



EFFORT FINAL REPORT

Publishable Summary

EFFORT: Ecology from Farm to Fork Of microbial drug Resistance and Transmission

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Glossary

Term	Explanation
2-HDA	2-hexadecynoic acid
2-AFAs	2-alkynoic fatty acids
ADS	Assessment and Decision Support
AMR	Antimicrobial Resistance
ARG	Antimicrobial Resistance Genes
COINs	Conjugation Inhibitors
DDDvet	Defined Daily Doses for animals
DCDvet	Defined Course Doses for animals
EAB	EFFORT External Advisory Board
EB	Executive Board
ECVPH	European College of Veterinary Public Health
ECW	EFFORT Collaborative Website
EDC	Electrostatic Dust fall Collector
EFSA	European Food Safety Authority
EMA	European Medicines Agency
ESBL	Microbial Extended Spectrum β -lactamase
ESVAC	European Surveillance of Veterinary Antimicrobial Consumption
FVE	Federation of Veterinarians in Europe
HHWI	Herd Health and Welfare Index
IFAH	International Federation of Animal Health
ILT	Infectious Laryngotracheitis
IRAS	Institute for Risk Assessment Sciences (UUVM)
KOM	Kick-Off meeting
KRPM	Kilobase Reference Per Million
MBACI	Multiple Before/After Control Intervention
MG	Metagenomics
MIC	Minimal Inhibitory Concentration
MLSb	Macrolide-Lincosamide-Streptogramin B
MS	Mass Spectroscopy
NCBI	National Center for Biotechnology Information (USA)
NGS	Next-Generation Sequencing
OIE	World Organization for Animal Health
PFGE	Pulse Field Electrophoresis
SAS	Statistical Analysis Software
SVEPM	Society of Veterinary Epidemiology and Preventive Medicine
TC	Telephone Conference
TI	Treatment Incidences
WGS	Whole Genome Sequencing

I. Final publishable summary report

A. Executive Summary

The use of antimicrobials invariably leads to selection of bacteria that are resistant against the substance used. Resistance can then spread in populations and the environment. In human medicine, antimicrobial resistance (AMR) leads to increased morbidity and mortality. Also in veterinary medicine the efficacy of antimicrobial treatments is declining and from several bacterial diseases multidrug resistant strains are circulating making these infections untreatable resulting in a high economic burden for the producers and animal welfare problems. The problem of treatment failure is amplified by the fact that new and effective antimicrobials are not currently being developed at a sufficient rate nor will they be developed at a higher rate in the near future. Antimicrobial resistance therefore poses a major threat to the continued efficacy of antimicrobial agents in both human and veterinary medicine.

The EFFORT project was a multi-disciplinary research programme which investigated the epidemiology and ecology of antimicrobial resistance in food-producing animals, the (farm) environment, and food of animal origin, companion animals and wildlife to evaluate and quantify the AMR exposure pathways for humans. This was conducted by a combination of epidemiological and ecological studies using newly developed molecular and bio-informatics technologies. EFFORT included an exposure assessment of humans from animal and environmental sources. The ecological studies on isolates were verified by in vitro and in vivo studies. Moreover, real-life intervention studies were conducted with the aim to reduce the use of antimicrobials in veterinary practice.

Focus was on understanding the eco-epidemiology of antimicrobial resistance from animal origin and based on this, predicting and limiting the future evolution and exposure to humans of the most clinically important resistance by synthesising different sources of information in our prediction models. The work was performed in nine countries in pig and poultry farms, and in selected countries on turkey, veal, and fish farms, in wildlife and companion animals. In two countries studies in pig and poultry slaughterhouses were included.

The outcome of the project comprises several topics, amongst them, without being complete, AMU and AMR in participating countries with the identification of risk factors for AMR, approaches (supported by coaching) to explore and perform on-farm interventions to reduce AMU, the economic and animal welfare aspects of AMU and their relation with the interventions to reduce AMU, the use of metagenomics for source attribution, the use of metagenomics for research and future surveillance, and the spread of genes and plasmids. An important deliverable was a course on metagenomics that is on-line available.

Most relevant results were either published or are in preparation for publication in scientific journals, and are made public through publicly available deliverables.

B. Description of the Project Context and Objectives

(a) Work strategy and general description

The EFFORT project studies the complex epidemiology and ecology of antimicrobial resistance and the interactions between bacterial communities of commensals and pathogens in animals, the food chain and the environment.

This is conducted by a combination of epidemiological and ecological studies using newly developed molecular and bio-informatics technologies. EFFORT includes an exposure assessment of humans from animal and environmental sources. The ecological studies on isolates are verified by *in vitro* and *in vivo* studies. Moreover, real-life intervention studies are conducted with the aim to reduce the use of antimicrobials in veterinary practice. Focus is to understand the eco-epidemiology of antimicrobial resistance from animal origin and based on this, to predict and limit the future evolution of AMR and exposure to humans of the most clinically important resistance determinants by the inclusion of different sources of information in our prediction models.

Through its results, EFFORT provides scientific evidence and high-quality data that will inform decision makers, the scientific community and other stakeholders about the consequences of Antimicrobial Resistance (AMR) in the food chain, in relation to animal health and welfare, food safety and economic aspects. These results can be used to support political decisions and to prioritise risk management options along the food chain.

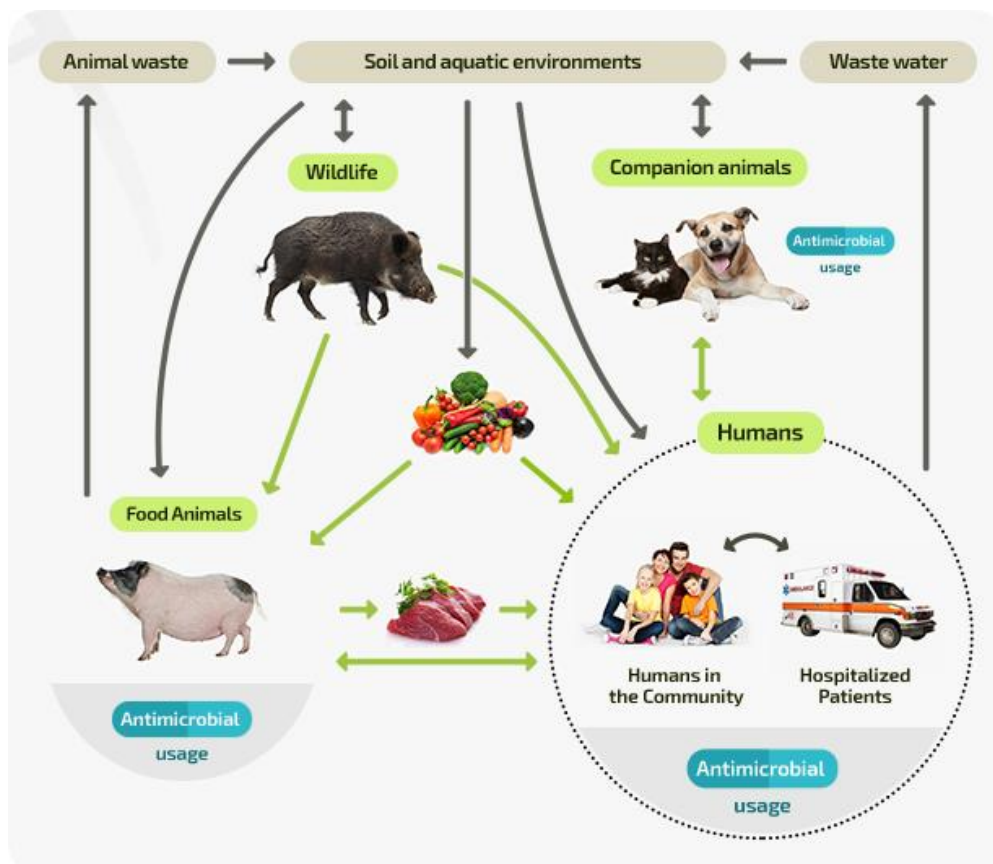


Figure 1. Schematic drawing of antimicrobial resistance gene movements across the food chain.

(b) *Objectives of the Project*

The main objectives and important innovative aspects of EFFORT were as follows:

- ✓ The characterization of the resistome of production animals as determined by metagenomics and assessing the added value of genomic analysis of isolates and metagenomic analysis of bacterial communities in comparison with the conventional used methods for EU based surveillance of antimicrobial resistance using indicator organisms.
- ✓ The quantification and characterisation of herd level antimicrobial usage in different animal species.
- ✓ The comprehensive and multinational/multispecies datasets collected and built will allow determination of the complex associations between risk factors including antimicrobial usage, and the occurrence of resistance (resistome and conventional data).
- ✓ An estimation of the relative contribution of different sources and various transmission routes on antimicrobial resistance in humans in the general population as well as in selected occupational risk groups. This would also allow for an estimation of the effect of reduced antimicrobial use and other specific interventions on human exposure.
- ✓ The determination of genetic characteristics involved in the success of high-risk clones and mobile genetic elements in the epidemiology of AMR and the estimation of the relative impact on human infection risks caused by transfer of antimicrobial resistance determinants between commensals and pathogenic organisms.
- ✓ The implementation of on-farm interventions (e.g. restricted usage of antimicrobials) in multiple European countries and animal species following a common approach, including an analysis of the economic effects, animal welfare consequences and resistance levels.
- ✓ The use of novel statistical analytical approaches for rich meta-genomic data to obtain a “fingerprint” of resistance patterns for different populations (humans, animals) and the environment. Simultaneously these fingerprint patterns will be associated to determinants of antimicrobial resistance of relevance for human and animal health.

(c) *Management structure and procedures*

The Project Coordinator ensured the smooth operation of the project and guaranteed that all efforts were focused towards the objectives. He submitted all required progress reports, deliverables, financial statements to the European Commission, and, with the assistance of his team and ARTTIC he was responsible for the proper use of funds and their transfers to participants. The Project coordination team was composed of Jaap Wagenaar, EFFORT Coordinator and Haitske Graveland, EFFORT Project Manager, both based at the Utrecht University, the Netherlands. He was supported by the Project Office composed of Dominique Wasquel, Project Manager, and Carlos Triay, Senior Consultant, both based at ARTTIC Paris. The Coordination Team was concerned with the scientific management and the co-ordination of all research activities while the Project Office at ARTTIC was responsible for administrative, financial and contractual management and the organisational co-ordination of the project activities.

Besides, the General Assembly was in charge of the political and strategic orientation of the project and acted as the arbitration body. It met physically once a year. The Project Executive Board consisted

of all work package leaders and the Coordinator was in charge of monitoring all activities towards the objective of the project in order to deliver the expected results as promised, in due time and in the budget. The Project Executive Board had regular audio meeting every three months during the funding period. Furthermore, an external advisory board was implemented to ensure a high standard of research and monitor the progress of the project by taking part in the annual General Assembly meetings

C. Description of the Main S&T Results/Foregrounds

The description of the main results / foregrounds here below is based on the EFFORT presentations made during the EFFORT Conference held end of November 2018. It gives an overview of the outcomes of the project and also summaries the EFFORT key messages presented at the EFFORT event.

(a) *Prudent Use of antimicrobials in practice: all about metagenomics:*

The major developments in sequencing technologies and especially the introduction of the so-called next-generation sequencing (NGS) technologies during the last two decades has made NGS an applicable technology for a very large number of purposes. Microbial Metagenomics utilising NGS has emerged as an important scientific methodology to simultaneously reveal microbial community composition including, taxonomic affiliation, diversity, functions, and interactions of microbiomes across all domains of life. In metagenomics the complete DNA in a given sample is purified and often millions of small pieces (50-250 base-pairs) of DNA randomly sequenced. Each sequence is then compared to reference databases and the number of reads mapping to a specific reference-sequence, such as a specific antimicrobial resistance gene is then equivalent to the prevalence (or abundance) of that gene in the specific sample.

There are however, multiple individual steps in the generation of gene abundance profiles, including sample collection, sample storage, DNA isolation, library preparation, sequencing, as well as data analysis.

The effect of sample processing seems to be a major explanation for the differences observed between studies. Thus, in EFFORT we as a first step evaluated a number of different protocols for DNA-purification to obtain a final protocol that could be applied across the different participants (Knudsen et al. 2016). A standard work-flow has been developed and published. However, even using a standardised protocol a ringtrial between partners, showed so large variation, that it for this study was deemed necessary to centralise the purification and sequencing.

Compared to DNA-purification and sequencing, standardization of storage conditions can be difficult because sample collection may be performed over a long period of time, suitable storage facilities may be unavailable for instance in field studies, and proper shipping conditions can be limited when samples need to be transported to a central laboratory for analysis. Currently, the procedure introducing least variation seems to be to freeze the samples as soon as possible and then only thaw them once when used for DNA-purification.

Variation might also be introduced when applying different bioinformatic analytic procedures (Petersen et al. 2017). However, in comparison to the data-generation procedures, this is considered less critical since it would be possible to re-analyse available raw data.

(b) *The current situation regarding AMU and AMR*

✓ **Building the integrated evidence base for the food chain**

In the effort project we managed to build a huge database on antimicrobial use (AMU) and resistance (AMR) data as well as herd characteristics of no less than 180 pig farms and 181 broiler farms from 9 different European countries (Belgium, Bulgaria, Denmark, France, Germany, Italy, Poland, Spain, The Netherlands). In each of these countries 20 conventional farms per species (and an additional pilot broiler farm in Belgium) were selected, which are representative for commercial pig and broiler breeding in Europe. Given the selection of only 20 farms per country the obtained results are not necessarily fully representative of each specific country. Yet, the total database does give a good overview of the situation in Europe and the variation between farms throughout Europe. Besides pigs and broilers we have also built smaller databases on 60 turkey farms originating from France, Germany and Spain (20 farms per country); 60 veal calf farms from France, Germany and The Netherlands, and 60 trout production sites from France, Poland and Spain. In each of the sampled farms we have recorded farm characteristics such as farm size, location, production type, duration of production rounds. Next, we also quantified the antimicrobial use both through registration of collective group treatments in the sampled batches as through antimicrobial purchase data of the preceding year. The biosecurity status of each of the farms was quantified by means of the biocheck.ugent® methodology. This is a risk based scoring system which allows to measure the biosecurity situation objectively and divides it into an internal and external biosecurity score. External biosecurity comprises all measures taken to prevent the introduction of disease into a farm whereas the internal biosecurity comprises all measures taken to reduce the spread of infectious agents within the farm. Before the start of the EFFORT project this scoring system was available for pigs and broilers, for veal and turkey a new version was created. For pigs and broilers also the animal welfare was evaluated by means of the herd health and welfare index which provides a welfare score based on a number of animal observations. Besides the production animals we also collected data from 150 dogs and 150 cats from Belgium, Italy and The Netherlands (50 per species per country). In these species, both AMU and AMR was determined. Finally, also 300 wild boars (100 per country), as representatives of the wild animal population, from Germany Italy and Poland were sampled for AMR determination.

The quantification of the AMU was done by means of the determination of the treatment incidence per 100 days. This number represents the average number of days that the animal has been treated with antimicrobials in a theoretical lifespan of 100 days. Therefore this is a reflection of the percentage of the lifetime an animal is treated with antimicrobials. In order to be able to calculate this treatment incidence the amount of antimicrobials administered (group treatments) or purchased was collected. This info was then combined with a defined daily dose (DDDvet) determined for each antimicrobial class and administration route. Most of the DDDvet's could be retrieved from the European ESVAC list. However, for the minor species such as Turkey and veal calves we had to determine the DDD's ourselves. Besides we had to determine the duration of the production periods and the number of animals at risk for treatment in each group. Standardization of the data collection of the used antimicrobials throughout the nine countries has proven to be a huge challenge, especially in those countries where no herd level data collection are yet installed.

As the antimicrobial resistance is concerned, 10 pooled faecal samples were collected in every sampled farm. The samples were always collected from animals close to slaughter age. From these faecal

samples 10 randomly selected *E.coli* were isolated. Antimicrobial resistance in these *Coli*'s was determined by means of broth microdilution and sensitive methodology. The panel of antimicrobials tested for was based on the EFSA determined panel. Epidemiological cutoff values were used to differentiate between wild type and non-wild type isolates. Besides MIC determination on *E. coli* also the full resistome was determined by means of DNA extraction and sequencing of 1 pool of 25 fresh droppings per farm. These results were then further analyzed by means of bioinformatics processing. Finally also qPCR was performed on 7 fresh faecal droppings per farm to determine the abundance of 4 selected resistance genes.

✓ **Why we need to (further) rethink our antimicrobial use in European food animal production**

Quantification of AMU on the participating farms showed that on the median pig and broiler farm, animals were treated during 9% of their lifespan. For pigs this equaled 18 days out of a standardized period of 200 days. For broilers this equaled 4 days out of 42 days of production. However, there was a big variation in AMU on farm level within each country and between countries for both species. TI on the broiler farms ranged from 0 to 127. Zero, meaning no usage at all and 127, indicating multiple treatments at the same time and/or used doses that were much higher than the corresponding DDDvet value. At least 1 broiler farm per country did not use antimicrobials in the participating house. In total there were 67 rounds that did not report any AMU, showing that it is feasible to rear broilers without AMU. For pigs, 21 batches did not report any collective group treatments and TI ranged from 0 to 114. AMU on the median turkey farm (TI = 10) was similar to the median broiler farm (TI = 9). Also on the turkey farms, 12% of the participating farmers succeeded in rearing their animals without any AMU. Although TI on the median veal calf farm (TI=13) was only slightly elevated compared to the other species, none of the farmers succeeded in rearing these animals without the use of antimicrobials. Finally, AMU in healthy cats and dogs was investigated. Results showed that 19% of the healthy cats and dogs participating in the study, were treated with antimicrobials in the year preceding data collection.

In all different animal production systems, antimicrobial usage seemed to peak early on in production and at strategic time points. On the pig farms, there were three peaks in AMU during production. A first one at the beginning of production, a second and a third peak at weaning and start-up of the fattening period, respectively. In broiler, turkey and veal calf production we saw a similar first peak in AMU. On the broiler farms, 47% of the total AMU was administered in the first week of production, on the turkey farms 91% of the total AMU was administered during the first half of the rearing period. In addition, a positive association was found for the AMU between age categories on the pig farms, meaning that a higher usage in an early production stage is associated with a higher usage later on in production.

A qualitative analysis of AMU on the pig farms showed a higher used daily dose compared to the "recommended" daily dose from ESCVAC. Suggesting overdosing or too low DDDvet values. In general, treatments on pig farms were also applied too long, with 10% of the oral treatments being administered for at least 21 consecutive days. Looking into more detail at dosing, we saw a big variation in the used daily dose (UDD) in all species for certain active substances. For example, UDD for oxytetracycline products administered orally on turkey farms, ranged from 5.5mg to 80 mg per kg of animal. A similar variation is seen between the recommended dosages, written in the summary of

product characteristics, of all oxytetracycline products registered in Spain, France or Germany for oral administration in turkeys. Next, when looking at the choice of antimicrobials we see a focus on certain antimicrobial classes. On the broiler farms, extended-spectrum penicillins, polymyxins and fluoroquinolones represented 70% of all AMU. More importantly 44% of AMU is represented by polymyxins and fluoroquinolones which are critically important antimicrobials for human medicine. These drugs should be used as little as possible in veterinary medicine, to safeguard their effectiveness against resistant infections in human medicine, where they are a last resort drug. Also in cats and dogs, antimicrobials that are on the WHO's CIA list were still frequently used, with 3th generation cephalosporins and fluoroquinolones representing 8% and 12% of total AMU in cats and dogs, respectively. Finally, we saw a variation in choice of antimicrobial class to treat a similar indication. A pattern that showed up in all species. While in country A only 3 different classes were used to treat intestinal disorders in pigs, in country D treatments of pigs with intestinal disorders covered a range of 13 different antimicrobial classes.

These findings are proof that a substantial further reduction of antimicrobial use is possible. The focus of this reduction needs to lie on strategic use of antimicrobials and AMU in the early stage of production. However, there is also a need for evidence based research and guidelines regarding harmonization of dose, dosage and choice of active substance to promote a more responsible AMU.

✓ **Uncovering the metagenomic AMR landscape in European livestock**

In order to quantify AMR in the livestock herds, we opted for a metagenomic approach that had previously proved useful in a Danish pilot study among ten pig herds (Munk et al., 2016). Instead of attempting to culture individual species of bacteria, DNA was extracted directly from the faecal samples and prepared for sequencing on Illumina HiSeq instrument to approximately 50 million fragments per sample. The herd-level resistomes (from 20 pig and 20 poultry herds from each of the nine sampled countries) were analysed in detail.

More than 5,000 billion basepairs of DNA were sequenced from 359 herd-level pools originating from over 9,000 animals. This makes it the largest metagenomic livestock study to date. The data was bioinformatically processed to determine the taxonomic distribution and resistance gene distribution among the pig and poultry herds and was published in Nature microbiology (Munk et al., 2018).

To validate the entire approach from sampling through in silico analysis, we performed triplicate sampling rounds in two of the pig farms. Each round of sampling 25 faecal samples, resulted in a herd-level resistome. As expected, the farm resistomes clustered together in triplicates, according to herd of origin.

The total AMR load varied approximately 20-fold from the highest to the lowest samples and was generally higher in pigs when considering all the ResFinder genes. Danish poultry had the lowest mean AMR load, with several samples below 500 fragments per Kilobase reference per million bacterial fragments (FPKM). Italian pigs on the other hand had the highest load with several samples above 10,000 FPKM.

We identified more than 400 different AMR genes across the samples, but their relative contribution and number of different genes per samples varied drastically. The AMR gene richness (unique AMR genes) was mirrored significantly between the two animal species, meaning that countries with many unique AMR genes in one species also tended to have many in the other species.

Using different techniques, we clustered samples according to their resistome similarity (beta-diversity). Pig and poultry samples were entirely different clusters with no overlap. There was a significant country effect within both animal species, but the country effect was stronger in pigs.

In addition to the country effect, we found that the underlying bacterial distribution in the samples was significantly associated with the resistome profiles. This suggests that what differs from sample to sample is not simply the proportion of members of a taxa carrying AMR genes, but the proportion of different taxa driving a significant part of the resistome variation. This could indicate that modulating the bacteriome e.g. through changing feed, could work by proxy in reducing AMR load.

We tested for individual AMR genes that differed in abundance between countries within each animal species. Several AMR genes of medical importance were found to vary significantly across the European countries. This includes the linezolid AMR gene *optrA*, which was abundant in Bulgarian and Italian pigs. The colistin AMR gene *mcr-1* was also most abundant in poultry in the same two countries. *blaCTX-M* was found to vary significantly in poultry with Spain having the highest median abundance. In pigs however, it was barely detectable, though we know it should be present. This indicates the detection limit of our metagenomic approach is lower.

In summary, our metagenomic analysis generated a wealth of information on the current AMR landscape in the most important European livestock animals. At some cost to sensitivity, our method has a much wider reach compared to traditional methods that focus on isolating a few species. The sequence dataset produced is an invaluable resource and allows for both quantifying future AMR genes and novel uses. An example includes our ongoing bioinformatics work to rebuild the bacterial genomes from the samples, discover new microbes and assigning AMR genes to bacterial species.

(c) *Changing practices to reduce AMU and AMR*

✓ **The antimicrobial resistome in relation to antimicrobial use and biosecurity in pig and broiler farming**

Objectives: Previous studies in food-producing animals have shown associations between antimicrobial use (AMU) and resistance (AMR) in specifically isolated bacterial species. Multi-country data is scarce and only describes between-country differences. Here we investigate associations **between the pig faecal mobile resistome and characteristics at farm-level across Europe.**

Methods: A cross-sectional study was conducted among 176 conventional pig farms from nine European countries. Twenty-five faecal samples from fattening pigs were pooled per farm and acquired resistomes were determined using shotgun metagenomics and Resfinder reference database, i.e. the full collection of horizontally acquired antimicrobial resistance genes (ARGs). Normalized Fragments resistance genes Per Kilobase reference per Million bacterial fragments (FPKM) were calculated. Specific farm-level data (AMU, biosecurity) was collected. Random-effects meta-analyses were performed by country, relating farm-level data to relative ARG abundances (FPKM).

Results: Total AMU during fattening was positively associated with total ARG (total FPKM). Positive associations were especially observed between widely used macrolides and tetracyclines, and ARGs corresponding to the respective antimicrobial classes. Significant AMU-ARG associations were not found for β -lactams and only few colistin ARGs were found, despite being the antimicrobial classes used highest in younger pigs. Increased internal biosecurity was directly related to higher abundances of ARGs mainly encoding macrolide resistance. These effects of biosecurity were independent of AMU in mutually adjusted models.

Conclusions: Using resistome data in association studies is unprecedented and adds accuracy and new insights to previously observed AMU-AMR associations. Major components of the pig resistome are positively and independently associated with on-farm AMU and biosecurity conditions.

✓ **The antimicrobial resistome in relation to antimicrobial use and biosecurity in broiler farming**

Objectives: To determine associations between farm and flock level antimicrobial usage (AMU), farm biosecurity status, and the abundance of faecal antimicrobial resistance genes (ARGs) on broiler farms.

Methods: In the cross-sectional pan-European EFFORT study, conventional broiler farms were visited and faeces, AMU and biosecurity records were collected. The resistomes of pooled faecal samples were determined by metagenomics analysis for 176 farms. A meta-analysis approach was used to relate total and class-specific ARGs (expressed as Fragments Per Kilobase reference per Million bacterial fragments, FPKM) to AMU (Treatment Incidence per Defined Daily Dose, TIDDDvet) per country and subsequently across all countries. In a similar way, the association between biosecurity status (Biocheck.UGent) and the resistome was explored.

Results: Sixty-six (38%) flocks did not report group treatments but showed a similar resistome composition and roughly similar ARG levels as AM-treated flocks. Nevertheless, we found significant positive associations between beta-lactam, tetracycline, macrolide and lincosamide, trimethoprim and aminoglycoside antimicrobial flock treatments and ARG clusters conferring resistance to the same class. Similar associations were found with purchased products. In a gene level analysis for beta-lactams and MLS, a significant positive association was found with the most abundant gene clusters *bla*_{TEM} and *ermB*. Little evidence was found for associations with biosecurity.

Conclusions: The faecal microbiome in European broilers contains a high diversity of ARGs, even in the absence of current antimicrobial selection pressure. Despite this, the relative abundance of genes and composition of the resistome is positively related to AMU in European broiler farms for several antimicrobial classes.

✓ **Value of farm-level interventions**

On-farm antimicrobial usage results of the complex interaction of multiple factors leading to a balance between animal diseases, health and welfare. The Veterinarian is the main actor in a good antimicrobial stewardship based on its knowledge of the animal diseases, epidemiological context of

their emergence and the influence of different technical parameters as risk factors. In the project, we defined farm-level intervention as any veterinary coaching done by a veterinarian to mitigate the on-farm use of antimicrobials.

To study the effect of intervention to reduce antimicrobial use in broiler and pig production, two longitudinal epidemiological studies were performed to assess the impact of intervention actions on antimicrobial usage, resistance and zootechnical performance. In both studies, the intervention was tailored to each local farm situation assessed by the veterinarians involved, who proposed a series of actions to participating farmers, who decided whether or not to apply them. The veterinarians regularly reviewed their implementation and adapted action plans throughout the study. For both species, retrospective data were collected for at least one year before the intervention and prospective data collection was implemented. The study designs were a multiple before-after control-intervention and a stepped wedge design respectively in broiler and farrow-to-finish pig production. In both studies, faecal material was sampled twice to measure the effect of interventions on antimicrobial resistance.

In three countries (Belgium, France and Spain), 109 broiler farms were recruited by 25 veterinarians and followed between mid-2015 and the end of 2017. The action plans collected concern a diversity of actions dedicated to farm management (climate, feed, water, batch), disease management (diagnosis, treatment, stewardship, biosecurity) and farmers (training, scoring and monitoring). Antimicrobial use declined significantly in control and intervention farms over time. Intervention had a significant additional effect on the reduction of antimicrobial use. No effect was observed on the main zootechnical parameters (mortality rate, average daily gain, feed conversion rate) while a reduction in antimicrobial resistance was observed over time for ampicillin, gentamicin, ciprofloxacin, sulfamethoxazole and tetracycline. Intervention effects were found to be significant for the latter three.

In two countries (France and the Netherlands), 59 farrow-to-finish pig farms were recruited and monitored by 22 veterinarians. Fifty farms were included in the antimicrobial usage analysis. The action plans contained four domains (disease management, drug stewardship, farm management, farmer knowledge) and covered different stages (sows and gilts, suckling piglets, post-weaning pigs, fatteners). Most of the proposed actions concerned sows and gilts, piglets and post-weaning pigs. A significant reduction in antimicrobial usage was observed over time and after the implementation of action plans. No intervention-, time- or country-related fixed effect was found to have a significant effect on the mortality rates recorded either in weaners or fatteners. Unfortunately, the difference in the recording systems for animal production parameters between the two countries impaired our capacity to analyse other zootechnical data. Antimicrobial resistance did not change significantly between the two sampling times.

The two studies have shown that antimicrobial usage is affected by the tailored intervention proposed and followed up by veterinarians without impairing the zootechnical indicators monitored. An overall reduction in antimicrobial usage was observed on all farms throughout the study period. For further studies, the implementation of information tools to collect, share and analyse the data between the different stakeholders should improve these tailor-made interventions. The development of a time series design and data analysis should also be promoted to extract new knowledge from each individual case.

Regarding the economic value of AMU, research showed that broiler production with low AMU without compromising the farms' economic performance is possible. This offers prospects for reducing AMU on a large (e.g. sector-wide) scale. However, it was also observed that there exists a large variety in both technical performance and economic performance. Moreover, it seems likely that high farm performance associated with high AMU can have at least two explanations: either overuse of AM because of farmer related aspects (e.g. risk-attitude) or required high AMU to compensate for management deficiencies. In the first case, interventions should be primarily directed towards the farmer him/herself (e.g. persuasive interventions), whereas in the latter case, farm set-up and biosecurity are prime focuses of interventions. These different intervention approaches of course have different economic (i.e. cost) implications.

Regarding economic impacts of intervention, the results were ambivalent: for some farmers economic performance and/or AMU increased respectively was reduced; hence, this could be regarded as a positive effect. Other farms however showed a contradictory effect: reduced economic performance and/or increased AMU. The specific causes of these observations could not be explained by the study.

The studies discussed above were focused at individual farmers, where the veterinarian tried to help the farmer with reducing AMU. Hence, farm-specific issues, such as farm and production context, were dominant. Other issues, such as collective or national actions were considered nevertheless in this study for their effect on control and intervention farms. Besides farm-specific issues affecting AMU, also other phenomena can be of importance. For instance, with regard to broiler production, recently slower growing breeds have been introduced in e.g. The Netherlands or new marketing rules have been proposed such as additional conditions on animal welfare in e.g. France. Few cases were reported in the farms monitored in our study. The countrywide introduction of new marketing concepts based on these slower growing animals coincided with a reduction in average AMU. These examples show that a broader approach of aiming to reduce AMU than just animal-disease related issues can offer prospects for the future.

(d) *Risk and risk management*

✓ **Complex linkages: Analysis of AMR patterns in humans and the environment**

The faecal resistome and bacteriome composition differs between & within occupationally exposed human populations ranging from primary production and slaughterhouse workers. At least part of these differences are animal exposure related. Initial analyses suggest that the human faecal bacteriome and resistome are interrelated.

✓ **Quantifying exposure to AMR: transmission routes and risk for public health**

The transmission of AMR determinants (or AMR genes (ARGs)) between animals and humans was explored in the project in different forms, using either qPCR measurements of two specific ARGs – tetW and ermB – or the relative occurrence of known acquired ARGs in the gut microbiota (resistome) determined by shotgun metagenomic sequencing. qPCR results were obtained both for food samples collected at retail and for human, environmental and animal samples collected in broiler and pig farms and slaughterhouses. Metagenomic data was obtained for all (pooled) animal samples collected at the farms and for some human samples collected in farms and slaughterhouses.

Human exposure to the ARGs tetW and ermB was assessed both for the transmission routes of food consumption and occupational exposure. Association between human and animal resistomes was determined at the reservoir (farm) level.

The results achieved were not absolute quantifications of human exposure, but relative: 1) relative consumer exposure through different food types, 2) relative human exposure from different slaughterhouse work areas, and 3) relative overlap between species-specific resistome markers.

Transmission through food consumption: A stochastic comparative exposure model was developed to estimate the relative potential exposure of consumers to the two ARGs tetW and ermB through consumption of different foods, including all food products that were sampled at retail level in the project. To our knowledge, this is the first study that assessed the relative consumer exposure to ARGs through retail food products across several (9) EU countries. Both tetW and ermB were more prevalent and abundant in chicken and turkey meat than in other food types. Accordingly, all countries showed higher relative human exposure through chicken than through pork. The within-country ranking of different food products was overall similar between countries, but the magnitude of the difference in exposure through different products varied. In the countries, where turkey was sampled, relative exposure followed that from chicken. Veal only relatively contributed to exposure in one of the countries where it was sampled, and trout had a negligible relative contribution to exposure in all three sampled countries. In a farm-to-fork approach to exposure assessment, steps downstream from retail such as home transport, home storage and cooking will also have an impact on the load of AMR bacteria to which consumers are exposed, therefore the results in this study do not represent estimates of the actual exposure, but a proxy estimate of relative potential exposure, should risky circumstances occur prior to consumption. In order to model real exposure to AMR determinants, several parameters describing any molecular changes in AMR bacteria (such as horizontal gene transfer, mutations, gene expression and death) throughout the food chain would be necessary. To our knowledge, such parameters have not been determined yet. Additionally, the multiple import origin of foods, as the one observed for turkey supply among the sampled countries, may have a

potentially large impact on the exposure of consumers to foodborne AMR bacteria. To determine a population's exposure to ARGs, it is important to consider both the place of food production and the place of food consumption.

Transmission through occupational exposure: qPCR measurements of *tetW* and *ermB* within broiler slaughterhouses allowed to investigate associations between the abundance of the two genes in workers and variables characterizing the work environment, using multivariable regression analysis. The response variable was the normalized quantity of the target gene carried by workers, and candidate predictor variables included all variables that characterized the individual work environment and practices: slaughter-line work position, sources of contamination the worker was exposed to (i.e. non-human sample types: animal faeces, gloves, carcasses, meat), use of knife during work, frequency of handwashing, frequency of changing gloves, use of protection equipment (i.e. apron, gloves, mask, cutting gloves) and number of work hours. Overall, both regression analyses showed a significant decrease in ARG carriage by the workers from the dirty area (hanging, bleeding, defeathering) to the clean area (evisceration and cutting) of the slaughter line (a comparable significant effect was also observed in a preliminary similar analysis for pig slaughterhouses). Additionally, human *tetW* carriage was lower in the cutting area than in the evisceration area. Among other tested predictors, the use of an apron showed a significantly lower carriage of both genes, although this effect was partially masked by the specific work area. Carriage of *ermB*, additionally, depended on the number of work hours (higher carriage with longer work weeks), the use of knife (higher carriage with knife use) and the frequency of changing gloves (higher carriage with higher daily frequency). All of these effects were also partially related to the worker's position in the slaughter line. In conclusion, there was a clear association between the work position along the slaughter line, and consequently the level of AMR contamination the worker is exposed to, and the worker's carriage of these two ARGs in the gut. These findings highly suggest the occurrence of occupational exposure to ARGs. Additionally, depending on the ARG analysed, associations with different characteristics of the work environment were observed. ARG-specific differences in associations may be due to factors related to the background of the worker and not to the work environment. Further studies are needed to investigate this hypothesis.

The impact of farm interventions on human exposure: An association study between antimicrobial use (AMU) and AMR in broiler farms within EFFORT showed a positive relationship between the lifetime macrolide, lincosamide and streptogramin (MLS) use and the relative abundance of *ermB*. Even though a direct link cannot be established between this study and studies 1 and 2 described above, their integrated interpretation suggests that a lower AMU in broiler farms would probably lead to a reduction in the presence of *ermB* in the animals' guts community and consequently to a lower exposure of both slaughterhouse workers and consumers to this particular ARG.

Reservoir-level, resistome-based source-attribution of human AMR: The metagenomic results from farms of different food-animal species were applied in a supervised machine-learning approach to investigate a country-independent cluster of AMR genes that allows to predict the origin of a sample based on its resistome. We identified a cluster of ARGs that allowed distinguishing between four different animal reservoirs (pig, broiler, veal, turkey), independently of the country of origin. Based on these selected resistome markers, preliminary results show some association between the resistome of humans and pigs, for humans with occupational exposure to pigs. We conclude, therefore, that this methodology is a significant step forward to define a potential predictor variable for the development

of a future source-attribution model, to attribute human resistomes to different reservoirs, in a probabilistic manner.

The joint analysis of the EFFORT results on the assessment of human exposure to AMR determinants by different transmission routes confirms the existence of transmission from food animals to humans. The relationship between AMR in animals (or animal-related sources) and humans in the context of occupational exposure is evident, whereas the magnitude of the real contribution of transmission through food consumption needs further investigation.

✓ **Management of resistance risk in food**

The availability of antibiotics to treat infectious diseases in both humans and animals has radically improved the health of both. However, paradoxically, the successful application of antibiotics for clinical and/or agricultural purposes threatens their long term use, particularly given the emergence of antibiotic resistance among microorganisms impacting animal health.

Food processing and preservation methods are applied to ensure the safety and/or extend the shelf life of foodstuffs. Antibiotic resistant foodborne pathogens may be considered a subset of foodborne pathogens and therefore it may be assumed that biophysical parameters designed to mitigate the contamination of food with pathogens should also ensure the absence of antibiotic resistant pathogens. However, there is limited scientific data to verify this other than HACCP- based systems, which require the implementation of validated food pathogen inactivation step(s). Scientific evidence was sought for differences in antibiotic resistant pathogen phenotypes behaving differently from antibiotic-sensitive food pathogens when subjected to various biophysical processes. A wide variety of processes were considered, including thermal, non-thermal, preservation, multi-hurdle approaches and treatments which may be considered minimal processing. Although, there are several publications investigating the effects on bacterial resistance of these industry processes, data in relation to their influence/impact on antibiotic resistance transfer or susceptibility is lacking.

The majority of the studies (with evidence available) investigating this phenomenon were performed at laboratory-scale and hence may be considered as 'low strength studies', as previously defined in EFSA report. We have shown that several processed, included in the low processing methods used today, enhance transfer of antimicrobial resistance genes between bacteria. Specially, increase in 1% NaCl and temperature shifts as subtle as 5 degrees lead to logarithmic transfer of plasmids of the IncL, IncF and IncK2 families, respectively. Thus, transfer of AMR genes should be taken into consideration when applying these apparently minor changes in the food chain.

Good Manufacturing Practices and Good Hygiene Practices are indispensable to the production of safe food. Key parameters in these practices include equipment, hygienic design, facility zoning, cleaning and sanitation. Cleaning /sanitation regimes and the use of biocides have received considerable attention in the literature, with a number of studies (laboratory scale and mostly rated of 'low strength') demonstrating reduced susceptibility to antibiotics after application of these processes. There is no common antibiotic substance group that always showed the same results on resistance in tested strains.

Conjugation of AMR genes can be hindered *in vivo*, using novel fatty-avoid derivatives in the gastrointestinal tract of mammal and fish. This opens the avenue to the development of further molecules that can ultimately be used in food as a food additive to decrease spread of AMR genes.

(e) *Future surveillance*

In general, surveillance is conducted for the purpose to inform decision makers on emergence of and trends in resistance and AMU. Moreover, data from the surveillance is used to evaluate the effectiveness, progress and general suitability of interventions. Regarding antimicrobial resistance, decisions are targeted at the reduction of resistance in relevant bacteria. As second component to be considered are decisions targeted at reducing or optimising the use of antimicrobials in animals (and people) as the AMU is biggest driver for the selection of AMR. EFFORT aimed to inform surveillance decisions for both components.

Starting with surveillance of usage, the EFFORT project developed and implemented a harmonised method for data collection at farm level and analysis of these data across several countries in Europe. Although there are a number of guidelines and recommendations on these activities issued by organisations such as the FAO, WHO and the OIE, EFFORT achieved a milestone in implementing such guidelines at farm level across countries in Europe. Based on the findings of the EFFORT project, a number of recommendations can be made for the collection and analysis of usage data. First and foremost, this activity is technically demanding. In the EFFORT project, substantial resources were needed to implement a harmonised process and to manage the usage data. A standardised approach for the measurement units in which usage is recorded is of crucial importance. Although many technical usage can be converted into each other, a minimum set of harmonised variables is needed. During the EFFORT project, a number of European countries are introducing or have introduced databases for capturing usage in food animals at animal species level. Such tools should definitely facilitate data capture and subsequent analysis. It was also remarked that countries that have already experience with herd level AMU data collection systems can generally produce better data, indicating that there is a clear increase in experience in the data collection process.

The data showed a huge variability in both in the total AMU as well as the different types of antimicrobials used in the farms included in the EFFORT sample. This variability was present both within and between countries. Some of the variation can be explained, but other aspects are very difficult to rationalise. It is therefore recommended to revisit prudent use principles and increase efforts to implement prudent usage principles usage best available and appropriate evidence in each country and livestock species. While a variability of the health status between national populations can be expected, first choice treatment options for indications should be compared and aligned where possible. Optimisation is also possible regarding choices of specific products, dosages and routes of administration. Differences in the licensing of the same substance for the same species and identical indication should ideally be challenged and corrected.

In Europe, there is currently a range of usage indicators and targets used. Some countries have defined reduction targets, often measured in %, which are either applied across species or animal species specific. The reduction targets mainly concern all antibiotic substance categories but sometimes are also specifically targeting specific molecule. Other countries apply more weight to substances that are of critical importance. Also, reduction targets can be based on rolling averages or on fixed values for a

certain period. EFFORT did not specifically evaluate this aspect of risk management. However, in the final EFFORT conference, this topic was debated and the motivational value of using reduction targets was clearly recognized. At the same time a call for evidence-based reduction targets was voiced.

Regarding the monitoring of resistance, the number of available microbiological approaches is increasing. Three broad categories were considered within EFFORT, namely phenotypic resistance measures (MIC) – the current internationally used reference method – as well as whole genome sequencing (WGS) and metagenomics (MG). Based on EFFORT results, we concluded that there is high agreement between MIC and WGS. As the latter offers additional advantages in terms of epidemiological information, this is an attractive alternative. Also, when using WGS pathogen detection, molecular sub-typing, virulence and resistance testing can be conducted in the same step thus increasing efficiency. However, protocols and interpretation need further harmonisation and sufficient laboratory performance standards need to be assured. A single curated reference database should be agreed.

Regarding the use of WGS in the context of surveillance, EFFORT provided new and important progress highlighting both the power of this approach as well as its limitations. Although WGS provides an unbiased account of the diversity of genes present in an isolate and would as such directly be applicable in a multi-country surveillance context. The approach is currently not fully standardised for metagenomic analysis and MG-results therefore need to be compared and analysed with caution. This is a limitation for MG-surveillance conducted in an international context. An important issue is the low sensitivity of MG for rare or less commonly occurring genes and for matrices with low gene concentrations, such as food products. Additional challenges exist in the analysis of the highly complex data produced by MG and the need for technical experts to analyse and interpret such data. Finally, the communication of the resulting data is a further challenge and the link between gene presence and public or animal health risk yet needs to be established. Specifically, for AMR, some findings in the EFFORT data are difficult to interpret. For example, a very strong country effect was detected, the drivers of which can lie in the production pyramid, animal trade, animal feeding, general hygiene and environmental differences. More work is needed to fully understand such differences in the resistome.

The challenges extend also to aspects of data ownership, costs involved (particularly for MG) and the lack of a regulatory basis for using WGS. Still, the EFFORT consortium advocates the implementation of WGS of isolates in the near future. However, it also concludes that the strength of MG is currently in a research-context and more work is needed before it can be integrated in routine surveillance.

D. Potential Impact

Socio-economic impact and the wider societal implications of the project

(a) *Contribution to Community and social objectives*

The community and social objectives of the European Union relate to the general functioning of the common market, sustainable development, economic growth and general competitiveness in the global context. Employment and social progress as well as the elimination of inequality are also relevant.

Antimicrobial resistance (AMR) is one of the key challenges identified and prioritised by the United Nations. It is relevant to five of the UN Millenium Development Goals, namely reduction of child morbidity, improvement of maternal health, combating infectious diseases, ensuring environmental sustainability and developing global partnerships for development (Asokan & Kasimanickam, 2013). The EFFORT project focused on the food chain and therefore prioritised the goals related to infectious disease prevention and public health in general, but also to sustainability.

Food production and trade is an economically important sector in Europe. As intra-community trade is unrestricted, biological hazards such as AMR play an important role in assuring the undisrupted supply of sufficient and high-quality food, including animal-derived food. Under World Trade Organisation principles, and specifically under Codex Alimentarius standards, AMR is currently not generally accepted as a trade-limiting hazard. It is however considered in the context of general principles of food production, and several documents are currently being developed in the international community. Although AMR is currently not commonly accepted as a hazard relevant to trade, individual European countries have used sanitary arguments in the context of AMR to restrict trade of animal-derived food containing specific bacteria and/or resistance determinants. In this context, EFFORT has contributed to the joint development of methods and technologies used for establishing the extent of AMU and AMR across Member States. This provides an important platform for establishing the presence of this hazard in the EU and to compare like-for-like across trade partners. It also helps harmonising the relevant standards and approaches and to establish a joint understanding of the risk and suitable risk management measures.

Regarding the economics of AMR, EFFORT results have specifically contributed to the development of farm-level interventions. Based on the studies conducted as part of WP6 and WP8, it was shown that measures to reduce AMU can be effective as well as economically attractive. This is a key message for the agricultural industry who may be reluctant to abandon a tool that they have perceived as essential for animal health in the past. Farms have, however, unique combinations of technical performance and AMU and therefore require farm-specific strategies to successfully reduce AMU. Interventions should address aspects of the farm, the farmer and the animals in combination. The risk attitude of the farmer has also an important economic effect (damage abatement). WP8 showed that production system improvements can improve the damage abatement effect and reduce the need for AMU. EFFORT results therefore provide tangible results and objective arguments that will help change this perception and lead to a reduction of AMU across the EU.

(b) *Main dissemination activities and exploitation of results*

Main Dissemination activities

At the beginning of the EFFORT project, a comprehensive dissemination plan was developed. All activities listed there were fully implemented as planned. A key activity was the stakeholder mapping conducted at the very beginning of the project. This provided an important tool for all other activities. As originally planned, EFFORT applied a range of dissemination formats to reach the stakeholder groups. The key activities are highlighted below. Also, a poster was composed on all dissemination activities (see below).

Dissemination activities were supported by regular interaction with the EFFORT **Stakeholder Panel**. Panel members were recruited at the beginning of the project and included food and livestock industry representatives, members of consumer organisations, research and policy experts. Interaction with the stakeholder panel were conducted by email and via web conferencing. A key role of the stakeholder panel was also to provide feedback on results in the final phase of the project. The presentations for the final conference were reviewed by the stakeholder panel. Also, members of the policy session in the final conference were recruited from among the stakeholder panel.

Additional facilitation of dissemination activities was provided by member of the **External Advisory Board** of EFFORT. The board included members of key organizations like the World Health Organization (WHO), World Organisation for Animal Health (OIE), the Federation of Veterinarians in Europe (FVE) and the European Food Safety Association (EFSA), and two scientists who are experts in the field of AMR. In addition to their role of providing feedback to the scientific work, the board members also helped identify target groups and messages for dissemination. Selected members of the Scientific Advisory Group were featured in the newsletter (see below).

The EFFORT **web-site** was one of the key dissemination channels of both general information on the project as well as specific dissemination of outputs and results. The web site traffic was continuously monitored and reported at each management meeting and reaching >3000 visitors in the final year of the project alone. The web site had considerable global reach.

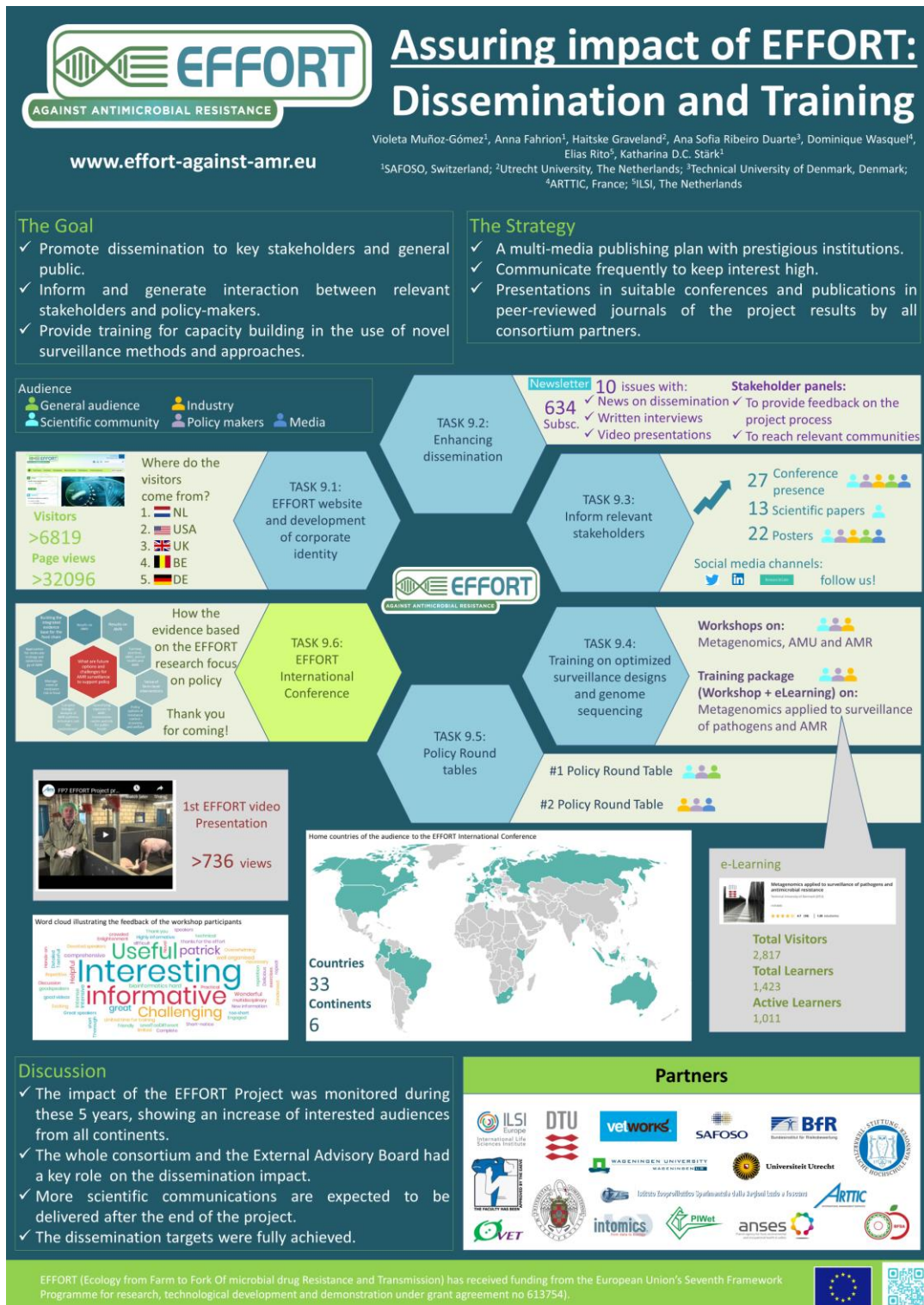
An important dissemination activity was the quarterly **newsletter** published by the EFFORT consortium. The newsletter contained regular updates on project activities. Each issue featured a specific work package. It was also used as a platform to promote contributions from junior scientists. To achieve that, each newsletter included a video of a researcher talking about his or her work in EFFORT. These videos were also distributed via the web-site and via YouTube channels. A total of ten newsletter issues were published. By the end of the project, the newsletter reached 634 subscribers per issue.

To reach a scientific audience, EFFORT partners presented results at a range of scientific meetings both within Europe as well as meetings with a global reach. With the increasing availability of outputs in the final phase of the project, also **conference presentations** increased. In addition, the project **roll-up banner** was used to increase EFFORT's visibility at meetings and conferences where EFFORT partners were present. The roll-up constantly travelled throughout the years and was present at >20 meetings.

To reach a less scientific audience, EFFORT attended industry and consumer-focused **meetings and conferences**. This was significantly facilitated by EFFORT partners ILSI and VION. Also, the final

conference was open to members of the public.

Another channel for frequent dissemination to scientific and non-scientific audiences was **Twitter and LinkedIn**. These accounts were actively managed through the dissemination team with contributions from all partners. A total of 219 followers and 273 group members was achieved, respectively. In the last phase of the project, an account on ResearchGate was added, which achieved 221 reads.



During the EFFORT project duration, **two policy round tables** were organised of which the first was virtual (online) and the second was integrated in the final conference (see below). The objective of these activities was to present EFFORT work and results to policy makers, particularly to inform on any findings relevant to standard development and future legislation. As risk managers, policy makers were a key target group of the project.

For specific dissemination of technical knowledge present in and generated by the EFFORT consortium, three **training events** were organised, two of which focused on antimicrobial usage (part of WP5) and one focused on resistance. While the former two were half-day events associated with professional meetings of relevant organisations (i.e. Society of Veterinary Preventive Medicine and European College of Veterinary Public Health), the latter was an independent event organised by EFFORT. It consisted of two components, an e-learning element and a physical classroom teaching element. The latter was important to obtain feedback also on the e-learning to improve it. The online training continues to be available on the Coursera platform. To date, >1,000 learners have actively participated in this training.

A highlight among dissemination activities was the **final EFFORT conference** held in Utrecht, November 26-28, 2018. The conference featured well-known external speakers from international organizations (i.e. FAO, WHO, OIE and EMA) as well as EFFORT researchers. An overview about antimicrobial stewardship in future animal production, current risk management tools and the use of the guidelines for the prudent use of antimicrobials in practice were provided. The EFFORT consortium presented the results of the project giving an outline of its research during the last five years. At the end of the conference, a policy round table which aimed to translate the EFFORT results into policy took place giving place to engaging discussions. Almost 250 participants from 34 countries registered for the conference.

Exploitation of results

At the start of the EFFORT project, partners and mainly SMEs foresaw that the project research activities could lead to products that could be marketed beyond the duration of the project. The following table provides here an overview of the exploitation results:

Partner	Planned use of project results	Exploitation results with possible estimated market potential (€)
SAFOSO	External use: Improved expertise in advising clients on risks related to antimicrobial resistance	<u>Exploitation result N°1</u> SAFOSO exploited EFFORT results mainly in a general way, integrating the knowledge from the research outputs in its advice provided to clients. The originally expected market potential of 100,000 € was reached by participation in other research consortia with a focus on antimicrobial resistance as well as provision of specialist advice to clients such as the Swiss Federal Administration and industry clients. SAFOSO AG has established itself as an expert in this field. Its staff members are being recruited into international expert committees. This expertise is also featured on SAFOSO's web site.
VION	Improve hygiene in the production by innovation	<u>Exploitation result N°2</u> Identification of the critical points for AMR contamination of carcasses (see also Exploitation N°3) led to the implementation of vacuum cleaning with steam of the carcasses after the critical step. Furthermore, an innovative disinfection method of knives based on Inस्पेक्स rather than 82°C water was implemented.
	Identify the critical points in the chain to reduce human exposure	<u>Exploitation result N°3</u> Critical steps for containing AMR along the slaughter lines were shown in EFFORT to be identical to other foodborne bacteria. As such, consequent and structural implementation of hygiene measures was stressed again with employee training and emphasized in all production facilities of Vion. The results are also communicated directly to stakeholders and through Vion's Corporate Social Responsibility report as part of increasing transparency in the food chain and further build confidence in food safety.
Intomics	Services around and commercialisation of the pipeline for analysis of genomics data from metagenomics samples Novel and improved algorithms for NGS data analysis will enhance internal data analysis capabilities	<u>Exploitation result N°4</u> Expected market potential: 0.1-5 million Euro in annual revenue metagenomic analyses and 0.2-2 million Euro in annual revenue from additional knowhow on and algorithms for NGS data.

Partner	Planned use of project results	Exploitation results with possible estimated market potential (€)
NVRI	Improvement of national antimicrobial resistance monitoring schemes and prudent use of antimicrobials	<u>Exploitation result N°5</u> Unestimated costs: safer, better food, animal welfare reduced public health implications of antimicrobial usage in animals.
ILSI	Promotion of EFFORT project and results to relevant stakeholders engaged in ILSI Europe committees.	<u>Exploitation result N°6</u> A better understanding of the current state of the art in AMR development, current challenges, and knowledge gaps about the consequences of AMR in the food chain will trigger new research projects and support risk managers in enhancing confidence in food safety.

(c) *Outlook and future research*

EFFORT realised the first multi-country harmonised data collection on AMU and AMR in livestock production chains and food. Despite the technical complexity of the implementation of such an activity, the resulting data set is unique and my no means completely exploited. Many more analyses are possible. We have already been contacted by a follow-up H2020 project OH-EJP. Although full release of the data is planned, certain restrictions are currently applied due to pending publications by the consortium.

The results revealed substantial differences between AMU between countries. The factors driving these differences could not be identified in this project because the number of farms per country was too limited. However, certain hypotheses can be formulated based both on the differences in the amount of AMU as well as in terms of the relative importance of substances used.

EFFORT has explored the use of genomic methodologies for AMR surveillance purposes and to quantify AMR in complex bacterial communities in gastro-intestinal tract and environment. For this aim EFFORT invested in standardisation that is crucial for sample collection and handling, DNA-extraction methods and NGS-methodologies including depth of sequencing required. This knowledge provided important new insights in AMR-research and is crucial for future research projects that study the epidemiology and evolution of AMR in a One-Health context. Moreover, EFFORT has demonstrated that complex genomic data can be used to quantify AMR at farm and country level, which supports the understanding of factors that determine difference in AMR levels.

The application of a metagenomic approach will be important in future research to understand the complex interactions between bacterial species, plasmids and other mobile genetic elements and resistance genes in bacterial communities in the gut or other niches that determine evolution and epidemiology of AMR.

In terms of farm-level interventions to reduce AMR, EFFORT confirmed that tailored interventions should be used that consider characteristics of the farm, the farmer and the animals themselves. Strategies based on the use of veterinary consulting and advise which is already used in several EU Member States were confirmed to be promising and economically attractive. The central role of motivation, technical knowledge and benchmarking should be central elements of effective reduction strategies.

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