD, Sjölund M, Backhans A, Beilage EG, Lösken S, Belloc C, Collineau L, Iten D, Visschers V, Nielsen EO, Dewulf J, 2015. MINAPIG Consortium. Alternatives to the use of antimicrobial agents in pig production: A multi-country expert-ranking of perceived effectiveness, feasibility and return on investment. Prev Vet Med. 118(4):457-466.

Putier F, 2010. Evaluation of cross-contamination in livestock feed delivery trucks. Technics. Kraftfutter/Feed magazine 9-10/2010.

Rasschaert G, Lander C, Daeseleire E, Herman L, Heyndrickx M, 2017. Antibiotic resistant Salmonella and E. coli from pig and veal manure In: Proceedings of the 22th Conference on Food Microbiology. Brussels, 21 September2017.

Sandegren L, 2014. Selection of antibiotic resistance at very low antibiotic concentrations. Ups J Med Sci. 119(2):103–107.

Smet E, Debruyne J, Deckx J, Deboosere S, 2003. Manure treatment according to the Trevi-concept. Commun Agric Appl Biol Sci. 68(2):125-131.

Stevens M, Piepers, Supré K, Dewulf J, De Vliegher S, 2016. Quantification of antimicrobial consumption in adult cattle on dairy herds in Flanders, Belgium, and associations with udder health, milk quality, and production performance. J Dairy Sci. 99(3):2118-2130.

Stolker AAM, Manti V, Zuidema T, van Egmond H, Deckers ER, Herbes R, Hooglugt J, Olde Heuvel E, de Jong J, 2013. Carry-over of veterinary drugs from medicated to nonmedicated feeds in commercial feed manufacturing plants. Food Addit Contam Part A. 30:1100–1107.

Szatmári I, Laczay P, Borbély Z, 2011. Degradation of doxycycline in aged pig manure. Acta Vet Hung. 59(1):1-10.

Tiong J, Loo J, Mai CW, 2016. Global Antimicrobial Stewardship: A Closer Look at the Formidable Implementation Challenges. Front Microbiol. 7:1860.

Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R, 2015. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci USA. 112(18):5649-5654.

Van den Meersche T, Rasschaert G, Haesebrouck F, Van Coillie E, Daeseleire E, Heyndrickx M, 2017. The Effect of Biological Treatment of Manure on the Presence of Antibiotic Residues, Antibiotic Resistance Genes, and Zoonotic Pathogens. Book of abstracts IAFP's European symposium on food safety, International Association for Food Protection, European symposium on food Safety. Brussels, 29 March 2017.

Varel VH, Wells JE, Shelver WL, Rice CP, Armstrong DL, Parker DB, 2012. Effect of anaerobic digestion temperature on odour, coliforms and chlortetracycline in swine manure or monensin in cattle manure. J Appl Microbiol. 112(4):705–715.

(VCM) Flemish coordination centre for manure processing, 2018. VCM Vision statement on the transition in manure processing.   https://www.vcm-mestverwerking.be

(VLM) Vlaamse Landmaatschappij, 2017. Mestrapport 2016. p1-195. https://www.vlm.be/nl

Wang J, Han JZ, 2008. Effects of heavy metals and antibiotics on soil and vegetables. J Ecol Rural Environ. 24(4):90–93.

Weiland P, 2010. Biogas production: current state and perspectives. Appl Microbiol Biotechnol. 85:849.

Whitehead TR, Cotta MA, 2013. Stored swine manure and swine faeces as reservoirs of antibiotic resistance genes. Lett Appl Microbiol. 56(4):264-267.

Widyasari-Mehta A, Hartung S, Kreuzig R, 2016. From the application of antibiotics to antibiotic residues in liquid manures and digestates: A screening study in one European center of conventional pig husbandry. J Environ Manage. 177:129–137.

 (WHO) World Health Organization, 2017a. WHO guidelines on use of medically important antimicrobials in food-producing animals. Geneva. p1-88. http://www.who.int

(WHO) World Health Organization, 2017b. Critically important antimicrobials for human medicine – 5th rev 2016. Geneva. p1-48. http://www.who.int

Youngquist CP, Mitchell SM, Cogger CG, 2016. Fate of Antibiotics and Antibiotic Resistance during Digestion and Composting: A Review. J Environ Qual. 45(2):537-545.

ANNEX

FACULTEIT
DIERGENEESKUNDE
accredited by EAEVE

QUESTIONNAIRE SUR L'EAU POTABLE ET LES ALIMENTS POUR LES FERMES MODERNES

Renseignements Généraux

1. nombre de truies présentes ....………………………………………………

2. nombre de porcelets sevrés présents ....………………………………………………

3. nombre de porcs d'abattage (cochons) présents ....………………………………………

Question	L'eau potable	L'alimentation
Construction d'eau potable/système d'alimentation		
4. quel type d'eau et d'aliments utilisez-vous pour vos **truies**? (plusieurs réponses sont possibles)	☐ eau du robinet/eau de distribution/eau courante (**leidingwater**) ☐ eau de source de puits d'eau (**waterput**)/source de profondeur/le puits profonde/le puits de forage quelle est **la profondeur** du puits…………………………………………….. ☐ eau de **drainage** ☐ eau de **surface** ☐ eau de **pluie**	☐ Meel ☐ Korrels/pellets ☐ Wet/dry voeder (varkens mengen zelf water in hun droogvoeder) ☐ Brijvoeder (nat voeder) ☐ Andere, nl. ……………………………………………………… ………………………………………………………

Question	L'eau potable	L'alimentation
5. Quel type d'eau et d'aliments utilisez-vous pour vos **porcelets/cochonnets/gporets/conchons de lait**? (plusieurs réponses sont possibles)	☐ eau du robinet/eau de distribution/eau courante (**leidingwater**) ☐ eau de source de puits d'eau (**waterput**)/source de profondeur/le puits profonde/le puits de forage quelle est **la profondeur** du puits……………………………………………….. ☐ eau de **drainage** ☐ eau de **surface** ☐ eau de **pluie**	☐ Meel ☐ Korrels/pellets ☐ Wet/dry voeder (varkens mengen zelf water in hun droogvoeder) ☐ Brijvoeder (nat voeder) ☐ Andere, nl. …………………………………………………………… ……………………………………………………………
6. quel type d'eau et d'aliments utilisez-vous pour vos **cochons**? (plusieurs réponses sont possibles)	☐ eau du robinet/eau de distribution/eau courante (**leidingwater**) ☐ eau de source de puits d'eau (**waterput**)/source de profondeur/le puits profonde/le puits de forage quelle est **la profondeur** du puits……………………………………………….. ☐ eau de **drainage** ☐ eau de **surface**	☐ Meel ☐ Korrels/pellets ☐ Wet/dry voeder (varkens mengen zelf water in hun droogvoeder) Brijvoeder (nat voeder) ☐ Andere, nl. …………………………………………………………… ……………………………………………………………

Question	L'eau potable	L'alimentation
	eau de **pluie**	
Question	**L'eau potable**	**L'alimentation**
7. **Quel âge** a la partie la **plus ancienne** de l'installation eau / alimentation?	……………………………………………………………..	……………………………………………………………
8. … et la partie la plus **jeune**?	……………………………………………………………..	……………………………………………………………
9. Avez-vous un compteur d'eau?	☐ Oui ☐ Non	/
10. Les **lignes principales/ les tuyaux principaux (du circuit de l'eau / alimentation)** sont faites de quels matériaux?	☐ Fer ☐ Fer galvanisée ☐ PVC (plastique) ☐ acier inoxydable ☐ acier avec revêtement par courriel ☐ autres: ………………………………	☐ Fer ☐ Fer galvanisée ☐ PVC (plastique) ☐ acier inoxydable ☐ acier avec revêtement par courriel ☐ autres: ……………………………… ☐
11. Les **lignes/ les tuyaux des truies (du circuit de l'eau / alimentation)** sont faites de quels matériaux?	☐ Fer ☐ Fer galvanisée ☐ PVC (plastique) ☐ acier inoxydable ☐ acier avec revêtement par courriel ☐ autres: ………………………………	☐ Fer ☐ Fer galvanisée ☐ PVC (plastique) ☐ acier inoxydable ☐ acier avec revêtement par courriel ☐ autres: ………………………………

Question	L'eau potable	L'alimentation
12. Les **lignes/ les tuyaux des porcelets (du circuit de l'eau / alimentation)** sont faites de quels matériaux?	☐ Fer ☐ Fer galvanisée ☐ PVC (plastique) ☐ acier inoxydable ☐ acier avec revêtement par courriel ☐ autres: …………………………………	☐ Fer ☐ Fer galvanisée ☐ PVC (plastique) ☐ acier inoxydable ☐ acier avec revêtement par courriel ☐ autres: …………………………………
13. Les **lignes/ les tuyaux des cochons (du circuit de l'eau / alimentation)** sont faites de quels matériaux?	☐ Fer ☐ Fer galvanisée ☐ PVC (plastique) ☐ acier inoxydable ☐ acier avec revêtement par courriel autres: …………………………………	☐ Fer ☐ Fer galvanisée ☐ PVC (plastique) ☐ acier inoxydable ☐ acier avec revêtement par courriel ☐ autres: …………………………………
Purification de l'eau, qualité de l'eau et acidification		
14. Si vous **purifiez ou traitez** l'eau potable, comment faites-vous cela?	…………………………………………………………………………… …………………………………………………………………………… …………………………………………………………………………… ……………………………………………………………………………	/

Question	L'eau potable	L'alimentation
15. Prenez-vous **des échantillons** d'eau pour vérifier la qualité de l'eau?	☐ Jamais ☐ Oui → à **quelle fréquence** faites-vous cela? ………………………………………………………………	/
Question	**L'eau potable**	**L'alimentation**
16. Si vous prenez des échantillons, **où prenez-vous votre échantillons** (plusieurs responses possibles)?	☐ **à la source** (où l'eau entre dans la ferme) ☐ **au réservoir** d'eau (watertank) ☐ Aux **bassins / tétines**	/
17. Ajoutez-vous de **l'acide** à l'eau des truies? Quels produits utilisez-vous?	……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ………………………………………………………………	/
18. Ajoutez-vous de **l'acide** à l'eau des porcelets? Quels produits utilisez-vous?	……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ………………………………………………………………	/
19. Ajoutez-vous de **l'acide** à l'eau des cochons? Quels produits utilisez-vous?	……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ………………………………………………………………	/

Question	L'eau potable	L'alimentation
Nettoyage et désinfection		
20. **À quelle fréquence** (combien de fois) nettoyez ou désinfectez-vous les parts suivants?	☐ Des conduites d'eau ……………………………………………………… ☐ Réservoir de l'eau ……………………………………………………… ☐ Aux bassins / tétines ………………………………………………………	☐ silo ……………………………………………………… ☐ Réservoir d'alimentation (si il y en a un) ……………………………………………………… ☐ Boîte d'alimentation ………………………………………………………
21. Avec **quels produits** sont ces parts nettoyés et désinfectés? (Plusieurs réponses sont possibles)	☐ peroxyde d'hydrogène ☐ Mélange d'acides organiques ☐ dioxyde de chlore ☐ autres: ………………………………………	☐ peroxyde d'hydrogène ☐ Mélange d'acides organiques ☐ dioxyde de chlore ☐ autres: ……………………………………….
Traitement de groupe par l' alimentation et par l' eau		
22. **Quels compartiments peuvent recevoir des medicaments** par l'eau potable ou par l' alimentation?	☐ Truies ☐ Porcelets ☐ cochons ☐ Je n'utilise jamais de médicaments pour mélanger dans l'eau	☐ Truies ☐ Porcelets ☐ cochons ☐ Je n'utilise jamais de médicaments pour mélanger dans l'alimentation

Question	L'eau potable	L'alimentation
23. Utilisez-vous parfois des **combinaisons** de médicaments à l'eau potable / à l'aliment?	☐ Oui, ils sont: …………………………………………………………… …………………………………………………………… …………………………………………………………… ……………………………………………………… ☐ Non, jamais	☐ Oui, ils sont: …………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… ………………………………. ☐ Non, jamais
24. Quels groupes de truies peuvent recevoir **séparément (individuellement) de l'eau / alimentation médicamentée?**	☐ Par cabine (per hok) ☐ Par compartiment ☐ Par stable	☐ Par cabine ☐ Par compartiment ☐ Par stable
25. Quels groupes de porcelets peuvent recevoir **séparément de l'eau / alimentation médicamentée?**	☐ Par cabine (per hok) ☐ Par compartiment ☐ Par stable	☐ Per hok ☐ Per compartiment ☐ Per stal
26. Quels groupes de nourrins peuvent recevoir **séparément de l'eau / alimentation médicamentée?**?	☐ Par cabine (per hok) ☐ Par compartiment ☐ Par stable	☐ Par cabine (per hok) ☐ Par compartiment ☐ Par stable
27. Existe-t-il **une conduite d'eau / d'alimentation séparée** pour l'eau et les aliments médicamenteux et pour l'eau / les aliments auxquels aucun médicament n'a été ajouté?	☐ Oui ☐ Non	☐ Oui ☐ Non

Question	L'eau potable	L'alimentation
Préparer d' eau/alimentation médicamentée		
28. Sont **les tuyaux nettoyés avant d'ajouter** des médecines à l'eau potable ou à l'alimentation?	☐ toujours ☐ parfois ☐ jamais	☐ toujours ☐ parfois ☐ jamais
29. Sont **les tuyaux nettoyés avant après** la therapie avec l'eau ou l'alimentation médicamentées?	☐ toujours ☐ parfois ☐ jamais	☐ toujours ☐ parfois ☐ jamais

30. utilisez-vous une **pompe doseuse ou un réservoir d'eau** potable pour ajouter des medicines dans l'eau?
 	
Comment ajoutez-vous le médicament à l'alimentation?
	Avec une pompe doseuse
	    ☐ electrique (Digi Doser, Aquados, MSeasydoser,…)
	    ☐ mechanique (Dosatron, Dosmatic,…)
	par le réservoir d'eau potable
	    ☐ Le réservoir d'eau potable est à la source
	    ☐ Le réservoir d'eau potable est dans le département
		☐ J'achète un aliment médicamenté du fabricant de nourriture pour animaux
		☐ Topdressing (Couche de finition) (poudre sur le dessus du chargeur)
		☐ par un doseur de ligne d'alimentation (par exemple KMD-4L de Raijmakers Agro) (« un moulin »)

Question	L'eau potable	L'alimentation
31. **Comment mélangez-vous le médicament avec l'eau ou la nourriture?** (Par exemple, je mélange d'abord la poudre dans un seau d'eau puis ajoute l'eau du seau au réservoir d'eau, j'ajoute la poudre directement au réservoir d'eau, etc.)	…………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… ………………………………………………………… ……………………………………………………………	…………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… ……………………………………………………………
32. Si vous utilisez le médicament, **ajoutez**-vous quelque chose (par exemple de l'acide ou du bicarbonate de sodium) pour rendre le médicament **plus soluble**? Si oui, alors?	…………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… ……………………………………………………………	/
33. Lorsque vous préparez de l'eau potable / des médicaments pour aliments: **remuez ou mélangez**-vous dans la pré-solution?	☐ Oui, tant que c'est dans le dépliant ☐ Parfois ☐ Jamais	☐ Oui, tant que c'est dans le dépliant ☐ Parfois ☐ Jamais
34. **Comment prenez- vous la quantité** de médicaments dont vous avez besoin?	☐ Avec une tasse à mesurer ☐ Dans un seau ☐ Dans un plat doseur avec répartition de l'échelle ☐ Avec une échelle ☐ J'évalue combien j'ai besoin	☐ Avec une tasse à mesurer ☐ Dans un seau ☐ Dans un plat doseur avec répartition de l'échelle ☐ Avec une échelle ☐ J'évalue combien j'ai besoin

Question	L'eau potable	L'alimentation
35. **À quelle fréquence** (combien de fois) produisez-vous des aliments/ de l'eau médicamenteux? (par exemple une fois par jour)	…………………………………………………………… …………………………………………………………… …………………………………………………………… ……………………………………………………………	…………………………………………………………… …………………………………………………………… …………………………………………………………… ……………………………………………………………
36. Donnez-vous de l'eau / de la nourriture médicamentée **toute la journée ou quelques heures par jour**? (par exemple: eau médicamentée continue pendant 5 jours)	☐ Toute la journée ☐ Quesques heures par jour	☐ Toute la journée ☐ Quesques heures par jour
37. Si vous avez une pompe doseuse ou un doseur sur la ligne d'alimentation, quand a été **la dernière fois qu'un entretien / étalonnage a été effectué**?	…………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… ……………………………………………………………	…………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… ……………………………………………………………
38. Portez-vous **des gants** quand vous prenez de l'eau ou des médicaments?	☐ Toujours ☐ Parfois ☐ Jamais	☐ Toujours ☐ Parfois ☐ Jamais
39. Portez-vous **un masque** quand vous prenez des médicaments?	☐ Toujours ☐ Parfois ☐ Jamais	☐ Toujours ☐ Parfois ☐ Jamais

Question	L'eau potable	L'alimentation
Nettoyer et desinfecter après la therapie		
40. **Nettoyez-vous les outils utilisés** (par exemple le seau) après avoir pris des médicaments?	☐ Toujours ☐ Parfois ☐ Jamais	☐ Toujours ☐ Parfois ☐ Jamais
41. **Arrêtez-vous la désinfection** de l'eau si vous ajoutez des médicaments à l'eau potable?	☐ Oui ☐ Non ☐ Je n'utilise jamais de médicaments dans l'eau	/
Expérience personnelle avec de l'eau potable / des médicaments		
42. Avez-vous parfois des **problèmes avec la facilité d'utilisation** des aliments pour animaux / des médicaments pour l'eau potable? (Par exemple, la quantité de travail est-elle trop grande?) (gebruiksgemak)	………………………………………………………………… ………………………………………………………………… ………………………………………………………………… ………………………………………………………………… …………………………………………………………………	………………………………………………………………… ………………………………………………………………… ………………………………………………………………… ………………………………………………………………… …………………………………………………………………
43. Avez-vous parfois **des problèmes pratiques** dans l'exécution de la thérapie? (Par exemple colmatage, précipitation, ne se dissolvent pas bien, pas savoureux, tourné les mauvaises grues, ...)	………………………………………………………………… ………………………………………………………………… ………………………………………………………………… ………………………………………………………………… …………………………………………………………………	………………………………………………………………… ………………………………………………………………… ………………………………………………………………… ………………………………………………………………… …………………………………………………………………

Question	L'eau potable	L'alimentation
44. **Quelle est l'efficacité** de votre thérapie? (Par exemple, sont les porcs toujours mieux après la thérapie?)	……………………………………………………… ……………………………………………………… ……………………………………………………… ……………………………………………………… ………………………………………………………	……………………………………………………… ……………………………………………………… ……………………………………………………… ……………………………………………………… ………………………………………………………
45. **Préférez-vous** un type particulier d'eau potable / de médicaments et **pourquoi**?	……………………………………………………… ……………………………………………………… ……………………………………………………… ……………………………………………………… ………………………………………………………	……………………………………………………… ……………………………………………………… ……………………………………………………… ……………………………………………………… ………………………………………………………

Merci beaucoup pour votre temps! Puis-je vous contacter plus tard pour prendre des échantillons d'aliments et / ou d'eau?

☐ **Oui**

☐ **Non**

ENQUETE OMTRENT DRINKWATER- EN VOEDERSYSTEMEN IN DE VARKENSHOUDERIJ

Kruis aan wat past. Als het in de enquête gaat over zeugen, worden de zeugen in groepshuisvesting bedoeld.

Algemene bedrijfsinformatie

46. Aantal zeugen nu aanwezig   …………………….……………………………………

47. Aantal gespeende biggen nu aanwezig   …………………….……………………………………

48. Aantal vleesvarkens nu aanwezig   …………………….……………………………………

Vraag	Drinkwater	Voeder
Opbouw drinkwater-/voedersysteem		
49. Wat voor water/voeder gebruikt u voor uw **zeugen**? (Meerdere antwoorden zijn mogelijk)	☐ Leidingwater/stadswater ☐ Bronwater uit waterput/dieptebron/boorput/pulsput Wat is de diepte van de put? …………………………………………………………… ☐ Drainagewater ☐ Oppervlaktewater ☐ Regenwater (hemelwater)	☐ Meel ☐ Korrels/pellets ☐ Wet/dry voeder (varkens mengen zelf water in hun droogvoeder) ☐ Brijvoeder (nat voeder) ☐ Andere, nl. …………………………………………………… ……………………………………………………

Vraag	Drinkwater	Voeder
50. Wat voor water/voeder gebruikt u voor uw **biggen**? (Meerdere antwoorden zijn mogelijk)	☐ Leidingwater/stadswater ☐ Bronwater uit waterput/dieptebron/boorput/pulsput Wat is de diepte van de put? ………………………………………………………………… ☐ Drainagewater ☐ Oppervlaktewater ☐ Regenwater (hemelwater)	☐ Meel ☐ Korrels/pellets ☐ Wet/dry voeder (varkens mengen zelf water in hun droogvoeder) ☐ Brijvoeder (nat voeder) ☐ Andere, nl. ………………………………………………………… …………………………………………………………
51. Wat voor water/voeder gebruikt u voor uw **vleesvarkens**? (Meerdere antwoorden zijn mogelijk)	☐ Leidingwater/stadswater ☐ Bronwater uit waterput/dieptebron/boorput/pulsput Wat is de diepte van de put? ………………………………………………………………… ☐ Drainagewater ☐ Oppervlaktewater Regenwater (hemelwater)	☐ Meel ☐ Korrels/pellets ☐ Wet/dry voeder (varkens mengen zelf water in hun droogvoeder) Brijvoeder (nat voeder) ☐ Andere, nl. ………………………………………………………… …………………………………………………………
52. Hoe oud is het **oudste** deel van de water-/voedervoorziening ongeveer?	…………………………………………………………………	…………………………………………………………
53. Hoe oud is het **nieuwste** deel van de water-/voedervoorziening ongeveer?	…………………………………………………………………	…………………………………………………………

Vraag	Drinkwater	Voeder
54. Hebt u een **watermeter**?	☐ Ja ☐ Neen	
55. Uit welk materiaal zijn de **hoofdleidingen** gemaakt (meerdere antwoorden zijn mogelijk)	☐ Ijzer ☐ Gegalvaniseerd ijzer ☐ PVC ☐ RVS (roestvrij staal) ☐ Andere, namelijk ……………………………………	☐ Ijzer ☐ Gegalvaniseerd ijzer ☐ PVC ☐ RVS (roestvrij staal) ☐ Staal met coating van email ☐ Andere, namelijk …………………………………
56. Uit welk materiaal zijn de leidingen in het **zeugencompartiment** gemaakt?	☐ Ijzer ☐ Gegalvaniseerd ijzer ☐ PVC ☐ RVS (roestvrij staal) ☐ Andere, namelijk ……………………………………	☐ Ijzer ☐ Gegalvaniseerd ijzer ☐ PVC ☐ RVS (roestvrij staal) ☐ Staal met coating van email ☐ Andere, namelijk …………………………………
57. Uit welk materiaal zijn de leidingen in het **biggencompartiment** gemaakt?	☐ Ijzer ☐ Gegalvaniseerd ijzer ☐ PVC ☐ RVS (roestvrij staal) ☐ Andere, namelijk ……………………………………	☐ Ijzer ☐ Gegalvaniseerd ijzer ☐ PVC ☐ RVS (roestvrij staal) ☐ Staal met coating van email ☐ Andere, namelijk ………………………………..

Vraag	Drinkwater	Voeder
58. Uit welk materiaal zijn de leidingen in het **vleesvarkencompartiment** gemaakt?	☐ Ijzer ☐ Gegalvaniseerd ijzer ☐ PVC ☐ RVS (roestvrij staal) ☐ Andere, namelijk ………………………………………	☐ Ijzer ☐ Gegalvaniseerd ijzer ☐ PVC ☐ RVS (roestvrij staal) ☐ Staal met coating van email ☐ Andere, namelijk ……………………………………
Waterzuivering, waterkwaliteit en aanzuren		
59. Indien u het drinkwater zuivert, hoe doet u dit?	…………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………	/
60. Laat u een waterstaal onderzoeken?	☐ Nooit ☐ Ja → **hoe vaak** doet u dit? ………………………………………………………… ………………………………………………………… …………………………………………………………	/

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Vraag	Drinkwater	Voeder
61. Indien u een waterstaal laat onderzoeken, **waar** neemt u dan de stalen (meerdere antwoorden mogelijk)?	☐ Aan de bron (het water dat het bedrijf binnenkomt) ☐ Aan de watertank ☐ Aan de drinkbakken/drinknippels	/
62. Zuurt u het water van de **zeugen** aan? Welke producten gebruikt u?	…………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………	/
63. Zuurt u het water van de **biggen** aan? Welke producten gebruikt u?	…………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………	/
64. Zuurt u het water van de **vleesvarkens** aan? Welke producten gebruikt u?	…………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………	/

Vraag	Drinkwater	Voeder
Reiniging en ontsmetting		
65. **Hoe vaak** wordt het volgende gereinigd en/of ontsmet? (Bijvoorbeeld: 1 keer per week worden de leidingen gereinigd)	☐ Waterleidingen ……………………………………………………………….. ☐ Watertank ……………………………………………………………….. ☐ Drinkbakken/nippels ………………………………………………………………..	☐ Silo ………………………………………………………………… ☐ Voedermengtank (indien aanwezig) ………………………………………………………………… ☐ Voederbakken …………………………………………………………………
66. Met **welke producten** wordt de bovenstaande onderdelen gereinigd en ontsmet? (Meerdere antwoorden zijn mogelijk)	☐ Waterstofperoxide ☐ Mengsel van organische zuren ☐ Chloordioxide ☐ Andere, namelijk ………………………………………………………………..	☐ Waterstofperoxide ☐ Mengsel van organische zuren ☐ Chloordioxide ☐ Andere, namelijk ……………………………………………………………….
Groepsbehandeling via voeder en water		
67. Welke compartimenten kunnen **medicatie** via het drinkwater of het voeder krijgen?	☐ Zeugen ☐ Biggen ☐ Vleesvarkens ☐ Ik gebruik nooit drinkwatermedicatie	☐ Zeugen ☐ Biggen ☐ Vleesvarkens ☐ Ik gebruik nooit voedermedicatie

Vraag	Drinkwater	Voeder
68. Worden er soms **combinaties van geneesmiddelen** aan het drinkwater/voeder toegevoegd?	☐ Ja, nl. …………………………………………………………… …………………………………………………………… …………………………………………………………… ………………………………………………………… ☐ Neen, nooit	☐ Ja, nl. …………………………………………………………… …………………………………………………………… …………………………………………………………… …………………………………………………………… ☐ Neen, nooit
69. Welke groepen **zeugen** kunnen **apart** (onafhankelijk van elkaar) drinkwater-/voedermedicatie krijgen?	☐ Per hok ☐ Per compartiment ☐ Per stal	☐ Per hok ☐ Per compartiment ☐ Per stal
70. Welke groepen **biggen** kunnen **apart** (onafhankelijk van elkaar) drinkwater-/voedermedicatie krijgen?	☐ Per hok ☐ Per compartiment ☐ Per stal	☐ Per hok ☐ Per compartiment ☐ Per stal
71. Welke groepen **vleesvarkens** kunnen **apart** (onafhankelijk van elkaar) drinkwater-/voedermedicatie krijgen?	☐ Per hok ☐ Per compartiment ☐ Per stal	☐ Per hok ☐ Per compartiment ☐ Per stal
72. Is er een aparte water-/voederleiding voor gemedicineerd water/voeder en voor water/voeder waaraan geen medicatie werd toegevoegd?	☐ Ja ☐ Neen	☐ Ja ☐ Neen

Vraag	Drinkwater	Voeder
Bereiding van voeder- en watermedicatie		
73. Worden de leidingen gereinigd **vooraleer** de drinkwater/-voederbehandeling te starten?	☐ Altijd ☐ Soms ☐ Nooit	☐ Altijd ☐ Soms ☐ Nooit
74. Worden de leidingen gereinigd **na** het toevoegen van drinkwater-/voedermedicatie?	☐ Altijd ☐ Soms ☐ Nooit	☐ Altijd ☐ Soms ☐ Nooit

75. Via welke weg mengt u medicatie in?	☐ Via een doseerpomp
	    ☐ Elektrische doseerder (Digi Doser, Aquados, MSeasydoser,…)
	    ☐ Mechanische doseerder (Dosatron, Dosmatic,…)
	☐ Via de drinkwatertank
	    ☐ Deze staat aan de bron
	    ☐ Deze staat aan de afdeling
		☐ Ik koop gemedicineerd voeder aan bij de mengvoederfabrikant
		☐ Topdressing (poeder bovenop het voeder)
		☐ Via een doseerder op voederlijn (bijvoorbeeld KMD-4L van Raijmakers Agro)

Vraag	Drinkwater	Voeder
76. Hoe doet u het **inmengen** zelf? (Bijvoorbeeld: ik meng het poeder eerst in een emmer water en dan voeg ik het water van de emmer toe aan de watertank, ik voeg het poeder rechtstreeks toe aan de watertank, enz.)	……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ……………………………………………………………… …………………………………………………………… ……………………………………………………………	……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ……………………………………………………………
77. Als u medicatie toevoegt aan drinkwater, **voegt u dan iets toe** (bijvoorbeeld zuur of natriumbicarbonaat) om het geneesmiddel **beter oplosbaar** te maken? Zo ja, wat dan?	……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ……………………………………………………………… ………………………………………………………………	/
78. Bij het maken van drinkwater-/voedermedicatie: **roert of mixt** u in de vooroplossing?	☐ Ja, zolang als in de bijsluiter staat ☐ Soms ☐ Nooit	☐ Ja, zolang als in de bijsluiter staat ☐ Soms ☐ Nooit
79. Hoe neemt u de **hoeveelheid** medicatie die u nodig heeft?	☐ Met een maatbeker ☐ In een emmer ☐ In een doseervat met schaalverdeling ☐ Met een weegschaal ☐ Ik schat op het zicht hoeveel ik nodig heb	☐ Met een maatbeker ☐ In een emmer ☐ In een doseervat met schaalverdeling ☐ Met een weegschaal ☐ Ik schat op het zicht hoeveel ik nodig heb

Vraag	Drinkwater	Voeder
80. Hoe **frequent** maakt u meestal gemedicineerd voeder/water aan? (bijvoorbeeld 1x per dag)	…………………………………………………… …………………………………………………… …………………………………………………… …………………………………………………… ……………………………………………………	…………………………………………………… …………………………………………………… …………………………………………………… …………………………………………………… ……………………………………………………
81. Geeft u het gemedicineerd water/voeder de hele dag door, of een paar uur per dag? (bijvoorbeeld: continu gemedicineerd water gedurende 5 dagen)	☐ De hele dag door ☐ Enkele uren per dag	☐ De hele dag door ☐ Enkele uren per dag
82. Als u een doseerpomp/doseerder op de waterlijn/voederlijn heeft: wanneer was de laatste keer dat hier een **onderhoud/kalibratie** op werd uitgevoerd?	…………………………………………………… …………………………………………………… …………………………………………………… ……………………………………………………	…………………………………………………… …………………………………………………… …………………………………………………… ……………………………………………………
83. Gebruikt u **handschoenen** bij het nemen van water-/voedermedicatie?	☐ Altijd ☐ Soms ☐ Nooit	☐ Altijd ☐ Soms ☐ Nooit
84. Gebruikt u een **masker** bij het nemen van water-/voedermedicatie	☐ Altijd ☐ Soms ☐ Nooit	☐ Altijd ☐ Soms ☐ Nooit

Vraag	Drinkwater	Voeder
Reinigen en ontsmetten na medicatie		
85. Reinigt u het gebruikte **gereedschap** (bijvoorbeeld de emmer) nadat u medicatie genomen heeft?	☐ Altijd ☐ Soms ☐ Nooit	☐ Altijd ☐ Soms ☐ Nooit
86. Wordt de drinkwater**ontsmetting** gestopt als u medicatie in het drinkwater doet?	☐ Ja ☐ Neen ☐ Ik gebruik geen drinkwaterontsmetting	/
Eigen ervaring met drinkwater-/voedermedicatie		
87. Ondervindt u soms problemen met het **gebruiksgemak** van voeder-/drinkwatermedicatie? (Bijvoorbeeld problemen met de werkdruk)	………………………………………………………………… ………………………………………………………………… ………………………………………………………………… ………………………………………………………………… …………………………………………………………………	………………………………………………………………… ………………………………………………………………… ………………………………………………………………… ………………………………………………………………… …………………………………………………………………
88. Ondervindt u soms problemen met het **uitvoeren** van de therapie? (Bijvoorbeeld verstoppen, neerslag, niet goed oplossen, niet smakelijk, verkeerde kranen opengedraaid, …)	………………………………………………………………… ………………………………………………………………… ………………………………………………………………… ………………………………………………………………… …………………………………………………………………	………………………………………………………………… ………………………………………………………………… ………………………………………………………………… ………………………………………………………………… …………………………………………………………………

Vraag	Drinkwater	Voeder
89. Hoe **effectief** vindt u de behandeling? (Bijvoorbeeld zijn de varkens na de behandeling altijd beter?)	…………………………………………………………………… …………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………	…………………………………………………………………… …………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………
90. Hebt u een **voorkeur** voor een bepaalde soort drinkwater-/voedermedicatie en waarom?	…………………………………………………………………… …………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………	…………………………………………………………………… …………………………………………………………………… …………………………………………………………………… …………………………………………………………………… ……………………………………………………………………

Tot slot

Heel erg bedankt voor uw tijd! Mogen we later nog contact met u opnemen om voeder- en/of waterstalen te nemen?

☐ **Ja**

☐ **Neen**

SUMMARY

Antimicrobial treatment administered to pigs through feed and water group medication can lead to the presence of antimicrobial residues in the gastro-intestinal tract and manure of pigs. Antimicrobial residues in these matrices (gastro-intestinal tract of pigs and pig manure), may cause a potential for selection and dissemination of antimicrobial resistance. The aim of this doctoral thesis (**Chapter 2**) was to fill a number of research gaps, regarding different aspects related to oral group antimicrobial medication of pigs, in a quantitative manner.

First, in order to estimate the amount of antimicrobials used in Belgium per species (i.e. pigs, poultry and veal calves), an extrapolation of farm-level data to national-level data was performed according to the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) methodology (**Chapter 3**). According to this extrapolation attempt, pigs are estimated to consume most antimicrobials in absolute values at the national level in Belgium, i.e. 159.40 tons annually (52.4% of total use), followed by broilers (26.53 tons; 8.7%) and white veal calves (25.18 tons; 8.3%). According to the gathered data and estimations, the use of antimicrobials in pigs, broilers and veal calves corresponds to 69.4% of the total use in Belgium. Based on the data used for this extrapolation, oral treatments were more common than injectable ones for fattening pigs and weaned piglets (TI_{UDDA}, oral =176.5, while TI_{UDDA}, injectable =24.2). Taking into account that the antimicrobial use in sows could not be quantitatively estimated at this stage and were therefore not taken into account in these estimations, this extrapolation places the pig population on the top of high consumers of antimicrobials in Belgium, in absolute values and possibly also in terms of TI, especially for piglets.

Since oral group medication through feed and water is the most common way of antimicrobial administration to the majority of the Belgian pigs (especially fattening pigs and piglets), a field study was performed in 52 pig farms in Belgium, to examine the level of use of these two methods (feed and water medication), and the conditions of preparation and administration of antimicrobials via the feed and water (**Chapter 4**). In the farms visited, it was observed that water medication was used more (n=47 farms) than in-feed medication (n=36). Per production stage, water medication was used more in piglets (87.5%) and fattening pigs (91%), while top dressing was mainly used in sows (55%). The farmers generally reported a good experience in terms of ease of use and effectiveness for both methods. A number of farmers suggested that the preparation of water medication requires more labor and some issues are met when used (e.g. precipitation). However, more research is needed to address other important issues, such as homogeneity and stability of antimicrobials in feed and water, in order to be able to fully compare the two methods of oral group medication.

Focusing specifically on oral antimicrobial group medication of pigs via feed, these treatments can further lead to cross-contamination of non-medicated feed with residues of antimicrobials. These

residues also end up in the gastro-intentional tract of pigs and in pig manure, adding up to the amount of residues from intended antimicrobial administration. The model developed and presented in **Chapter 5**, estimated that, when antimicrobial medicated feed represents a hypothetical x_i=2% of the total feed produced in a country per year (as it is the case for Belgium), then around 5.5% (95%CI 3.4% ; 11.4%) of the total feed produced in a year could be cross-contaminated with different levels of antimicrobials due to practices related to the production, transport and storage of medicated feed. In detail, 1.80% (95%CI 0.2% ; 7.7%) of the total feed produced in such a country would be cross-contaminated due to antimicrobial carry-over occurring at the feed mill level, 1.83% (95%CI 1.3%; 2.0%) at the transport truck level and 1.84% (95%CI 1.2% ; 2.0%) at the farm level. The model also demonstrated that, even in cases where antimicrobial medicated feed would be produced in end-of-line mixers or fine dosing system on trucks, the risk of cross-contamination would not be negligible; the percentage of cross-contaminated feed produced in a country (where x_i=2%) per year would be 3.7% (95%CI 2.9% ; 4.0%) and 2.4% (95%CI 1.6% ; 2.7%), respectively.

In **Chapter 6**, a probabilistic model is presented which shows the potential extent of the effect of cross-contaminated pig feed on resistance selection. The results of the model include estimations of the proportion of pigs per production stage (i.e. piglets, sows, fattening pigs) with residues of doxycycline, chlortetracycline, sulfadiazine and trimethoprim in their intestinal contents from caecum and colon, as a result of exposure to cross-contaminated feed with different carry-over levels (i.e. 1% and 3%), in Belgium. By using a semi-quantitative approach, these estimations were combined with experimental data on antimicrobial concentrations associated with potential for resistance selection pressure. Based on this model, it is estimated that 7.7% (95%CI 1.6% ; 36.9%) of sows, 4.2% (95%CI 1.0% ; 18.7%) of piglets and 2.8% (95%Cl 0.5% ; 14.9%) of fatteners in Belgium have residues of doxycycline in their intestinal tract due to consumption of feed with at least 1% carry-over. These values were estimated to be almost triple for sulfadiazine, but substantially lower for chlortetracycline and trimethoprim. Doxycycline concentrations as low as 1 mg/L (corresponding to consumed feed with at least 1% carry-over) can select for resistant porcine commensal *E. coli in vitro* and *in vivo*. Research is needed to specify the minimum residual concentrations of more antimicrobials associated with resistance development, to be able to draw conclusions on this risk for the other antimicrobials. However, since the possibility of resistance mechanisms (e.g. co-selection) occurring cannot be excluded, the results of this model highlight that the use of antimicrobial medicated feed should be minimized where possible.

Chapter 7 presents a model estimating the level of antimicrobials commonly administered to pigs orally, that is present in manure at the end of common storage durations in pits and, thus, readily applied onto soil. The data used in this model are based on a literature review which focused on the

bioavailability and extent of *in vivo* biotransformation of twelve antimicrobials, and on the level of their persistence in manure. The model estimated that at least 18.4% of the total amount of these orally administered antimicrobials is present in manure which is readily applied onto soil. Based on sales of antimicrobials, it was estimated that this percentage corresponds to over 25,000 kg of antimicrobial residues in pig manure in Belgium. If export and processing are considered, the aforementioned percentage is reduced to at least 9.7%. From the studied antimicrobials, the highest level of residues in stored manure was estimated for doxycycline, as a combining result of its high use in pigs, low bioavailability and high stability in manure. However, even for antimicrobials not expected to be found in manure at the end of maximum storage, certain levels of these can be present in fractions of manure stored for shorter duration. Other antimicrobials are readily degraded and therefore pose less threat.

The results of this thesis highlight the importance of reducing the amount and frequency of antimicrobial treatments of pigs. The prohibition of prophylactic antimicrobial treatments, which are mainly administered orally, as a first step, would be expected to have a considerable effect towards this direction.

SAMENVATTING

Behandeling van varkens met antimicrobiële middelen door massa medicatie via voeder en water kan leiden tot de aanwezigheid van antimicrobiële residuen in het gastro-intestinaal stelsel en in de mest van varkens. Antimicrobiële residuen in deze matrices (gastro-intestinaal stelsel en mest), kan potentieel zorgen voor selectie en verspreiding van antimicrobiële resistentie. Het doel van deze doctoraatsthesis (**Hoofdstuk 2**) was om een aantal onderzoeksvragen, met betrekking tot aspecten die gerelateerd zijn aan orale massa medicatie van antimicrobiële middelen bij varkens, op te vullen op een kwantitatieve manier.

Om de hoeveelheid antimicrobiële middelen gebruikt in België per species (nl. varkens, pluimvee en vleeskalveren) in te schatten, werden data op bedrijfsniveau naar nationaal niveau geëxtrapoleerd volgens de methodologie van de *European Surveillance of Veterinary Antimicrobial Consumption* (ESVAC) (**Hoofdstuk 3**). Volgens deze poging tot extrapolatie wordt geschat dat varkens de meeste antimicrobiële middelen in absolute waarden consumeren op nationaal niveau in België, nl. 159.40 ton jaarlijks (52.4% van het totale gebruik), gevolgd door braadkippen (26.53 ton; 8.7%) en witvlees kalveren (25.18 ton; 8.3%). Volgens de verzamelde data en veronderstellingen, komt het gebruik van antimicrobiële middelen bij varkens, braadkippen en vleeskalveren overeen met 69.4% van het totale gebruik in België. Gebaseerd op de data die gebruikt werden voor deze extrapolatie, kwamen orale behandelingen meer voor dan parenterale behandelingen voor vleesvarkens en voor gespeende biggen (TI$_{UDDA}$, oraal =176.5, terwijl TI$_{UDDA}$, parenteraal =24.2). Als er rekening wordt gehouden met het feit dat het gebruik van antimicrobiële middelen bij zeugen niet kwantitatief kon geschat worden in dit stadium, en daarom dus ook niet in rekening gebracht werd in deze inschattingen, zorgt deze extrapolatie ervoor dat de varkenspopulatie bovenaan staat bij de grootverbruikers van antimicrobiële middelen in België, en dit zowel in absolute waarden als waarschijnlijk ook in termen van TI, vooral voor biggen.

Vermits orale massa medicatie via voeder en water de meest gebruikte manieren zijn om antimicrobiële middelen toe te dienen bij de meerderheid van de Belgische varkens (in het bijzonder vleesvarkens en biggen), werd een veldstudie uitgevoerd op 52 varkensbedrijven in België om het gebruik van deze twee methoden (medicatie via voeder en water), de voorbereidingsomstandigheden en de toediening van antimicrobiële middelen via het voer en water te onderzoeken (**Hoofdstuk 4**). Op de bezochte bedrijven werd vastgesteld dat medicatie via het water meer werd gebruikt (n=47 bedrijven) dan medicatie via het voeder (n=36). Wanneer er gekeken werd per productiefase, werd medicatie via water meer gebruikt bij biggen (87.5%) en vleesvarkens (91%) terwijl topdressing voornamelijk gebruikt wordt bij zeugen (55%). Over het algemeen meldden de boeren een goede ervaring op vlak van gebruiksgemak en effectiviteit over beide manieren. Een aantal boeren stelden

dat de voorbereiding van gemedicineerd water meer werk vereist en dat er enkele problemen optreden bij het gebruik (bv. neerslag). Er is echter nood aan meer onderzoek om andere belangrijke problemen te bestuderen, zoals homogeniciteit en stabiliteit van antimicrobiële middelen in het voeder en water, om beide methoden van orale massa medicatie volledig te kunnen vergelijken.

Wanneer specifiek wordt gefocust op het toedienen van orale antimicrobiële massa medicatie via varkensvoer, kunnen deze behandelingen leiden tot kruiscontaminatie van niet gemedicineerd voeder met residuen van antimicrobiële middelen. Deze residuen eindigen in het gastro-intestinaal stelsel van de varkens en de varkensmest, dit komt bovenop de residuhoeveelheid van de bedoelde antimicrobiële toediening. Het ontwikkelde model, voorgesteld in **Hoofdstuk 5**, schat in dat, wanneer antimicrobieel gemedicineerd voeder een hypothetische x_i=2% van de totale voederproductie vertegenwoordigt (zoals in België), het mogelijk is dat ongeveer 5.5% (95%CI 3.4% ; 11.4%) van de totale voederproductie in een jaar kruis gecontamineerd zou kunnen zijn, met verschillende hoeveelheden van antimicrobiële middelen, omwille van processen gerelateerd aan de productie, transport en opslag van het voeder. In detail, 1.80% (95%CI 0.2%; 7.7%) van het voeder geproduceerd in een gelijkaardig land zou kruis gecontamineerd zijn door antimicrobiële overdracht op niveau van de voederfirma, 1.83% (95%CI 1.3%; 2.0%) op niveau van transport en 1.84% (95%CI 1.2% ; 2.0%) op niveau van het bedrijf. Het model toont ook aan dat, zelfs in gevallen waar antimicrobieel gemedicineerd voeder zou worden geproduceerd met mixers op het einde van de lijn of met fijndoseersysteem op vrachtwagens, het risico op kruiscontaminatie niet te verwaarlozen is; het percentage van kruis gecontamineerd voeder in een land (waar x_i=2%) zou per jaar 3.7% (95%CI 2.9%; 4.0%) en 2.4% (95%CI 1.6% ; 2.7%) zijn, respectievelijk.

In **hoofdstuk 6** is een waarschijnlijkheidsmodel voorgesteld dat de mogelijke omvang weergeeft van het effect van kruis gecontamineerd varkensvoeder op selectie van resistentie. De resultaten van het model omvatten inschattingen van het aantal varkens per productiefase (nl. biggen, zeugen, vleesvarkens) met residuen van doxycycline, chloortetracycline, sulfadiazine en trimethoprim in hun darminhoud (blinde darm en dikke darm), als gevolg van blootstelling aan kruis gecontamineerd voeder met verschillende gradaties van versleping (nl. 1% en 3%), in België. Door een semi-kwantitatieve aanpak te gebruiken, werden deze inschattingen gecombineerd met experimentele data over antimicrobiële concentraties geassocieerd met het potentieel voor selectiedruk op resistentie. Gebaseerd op dit model wordt geschat dat 7.7% (95%CI 1.6% ; 36.9%) van de zeugen, 4.2% (95%CI 1.0% ; 18.7%) van de biggen en 2.8% (95%CI 0.5% ; 14.9%) van de vleesvarkens in België residuen van doxycycline in hun gastro-intestinaal stelsel hebben door consumptie van voeder met minstens 1% versleping. Deze waarden zijn voor sulfadiazine vermoedelijk 3 keer hoger, maar wezenlijk lager voor

chloortetracycline en trimethoprim. Doxycycline concentraties van 1 mg/L (overeenkomstig met geconsumeerd voeder met minstens 1% versleping) kunnen reeds selecteren voor resistente porciene commensale *E. coli in vitro* en *in vivo*. Onderzoek is noodzakelijk om de minimum concentraties aan residuen van meer antimicrobiële middelen, geassocieerd met ontwikkeling van resistentie, te specifiëren. Aangezien het voorkomen van resistentiemechanismen (bv. co-selectie) niet uitgesloten kan worden, benadrukken de resultaten van dit model dat het gebruik van gemedicineerd diervoeder moet geminimaliseerd worden waar nodig.

Hoofdstuk 7 stelt een model voor waarbij een inschatting wordt gemaakt van de hoeveelheid frequent oraal toegediende antimicrobiële middelen, aanwezig in mest op het einde van de gebruikelijke bewaartermijn in putten, en dus in deze hoedanigheid op de bodem gebracht worden. De data die in dit model gebruikt worden zijn gebaseerd op een literatuuroverzicht dat zich focust op de biologische beschikbaarheid en de omvang van *in vivo* biotransformatie van 12 antimicrobiële middelen, alsook op hun niveau van persistentie in mest. Het model schatte dat minstens 18.4% van de totale hoeveelheid oraal toegediende antimicrobiële middelen aanwezig zijn in mest die in die hoedanigheid op de bodem aangebracht worden. Gebaseerd op de verkoop van antimicrobiële middelen, werd geschat dat dit percentage overeenkomt met meer dan 25,000 kg antimicrobiële residuen in varkensmest in België. Als export en verwerking in acht genomen worden, wordt het bovenstaande percentage gereduceerd naar minstens 9.7%. Van de bestudeerde antimicrobiële middelen wordt voor doxycycline de hoogste concentratie aan residuen in opgeslagen mest geschat, door een combinatie van het hoge verbruik bij varkens, de lage biologische beschikbaarheid en hoge stabiliteit in mest. Ook voor antimicrobiële middelen die niet verwacht worden in mest op het einde van de bewaartermijn, kunnen bepaalde niveaus aan residuen van deze antimicrobiële middelen gevonden worden in fracties van mest die voor kortere duur zijn opgeslagen. Andere antimicrobiële middelen worden gemakkelijker afgebroken en vormen daardoor minder bedreiging.

De resultaten in deze thesis benadrukken het belang van de reductie van de hoeveelheid en de frequentie van behandelingen met antimicrobiële middelen bij varkens. Het verbod op profylactische behandelingen met antimicrobiële middelen, die voornamelijk oraal worden toegediend, als een eerste stap, zou waarschijnlijk een aanzienlijk effect in deze richting hebben.

CURRICULUM VITAE

Marilena Filippitzi was born on January 22 1988 in Volos, Greece. She received her veterinary degree from the Aristotle University of Thessaloniki in 2011, where she particularly collaborated with the Laboratory of Microbiology, Immunology and Infectious Diseases of the faculty. She holds an MSc degree in Control of Infectious Diseases in animals, from the Royal Veterinary College (RVC), London. During her Masters studies, she collaborated with the Centre for Epidemiology and Risk Analysis of the UK Animal and Plant Health Agency. After that, she completed an in-service traineeship at the European Food Safety Authority in Parma, placed at the Scientific Committee and Emerging Risks Unit. Since 2014, Marilena was based at the Veterinary Epidemiology Unit of Ghent University, as a resident in specialization training (under the supervision of Prof Dr Jeroen Dewulf and Prof Dr Dominiek Maes) and a PhD student (under the supervision of Prof Dr Jeroen Dewulf and Prof Dr Mathias Devreese). In 2017, she obtained the title of Diplomate of the European College of Veterinary Public Health (ECVPH) in Population Medicine. During these years, she worked on various research projects and completed scientific externships at the French Agency for Development in Paris, and the veterinary public health consultancy firm Epi-Interactive® in Wellington, New Zealand. As a postgraduate student, she acted as representative of the ECVPH Residents and of the Masters students at the RVC.

Since April 2018, Marilena is working as veterinary epidemiologist at the Belgian Federal Institute Sciensano in Brussels. She represents Belgium in the Management Committee of the EU COST Action Network for Evaluation of One Health (NEOH) and she further represents NEOH in the EU COST Network of Science Communicators. She is also external collaborator with the National School of Public Health in Athens. She is author and co-author of several publications, with active participation in national and international conferences and organization of workshops.

BIBLIOGRAPHY

Publications in international journals

- Filippitzi M.E., Chantziaras I., Devreese M., Dewulf J. Probabilistic risk model to assess the potential for resistance selection following the use of antimicrobial medicated feed in pigs. Food Additives and Contaminants, Part A. [Epub ahead of print].

- Filippitzi M. E., Brinch Kruse A., Postma M., Sarrazin S., Maes D., Alban L., Nielsen L.R., Dewulf J. 2017. Review of transmission routes of 24 infectious diseases preventable by biosecurity measures and comparison of the implementation of these measures in pig herds in six European countries. Transboundary and Emerging Diseases. 65(2):381-398.

- Falzon L.C., Lechner I., Chantziaras I., Collineau L., Courcoul A., <u>Filippitzi M.E.</u>, Laukkanen-Ninios R., Peroz C., Pinto Ferreira J., Postma M., Prestmo P.G., Phythian C., Sarno E., Vanantwerpen G., Vergne T., Grindlay D., Brennan M. The quantitative outcomes of a "One Health" approach to study global health issues: a systematic review. Ecohealth. doi: 10.1007/s10393-017-1310-5. [Epub ahead of print].

- Ruegg S., McMahon B., Häsler B., Esposito R., Rosenbaum Nielsen L., Speranza C., Ehlinger T., Peyre M., Aragrande M., Zinsstag, Davies P., Mihalca A., Buttigieg S., Rushton J., Carmo L., De Meneghi D., Canali M., <u>Filippitzi M.E.</u>, Goutard F., Ilieski V., Milievi D., O'Shea H., Radeski M., Kock R., Staines A., Lindberg A., 2017. A blueprint to evaluate One Health. Frontiers in Public Health. 5:20.

- Filippitzi M. E., Goumperis T., Robinson T., Saegerman C., 2016. Microbiological zoonotic emerging risks, transmitted between livestock animals and humans (2007-2015). Transboundary and Emerging Diseases. 64(4):1059-1070.

- Filippitzi M. E., Sarrazin S., Imberechts H., Smet A., Dewulf J., 2016. The risk of cross-contamination due to the use of antimicrobial medicated feed throughout the trail of feed from the feed mill to the farm. Food Additives and Contaminants, Part A. 33(4):644-55.

- Filippitzi M. E., Callens B., Pardon B., Persoons D., Dewulf J., 2014. Antimicrobial use in pigs, broilers and veal calves in Belgium. Vlaams Diergeneeskundig Tijdschrift. 83(5). p.215-224.

- Filippitzi M.E., Devreese M., Broekaert K., Rasschaert G., Daeseleire E., Meirlaen J., Dewulf J. Quantitative risk model to estimate the level of antimicrobial residues that can be transferred to soil via manure, due to oral treatments of pigs. Submitted manuscript.

- Chantziaras I., Smet A., <u>Filippitzi M. E</u>., Haesebrouck F., Boyen F., Dewulf J. Use of a seeder-sentinel *in vivo* animal model to evaluate the effect of a commercial competitive exclusion product on the selection and spread of enrofloxacin resistance in commensal *E. coli* in broilers. Avian Pathology. [Epub ahead of print].

- Vandael F., <u>Filippitzi M.E.</u>, Dewulf J., Devreese M., Croubels S. Patterns of antimicrobial use in pigs via feed and water: A field-study in pig farms in Belgium. In preparation.

- Filippitzi M.E., Chantziaras I., Saegerman C. A quantitative release assessment of the risks associated with the mechanical transport of Rift Valley Fever virus-carrying mosquitoes into previously unaffected areas. In preparation.

Book chapters

- Dewulf J., Postma M., Vanbeselaere B., Maes D., <u>Filippitzi M.E.,</u> 2018. Transmission of pig diseases and biosecurity in pig production. Chapter 12. In: Biosecurity in animal production and veterinary medicine. Acco.
- Filippitzi M.E. A One Health Evaluation Framework: Impact evaluation. Chapter 3. In: A handbook for evaluation of One Health. Wageningen Academic Publishers.

Oral presentations

- Two probabilistic risk models to assess the potential for resistance selection and residues transferred to soil due to oral antimicrobial treatment of pigs. 8th International Symposium on hormone and veterinary drug residue alaysis (VDRA), Ghent, Belgium. 05.2018.
- Probabilistic risk modelling. VEE Workshop, Belgium. 05.2018.
- Quantitative risk modelling. The example of a quantitative exposure model of pigs to cross-contimanted feed with residues of antimirobials. ECVPH Workshop, Liège, Belgium. 09.2017.
- The risk of introducing Rift Valley fever virus into the UK : A quantitative risk analysis. ECVPH Workshop, Liège, Belgium. 09.2017.
- One Health : Metrics and Methods. National School of Public Health (NSPH), Athens. 01.2017.
- The risk of cross-contamination due to the use of antimicrobial medicated feed: A quantitative exposure model from the feed mill to the farm. 25 years AESA International Congress, Liège, Belgium. 09.2016.
- Biocheck.ugent®: A risk based biosecurity scoring system. Science Week of Australian and New Zealand College of Veterinary Scientists. Epidemiology Chapter, Gold Coast, Australia. 07.2016.
- The risk model for the cross-contamination due to the use of antimicrobial medicated feed. Science Week of Australian and New Zealand College of Veterinary Scientists. Epidemiology Chapter, Gold Coast, Australia. 07.2016.
- Network for Evaluation of One Health (NEOH): Developing a standardized methodology to evaluate One Health activities. Science Week of Australian and New Zealand College of Veterinary Scientists. Epidemiology Chapter, Gold Coast, Australia. 07.2016.

- Risk communication in Food Safety. National School of Public Health (NSPH), Athens. 03.2016.
- Risk communication for Public Health harards. National School of Public Health (NSPH), Athens. 04.2015.
- Emerging Public Health risks. National School of Public Health (NSPH), Athens. 04.2015.
- Biosecurity and antimicrobial usage. ECVPH Workshop, Bakum, Germany. 02.2014.
- A Quantitative Release and Spatial Exposure Assessment of the Risks associated with Rift Valley Fever-Infected Mosquitoes arriving into Great Britain aboard Commercial Aircraft. AESA Symposium, Liège, Belgium. 10.2013.

Poster prizes

- Filippitzi M. E., Sarrazin S., Dewulf J. The risk of cross-contamination due to the use of antimicrobial medicated feed. VEE Annual Conference, Belgium. 10.2015. 1st Poster prize.
- Filippitzi M. E., Carmo L. P., De Meneghi D., Rosenbaum Nielsen L., Häsler B. Transdisciplinary collaboration across Europe to promote Global Health: Network for Evaluation of One Health (NEOH). ECVPH Annual General Meeting and Conference, Belgrade, Serbia. 10.2015. 1st Poster prize.

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Ουδέν οίδα ούτε διδάσκω τι,   *I know nothing, I teach nothing,*

αλλά διαπορώ μόνον   *I only question*

Σωκράτης, 469-399 π.Χ., φιλόσοφος   Socrates, 469-399 B.C., philosopher

Bruno, for occasionally taking me out for a walk. Alexandros, thank you for being a constant source of strength and for making these years even more beautiful.

Writing is a lonely path and, therefore, I feel grateful that a part of this book has been written with the view of the Mediterranean sea, inspired by the Greek sun and in the company of beautiful music.

Marilena

Ghent, June 2018